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DISTRIBUTION OF ANTHOCYANINS IN WILD AND CULTIVATED BANANA VARIETIES

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IN MEMORY OF TONY SWAIN, 1922–1987

Key Word Index—*Musa acuminata*; *M. balbisiana*; Musaceae; banana; cultivars; chemotaxonomy; domestication; anthocyanins.

Abstract—An examination of bract anthocyanins in 59 banana varieties separated them into five main chemotypes, based upon the presence/absence of methylated compounds and/or the ratio between delphinidin and cyanidin derivatives. The data suggest the occurrence of two centres of domestication for the *acuminata* cultivars in southeast Asia; the origins of some hybrid cultivars are also discussed.

INTRODUCTION

Musa acuminata and *Musa balbisiana* are the main representatives of the genus *Musa* (Fig. 1); almost all the edible bananas are originated from these two wild species [1, 2]. Among these edible bananas, the different cultivars are classified according to their genomic constitution and ploidy level: diploid *acuminata* cultivars AAcv (cv to differentiate from AAw, wild forms of *M. acuminata*); triploid and tetraploid *acuminata* cultivars AAA, AAAA and triploid and tetraploid hybrid cultivars AAB, ABB, AAAB, AABB, ABBB.

A previous survey of anthocyanins in the bracts of banana has been made by Simmonds [3]: *M. balbisiana* was characterized by various mixtures of delphinidin and cyanidin, the latter being predominent; and *M. acuminata* contained various combinations of cyanidin, delphinidin and their methylated derivatives, peonidin, petunidin and malvidin. The distribution of these various anthocyanidins among the different banana cultivars gave good support to the taxonomic ordering within this genus.

With respect to the glycosylation pattern, Simmonds [3] showed that each aglycone occurred in a single glucosidic form: probably a 3-*O*-diglucoside; a possible exception was the aglycone cyanidin in *M. balbisiana* which occurred as two unidentified diglycosides. Williams and Harborne [4], in a review on the Zingiberales, reported in the leaves of *M. acuminata* subsp. *zebrina* the identification of cyanidin and peonidin 3-rutinosides.

In the present paper, we describe the distribution of anthocyanins in the bracts of banana collection from I.R.F.A. (Institut de Recherches sur les Fruits et Agrumes, Guadeloupe, France).

RESULTS AND DISCUSSION

Anthocyanins

Nine pigments separated by HPLC were numbered from 1 to 9, according to increasing retention times; the relative amounts of compounds 1, 2 and 9 were weak, so that only compounds 3–8 could be identified by classical methods (Table 1). The absence of a shoulder around 330 nm, and the fact that no migration occurs on paper electrophoresis, indicates absence of acylation. The presence of a shoulder in the visible spectrum at 440 nm and the R_f values obtained on TLC indicate the presence of 3-rutinosides. Compound 4 with an unusual chromatographic behaviour could not be further characterized.

Ontogeny of bract pigmentation

The colour of the bracts in the male inflorescence progressively vanishes, from the outside bract (bract number 1, the oldest) to the inner uncoloured bracts; Simmonds [5], following the synthesis of anthocyanins in the bracts of *M. acuminata* subsp. *malaccensis*, pointed out that the methylated pigments appeared at the same time as cyanidin and delphinidin glycosides; moreover, the malvidin glucoside seemed to appear several days before the peonidin glucoside when both pigments were present.

Our results are presented in Table 2. It can be observed that, whichever variety, the relative distribution of the anthocyanin pattern is remarkably stable from the outer bracts to the inner bracts. Such behaviour constitutes a particularly interesting morphogenetic situation.

Chemotaxonomic definition of banana varieties

Fifty-nine varieties, each represented by one to four individuals, were studied for their anthocyanin patterns; for each variety, a fingerprint was defined for the nine anthocyanins, by their relative amounts expressed as percentages.

The data matrix (Table 3) so obtained was then treated by Principal Components Analysis. The distribution of the individuals on the plan defined by the first two factorial axes is shown in Fig. 2. Five groups, 1–5 were

