Differentiating the Volcaniform Phytoliths of Bananas: *Musa acuminata*

L. Vrydaghs, T. Ball, H. Volkaert, I. van den Houwe, J. Manwaring and E. De Langhe

Research

Abstract

Banana phytoliths are considered a suitable tool in archaeology to track the history of the human populations involved in banana cultivation and dispersal throughout the tropical world. This study is confined to an initial investigation of the species *Musa acuminata* Colla and of its edible diploid and triploid derivatives. Slight morphological and/or morphometrical differences of the volcaniform phytoliths can be expected because of the very complex and bi-specific phylogeny of the edible banana. A stepwise procedure in the analysis of these phytoliths is therefore required.

Analysis of 21 samples covering a wide spectrum in genetic diversity, shows that banana phytolith diversity is linked to phylogeny. The results suggest that precise and reliable identification of phytoliths in archaeological contexts is possible, but that the examination of an additional set of samples is necessary to fully understand the extent of morphotypic variation and traits for diagnostic discrimination.

Introduction

Humanity played a dominant role in the diffusion of the edible banana over the tropical world. Only with the help of mobile and/or intercommunicating human populations could this seedless, and thus vegetatively propagated, crop have reached Africa, the Pacific and America, starting from its primary diversity center in Southeast Asia and Melanesia. Traces of edible banana in archaeological contexts have the potential to document the movement and/or contacts of humans in ancient times (De Langhe *et al*. 2009, Vrydaghs & De Langhe 2003).

Edible banana plants are not woody, and seeds and pollen are rarely produced. Therefore, their history has been very difficult to trace in the archaeobotanical record. However, recent research shows that the relatively resistant silica bodies of phytoliths have the potential to become an efficient tool for tracking the banana in different archaeological contexts (see archaeobotanical reviews in Donohue & Denham 2009, Vrydaghs *et al*. 2003). Indeed, archaeologists working at sites of different ages in a variety of different regions have recovered phytoliths from banana plants which reflect different ecological and historical processes (for a review of this evidence see Donohue & Denham 2009). Hence, there is a need for the differentiation of phytoliths produced by particular banana groups, both wild and cultivated.

In a previous publication we focused on differences in volcaniform phytolith crater width (CW) between the two basic species *Musa acuminata* Colla (AA) and *Musa balbisiana* Colla (BB) (Ball *et al*. 2006). Our data suggested that initial domestication did not seem to affect phytolith size, since no significant difference was found in CW between wild and edible AA diploids (Ball *et al*. 2006). When

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Ethnobotany Research & Applications 7:239-246 (2009)

Published: July 30, 2009

www.ethnobotanyjournal.org/vol7/i1547-3465-07-239.pdf

we subsequently investigated phytoliths from the triploid cultivars with the corresponding genome configurations AAA, AAB and ABB, a rather complex pattern emerged. Consequently we have found it necessary to undertake more analyses incorporating a larger sample of wild AA to examine the possible effect of triploidy in the AA-AAA sequence.

Table 1. The geographical distribution of *Musa acuminata* Colla subspecies.

***M. acuminata* subspecies**	**Geographical area**
banksii (F. Muell.) Simmonds	New Guinea and around
errans (Blanco) R.V.Valmayor	Philippines
microcarpa (Becc.) Simmonds	Borneo (and Sulawesi?)
zebrina Van Houtte ex J.É. Planch.	southwest Indonesia
malaccensis (Ridl.) Simmonds	Malaysia and Thailand (dominant in the south)
truncata (Ridl.) Kiew.	Malaysia and southernThailand: higher altitudes
siamea Simmonds	Thailand, Vietnam, southern China
burmannica Simmonds	western Thailand, Myanmar, India

Phylogenetic background of taxa analyzed

Morphological diversity within the species *M. acuminata* is broadly linked to geographic distribution (Table 1, De Langhe *et al*. 2009). Molecular analyses indicate that *M. acuminata* ssp. *siamea* and *M. acuminata* ssp. *burmannica* probably form one subspecies (Perrier *et al*. 2009), which confirms morphological observations in Thailand (Volkaert & De Langhe, fieldwork). Consequently, they are referred to as the *burmannica-siamea* complex here.

