

# Response to selection of uneven degreening in the clones of a Cavendish banana cultivar (*Musa cv. AAA*) in Taiwan

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## Response to selection of uneven degreening in the clones of a Cavendish banana cultivar (*Musa cv. AAA*) in Taiwan.

**Abstract — Introduction.** 'Uneven degreening' is a kind of ripening disorder seriously affecting the export quality of Cavendish bananas in Taiwan. This study aims to investigate the variation in the frequency of uneven degreening in the selected clones of a Cavendish cultivar, 'Pei-Chiao', and to access the feasibility of controlling this ripening disorder through clonal selection. **Materials and methods.** A pilot experiment was conducted at two locations in southern Taiwan. Micropropagated plants derived from selected 'normal' and 'disordered' plants were evaluated for the occurrence of uneven degreening at the time of ripening. A 2-year follow-up experiment was carried out in two trials, each consisted of 6 'normal' and 6 'disordered' clones. Correlation of the ripening pattern and seven other horticultural traits were also investigated. **Results and discussion.** In the pilot experiment, the mean percentage of progenies showing uneven degreening was higher in the disordered group (83.2%) than in the normal group (44.5%). Similar results were observed in the two follow-up experiments. It was also found that among 12 normal clones, 66.7% did not produce progenies showing the severe type of disorder in two cycles of experiment. However all the disordered clones tested produced some progenies belonging to the severe type of disorder. Thus, it is possible to reduce the occurrence of uneven degreening gradually in the 'Pei-Chiao' population by continuous elimination of disordered plants in the stock nursery which supplies source materials for the banana micropropagation program. (© Elsevier, Paris)

Taiwan / *Musa acuminata* / selection responses / postharvest physiology / ripening / defects

## Sélection parmi des bananiers Cavendish (*Musa cv. AAA*) à Taïwan, pour lutter contre l'anomalie du déverdissement hétérogène.

**Résumé — Introduction.** Le « déverdissement hétérogène » est une anomalie de maturation qui affecte gravement la qualité des bananes Cavendish exportées de Taïwan. L'étude présentée a porté sur une évaluation de la fréquence d'observation du phénomène parmi des clones sélectionnés du cultivar Cavendish « Pei-Chiao » et a cherché à vérifier la possibilité d'un contrôle de cette anomalie au travers d'une sélection clonale. **Matériel et méthodes.** Une expérimentation pilote a été conduite sur deux sites au sud de Taïwan. Des plants issus de la micropropagation de plants « normaux » et de plants « anormaux » ont été observés quant à la régularité du déverdissement de leurs fruits au moment de la maturation. Une autre expérimentation a été suivie par ailleurs pendant 2 années sur deux essais, chacun d'eux consistant en l'observation de la production de six clones normaux et six clones anormaux. Une corrélation a été recherchée entre le comportement de maturation et la manifestation de sept autres caractères agronomiques. **Résultats et discussion.** Dans l'expérimentation pilote, le taux moyen de descendances montrant l'anomalie a été plus élevé dans le groupe des plants anormaux (83,2 %) que dans celui des plants normaux (44,5 %). Des résultats comparables ont été obtenus dans les deux essais suivis par ailleurs : parmi les 12 clones de plants normaux étudiés, 66,7 % n'ont pas produit, au cours des deux cycles d'expérimentation, de descendance exprimant sérieusement l'anomalie. Cependant, tous les clones de plants anormaux ont produit des descendances fortement affectées par le déverdissement hétérogène. Il serait donc possible de réduire peu à peu l'impact de l'anomalie, dans la population de Pei-Chiao, en éliminant systématiquement les plants anormaux dans le stock des plants mères utilisés dans le programme de micropropagation du bananier. (© Elsevier, Paris)

