Annals of Botany **106**: 849–857, 2010
doi:10.1093/aob/mcq187, available online at www.aob.oxfordjournals.org

ANNALS OF BOTANY
Founded 1887

VIEWPOINT

Did backcrossing contribute to the origin of hybrid edible bananas?

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Received: 26 April 2010 Returned for revision: 21 May 2010 Accepted: 25 August 2010 Published electronically: 20 September 2010

• *Background* Bananas and plantains (*Musa* spp.) provide a staple food for many millions of people living in the humid tropics. The cultivated varieties (cultivars) are seedless parthenocarpic clones of which the origin remains unclear. Many are believed to be diploid and polyploid hybrids involving the A genome diploid *M. acuminata* and the B genome *M. balbisiana*, with the hybrid genomes consisting of a simple combination of the parental ones. Thus the genomic constitution of the diploids has been classified as AB, and that of the triploids as AAB or ABB. However, the morphology of many accessions is biased towards either the A or B phenotype and does not conform to predictions based on these genomic formulae.

• *Scope* On the basis of published cytotypes (mitochondrial and chloroplast genomes), we speculate here that the hybrid banana genomes are unbalanced with respect to the parental ones, and/or that inter-genome translocation chromosomes are relatively common. We hypothesize that the evolution under domestication of cultivated banana hybrids is more likely to have passed through an intermediate hybrid, which was then involved in a variety of backcrossing events. We present experimental data supporting our hypothesis and we propose a set of experimental approaches to test it, thereby indicating other possibilities for explaining some of the unbalanced genome expressions. Progress in this area would not only throw more light on the origin of one of the most important crops, but provide data of general relevance for the evolution under domestication of many other important clonal crops. At the same time, a complex origin of the cultivated banana hybrids would imply a reconsideration of current breeding strategies.

Key words: Backcrossing, banana, breeding, genotype, hybrids, *Musa*.

INTRODUCTION

Vegetatively propagated crop plants have a significant role in human nutrition and livestock feed, and provide raw materials for industrial uses. Among others, they are an important source of carbohydrates in the human diet: potato in temperate climate zones, cassava, banana, sweet potato, taro and yam in warm and tropical regions. Despite the vital importance of clonal crops for the humankind, surprisingly little is known about their origins. Until recently, domestication of clonally propagated crops has been considered a simple process dubbed 'single-step domestication' in which spontaneous variants were picked up by early farmers and since then propagated vegetatively (Zohary, 2004). Although the knowledge on the evolution of clonally propagated crops under domestication remains poor, growing evidence disproves this oversimplified view (reviewed by McKey *et al.*, 2010). The observation of traditional farming practices and the analysis of genetic structure of local landraces provided evidence that clonal crops are often grown as mixed clonal–sexual systems. The praxis of including the so called 'volunteer seedlings' originating from intra- or even inter-specific hybridizations into the stocks of clones has been observed for a number of species, including cassava (Elias *et al.*, 2001), ensete (Shigeta, 1996), potato (Johns and Keen, 1986), taro (Caillon *et al.*, 2006) and yam (Scarcelli *et al.*, 2006). McKey *et al.* (2010) argue that the mixed clonal–sexual systems provided many opportunities for accumulation of domesticated traits.

Bananas and plantains (*Musa* spp., here collectively called bananas) provide a staple food for many millions of people living in the humid tropics and are believed to be one of earliest plant species to be domesticated (Denham *et al.*, 2003). The cultivated banana is a sterile, parthenocarpic plant (Heslop-Harrison and Schwarzacher, 2007) selected by early farmers in south-east Asia, and thereafter maintained by vegetative propagation. Most of cultivated banana accessions are diploid or triploid and it is believed that they originated from intra- and inter-specific hybridizations between seed-bearing subspecies of *M. acuminata* (A genome donor) and *M. balbisiana* (B genome donor) (Cheesman, 1948; Simmonds and Shepherd, 1955). As a result, the various types can be classified on the basis of their genome constitution, as AA and AB (diploids), and AAA, AAB and ABB (triploids). Simmonds (1962) attempted to further classify the triploid types based on crosses with the parental species, arguing that a given AAB accession could have arisen via either the cross (AB) × AA, or the cross (AA) × BB (parentheses indicate the source of female meiotic restitution). Thus,

the extant triploid represents the fusion of a diploid female gamete and a male A or B genome gamete.

However, the morphology of many banana varieties presumably originating from crosses between the A and B genome donors shows a bias towards the A or B phenotype, and does not correspond to the simple genome formulas proposed by Simmonds and Shepherd (1955). This may indicate that the origin of cultivated banana varieties was not a single-step affair and that the domestication involved a set of backcrosses and human selection leading to a modern-day crop. The idea that selected plants grown by early humans produced seed progeny after backcrossing is supported by the observation of residual fertility in most of clonally propagated banana varieties (De Langhe *et al.*, 2009). Moreover, in analogy with the praxis observed in some places until today (Johns and Keen, 1986; Shigeta, 1996; Elias *et al.*, 2001; Caillon *et al.*, 2006; Scarcelli *et al.*, 2006), one may speculate that the early cultivation of banana comprised mixed clonal–sexual systems. Cultivation of banana by the early farmers in tropical rainforest gaps (Groube, 1989) could provide a favourable environment for intra- and interspecific hybridizations and growth of hybrid volunteer seedlings, which were subject to human selection.

Here, we present a backcross hypothesis on the origin of cultivated banana varieties, and suggest experimental approaches designed to verify it. Approval of our proposal on the origin of cultivated banana clones through one or more backcrosses would fill an important gap in the history of humankind, tropical agriculture in particular, provide an argument to support the concept of a more complicated origin of clonal crops and, last but not least, call for reconsideration of strategies used in the current banana improvement programmes.