One of the examined edible AA samples, 'Guyod' (Table 2, number 18) is from the Philippines, where the subspecies *errans* occurs naturally. However, the exact status of *errans* as a subspecies is unclear (Carreel *et al*. 1994). Based on the similarities of morphologic traits, the *errans*

Table 2. Morphometric data of *Musa acuminata* samples. Notes: ITC = International Transit Centre (IPGRI, KULeuven); PNG = Papua New Guinea; Volkaert = leaf samples collected in situ; ST = southern Thailand; WT = western Thailand; NT = northern Thailand; ET = eastern Thailand; NST = Nakornsrithammarat Province; CW = crater width in µm

#	Taxon	Name	Origin	Mean CW (µm)	Range	S^2
WILD						
1	*Musa acuminata* ssp. *banksii*		PNG, ITC 0955	5.98	3.6-8.9	1.0
2	*Musa acuminata* ssp. *malaccensis*	SriLanna, ChiangMai	NT, Volkaert	7.10	4.8-10.5	1.4
3		Maesot, Tak	NT, Volkaert	6.92	4.6-10.1	1.1
4		Kra, Ranong	ST, Volkaert	7.03	4.9-10.3	1.1
5		Namtok Thanto, Ranong	ST, Volkaert	6.86	4.6-9.8	0.7
6		HuaiYot, Trang	ST, Volkaert	6.53	3.9-11.8	2.0
7		Kapaang, NST	ST, Volkaert	6.62	4.5-10.9	1.2
8		Namtok Phnomlook, NST	ST, Volkaert	6.48	4.7-9.8	1.2
9	*Musa acuminata* ssp. *burmannica-siamea*	PangSida, Sakaew	ET, Volkaert	6.42	4.6-10.1	1.0
10		Khlung, Chanthaburi	ET, Volkaert	7.08	3.8-14.0	1.8
11		Pala-U, Prachuabkhirikhan	WT, Volkaert	6.53	4.2-10.1	1.4
12		Phrao, ChiangMai	NT, Volkaert	7.83	5.1-11.3	1.4
13		MaeWong, Kamphaengphet	WT, Volkaert	8.11	5.3-12.7	2.0
14		SaiYoke, Kanchanaburi	WT, Volkaert	7.17	4.1-10.3	1.2
15	*Musa acuminata*	Betong, Yala	ST, Volkaert	6.82	4.2-10.0	1.2
EDIBLE						
16	AA	Djum Tao	ITC 0292	5.72	3.9-8.1	0.8
17		Uwati	ITC 0373	5.85	3.7-8.7	1.1
18		Guyod	ITC 0299	5.96	3.7-8.4	1.0
19	AAA	Cavendish : Pte Naine	ITC 0654	7.59	4.7-11.9	2.4
20		Cavendish : P. Masak Hiyau	ITC 0340	7.13	4.2-10.2	1.1
21		Red	ITC 0403	7.11	4.2-10.8	1.1

population can be considered as part of the *M. acuminata* ssp. *banksii* complex. Although crossing behavior of *errans* with *banksii* has not yet been evaluated, molecular analysis (SSR markers) suggests a close association of *errans* with *banksii* (Perrier *et al*. 2009). Significantly, the cytoplasmic DNA of 'Guyod' does not reflect such an association; it is hypothesized that the cultivar is the product of a hybrid '*errans* x *banksii*' backcrossed to *banksii* (Perrier pers. comm. 2008). Consequently, the cultivar 'Guyod' is hereby classified as 'close-to-*banksii*', and considered together with two other AA cultivars from New Guinea, '**Djum Tao**' and '**Uwati**'.