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## 1. introduction

Banana is one of the most important fruit crops for export in Taiwan. In 1996, the area for banana production was 8 688 ha producing 140 997 t of bananas, among which 36.7% was for export. The major cultivar grown in Taiwan is 'Pei-Chiao' which belongs to Giant Cavendish. It is tall, vigorous, and widely adapted with good eating quality. Influenced by the subtropical climate, the fruit bunch develops slowly in winter (from December to February of the following year), hence bananas harvested between March and June in Taiwan are superior in flavor and texture compared with bananas produced in tropical regions. This special quality is one of the reasons that keeps Taiwan bananas competitive in the Japanese market. However, because of the subtropical environmental conditions, the postharvest qualities, such as the optimal ripening temperature and shelf-life of Taiwan bananas are different from those produced under tropical conditions [1]. One of the postharvest problems is 'uneven degreening' [2–3] which is a ripening disorder in which part of the banana degreens slower than the rest or, in severe cases, fails to degreen after ethylene treatment (*figure 1*). This is a major drawback as it complicates the ripening

process and reduces the profit of traders. Thus the occurrence of uneven degreening threatens the survival of the banana industry in Taiwan.

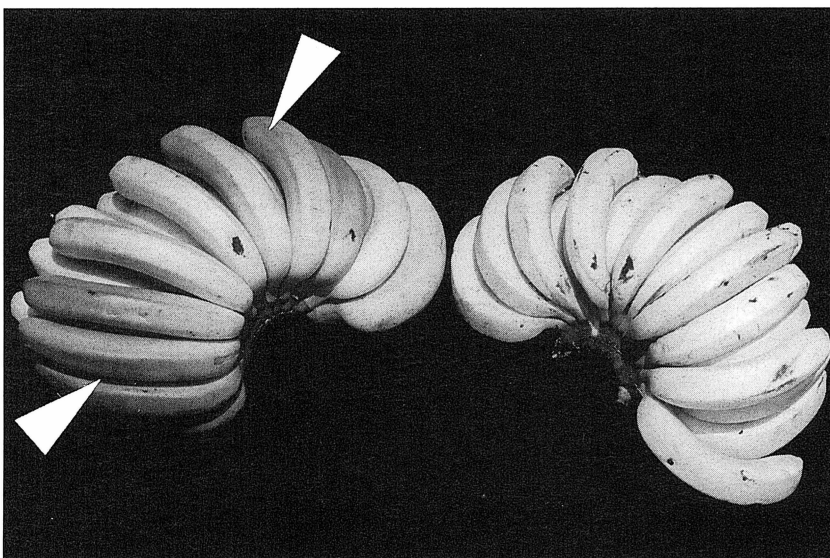
The occurrence of uneven degreening is seasonal with the peak season between mid-April and early June [2]. The severity of this disorder varies from year to year and also from orchard to orchard. Within a bunch, the proximal hands show higher frequency of abnormality than distal hands. Even within the same hand, the disorder occurs more often in the fingers of the outer row.

From the study of the post-harvest physiology of normal and disordered bananas [2], it was observed that the rates of respiration and ethylene production were lower in the unevenly degreened bananas, thus leading to a slower rate of ripening and degreening. However, the real cause of the phenomenon is still not understood. Low temperature at the early stage of flowering in January and February is suspected to be the cause of the disorder. The other possible causes, such as high level of nitrogen fertilizer [4] or latent infection of viruses [5] have not been proven to be related to the ripening disorder.

After several years of investigation, brown paper bag as bunch cover instead of blue polyethylene bag was found to be effective in reducing the severity of uneven degreening [3]. Since 1992, this method was recommended to farmers as a control measure but was not widely accepted because of the high cost of the paper covering. Recently, it was demonstrated that using newspaper to line the polyethylene covering could also reduce uneven degreening. However, this labor consuming measure was not widely accepted.

Ke et al. [6] studied the occurrence of uneven degreening in five somaclonal variants which are resistant to fusarial wilt (*Fusarium oxysporum* f. *cubense*, race 4) and morphologically different from the mother clone 'Pei-Chiao'. They observed variation in the degree of uneven degreening among the variants, with some more

**Figure 1.** Uneven degreening of banana. Right: normally degreened banana with uniform yellow colour; left: unevenly degreened banana with some fingers (arrow) remain green while others turned yellow.



pronounced than the others. In a later study, the frequency of uneven degreening in one of the fusarial wilt resistant clone GCTCV-215 was about 1–3% which was much lower compared to 10–16% in the local cultivar 'Pei-Chiao' [7].

