PENERBITAN PEGAWAI

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Phebe Ding, S. H. Ahmad, Abd. R. Abd. Razak, N. Shaari and M. T. M. Mohamed

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Plastid ultrastructure, chlorophyll contents, and colour expression during ripening of Cavendish banana (*Musa acuminata* 'Williams') at 18°C and 27°C

PHEBE DING[1]
S. H. AHMAD[1]
ABD. R. ABD. RAZAK[1]
N. SAARI[2]
M. T. M. MOHAMED[1]
[1]Department of Crop Science
[2]Department of Food Science
Universiti Putra Malaysia
43400 UPM Serdang, Selangor, Malaysia

Abstract When Cavendish banana (*Musa acuminata* 'Williams') is ripened at tropical ambient temperature (27°C) the peel fails to degreen although the pulp has softened. However, the peel will degreen to a yellow colour when the fruit is ripened at 18°C. The inability of the peel to degreen could be because of the retention of thylakoid membranes in the plastids and chlorophyll during the ripening process. A study was carried out to investigate the relationships between plastid ultrastructure, chlorophyll content, and peel colour of Cavendish banana ripened at 18±2°C (C18) and 27±2°C (C27). The peel of Cavendish banana underwent complete degreening when the fruit was ripened at a temperature of 18°C to produce a yellow fruit at ripening stage (RS) 6, after 9 days of treatment. In contrast, bananas exposed to 27°C failed to degreen. By day 5 after ripening initiation, the pulp had softened to eating-ripe in those fruit and brown specks appeared on the fruit peel indicating that senescence had begun. Transmission electron microscopy revealed that the grana-thylakoid membranes of peel chromoplasts had lysed by RS 6 in C18 fruit and only 40% of the total chlorophyll content from RS 1 was retained. In contrast, the grana-thylakoid membranes in C27 at day 5 were retained, along with retention of 57% of total chlorophyll content. Total chlorophyll content of C27 fruit correlated significantly with L*, C*, and h° colour values. The higher percentage of total chlorophyll retained in C27 compared with C18 fruit did not fully unmask the existing peel carotenoids, thus producing a pale-green fruit.

Keywords banana degreening; chloroplast; grana; thylakoid membrane; chromoplast

INTRODUCTION

Colour is a primary component of fruit quality and quality is a major determinant of cash value (Tourjee et al. 1998). Degreening or yellowing of peel is the most distinct external symptom during banana ripening (Lizada et al. 1990) and it is a good external indicator of internal fruit ripeness (Li et al. 1997). The colour is an important characteristic used by growers, wholesalers, retailers, exporters, importers, and researchers to determine whether the fruit is ripe or unripe.

Most Malaysian bananas degreen naturally under tropical temperature of 27±2°C. However, Cavendish banana (*Musa acuminata* 'Williams'), which is the most commercially important banana in global trade, does not degreen naturally under such tropical temperatures although the pulp softens quickly. Temperate temperatures of 18–20°C are needed to induce normal degreening. Malaysia produces Cavendish bananas for export as well as for the local market. To degreen Cavendish bananas under Malaysian conditions, cool ripening rooms have to be built to provide the temperature of 18–20°C. This has resulted in increased production costs for smallholders who are the major banana producers in Malaysia. Without such cool treatment, there is poor demand for green-ripe banana since a yellow-ripe Cavendish banana is more appealing to consumers. In the light of these difficulties, smallholders prefer to plant traditional varieties of bananas which can degreen naturally under normal tropical conditions and temperature of 27°C, although Cavendish banana plants have more desirable production characteristics, such as bigger and heavier fruit bunches.

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The external colour of banana fruit depends upon the relative amounts of individual pigments present in the peel (Meddlicott et al. 1992). Green and yellow colorations are imparted by lipid-soluble plastid pigments, chlorophylls and carotenoids. Degreening in banana during ripening is a consequence of chlorophyll degradation, with little or no increase in the total carotenoid level (Seymour et al. 1987). Chlorophylls usually degrade to a colourless chlorophyll catabolite during fruit maturity and ripening, thus unmasking the yellow and orange colours of carotenoid pigments (Matile et al. 1999). The rate of chlorophyll degradation in the Cavendish banana is temperature dependent (Turner 1997). The degradation of chlorophyll is maximal at 22°C, whereas very little chlorophyll degradation occurs at temperatures below 15°C or above 24°C. Retention of non-degraded chlorophyll resulted in non-degreening of banana peel even though the pulp had softened and become edible. The failure to degreen could be the result of the retention of thylakoid membranes in the chloroplast which relatively delayed chlorophyll (Chl.) *b* breakdown (Blackbourn et al. 1990a). Information on plastid ultrastructures, chlorophyll degradation, and colour expression with respect to ripening stage and temperature response in Cavendish bananas is lacking. The objective of this study was to investigate the plastid ultrastructures in relation to chlorophyll degradation, colour expression, and stages of ripening in Cavendish bananas exposed to 18°C and 27°C.