Molecular genetic studies strongly indicate that *banksii* is probably the major subspecies that contributed to domestication and the development of edible, parthenocarpic cultivars (Perrier *et al*. 2009). These studies also indicate that almost all AA cultivars (AAcvs) are the result of inter-subspecific hybridizations. However, the results of complementary analyses at cytoplasmic DNA level imply the presence of several *banksii* alleles in all AAcvs (Carreel *et al*. 2002). Consequently, the following historical hypothesis can be proposed: (1) in a first phase, and through cultural contact, initial inter-subspecific AAcvs were generated with a major contribution of *banksii*, and input from *microcarpa* and *zebrina*; (2) in a later phase, other cultural interactions brought the basic AAcvs into contact with the more 'northern' subspecies *malaccensis*, *errans*, *truncata* (?) and perhaps *burmannica/siamea*. Subsequently, over a long period of clonal propagation, the popular AA- and AAAcvs underwent frequent somaclonal mutations to form modern cultivar subgroups (Bakry *et al*. 2009). This hypothesis accounts for the presence of *banksii* DNA in many edible AA and AAA, including the Cavendish cvs, which is the primary commercial banana consumed worldwide (Perrier *et al*. 2009).

Material and methods

Phytoliths from the leaves of 21 banana accessions were examined during the current study (Table 2). Six leaf samples were supplied by the International Musa Transit Centre (ITC), Laboratory of Tropical Crop Improvement, K.U. Leuven, which hosts the *in vitro* world reference collection of Musaceae. The corresponding ITC plants were regenerated from the *in vitro* form in greenhouses with regular soil as substrate and the leaf samples were harvested and dried at about the 5th foliage leaf stage. The other 15 samples came from leaves collected during fieldwork in Thailand (H. Volkaert).

Phytoliths were extracted from leaf samples at the Brigham Young University, Provo, Microscopy Laboratory using wet ashing as described by Ball *et al*. (1993). They were described and named according to ICPN 1.0 (Madella *et al*. 2005). Morphotypic analysis of the extracted phytoliths (i.e., analysis of the variants of the volcaniform phytoliths produced by the taxa) was then conducted to determine if some studied taxa produced either distinctive volcaniform phytolith variants or distinctive frequencies of variants. Two direct and two derived morphometric parameters were examined: base length and crater width; "aspect product" (base length multiplied by crater width) and "aspect ratio" (base length divided by crater width) (Figure 1).

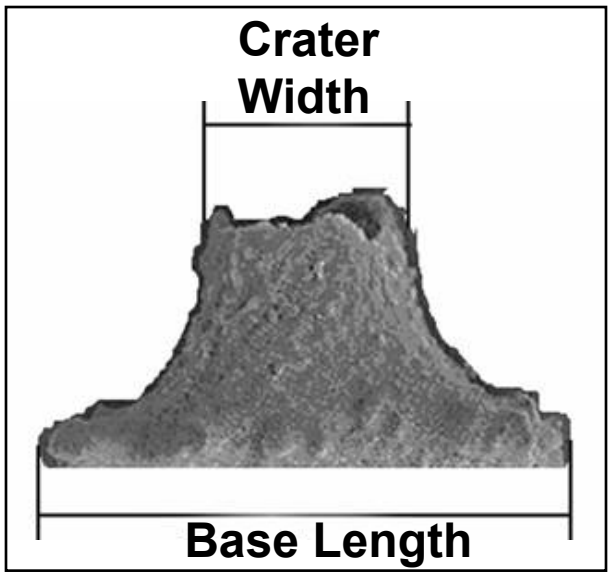

Figure 1. Parameters used for morphometric analysis of volcaniform banana phytoliths.

Statistical analysis of the data was conducted using the SAS System for Windows data analysis software (SAS Institute, Cary, North Carolina). Descriptive statistics by taxon of the mean, minima, maxima and variances were obtained for each of the morphometries evaluated. Shapiro-Wilk W tests for normality were performed to determine if the data could be confidently analyzed using parametric statistics.

Results

Morphotypology

Ball *et al*. (2006) identified eight variants of the volcaniform phytoliths produced by *Musa* (Table 3). Table 4 lists the frequency distribution of the variants observed in each of the accessions analyzed, based on a count of 100 phytoliths from each accession, with the exception of AAcv ITC 0373 (n = 70) and AAA ITC 0654 (n = 98).

The most frequent variants are 1 (volcaniform, regular base, central concave cone) and 3 (volcaniform, regular base, eccentric concave cone). The wild forms display a V1 and V3 percentage ranging from 66% (sample 9) to 99% (samples 6, 11, 13, 14, 15) while this percentage tends to be lower in the observed edible forms (Figure 2).