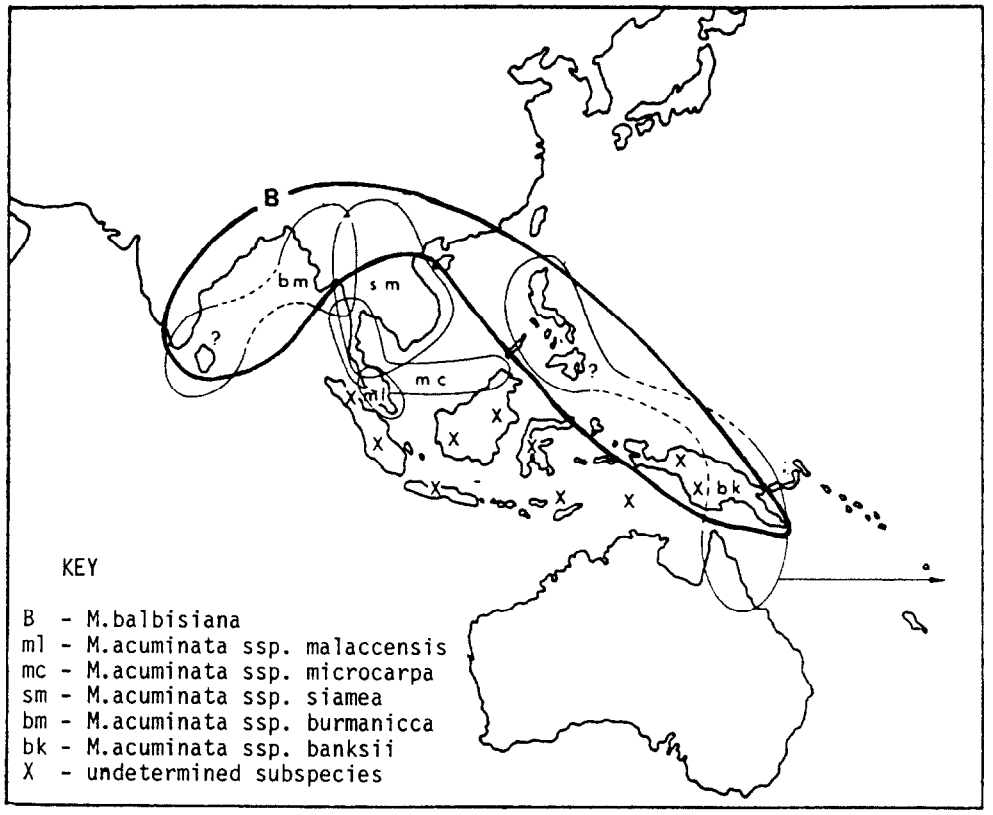

Fig. 1. Geographical distribution of *M. acuminata and M. balbisiana* [2].

Table 1. Anthocyanins of banana bracts

Compound number	Assigned structure	Abbreviation	HPLC retention times (min)	Observed R_f values* Syst. 1	Syst. 2
1			4.00		
2			13.00		
3	3-*O*-Rutinosyldelphinidin	De	14.70	0.32	
4	Cyanidin derivative	Cyd	15.40		
5	3-*O*-Rutinosylcyanidin	Cy	17.25	0.30	0.68
6	3-*O*-Rutinosylpetunidin	Pt	19.50		
7	3-*O*-Rutinosylpeonidin	Po	21.45	0.63	0.80
8	3-*O*-Rutinosylmalvidin	Ma	22.60	0.63	0.80
9			25.20		

*TLC was carried out on 0.1-mm cellulose layers; solvent compositions were concentrated hydrochloric acid–formic acid–water = 24.9:23.7:51.4 (system 1); 7.1:31.4:61.5 [6].

defined corresponding to five main chemotypes (Fig. 2).

Groups 1 and 2 diverge from groups 3–5 by their ability to methylate; therefore, the anthocyanin metabolism of these first two groups is characterized by the accumulation of peonidin and malvidin, the latter being predominent in group 1, the former in group 2. By contrast, groups 3–5 are characterized by the accumulation of simple anthocyanins based on the cyanidin and delphinidin. The discrimination between these three groups is based upon different ratios of these two main anthocyanin classes.

Group 1, with one exception, is constituted by wild *acuminata* diploids AAw. The mean chemotype of group 2 proceeds from a simple readjustment of the metabolic balance between methylated anthocyanins; it is composed of wild *acuminata* diploids AAw, diploid *acuminata* cultivars AAcv and 11 out of the 13 triploid *acuminata* cultivars.