MATERIALS AND METHODS

Mature green Cavendish bananas were obtained from a fruit distributor and transported to the laboratory. Five hands, each containing 30–36 fruits, with each fruit weighing 180–220 g, were placed in a box and gassed with acetylene from CaC_2 (with an equivalent of 10 g CaC_2 kg^{-1} fruit) at 18°C (±2°C) and 27°C (±2°C) for 24 h. Fruit that ripened at 18°C (C18) were assessed based on the following ripening stages (RS): 1 = mature green; 2 = tinge of yellow; 3 = more green than yellow; 4 = more yellow than green; 5 = yellow with green tips; and 6 = full yellow. The days to reach each RS by comparing to RS 1 was 0, 1.88, 3.67, 5.33, 7.07, and 8.92 days. Fruit ripened at 27°C (C27) were assessed from day 1 to day 5 after ripening initiation, since the peel of the fruit did not degreen completely. For study of plastid ultrastructure, each treatment consisted of one fruit and was replicated 3 times. For study of peel chlorophyll contents and colour, five fruit were used for each replicate and the experiments were replicated 4 times.

Study of plastid ultrastructure

For ultrastructural studies, peel tissue measuring 5 mm × 5 mm × 5 mm at RS 1, 3, and 6 (C18) and at days 2 and 5 after ripening initiation (C27), were cut from the mid region of the fruit, fixed in Karnovsky's fixative (Karnovsky 1965) and postfixed with 1% osmium tetroxide in 0.1*M* phosphate buffer (pH 6.8) for 2 h at 25°C. The tissues were dehydrated through a graded series of ethanol, then infiltrated and embedded in Agar resin. Ultrathin-sections (0.06–0.09 μm) of the peel were mounted on 200 mesh copper grids, stained with lead citrate and uranyl acetate and viewed under a LEO 912AB Energy Filter Transmission Electron Microscope (TEM) (Germany).

The dimension of 10 plastids from three fruit of RS 1, 3, and 6 (C18) and day 2 and 5 after the ripening initiation (C27) was determined based on the micrograph. The longest distance and the broadest dimension indicated the length and width, respectively, of the plastid. The length of grana and number of thylakoid/stack of a granum were recorded in 10 plastids from three fruit as above.

Determination of peel chlorophyll contents

Fruit peels were sampled from each treatment at every RS (C18) and day (C27). Chl. *a*, Chl. *b*, and total chlorophyll contents were determined by the method of Arnon (1949).

Determination of peel colour

Peel colour was determined at every RS (C18) and day (C27) using Minolta CR-300 Chroma Meter (Minolta Corp., Japan) using the Illuminate C (Commission Internationale de l'Eclairage (CIE) 1976) and results were expressed as lightness (L*), chroma (C*), and hue (h°). The L* coordinate indicated the lightness of colour with values ranging from 0 = black to 100 = white. C*, which refers to the vividness of colour, was computed from values of a* and b* i.e., $C^* = (a^{*2} + b^{*2})^{1/2}$ which represented the hypotenuse of a right triangle with values ranging from 0 = least intense to 60 = most intense. h°, referred to as colour, was the angle of tangent^{-1} b*/a* where 0° = red purple, 90° = yellow, 180° = bluish-green, and 270° = blue. Measurements at the stem end, mid region, and floral end of each face of the peel were made and a mean value was obtained from five fruit per replicate.