None of the variants exhibited the necessary morphotypical details to enable the discrimination of wild forms from edible ones, or of edible diploids from triploids. However, we should not exclude the possibility that the frequencies of Variants 1 and 3 may contribute to some extent to the differentiation of edible from wild forms. Therefore, we intend to investigate this aspect further by examining a wider range of edible AA and AAA samples.

Table 3. Summary of observed banana volcaniform morphotype variants.

Morphotype	Name
Variant 1	Volcaniform, regular base, central concave cone
Variant 2	Volcaniform, irregular base, central concave cone
Variant 3	Volcaniform, regular base, eccentric concave cone
Variant 4	Volcaniform, irregular base, eccentric concave cone
Variant 5	Volcaniform, regular base, central convex cone
Variant 6	Volcaniform, regular base, eccentric convex cone
Variant 7	Volcaniform, irregular base, central convex cone
Variant 8	Volcaniform, irregular base, eccentric convex cone

Morphometry

Shapiro-Wilk W tests for normality showed that the data distribution was normal for Crater Width but slightly skewed towards higher values for Base Length. The skewed Base Length frequency distribution may be an artifact of the analysis. The bases of volcaniform phytoliths are comparatively fragile bodies that connect to each other through the walls of the cells that contain them (Ball *et al*. 2006). During the extraction of phytoliths, these thin bases may, in some cases, break at the junction of the walls, thus producing artefacts with shorter Base Lengths than in reality. The apparently thicker and more robust part of the volcaniform phytoliths that forms the crater is not so susceptible to breakage. Due to these problems, the derived parameters “aspect product” and “aspect ratio” are biased due to obtained variable Base Length. Accordingly, only the analyses for Crater Width (CW) are henceforth used for analysis and interpretation.

Table 4. Percentage of banana volcaniform morphotype variants in each sample.

	Taxon	Name	Origin	n	1	2	3	4	5	6	7	8	1+3 (%)
WILD													
1	*banksii*		PNG, ITC0955	100	51	2	42	0	2	3	0	0	93
2	*malaccensis*	Sri Lanna	NT, Volkaert	100	43	3	44	0	4	5	0	1	87
3		Maesot	NT, Volkaert	100	20	0	76	0	0	4	0	0	96
4		Kra	ST, Volkaert	100	39	1	57	0	0	3	0	0	96
5		Namtok Thanto	ST, Volkaert	100	39	1	57	0	0	3	0	0	96
6		Huai Yot	ST, Volkaert	100	20	0	79	1	0	0	0	0	99
7		Kapaang	ST, Volkaert	100	19	1	73	2	1	4	0	0	92
8		Namtok Phnomlook	ST, Volkaert	100	15	1	83	1	0	0	0	0	98
9	*burmannica-siamea*	PangSida	ET, Volkaert	100	17	3	49	7	1	22	0	1	66
10		Khlung	ET, Volkaert	100	47	1	45	4	0	3	0	0	92
11		Pala-U	WT, Volkaert	100	14	0	85	0	0	1	0	0	99
12		Phrao	NT, Volkaert	100	17	0	78	5	0	0	0	0	98
13		Mae Wong	WT, Volkaert	100	14	1	85	0	0	0	0	0	95
14		Sai Yoke	WT, Volkaert	100	17	0	82	0	0	1	0	0	99
15	*truncata*	Betong’	ST, Volkaert	100	20	0	79	1	0	0	0	0	99
EDIBLE													
16	AA	Djum Tao	ITC0292	100	43	1	27	6	5	12	6	0	70
17		Uwati	ITC0373	70	46	2	15	1	2	3	1	0	87
18		Guyod	ITC0299	100	58	6	29	0	4	3	0	0	87
19	AAA	Cavendish: Pte Naine	ITC0654	98	25	8	48	6	2	9	0	0	74
20		Cavendish: P.Masak Hiyau	ITC0340	100	21	11	62	4	0	0	1	1	83
21		Red	ITC0403	100	34	7	55	1	1	2	0	0	89