The relationships so revealed by the anthocyanin fingerprints has to be compared with the hypothesis concerning the origin and evolution of these banana varieties: *acuminata* cultivars (AAcv and AAA) would

Table 2. Relative amounts of anthocyanins in the different bracts of male inflorescence of six banana varieties

Cultivars and varieties*	Ranks†	Anthocyanin								
		1	2	3 De	4 Cyd	5 Cy	6 Pt	7 Po	8 Ma	9
AAw	I			2		18	10	22	48	
	II			1		20	8	27	42	
	III			1		17	8	24	47	1
AAcv	I			1	1	23	4	39	31	
	II				1	21	2	48	27	
	III				1	21	2	31	44	
AAA	I			8	2	66	8	10	6	
	III			5	1	77	7	5	4	
AAB	I			14	1	82	2	1		
	II			18	1	79	1	1		
	III			27	1	70	2			
ABB	I			14	6	75	2	1		
	II			15	5	78	2	1		
	III		1	17	5	70	4	1	1	
BB	I			6	15	75	2	1		
	II			6	14	75	2	1		
	III			6	14	75	2	2		

*See experimental for variety identification.
†Ranks I: outer three ranks, i.e. ranks 1,2,3. Ranks II: ranks 4,5,6. Ranks III: inner ranks, i.e. 7,8,9.

Table 3. Anthocyanin patterns of 71 individuals representatives for 59 banana varieties; the relative amounts of each anthocyanin are expressed as percentages of the total pattern

Anthocyanin									Variety identification
1	2	3 De	4 Cyd	5 Cy	6 Pt	7 Po	8 Ma	9	
									AAw
0	0	0	0	16	7	26	51	0	*M. acuminata* subsp. *malaccensis* type Selangor
0	0	3	2	19	6	27	43	1	*M. acuminata* subsp. *malaccensis* type Selangor
0	0	4	0	17	9	21	48	0	*M. acuminata* subsp. *malaccensis* type Selangor
0	0	1	0	17	9	28	45	0	*M. acuminata* subsp. *malaccensis* type Selangor
0	0	0	1	16	3	44	35	0	*M. acuminata* subsp. *malaccensis* type Pahang
0	0	3	0	16	6	23	52	0	*M. acuminata* subsp. *microcarpa*
0	0	1	0	17	4	29	49	0	*M. acuminata* subsp. *microcarpa*
0	0	0	0	8	2	58	32	0	*M. acuminata* subsp. *microcarpa* type Borneo
1	0	1	0	14	4	28	51	1	*M. acuminata* subsp. *burmannica* type Long Tavoy
0	0	1	0	3	4	11	80	0	*M. acuminata* subsp. *burmannicoides* type Calcutta
0	0	0	2	7	3	31	56	1	*M. acuminata* subsp. *siamea*
0	0	0	0	2	0	61	36	0	*M. acuminata* subsp. *siamea* type Khae
0	0	1	0	75	5	14	5	0	*M. acuminata* type Ajoupa
0	0	2	1	74	5	12	5	0	*M. acuminata* type Ajoupa
0	0	0	2	31	1	59	8	0	*M. acuminata* EN13
0	0	0	3	11	7	43	37	0	*M. acuminata* IDO113
3	0	16	0	47	14	5	15	0	*M. acuminata* (unidentified)
0	0	3	0	19	7	25	46	0	*M. acuminata* hybrid Pahang
0	0	1	1	21	4	42	29	0	*M. acuminata* hybrid type II
1	0	2	0	18	3	42	33	0	*M. acuminata* hybrid type III
1	0	4	0	12	8	22	54	0	*M. acuminata* hybrid type X
0	0	0	0	25	0	22	53	0	*M. acuminata* hybrid type XI
0	0	0	0	9	4	31	55	1	Pa Musare No.3
0	0	1	0	14	4	28	53	0	Pa Song Kla