The objectives of the present study are to investigate the response to selection of uneven degreening in the clones of a Cavendish cultivar 'Pei-Chiao' and to assess the feasibility of controlling this ripening disorder through clonal selection.

## 2. materials and methods

### 2.1 pilot experiment

'Pei-Chiao' which belongs to Giant Cavendish was used in this study because of its importance in the banana industry in Taiwan. In April 1991, 20 plants from a 'Pei-Chiao' stock nursery in the experimental farm of Taiwan Banana Research Institute at Hai-fung, in southern Taiwan (lat. 22°42' N, long. 120°29' E, 100 m MSL), were selected randomly. Their fruits were harvested and treated with ethylene for ripening. Two groups were identified according to their ripening pattern: the 'normal group' ripened and degreened normally, and the 'disordered group' had serious problem of uneven degreening. Four to six suckers from each group were used in micropropagation by meristem culture method [8]. The medium consisted of MS basal salts [9], 5 mg·L<sup>-1</sup> BA (6-benzylaminopurine) and 2 mg·L<sup>-1</sup> IAA (indole-3-acetic acid). In April 1992, 100 tissue culture plantlets of each group were planted at each of the two locations, Kao-shu and Hai-fung, in Pingtung in southern Taiwan, as a pilot trial. The trial was harvested between March to May in 1993 and the occurrence of uneven degreening was recorded. In Hai-fung, further observation was made in the ratoon crop in 1994.

### 2.2. follow-up experiments

For further verification of the response to selection of uneven degreening in the

clones of 'Pei-Chiao', another set of experimental materials were selected in 1993 from two independent sources: in trial A, six normal and six disordered clones were identified from plants in the pilot experiment at Kao-shu; in trial B, same number of normal and disordered clones were obtained from 'Pei-Chiao' stock nursery at Hai-fung. Suckers from selected plants were multiplied by meristem culture method. The tissue cultured plants were established in the field in April 1994 at Hai-fung. The planting density was approximately 2 000 plants·ha<sup>-1</sup> with wide row 3 m, narrow row 1.5 m and 2.2 m between plants within row. These trials were conducted using randomized complete block design with two replications. Each plot consisted of 12 plants in one row. Randomization were made independently within each trial. The trial was harvested in April–May 1995 and was continued by ratoon cropping for further investigation in March–May 1996.

### 2.3. recording parameters and analysis

The method for the determination of uneven degreening was as follows: the uppermost hand of each plant was dehanded, labelled and placed in plastic baskets. They were stored in a cold room at 20 °C for 1 or 2 d, then followed by the induction of ripening by ethylene (1 000 ppm) for 24 h. The door was opened for gas exchange for 1 h, after which the bananas were kept at low temperature for degreening. The temperature was 18 °C on the first day and decreased gradually to 14 °C. After 4 to 5 d, the normal ripening process should reach 'stage 4' or 'stage 5' according to the classification of Stover and Simmons [10]. The colour of the bananas was examined and classified accordingly in three levels: (1) normal degreening: all fingers of the same hand degreened at the same rate; (2) moderate disorder: part of the fingers degreened slower than the rest, but, at a later time, all fingers turned yellow; and (3) severe disorder: some fingers degreened extremely slowly and remained green even after

other bananas were completely ripened. When banana plants started to shoot, the following horticultural traits were recorded: the date of shooting, pseudostem height (from soil surface to the point of intersection of the two upper most leaves), pseudostem circumference at 30 cm above ground, number of hands and fingers. At the time of harvest, the date of harvest and bunch weight were recorded. Comparison of the means of each trait between normal and disordered groups were made by using *t*-test.

### 3. results

#### 3.1. pilot experiment

In 1993, the overall occurrence of uneven degreening in the plant crop of the pilot experiment was 57.6%. Consistent differences were observed in various levels of ripening disorder between the normal and disordered groups in this experiment (*table D*). The percentage of progenies ripened normally was 63.3 and 21.7% in the normal and disordered group, respectively. On the other hand, the percentage of progenies showing moderate level of uneven degreening was

higher in the disordered group (70.4%) than in the normal group (35.4%). Progenies having severe level of disorder was 7.9% in the disordered group and 1.4% in the normal group. A similar trend was observed in the first ratoon crop harvested in 1994. The percentages of progenies showing normal ripening, moderate and severe uneven degreening in the normal group were 47.9, 34.7 and 17.4%, while, in the disordered group, were 11.9, 48.2 and 39.9%, respectively.