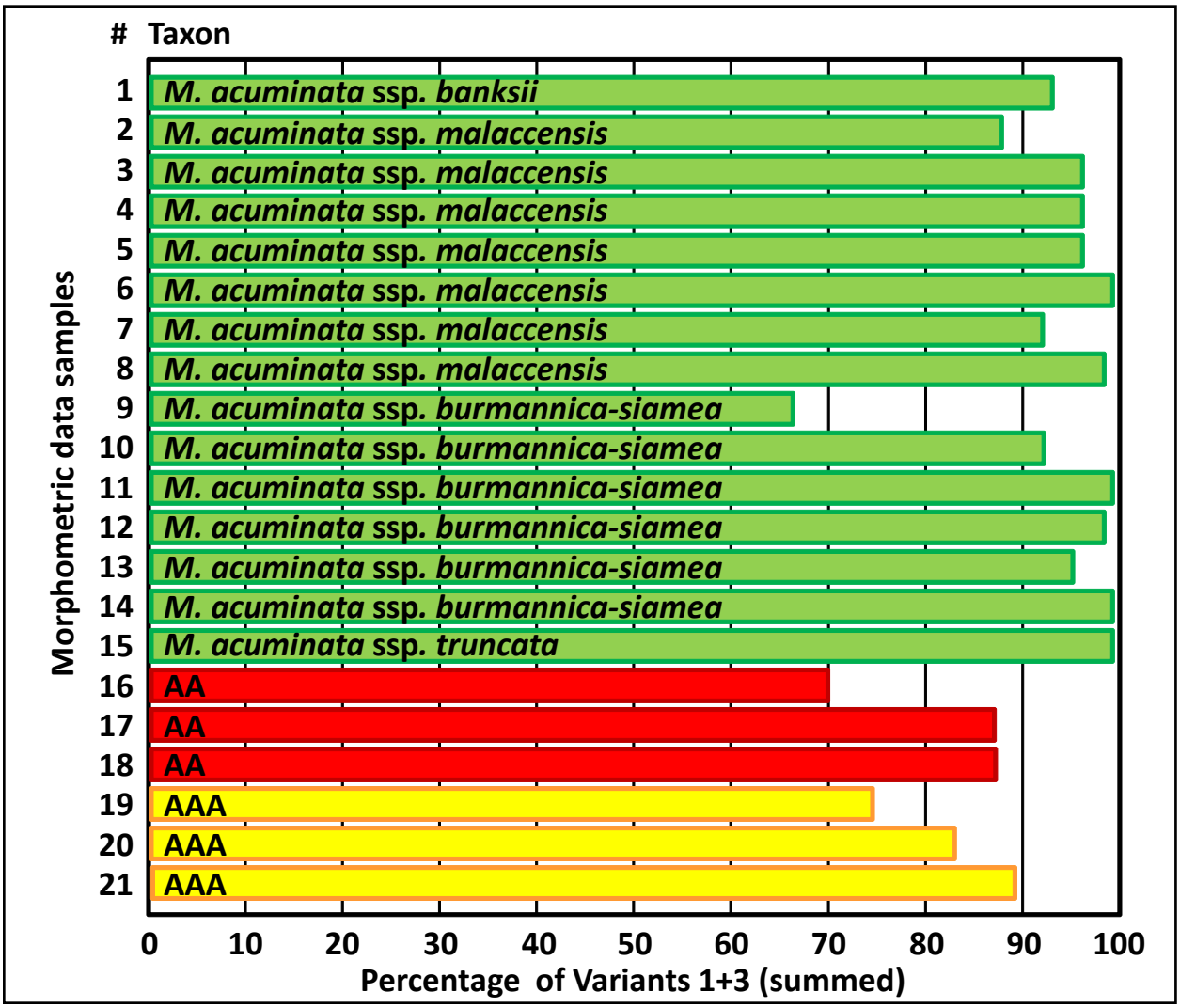

Figure 2. Percentages of morphometic variants 1+3 (summed) in wild (green), edible diploid (red), and edible triploid (yellow) banana varieties sampled.

Table 5 presents Tukey HSD test results for significant differences between sample CW means. The difference between the mean CW of each sample in the vertical "subspecies" column and the horizontal column is recorded in the matrix. Thus the mean of every sample is compared to the mean of every other sample in the study. For this data set, the difference between any two sample means is significant at $\alpha = 0.05$ when it exceeds an absolute value of 0.62.