Table 3. *Continued*

Anthocyanin									Variety identification
1	2	3 De	4 Cyd	5 Cy	6 Pt	7 Po	8 Ma	9	
									AAcv
0	0	2	0	23	3	41	31	0	P. Tongat
1	0	1	1	19	3	44	30	1	P. Lilin
1	0	0	1	18	2	47	29	1	P. Lilin
0	0	0	1	18	3	48	29	0	P. Lilin
0	0	0	1	19	2	49	28	0	Akondro Mainty
0	0	45	0	50	4	0	1	0	Sowmuk
0	1	51	0	46	3	0	0	0	Wudi Yali Yalua
1	0	32	0	54	9	1	3	0	SF 215
0	0	0	0	5	1	47	47	0	Toowoolee
0	0	27	0	54	12	1	6	0	Bie Yeng
0	1	58	0	32	7	0	2	0	Niyarma Yik
0	1	62	0	32	5	0	0	0	SN 2
0	0	2	0	12	7	21	56	1	SF 265
2	0	0	1	18	1	58	19	1	No. 110
0	0	1	1	44	5	23	27	0	P. Pipit
1	0	0	1	10	2	56	30	0	Rose
									AAA
0	0	0	0	26	0	40	34	0	Gros Michel cv Gros Michel sgp.
0	0	1	0	20	4	35	40	0	20 01 04 cv Gros Michel sgp.
0	0	6	0	71	13	5	11	0	No. 901 cv Cavendish sgp.
2	0	3	2	25	5	31	33	0	Figue Rose cv Red sgp.
0	0	0	0	7	0	54	39	0	Yangambi km.5 cv Ibota sgp.
0	0	0	0	52	0	24	24	0	Bolo Bigouyo cv Mutika sgp.
0	0	0	0	11	1	47	42	0	Khom
0	0	0	0	7	0	47	44	1	Khom
0	0	0	0	7	0	50	43	0	Khom Bao
0	0	0	0	6	1	53	39	1	Khai Thong Ruong
0	0	0	0	9	0	45	46	0	Type Yangambi
3	0	26	0	67	3	0	0	0	P. Perecet
2	0	0	0	10	2	56	30	0	P. Papan
0	0	0	0	7	2	49	42	0	P. Nangká
									AAAA
0	0	0	0	19	1	40	39	0	Champa Nasik
									AAB
0	0	25	0	71	3	1	0	0	French Sombre cv Plantain sgp.
4	0	28	0	64	2	1	1	0	Didiedi cv Plantain sgp.
2	0	28	0	66	2	1	0	0	Corne Type cv Plantain sgp.
1	0	26	0	47	14	3	9	0	Rajapuri India cv Pome sgp.
0	0	0	0	69	8	8	15	0	N'Sounga cv Pome sgp.
1	1	23	0	68	3	2	0	0	P.Pulut
									ABB
0	0	24	0	72	4	0	0	0	Poteau Geant cv Bluggoe sgp.
0	4	7	10	72	0	1	5	0	Muisa Tia cv P. Awak sgp.
4	4	12	10	62	2	1	5	0	Brazza II cv P. Awak sgp.
6	0	6	10	78	0	1	0	0	Brazza III cv P. Awak sgp.
3	0	5	12	80	0	0	0	0	Simili Radjah cv Peyan sgp.
2	2	41	0	46	5	1	2	0	Brazza IV cv Peyan sgp.
									BB
4	0	4	7	83	0	2	0	0	*M. balbisiana* type Cameroun
2	2	9	13	71	2	2	0	0	*M. balbisiana* type Honduras
3	0	4	11	81	0	1	0	0	*M. balbisiana* type Honduras
0	1	7	14	75	2	1	0	0	*M. balbisiana* type Honduras

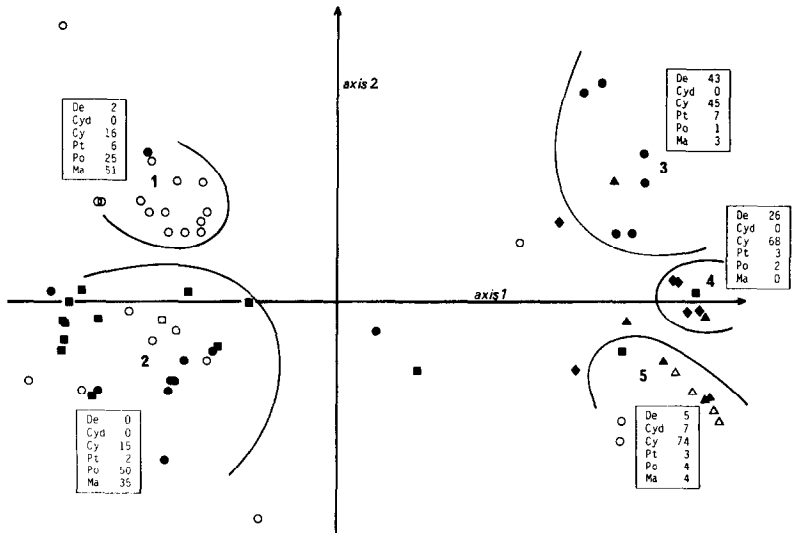

Fig. 2. Ordering of 71 individuals representatives for 59 banana varieties according to their anthocyanin patterns, treated by principal components analysis; the first two axis account for 88% of the total inertia.

Key: ○ AAw, wild *Musa acuminata* species; ● AAcv, edible *acuminata* diploids; ■ AAA, triploid *acuminata* cultivars; □ AAAA, tetraploid *acuminata* cultivars; ◆ AAB, triploid hybrid cultivars, predominantly *acuminata*; ▲ ABB, triploid hybrid cultivars, predominantly *balbisiana*; △ BB, wild *Musa balbisiana* species.

have emerged in the Malayan area, which is supposed to be the centre of origin of *M. acuminata*; the dispersal of cultivars in southeast Asia and towards Africa did occur subsequently, from human migrations. The different varieties of our collection corresponding to this evolutionary scheme, though they are accessions from various geographic origins (Indonesia, Cameroon, Honduras, etc), which are clustered in groups 1 and 2; so, it seems that domestication on one hand, and geographical movements on the other had little effect upon the anthocyanin metabolism.