#### 3.2. follow-up experiments

##### 3.2.1. variation in uneven degreening in normal and disordered clones

In order to verify the observation from the pilot experiment, several normal and disordered clones were selected from two independent sources. Their tissue-cultured progenies were evaluated in 1995 and 1996 in the two designed experiments: trial A and B. Overall occurrence of uneven degreening in the follow-up experiments was 45.6 and 29.2% in 1995 and 1996, respectively. There were consistent differences in the occurrence of different levels of uneven degreening among progenies derived from the nor-

**Table I.**

Frequencies (%) of different levels of uneven degreening in progenies of two groups of 'Pei-Chiao' banana cultivar in a pilot experiment located in Pingtung (Taiwan) (means of two locations in 1993).

Year of harvest	Cycle	Group	Number of plants investigated	% Uneven degreening		
				None	Moderate	Severe
1993	Plant crop (Pc)	Normal	130	63.3	35.4	1.4
		Disordered	86	21.7	70.4	7.9
1994	First ratoon (Fr)	Normal	42	47.9	34.7	17.4
		Disordered	21	11.9	48.2	39.9
1993 and 1994	Pc + Fr average	Normal	172	55.6	35.1	9.4
		Disordered	107	16.8	59.3	23.9
Significance				**	*	**

\*, \*\*: significant at the 5% and 1% probability level, respectively, from a *t*-test with *df* = 5.



mal and disordered clones in the two-year experiment (*table II*). In trial A, the mean frequencies of progenies without uneven degreening was higher in the normal clones (83.5%) than that in the disordered clones (52.5%). But for the percentages of unevening degreening, it was higher in the disordered clones (47.6%) than in the normal clones (16.5%). A similar trend was observed in trial B (*table II*). The occurrence of severe type of disorder in the progenies was even higher (43.5%) than in trial A (22.3%).

In these two trials, the severe type of uneven degreening occurred in progenies of all disordered clones, however, eight out of twelve (66.7%) normal clones did not produce progenies with the severe type of ripening disorder in the experiment.

### 3.2.2. correlation between horticultural characteristics and ripening pattern

Though the differences were not always significant, there was a tendency in the two follow-up experiments that the height and circumference of pseudostem, the days from planting to shooting and to harvest were taller, larger or longer in the disordered group than that of the normal group (*table III*). Significant differences were observed in the number of hands and fingers per bunch but different tendencies were observed in trial A and B. No significant difference was observed in bunch weight between the two groups.

The frequency of progenies having normal ripening behavior was negatively correlated with the height and circumference of pseudostem, days from planting to shooting and to harvest, and number

**Table II.**

Frequencies (%) of different levels of disorder (uneven degreening) in progenies (from micropropagation) of two groups of 'Pei-Chiao' banana cultivar in experiments located in Pingtung (Taiwan). Trials A and B are composed of selected clones from two independent sources.

Cycle	Group	Number of plants investigated	% Uneven degreening <sup>1</sup>		
			None	Moderate	Severe
<b>a) Trial A harvested in 1995 (plant crop) and 1996 (first ratoon)</b>					
Plant crop (Pc)	Normal	119	79.7	20.3	0.0
	Disordered	97	45.7	29.5	24.8
First ratoon (Fr)	Normal	92	87.4	11.4	1.2
	Disordered	76	59.3	21.0	19.7
Pc + Fc average	Normal	211	83.5	15.9	0.6
	Disordered	173	52.5	25.3	22.3
Significance			**	*	**
<b>b) Trial B</b>					
Plant crop (Pc)	Normal	125	71.1	21.7	7.1
	Disordered	80	20.8	26.5	52.7
First ratoon (Fr)	Normal	107	94.7	4.5	0.8
	Disordered	83	41.8	23.8	34.3
Pc + Fc average	Normal	232	82.9	13.1	4.0
	Disordered	163	31.3	25.2	43.5
Significance			**	*	**

<sup>1</sup> Means of six clones.