Deductions

Sampling consistency

The abundance of samples analyzed for the subspecies *malaccensis* and *burmannica-siamea* was intended to assess consistency in the results. The samples were collected *in situ* from widely different places in Thailand in order to evaluate environmental influence on CW. Of the seven samples of *M. acuminata* ssp. *malaccensis* analyzed only the extreme values of samples 2 and 8 had significantly different CW means. This finding suggests that environmental influences may not play a significant role in determining phytolith CW for this subspecies. The results provide circumstantial evidence that the method and techniques used are reliable, since they correspond to morphologic and genetic findings of close affiliation within *malaccensis*.

In sharp contrast to *malaccensis*, the *burmannica-siamea* complex displayed a large CW spectrum, with 11 out of 15 pairs showing significant differences. The samples 12 and 13 are responsible for 8 of the 11 significant differences. If, as apparently with *malaccensis*, environmental influences are not the source of this wide spectrum, then

Table 5. Absolute differences between the mean crater widths (CW) of banana taxa analyzed. Those in **bold** font are significantly different (α = 0.05) according to Tukey HSD tests.

		Differences Between Means																				
Subspecies		***banksii***	***malaccensis***							***burmanicasiamea***						***truncata***	**Edible AA**			**Edible AAA**		
Sample #		**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**
Mean Crater Width (µm)		5.98	7.10	6.92	**7.03**	**6.86**	6.53	**6.62**	6.48	6.42	7.08	6.53	7.83	8.11	7.17	6.82	5.72	5.85	5.96	7.59	7.13	7.11
Sample #	**1**		**1.12**	**0.94**	**1.05**	**0.88**	0.55	**0.64**	0.50	0.44	**1.10**	0.55	**1.85**	**2.13**	**1.19**	**0.84**	0.26	0.13	0.02	**1.61**	**1.15**	**1.13**
	2	**1.12**		0.18	0.07	0.24	0.57	0.48	**0.62**	**0.68**	0.02	0.57	**0.73**	**1.01**	0.07	0.28	**1.38**	**1.25**	**1.14**	0.49	0.03	0.01
	3	**0.94**	0.18		0.11	0.06	0.39	0.30	0.44	0.50	0.16	0.39	**0.91**	**1.19**	0.25	0.10	**1.20**	**1.07**	**0.96**	**0.67**	0.21	0.19
	4	**1.05**	0.07	0.11		0.17	0.50	0.41	0.55	0.61	0.05	0.50	**0.80**	**1.08**	0.14	0.21	**1.31**	**1.18**	**1.07**	0.56	0.10	0.08
	5	**0.88**	0.24	0.06	0.17		0.33	0.24	0.38	0.44	0.22	0.33	**0.97**	**1.25**	0.31	0.04	**1.14**	**1.01**	**0.90**	**0.73**	0.27	0.25