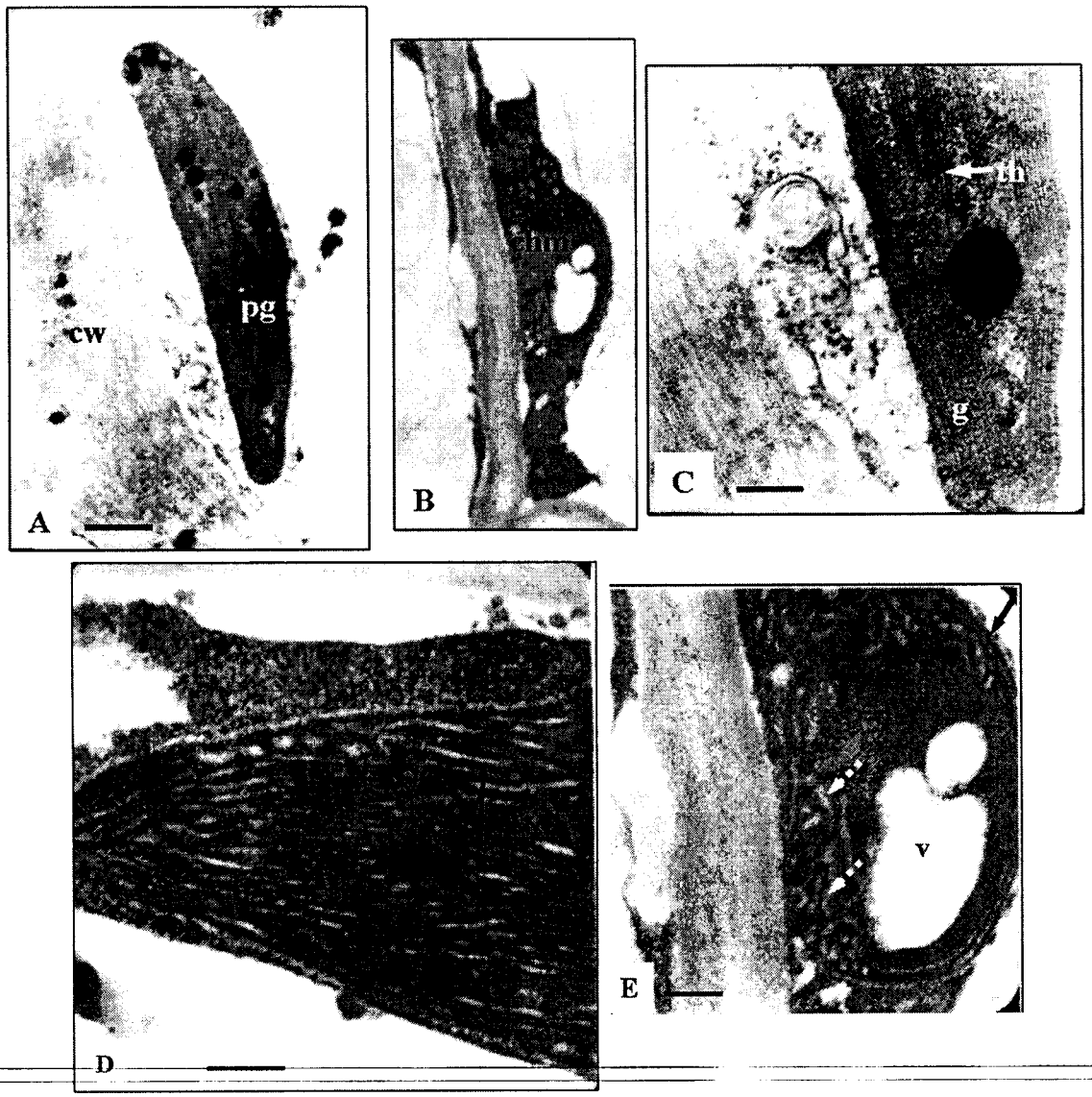

Fig. 1 Transmission electron micrograph of a Cavendish banana (*Musa acuminata* 'Williams') plastid ripened at 18°C. **A**, Ripening stage 1 chloroplast near to cell wall (cw) with randomly distributed plastoglobuli (pg). Bar = 0.6 μm. **B**, Ripening stage 6 chromoplast (chm). Shape of the plastid has transformed into an oval. Bar = 0.88 μm. **C**, Ripening stage 1 chloroplast showing tripartite structure of thylakoid (th) and granum (g) membranes. Bar = 0.17 μm. **D**, Ripening stage 3 chloroplast showing large perforation among thylakoids (single arrow) and tiny perforation among grana. Bar = 0.2 μm. **E**, Ripening stage 6 chromoplast showing disintegrated thylakoid-granal system. Granal-thylakoid is hardly seen and only short perforated thylakoids (dashed arrow) are noticed. Large vesicles (v) occupied much space.

Statistical analysis

The experimental design was a randomised complete block design with four replications of five fruit per replicate except for the study of plastid ultrastructure. The dimensions of plastids and grana were analysed statistically and standard deviations determined. The SAS ANOVA procedure was used for peel chlorophyll contents and colour (SAS 1988), and the mean separation was analysed by Duncan's multiple range test. Correlation analysis by means of Pearson's correlation matrix was performed to establish the association between peel chlorophyll contents and CIE colour values.