\*, \*\*: significant at the 5% and 1% probability level, respectively, from a *t*-test with *df* = 10.

**Table III.**

Comparison of several horticultural characteristics between normal and disordered banana clones (giving fruit uneven degreening) in two follow-up experiments carried out.

Cycle	Group	Plant height (cm)	Plant circumference (cm)	Planting to shooting (d)	Planting to harvest (d)	Nb of hands/bunch	Nb of fingers/bunch	Bunch weight (kg)
<b>a) Trial A (means of six clones in a two-years experiment)</b>								
Plant crop (Pc)	Normal	281	73.6	290	401	8.3	145	25.3
	Disordered	318	76.9	309	406	7.8	135	23.7
First ratoon (Fr)	Normal	310	78.4	238	368	9.3	163	28.9
	Disordered	322	78.3	252	377	8.5	147	27.7
Pc + Fc average	Normal	295	76.0	264	385	8.8	154	27.1
	Disordered	320	77.6	280	391	8.2	141	25.7
Significance		**	*	**	NS	**	*	NS
<b>b) Trial B</b>								
Plant crop (Pc)	Normal	279	71.8	291	400	8.3	142	25.1
	Disordered	292	74.5	320	411	9.5	167	25.3
First ratoon (Fr)	Normal	304	76.3	223	367	9.3	165	28.5
	Disordered	309	78.6	252	384	9.5	167	29.3
Pc + Fc average	Normal	292	74.1	257	383	8.8	154	26.8
	Disordered	300	76.6	286	398	9.5	167	27.3
Significance		NS	**	**	*	**	**	NS

\*, \*\*: significant at the 5% and 1% probability level, respectively, from a *t*-test with *df* = 10.  
NS: non-significant test.

**Table IV.**

Correlation analysis between the frequencies of different levels of disorder (fruit uneven degreening) and horticultural traits in banana groups of 'Pei-Chiao' in experiments carried out in southern Taiwan (pooled data, *n* = 24).

Levels of disorder	Plant height (cm)	Plant circumference (cm)	Planting to shooting (d)	Planting to harvest (d)	Nb of hands/bunch	Nb of fingers/bunch	Bunch weight (kg)
Normal	-0.49 *	-0.57 *	-0.73 **	-0.68 **	-0.48 *	-0.40 NS	-0.10 NS
Moderate	0.64 **	0.55 **	0.40 NS	0.38 NS	0.13 NS	0.09 NS	0.04 NS
Severe	0.35 NS	0.48 *	0.75 **	0.70 **	0.53 *	0.45 *	0.10 NS

\*, \*\*: significant at the 5% and 1% probability level, respectively.  
NS: non-significant test.

of hands per bunch (*table IV*). The frequency of progenies having the severe type of ripening disorder was positively correlated with all horticultural traits studied, except height of pseudostem and bunch weight. No correlation was found between bunch weight and ripening pattern.

#### 4. discussion

The severe type of uneven degreening has become a major problem in the banana industry in Taiwan. In the present study, variation in the frequencies of uneven degreening was found in different clones of 'Pei-Chiao'. Clones derived from plants showing severe type of disorder had a tendency to produce progenies having uneven degreening, especially the severe type. On the other hand, eight out of twelve (66.7%) clones selected for the absence of uneven degreening did not have the severe type of disorder in their progenies in the two-year study period. These findings indicate that clonal selection could be an effective way to reduce the occurrence of the severe type of uneven degreening in the 'Pei-Chiao' population.

The occurrence of uneven degreening is seasonal and varied from year to year. One of the implications is that selection of normal clones without ripening disorder should be done at the peak season of uneven degreening, i.e., from mid-April to June in Taiwan. Also, selection should be carried out more than one year or as a continuous process in the same population for several years in order to reduce the frequencies of uneven degreening in the population effectively. Though correlation between the levels of uneven degreening and some horticultural traits was observed, selection of normal clones without ripening disorder did not produce unfavorable effects on the horticultural traits of the population.