With regard to this simple evolutionary scheme, group 3 is questionable: effectively, it is mainly constituted by diploid *acuminata* cultivars, characterized by the quasi-absence of methylated anthocyanins, that is to say their fingerprints differ significantly from that of the other *acuminata* cultivars (group 2). This result seems to agree with the hypothesis that these *acuminata* types originated from Papouasia–New Guinea islands: indeed, on the basis of morphological and biometrical features, specialists recognize in this area a *M. acuminata* subsp. *banksii*, from which could have emerged a secondary domestication centre. This is in good agreement with the great divergence observed between chemotypes *acuminata* (group 2 and 3); the study of some representatives of the *banksii* subspecies would be very interesting.

Before considering the analysis of the hybrid cultivars, observation of the anthocyanin pattern of group 5 *M. balbisiana* is useful: The latter is characterized by a high cyanidin content (more than 80%) and the presence of the new compound Cyd. This is obviously a different metabolic type than the one of *M. acuminata* species. The high degree of anthocyanin divergence is noteworthy, taking into account the fact that the geographical distributions of *M. acuminata* and *M. balbisiana* overlap. In spite of this, *M. balbisiana* remains very homogeneous, morphologically and chemically.

The anthocyanin definitions of ABB hybrid cultivars are very close to *M. balbisiana*, for four out of six; the A genome mark is perceptible just for two varieties, by the presence of the delphinidin marker, indicative of a group 3 influence. This last characteristic appears more evidently within the hybrid cultivars AAB of group 4; these triploids, usually known as 'Plantains', would thus not have originated from a classical Malayan *M. acuminata*, but instead from a Papouasian *M. acuminata* subsp. *banksii* form.

Two AAB, known as 'Pome', are excluded from group 4: indeed, their anthocyanin pattern presents a relatively high percentage of methylated anthocyanins, as the *acuminata* mark of groups 1 and 2; this lead us to assume a more complicated *acuminata* origin for these two varieties.

In conclusion, it appears that anthocyanin data support the idea of two centres of domestication for the *acuminata* species, long ago isolated from each other, giving rise to very divergent cultivated forms, according to their pigment patterns. This chemotaxonomic approach provides a new understanding of the *acuminata* origins of hybrid cultivars.

EXPERIMENTAL

Plant material. Inflorescences were collected in the I.R.F.A. collection in Guadeloupe and air-posted to Lyon. The varieties used for anthocyanin determination were: AAw-*M. acuminata* subsp. *malaccensis* type Selangor; AAcv-Pisang Lilin cv; AAA-Grande Naine cv, Cavendish subgroup; AAB-French Sombre cv, Plantain subgroup; ABB-Poteau Géant cv, Bluggoe subgroup; BB-*M. balbisiana* from Honduras. The varieties used for taxonomic purposes are listed in Table 3.

Anthocyanins aglycones were identified according to

Harborne [7]; after hydrolysis in boiling 2 N HCl for 40 min, aglycones were analysed by 2D PC techniques on Whatman No. 3 MM paper, using Forestal as solvent for first dimension ($HOAc–HCl–H_2O$, 30:3:10) and BAW for second dimension (n-$BuOH–HOAc–H_2O$, 4:1:5, upper). Anthocyanin pigments were extracted in boiling MeOH–EtOH (1:1) for 40 min.; extracts were concd to 5 ml and filtered on glass wool.

HPLC was carried out using Nucleosil 5u-C18 column; solvents are A 5% aq. HCO_2H and B MeOH; initial composition 30% B in A, increasing 1% B per min for 15 min, 7% B per min for 5 min; flowrate 0.8 ml/min; detection 514 nm or 240 to 600 nm with scanning detector for spectral identification of the peaks; just before injection, anthocyanin extracts were diluted $\times 10$ in 0.1 N HCl. The aglycone moiety of the anthocyanin for each HPLC peak was identified according to the maxima of absorbance in the visible range (compounds 3, 6, 8: $\lambda_{max\ vis} = 530$, 535, 535 nm; compounds 4, 5, 7: $\lambda_{max\ vis} = 520$, 522, 522 nm; respectively) and to their RR_f (Table 1). Glycosidic patterns were determined by TLC (see Table 1).

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