RESULTS

The chloroplasts of RS 1 C18 before ripening were disc shaped (Fig. 1A) with mean size of 5.21 μm long and 1.07 μm wide (Table 1). As ripening progressed to RS 3, the chloroplasts of yellow-green C18 remained discoid in shape but the dimensions were slightly shorter and broader than those of RS 1 (Table 1), indicating a tendency towards a more spherical shape. At full yellow stage (RS 6), the chloroplasts became spherical (Fig. 1B, Table 1).

The thylakoids and grana lamellae filled up the stroma of chloroplasts of RS1 at C18. At greater magnification, the granal lamellae of RS 1 were clearly seen as tripartite structure (Fig. 1C). A granum stack consisted of eight thylakoid membranes and the mean length of each granum was 0.649 μm (Table 2). As ripening advanced to RS 3, the thylakoids and granum of chloroplasts started to disintegrate, seen by the occurrence of perforation in thylakoids and grana (Fig. 1D). The mean length of grana decreased by 25%, but the number of thylakoids/stack of granum increased by 75% as compared to RS 1 (Table 2). When the fruit reached RS 6, the granal-thylakoid lamellae had completely disintegrated and lysed, and were replaced by single and short perforated lamellae (Fig. 1E). These perforated and short lamellae were pushed to the periphery of chromoplasts by plastoglobuli.

The chloroplasts of C27 at day 2 after ripening initiation were elongated and swollen at one end (Fig. 2A). The mean length and width of chloroplasts was 3.22 μm × 1.17 μm (Table 1). After 2 days of ripening initiation, the thylakoids and grana started to disintegrate and were pushed to the periphery as a result of the existence of plastoglobuli at the centre of stroma. In Fig. 2B it was clear that the short and perforated thylakoids were distributed randomly in the stroma of chloroplast. A thin layer of cytoplasm surrounded the chloroplast, which was only observed in RS 3 and 6 of C18. This showed that 2 days after ripening initiation, the cells of C27 had started to undergo senescence. A stack of granum in the chloroplasts of C27 at day 2 after ripening initiation consisted of six thylakoids and the mean length was 0.319 μm (Table 2).

At day 5, the chloroplasts of C27 became shorter and rounder as compared to day 2. The thylakoids and grana lamellae of C27 chloroplasts were pushed aside. This was the result of filling of plastoglobuli or crystals in the centre (Fig. 2C). A granum of C27 chloroplasts at day 5 consisted of four thylakoids/stack with mean length of 0.396 μm, whereas in comparable C18 RS 6 plastids no granal-thylakoid stack was detected (Table 2). Electron microscopy revealed the persistence of the granal-thylakoid stack which could explain the failure of Cavendish bananas to degreen at tropical temperatures of 27°C.

Table 1 Plastid dimension of Cavendish bananas (*Musa acuminata* 'Williams') ripened at 18°C or 27°C. Dimensions were measured at ripening stages (RS) 1, 3, and 6 for banana ripened at 18°C whereas for those ripened at 27°C, were measured at day 2 and 5 after ripening initiation. Values are means of three fruits with ± standard deviation (n = 10).

	Ripening temperature				
		18°C		27°C	
	RS 1	RS 3	RS 6	Day 2	Day 5
Length (μm) ± SD	5.21±0.55	3.54±0.77	2.58±0.83	3.22±0.62	3.07±0.93
Width (μm) ± SD	1.07±0.03	1.36±0.35	1.64±0.51	1.17±0.29	1.32±0.34

Table 2 Length of grana and number of thylakoids/stack of a granum in 10 plastids of Cavendish bananas (*Musa acuminata* 'Williams') ripened at 18°C or 27°C. Grana length and number of thylakoids/stack were measured at ripening stages (RS) 1, 3, and 6 for banana ripened at 18°C whereas for those ripened at 27°C, chloroplast dimensions were measured at day 2 and 5 after ripening initiation. Values are means of three fruits with ± standard deviation (n=10).

	Ripening temperature				
		18°C		27°C	
	RS 1	RS 3	RS 6	Day 2	Day 5
Granum length (μm) ± SD	0.649±0.13	0.486±0.11	–*	0.319±0.08	0.396±0.13
No. of thylakoids/stack	8	14	–	6	4

*Grana could not be detected.

Both C18 and C27 fruit showed significant decreases in Chl. *a*, Chl. *b*, and total chlorophyll as ripening progressed (Table 3). During ripening, fruit of C18 took c. 9 days to ripen until RS 6. Fruit of C27 could be stored for only 5 days after ripening before senescence began. The total chlorophyll content of C18 reduced by 60% by RS 6. In C27, the total chlorophyll content was reduced by 43% in 5 days. In general, the rates of total chlorophyll breakdown over the ripening period were 0.101 and 0.132 μg g^{-1} FW h^{-1} in C18 and C27, respectively.