Somaclonal variation was observed among tissue-culture plants in different crops and was claimed to be one of the

sources of variation for plant improvement [11]. This kind of variation was also reported in bananas and plantains [12–14]. Most of these variations are stable and transmissible to progenies propagated asexually while some of these variations are unstable. Hwang [12] showed that only 15% of plants derived from a chimeric variant were chimeric themselves. Similar results were also found in a variant of multiple bunch [15]. The variation of uneven degreening can also be considered as a kind of somaclonal variation. In other words, some genetic factors are involved in controlling the occurrence of uneven degreening. These factors are probably unstable and their expression is subjected to environmental condition, thus explaining the seasonal and sporadic nature of uneven degreening.

Israeli et al. [16] classified somaclonal variation of bananas into two groups according to the phenotypic differences: exposed and unexposed. Somaclonal variants such as extra-long fingers, dwarf-type with no obvious choke-throat problem and *Fusarium* wilt resistance are examples of unexposed variants. Uneven degreening can also be classified as unexposed variant. The occurrence of this type of unexposed variants becomes a potential danger in the changing of the genetical constitution of a banana cultivar through in vitro propagation over some years. Thus a system of distribution and maintenance of banana cultivars is required, especially for those cultivars using in vitro methods for mass propagation. The system should consist of at least two levels: mother and multiplication stocks. The mother stock represents the true-to-type plants of the cultivar and should be maintained by the breeding institution releasing the cultivar. The multiplication stock should be derived from the mother stock, preferably by suckers, and should be maintained by the institution responsible for the multiplication and for supplying materials for in vitro propagation. This system of distribution and maintenance of banana cultivars has been adopted by the Taiwan Banana Research Institute for multiplication of millions of

banana plantlets of different cultivars annually. According to the results of this study, uneven degreening is one of the criteria for the elimination of off-type plants in the stock nursery. By this measure and by using brown paper bags for bunch covering, it is expected that the occurrence of unevening degreening can be brought under control in Taiwan bananas.

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### Selección contra la anomalía de la decoloración del verde heterogénea entre los plátanos Cavendish (*Musa* cv. AAA) en Taiwan.

**Resumen — Introducción.** La 'decoloración del verde heterogénea' es una anomalía de maduración que afecta gravemente la calidad de los plátanos Cavendish exportados de Taiwan. El estudio presentado abarcó una evaluación de la frecuencia de observación del fenómeno entre los clones seleccionados del cultivar Cavendish 'Pei-Chiao' e intentó verificar la posibilidad de un control de esta anomalía mediante una selección clonal. **Material y métodos.** Un experimento piloto fue llevado a cabo en dos sitios al sur de Taiwan. Se observaron plantas procedentes de la micro propagación de plantas 'normales' y de plantas 'anormales' en cuanto a la regularidad de la decoloración del verde de sus frutos en el momento de la maduración. Por otro lado se llevó a cabo otro experimento durante dos años en dos ensayos, cada uno de ellos consistiendo en observar la producción de seis clones normales y seis clones anormales. Se buscó una correlación entre el comportamiento de maduración y la manifestación de otros siete caracteres agronómicos. **Resultados y discusión.** En el experimento piloto, la tasa media de descendencias mostrando la anomalía fue más elevada en el grupo de las plantas anormales (83.2%) que en el de las plantas normales (44.5%). Se lograron resultados comparables en ambos ensayos vigilados. Por otro lado: entre los 12 clones de plantas normales estudiadas, un 66.7% no produjeron, durante los dos ciclos de experimento, descendencia expresando seriamente la anomalía. No obstante, todos los clones de plantas anormales produjeron descendencias fuertemente afectadas por la decoloración del verde heterogénea. Por lo tanto, resultaría posible reducir poco a poco el impacto de la anomalía, en la población de Pei-Chiao, al eliminar sistemáticamente las plantas anormales en las existencias de las plantas-madres utilizadas en el programa de micro propagación del plátano. (© Elsevier, Paris)

Taiwan / *Musa acuminata* / repuesta a la selección / fisiología postcosecha / maduramiento / defectos