	6	0.55	0.57	0.39	0.50	0.33		0.09	0.05	0.11	0.55	0	**1.30**	**1.58**	**0.64**	0.29	**0.81**	**0.68**	0.57	**1.06**	0.60	0.58
	7	**0.64**	0.48	0.30	0.41	0.24	0.09		0.14	0.20	0.46	0.09	**1.21**	**1.49**	0.55	0.20	**0.90**	**0.77**	**0.66**	**0.97**	0.51	0.49
	8	0.50	**0.62**	0.44	0.55	0.38	0.05	0.14		0.06	0.60	0.05	**1.35**	**1.63**	**0.69**	0.34	**0.76**	**0.63**	0.52	**1.11**	**0.65**	**0.63**
	9	0.44	**0.68**	0.50	0.61	0.44	0.11	0.20	0.06		**0.66**	0.11	**1.41**	**1.69**	**0.75**	0.40	**0.70**	0.57	0.46	**1.17**	**0.71**	**0.69**
	10	**1.10**	0.02	0.16	0.05	0.22	0.55	0.46	0.60	**0.66**		0.55	**0.75**	**1.03**	0.09	0.26	**1.36**	**1.23**	**1.12**	0.51	0.05	0.03
	11	0.55	0.57	0.39	0.50	0.33	0	0.09	0.05	0.11	0.55		**1.30**	**1.58**	**0.64**	0.29	**0.81**	**0.68**	.57	**1.06**	0.60	0.58
	12	**1.85**	**0.73**	**0.91**	**0.80**	**0.97**	**1.30**	**1.21**	**1.35**	**1.41**	**0.75**	**1.30**		0.28	**0.66**	**1.01**	**2.11**	**1.98**	**1.87**	**0.24**	**0.70**	**0.72**
	13	**2.13**	**1.01**	**1.19**	**1.08**	**1.25**	**1.58**	**1.49**	**1.63**	**1.69**	**1.03**	**1.58**	0.28		**0.94**	**1.29**	**2.39**	**2.26**	**2.15**	**0.52**	**0.98**	**1.00**
	14	**1.19**	0.07	0.25	0.14	0.31	**0.64**	0.55	**0.69**	**0.75**	0.09	**0.64**	**0.66**	**0.94**		0.35	**1.45**	**1.32**	**1.21**	0.42	0.04	0.06
	15	**0.84**	0.28	0.10	0.21	0.04	0.29	0.20	0.34	0.40	0.26	0.29	**1.01**	**1.29**	0.35		**1.10**	**0.97**	**0.86**	**0.77**	0.31	0.29
	16	0.26	**1.38**	**1.20**	**1.31**	**1.14**	**0.81**	**0.90**	**0.76**	**0.70**	**1.36**	**0.81**	**2.11**	**2.39**	**1.45**	**1.10**		0.13	0.24	**1.87**	**1.41**	**1.39**
	17	0.13	**1.25**	**1.07**	**1.18**	**1.01**	**0.68**	**0.77**	**0.63**	0.57	**1.23**	**0.68**	**1.98**	**2.26**	**1.32**	**0.97**	0.13		0.11	**1.74**	**1.28**	**1.26**
	18	0.02	**1.14**	**0.96**	**1.07**	**0.90**	0.57	**0.66**	0.52	0.46	**1.12**	0.57	**1.87**	**2.15**	**1.21**	**0.86**	0.24	0.11		**1.63**	**1.17**	**1.15**
	19	**1.61**	0.49	**0.67**	0.56	**0.73**	**1.06**	**0.97**	**1.11**	**1.17**	0.51	**1.06**	0.24	0.52	0.42	**0.77**	**1.87**	**1.74**	**1.63**		0.46	0.48
	20	**1.15**	0.03	0.21	0.10	0.27	0.60	0.51	**0.65**	**0.71**	0.05	0.60	**0.70**	**0.98**	0.04	0.31	**1.41**	**1.28**	**1.17**	0.46		0.02
	21	**1.13**	0.01	0.19	0.08	0.25	0.58	0.49	**0.63**	**0.69**	0.03	0.58	**0.72**	**1.00**	0.06	0.29	**1.39**	**1.26**	**1.15**	0.48	0.02	