The trend of reduction in Chl. *a* and Chl. *b* was consistent with that of total chlorophyll (Table 3). However, in C18, Chl. *a* was reduced to 67% as the fruit ripened to RS 6 in 9 days. Chl. *a* content of C27 was reduced by 45% after 5 days of ripening initiation. Chl. *a* was broken down at the rate of 0.046 and 0.056 μg g^{-1} FW h^{-1} in C18 and C27, respectively. Evidently, the breakdown period was longer in C18 (214 h) compared with C27 (120 h). The reduction in C18 Chl. *b* was very slow, occurring over a ripening period of 9 days, with 54% of the chlorophyll being reduced by RS 6. The reduction of Chl. *b* in C27 was 42% after 5 days of ripening initiation. The rates of Chl. *b* reduction were 0.052 and 0.076 μg g^{-1} FW h^{-1} in C18 and C27, respectively.

The CIE colour values of banana peels are presented in Table 4. The increase in L* of C18 peel was significant only until RS 3, and after that there was no significant increase in L*. For the peel of C27, the L* increased significantly each day after ripening initiation as the ripening progressed from day 0 to 5. Evidently, the pattern of changes in L* during ripening was different in each of the ripening temperatures studied. The peel C* values of C18 also increased significantly but only by 29% as the fruit ripened from stage 1 to 6, correlating with the more intense colour seen, whereas for C27 the peel C* increased by only 19% as the ripening progressed from 0 to 5 days after the ripening initiation. The increase of C* values by 29% in C18 indicated that the peel turned to a more vivid colour as the ripening progressed compared with an increase of 19% C* values in C27 peel. The peel h^{o} value of C18 decreased significantly at each RS, resulting in a change of peel colour from green to yellow. The h^{o} value of C18 was reduced by 23% in RS 6 compared with RS 1. However, the reduction in h^{o} of C27 peel was only 15%. Since the h^{o} values of C18 peel was 92°, the peel of these banana were perceived as yellow whereas C27 was still in the green colour range with h^{o} value of 102.58°.

Table 5 shows the correlation between chlorophyll and colour values of C18 and C27. Chl. *a* of C18 correlated significantly with Chl. *b* and total chlorophyll. However, there was no significant correlation between the chlorophyll and

Table 3 Effects of ripening temperature on chlorophyll (Chl.) *a*, Chl. *b*, and total chlorophyll of Cavendish bananas (*Musa acuminata* 'Williams') ripened at 18°C or 27°C. Banana ripened at 18°C were evaluated for six stages of ripening whereas for those ripened at 27°C were evaluated for 6 days after ripening initiation. (Mean separation in columns within banana cultivars and ripening temperatures followed by the same letter are not significantly different by Duncan's multiple range test, $P < 0.05$.) (FW, fresh weight.)

		Chl. *a* (μg g^{-1} FW)	Chl. *b* (μg g^{-1} FW)	Total chl. (μg g^{-1} FW)
Cavendish banana ripened at 18°C				
Ripening stage	Days to reach each ripening stage			
1	0	14.67 a	21.81 a	36.48 a
2	1.88	13.31 b	19.20 b	32.49 b
3	3.67	10.67 c	16.74 c	27.40 c
4	5.33	7.35 d	12.88 d	20.21 d
5	7.07	5.41 e	10.88 e	16.28 e
6	8.92	4.78 e	9.97 e	14.74 e
Cavendish banana ripened at 27°C				
	Days after ripening initiation			
	0	14.75 a	21.95 a	36.69 a
	1	14.16 ab	18.78 b	32.92 ab
	2	12.56 bc	17.38 bc	29.93 bc
	3	10.75 cd	15.92 cd	26.67 cd
	4	9.01 de	13.63 de	22.63 de
	5	8.08 e	12.81 e	20.87 e

the CIE colour values. The Chl. *a* of C27 correlated significantly with Chl. *b*, total chlorophyll, L*, C*, and h°. Chl. *b* correlated negatively with L* and C* values, but positively with h° values. A similar finding was also found in total chlorophyll.