these results suggest the *burmannica-siamea* complex is very heterogeneous.

Larger CW of the 'northern' acuminata subspecies

The mean CW of all the samples of the subspecies *malaccensis*, *burmannica-siamea* and *truncata* combined are 6.79 µm, 7.19 µm and 6.82 µm, respectively. Because the difference between these means is less than 0.62, they are not significantly different at α = 0.05. For reasons of convenience, we propose the collective term 'northern' for these subspecies, with the proviso that the area of *malaccensis* may well stretch from Thailand in the north to Sumatra in the south.

The general CW value of these northern *acuminata* subspecies (Table 2, numbers 2-15) of 7 µm is typically larger than the CW of the subspecies *banksii* and its edible derivatives (if 'Guyod' is included), which is slightly less than 6 µm (Table 2, numbers 1, 16-18). However, the CW of the single wild *banksii* accession is not significantly larger than that of the smallest non-*banksii* accession. Examination of other *banksii* accessions and derivatives should allow for a more precise evaluation of this slight overlap.

Nevertheless, the surprising general contrast in CW between the northern *acuminata* subspecies and the southeastern ssp. *banksii* deserves further investigation.

Triploidy enhances the CW by a factor 1.25?

The substantially larger size of the phytoliths from the AAA samples compared to those of the observed edible AA appears to reflect the analogous difference in the size of the hosting cells. There is no published, systematic comparative study of cell sizes across banana cultivars. The only reliable data concerns the prophase cell volume of male reproductive cells (Simmonds 1962:92, Table VI.1). Although the volume unit is not specified, the triploid cells are clearly larger than diploid cells, 34.7 compared to 17.6. Transformation to one dimension provides the values of 3.26 and 2.60, respectively, which indicates that the size of triploid cells is about 3.26/2.60 or 1.25 larger. Comparably, the ratio triploid/diploid for the CW of AAA versus AA phytoliths is 1.23, i.e., 7.28/5.89, and corresponds well with the cell size ratio. Whether this deduction can hold for all triploids is discussed below. However, the more systematic examination of many more samples should occur before any firm conclusion is reached.

Notably, the dwarfed Cavendish AAA cultivar, 'Petite Naine', has the same CW as the other two AAA cultivars. This is important because it indicates that cell dimensions may not be influenced by phenotypical differences, even spectacular ones.

Discussion

In a previous publication (Ball *et al*. 2006), edible AA diploids were not distinguishable from their wild ancestors using phytolith CW differences. This finding had a logical explanation: changes in fruit development and structure should not influence the basic anatomy of the vegetative plant parts. However, the presently observed CW variation among wild *acuminata* subspecies raises the question of why should all edible AA have similar CW if their wild ancestors are so different?

The difference in CW between the northern *acuminata* subspecies and southeastern *banksii* points to a corresponding difference in cell size. This is surprising for members of the same species and is a signal that variation within the *M. acuminata* species complex may be unusually large. The finding calls for study of the relevant tissue anatomies as well as of CW in subspecies that grow in intermediate regions, i.e., *microcarpa* and *zebrina*. Either a rough cline in CW would be observed from southeast to north over the entire *acuminata* domain, or the species would fall into two distinct entities with respect to phytolith dimension.

Variation in phytolith dimensions among *acuminata* subspecies has implications regarding the CW in triploid AAA cultivars, because they were generated from intersubspecific AAcvs. The three studied AAAs produce a similar CW with similar triploidy enhancement of the CW, if compared to the CW of *banksii* and its derivatives. This may be explained in part because most AAAs, and certainly the Cavendish (Table 2, numbers 19 and 20), have *banksii* alleles in their DNA. Some of these alleles could play a dominant role in phytolith expression. In this sense, the presence of northern *acuminata* DNA in AAAs would have no effect on CW. Incidentally, the Cavendish cultivars have most probably been generated in the northern part of the *acuminata* domain, but with the input of at least one edible AA 'from the south' (Perrier *et al*. 2009).

To verify these interpretations about domestication history, we intend to broaden the spectrum of the AA and AAA samples, principally adding other specimens that are supposed to have been generated in the northern zone, for example the members of the so-called AA '**Khai**' cluster and AAAs such as '**Ibota**' and '**Orotova**.' Such a spectrum would allow for the investigation of the possible effects of domestication on CW in diploids.

Conclusions

The intention of this research was to develop a method for the reliable differentiation and identification of wild and cultivated banana groups using phytoliths. This method can then be applied to the investigation of archaeologi-

cally derived phytolith assemblages in different regions of the tropics for a range of different time periods.

The findings reported here, while sharply focused on *M. acuminata* and its edible derivatives, indicate the need to examine many additional samples before we can propose a firm basis for banana differentiation and identification. If the hypothesis of a dominant role of *banksii* alleles in CW expression can be confirmed, the investigation of archaeological phytolith assemblages would be simplified for those regions where AA and AAA cultivars were important during the early stages of banana domestication, e.g., Indonesia, or during the later stages, e.g., the highlands of East Africa. Once this research has been undertaken the study can move to the hybrid edible groups (AB, AAB, ABB) wherein the *M. balbisiana* genome should interfere with CW expression.

Acknowledgements

We thank Dr Jaroslav Doležel, head of the Laboratory of Molecular Cytogenetics and Cytometry, Institute of Experimental Botany, Olomouc (Czech Republic), for assessing the ploidy of the samples from ITC. We greatly appreciate the helpful critical review by Xavier Perrier (CIRAD, France), especially regarding phylogenetic issues.

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