DISCUSSION

Similar observation of plastids underwent a chloroplast-to-chromoplast transition that involved the degradation of thylakoid membranes and the accumulation of plastoglobuli was also reported in Cavendish banana peel during ripening at 20°C (Blackbourn et al. 1990a) and wild-type tomato fruit (Barr et al. 2004). Plastoglobuli were believed to contain the lipids released from the degenerating thylakoid membranes (Barton 1966) and associated with carotenoid pool (xantophylls and carotenol fatty acid esters) (Merzlyak & Solovchenko 2002). It was suggested that chlorophyll degrading enzymes localised at the inner envelope of chloroplast

Table 4 Effects of ripening temperature on peel colours (L*, C*, and h°) of Cavendish banana (*Musa acuminata* 'Williams') ripened at 18°C or 27°C. Bananas ripened at 18°C were evaluated for six stages of ripening whereas for those ripened at 27°C were evaluated for 6 days after ripening initiation. (Mean separation in columns within banana cultivars and ripening temperatures followed by the same letter are not significantly different by Duncan's multiple range test, $P < 0.05$.)

		Peel colour		
		L*	C*	h°
Cavendish banana ripened at 18°C				
Ripening stage	Days to reach each ripening stage			
1	0	55.00 d	35.78 e	120.31 a
2	1.88	61.80 c	39.48 d	111.73 b
3	3.67	67.43 b	41.66 c	104.38 c
4	5.33	68.58 ab	42.96 c	99.66 d
5	7.07	69.17 ab	45.03 b	94.64 e
6	8.92	70.50 a	47.48 a	92.48 e
Cavendish banana ripened at 27°C				
	Days after ripening initiation			
	0	52.50 f	33.77 d	121.10 a
	1	57.94 e	35.32 cd	116.70 b
	2	59.71 d	36.65 bc	112.42 c
	3	62.50 c	37.94 abc	108.33 d
	4	65.06 b	38.89 ab	105.83 e
	5	67.56 a	40.37 a	102.58 f

Table 5 Correlation coefficient (r) for chlorophyll *a* (Chl. *a*), chlorophyll *b* (Chl. *b*), total chlorophyll (T chl.), L*, C*, and h° of Cavendish banana (*Musa acuminata* 'Williams') ripened at 18°C or 27°C. ($n = 24$.)

	Chl. *a*	Chl. *b*	T chl.	L*	C*	h°
Cavendish banana ripened at 18°C						
Chl. *a*	–					
Chl. *b*	0.99 $P \leq 0.01$	–				
T chl.	0.98 $P \leq 0.01$	0.99 $P \leq 0.01$	–			
L*	–0.10	–0.11	–0.10	–		
C*	0.03	–0.01	0.01	0.69 $P \leq 0.01$	–	
h°	0.05	0.07	0.06	–0.90 $P \leq 0.01$	–0.82 $P \leq 0.01$	–
Cavendish banana ripened at 27°C						
Chl. *a*	–					
Chl. *b*	0.95 $P \leq 0.01$	–				
T chl.	0.98 $P \leq 0.01$	0.99 $P \leq 0.01$	–			
L*	–0.86 $P \leq 0.01$	–0.84 $P \leq 0.01$	–0.86 $P \leq 0.01$	–		
C*	–0.73 $P \leq 0.01$	–0.69 $P \leq 0.01$	–0.72 $P \leq 0.01$	0.78 $P \leq 0.01$	–	
h°	0.89 $P \leq 0.01$	0.85 $P \leq 0.01$	0.88 $P \leq 0.01$	–0.96 $P \leq 0.01$	–0.84 $P \leq 0.01$	–

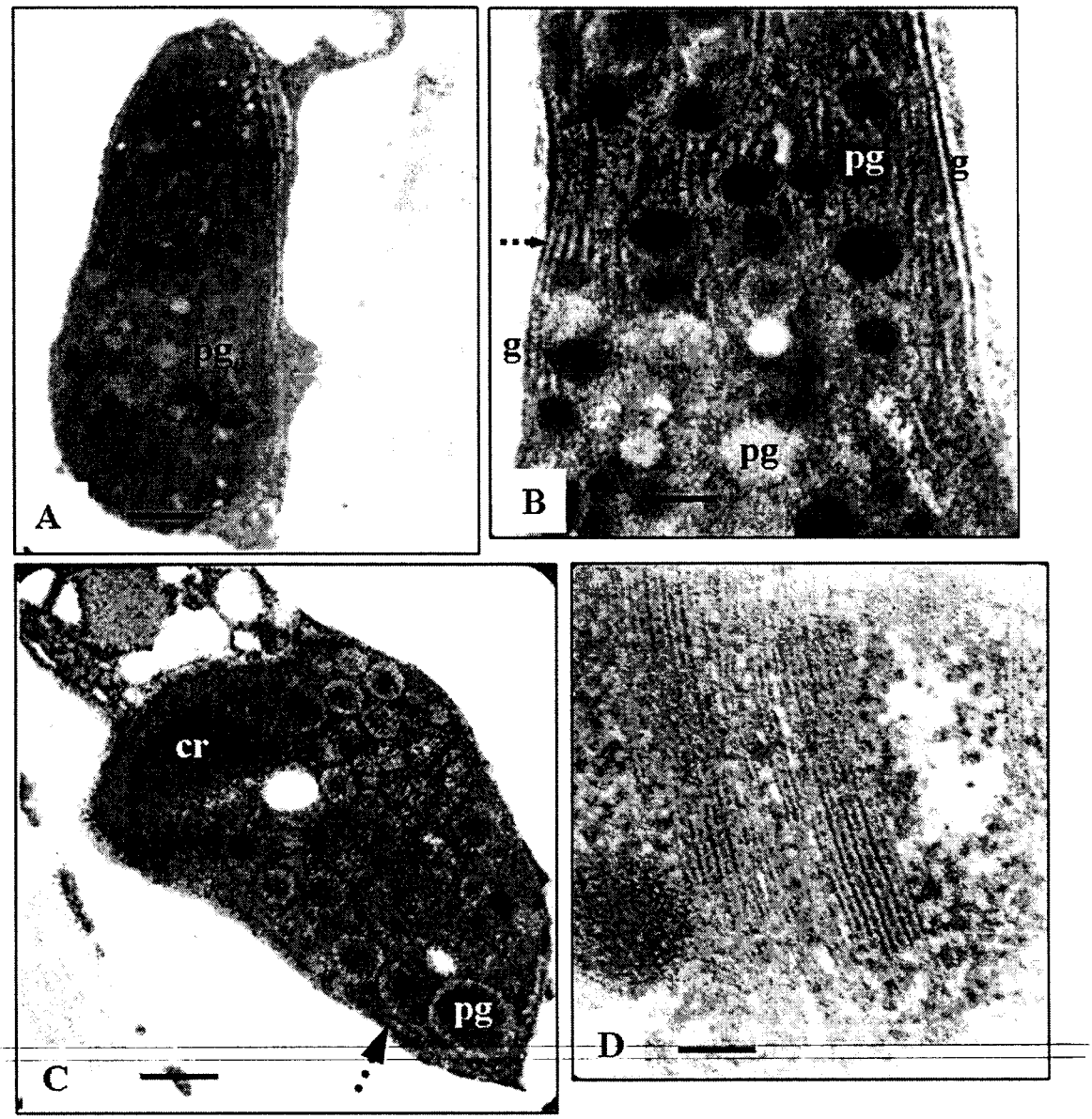

Fig. 2 Transmission electron micrograph of a Cavendish banana (*Musa acuminata* 'Williams') plastid ripened at 27°C. **A**, Chloroplast of day 2 after ripening initiation showing elongated and swollen shape of chloroplast with randomly scattered plastoglobuli (pg). Bar = 0.55 μm. **B**, Chloroplast of day 2 after ripening initiation showing grana (g) are pushed to the periphery, and the stromal thylakoid membranes are short and appear perforated (dashed arrow). Plastoglobuli with varying electron staining densities are present. Bar = 0.22 μm. **C**, Plastid of day 5 after ripening initiation showing crystal (cr), plastoglobuli, and perforated thylakoids (dashed arrow). Bar = 0.25 μm. **D**, Plastid of day 5 after ripening initiation showing tripartite structure of granal-thylakoid. Bar = 0.03 μm.

(Brandis 1996). Plastoglobuli could act as carriers to transport chlorophyll from thylakoids to other organelles (Guiamet et al. 1999). Therefore, entry of substrates by a carrier into the enzyme pathway is needed to degrade the chlorophyll.

As compared with C18, C27 contained a lot more of plastoglobuli and with varying electron staining densities (Fig. 2B,C). This was likely caused by osmium tetroxide staining, which reacts primarily with polar lipids and gave dense electron

stain (Bahr 1954). The non-electron density could possibly make up for apolar carotenoids which are solubilised and removed during sample processing. To our knowledge, the high number of plastoglobuli with varying electron staining densities in banana has not been reported elsewhere. The thylakoid membranes consist of c. 50% protein, 40% acyl lipid, and 10% pigment (by weight) (Gounaris et al. 1986). The galactolipids comprise 70–80% of the total lipid of thylakoid membranes. α-linolenic (18:3) or a combination of 18:3 and hexadecatrienoic (16:3) acids typically account for about two-thirds of all the thylakoid membrane lipid (Harwood 1982). Blackbourn et al. (1990b) have demonstrated that greater loss of galactolipids and a lower overall recovery of linolenic acid during Cavendish banana ripening at 35°C than at 20°C. As a result, Cavendish banana ripened at 35°C failed to degreen. Linolenic acid is a cofactor of chlorophyll bleaching activity *in vitro* (Luthy et al. 1986). The content of linolenic acid in the peel of C18 is higher than C27 (Isa unpubl. data). Although having more plastoglobluli in plastids of C27 than C18, lack of linolenic acid caused granal-thylakoid to fail to disintegrate. As a result, chlorophyll that retained in granal-thylakoid of C27 plastids masked the manifestation of yellow pigment. This was proved by higher retention of chlorophyll contents and h° values in C27 than C18 (Tables 3, 4). Similarly, in ripe tomato mutant *green flesh* fruit, a significant amount of granal-thylakoid stacks along with plastoglobuli, crystals, and undulating membranous structures remained in the plastids, giving rise to a rusty red fruit colour (Cheung et al. 1993). In plastids of wild type ripe tomato fruit only remnants of membranes remained and were red in colour.

The crystal found in the plastids of Cavendish banana (Fig. 2A,C) was believed to be phytoferritin, containing a non-toxic complex of protein and iron, derived from the cytochromes and the ferredoxin of the degraded thylakoids (Bonora et al. 2000). Very large phytoferritin aggregates are characteristics of mature and senescence chromoplasts. However, X-ray analysis needs to be carried out to confirm the composition of crystals found in Cavendish banana plastids.

Although the fruit of C18 and C27 were taken from the same hand of a fruit bunch and then ripened at different temperatures, the contents of chlorophyll in the peel were totally different at later stages of ripening. C27 retained 57% of the total chlorophyll whereas C18 retained 40% of total chlorophyll in the peel at the end of ripening. Plainsirichai et al. (2003) found that chlorophyll degradation in Cavendish banana ripened at 30°C was found only in the deeper region of peel. To degrade chlorophyll completely in the peel, C27 may require longer ripening duration as compared with C18. However, this is impossible for C27 as at day 5, senescence had taken place where the fruit pulp became watery and brown specks began to appear on the peel.

The finding of this work was concomitant with that of Blackbourn et al. (1990a). About 70% of the fruit chlorophyll disappeared after 2 days of ripening at 20°C and the peel of fruit started to turn yellow. After 4 days at 20°C, almost all the chlorophyll degraded and the bananas were bright yellow in colour. However, Cavendish bananas ripened at 35°C failed to lose all their chlorophyll even by day 4 and the peel appeared pale green. Similar results were reported by Dominguez & Vendrell (1993) and Marriott (1980) in Cavendish banana. Hertog (2003) stated that temperature is the main factor affecting all biochemical processes through its effects on activation enthalpy and entropy of the underlying reactions. Therefore, the inhibition of galactolipase activity and the subsequent retention of linolenic acid within the galactolipids would probably help to explain the incomplete chlorophyll degradation in Cavendish banana ripened at 27°C.

The gradual increase in the peel L* and C* in association with the decrease in h°, with a value of more than 90° (Table 4) describes the greenish yellow peel of C27 banana at the end of ripening. This was in agreement with the findings of Blackbourn et al. (1990a,b) who used ethylene to ripen Cavendish banana at 35°C and the peel remained green. Visual colour assessment and the CIE colour values correlate well with chlorophyll content (Li et al. 1997). The h° is also a good indicator of chlorophyll content except when chlorophyll content approaches zero. From the correlation analysis, it seemed that L*, C*, and h° values were not suitable to be used as indicators of degreening in C18 as the retention of total chlorophyll in the fruit was less than 57%. Probably CIE values could only be correlated significantly in the plant material with high retention of chlorophylls. Furthermore, the existence of carotenoids (Lizada et al. 1990; Seymour 1993) in the peel of RS 6 of C18 fruit could have interacted with chlorophyll and dictated the final colour. This was clearly found by Lancaster et al. (1994) in apple where the final red colour of the fruit was determined by the quantities of chlorophyll and carotenoid pigments present in the skin.

CONCLUSION

This study demonstrated that the incomplete disintegration of the thylakoid membrane system could possibly be responsible for the retention of chlorophyll in C27 fruit as compared with those ripened at 18°C. The total chlorophyll retained in C27 was 57% compared with only 40% in C18. Hence, the peel colour of C27 was greener than C18. It seemed that to manifest the yellow peel of banana, the total chlorophyll of the peel must be degraded by at least 60% with complete disintegration of thylakoid membrane system, and the CIE values could only be correlated significantly in the plant material with high retention of chlorophylls.

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