THE MORPHOLOGICAL SCORING SYSTEM AND ITS DIFFICULTIES

Simmonds and Shepherd (1955) selected a set of 15 morphological descriptors; each scored on a 1–5 scale, with a score of 1 indicating the *M. acuminata* and 5 the *M. balbisiana* form. Each of the traits was given an equal weighting to derive an aggregate score. The model was developed from an analysis of ten diploid and 31 triploid banana accessions, chosen to represent the phenotypic range of the edible banana. The overall scores were classified in four groups: 15–21, 26–41, 48 (only one cultivar examined) and 59–62·5, which agreed with the theoretical values of 15, 35, 45 and 55 for the AA/AAA, AAB, AB and ABB genome constitutions.

The rarity of edible AB types should be underlined. In mainland south-east Asia and the Philippines, where both wild *Musa* species are abundant, several edible AA – but not a single AB – are known, and the only two documented edible AB accessions ('Ney Poovan' and 'Kunnan') are confined to south India. The absence of any natural AB types across the centre of *Musa* diversity (from India to Papua New Guinea) diminishes the likelihood of a natural (AB) × AA cross occurring, suggesting instead that the (AA) × BB route is the more probable one. A few putative AB cultivars may have been found in Papua New Guinea, but their exact taxonomic status has not been established.

A major difficulty in the phenotypic scoring system relates to the range in aggregate score shown by the triploid accessions. For example, the expected range for AAB is 35–37, but the cultivars 'Pome' and 'Silk', both classified as AAB, scored 45·5 and 26, repectively (Simmonds and Shepherd, 1955). Such wide deviations can hardly be explained by experimental error. *Musa balbisiana* is hardly variable in its morphology, and not one of the many *acuminata* subspecies shows any of the *balbisiana* characteristics used by Simmonds and Shepherd, so that original variation in the wild ancestors cannot explain the deviations.

ORGANELLE GENOMES SUGGEST MORE COMPLEX ORIGIN OF HYBRID VARIETIES

The transmission of cytoplasmic DNA in two controlled crosses in *M. acuminata* showed that the chloroplast DNA (cpDNA) and the mitochondrial DNA (mtDNA) genomes were most probably inherited from the maternal and paternal parent, respectively (Fauré *et al.*, 1994). Thus the pattern of organelle inheritance could provide a simple diagnostic tool to distinguish the parental origins of the genomes present in interspecific hybrids, although whether this particular mode of transmission is shared throughout the entire *Musa* genus remains to be confirmed. For the present analysis, we have assumed, however, that this is the case. Characteristic cytotypes of wild and edible bananas were identified by Carreel (1994), Carreel *et al.*, (2002) and Boonruangrod *et al.* (2008), and these were used to construct a presumptive phylogeny (Table 1). For the sake of simplicity, the cpDNA polymorphisms identified by Boonruangrod *et al.* (2008) as Ca1–Ca3 have been grouped here as Ca (derived from wild *M. acuminata*), while Cb1 and Cb2 have been labelled as Cb (derived from *M. balbisiana*). Similarly, the mtDNA polymorphisms Ma1–Ma4 and Mb1–Mb3 have been combined as Ma and Mb, respectively. The clustering shows that the origin of AAA triploids is consistent with the Simmonds and Shepherd (1955) suggestion, but that, with only one exception ('Pisang Radjah'), the AB and AAB types all have the CaMa cytotype. ABB accessions have either a CaMb or a CbMb cytotype. The routes described below seek to account both for the lack of a cytoplasmic B signal in AB and AAB types, and the deviation from the expected aggregate morphology score among the AAB and ABB types.

BACKCROSS ROUTES

Dominant AAB hybrids with CaMa cytotype

To explain the absence of a B genome plastid and mitochondrion contribution to the cytotype of AB and AAB types, Carreel (1994) suggested that a fertile primary AB hybrid from a cross $AA_{female} \times BB_{male}$ with CaMb cytotype may have been pollinated by an AA donor of cytotype CaMa. This would have ensured that its AA or AB progeny were all CaMa. The pollination of an AB type by AA is known to produce viable diploid progeny, but their frequency is thought to be dependent on the genotype of the primary AB diploid's B genome progenitor (Shepherd, 1999). The secondary AB hybrid of cytotype CaMa could then produce ABA (AAB) offspring of CaMa cytotype when pollinated by an AA type.

TABLE 1. *Cytotypes of 51 diploid and triploid accessions (condensed from Boonruangrod* et al.*, 2008)*

CaMa	Wild *M. acuminata*	1. subspecies *microcarpa* Borneo Malaysia, S/E Borneo ITC0253
		2. ssp. *burmannicoides* Calcutta 4 India, Calcutta ITC0249
		3. ssp. *errans* Agutay Philippines ITC1028
		4. ssp. *siamea* Khae (Phrae) Thailand ITC0660
		5. ssp. *burmannica* Long Tavoy ITC0283
		6. ssp. *banksii* Paliama Papua New Guinea (PNG067) ITC0766
		7. ssp. *banksii* Banksii Papua New Guinea ITC0623
		8. ssp. *zebrina* Zebrina Indonesia ITC1177
		9. ssp. *zebrina* Maia Oa Hawaii ITC0728
		10. ssp. *malaccensis* Malaccensis Peninsular Malaysia ITC0250
	AA cultivars	11. Pisang jari buaya Pisang Jari Buaya Malaysia ITC0312
		12. Sucrier Pisang mas Malaysia ITC0653
		13. Cooking AA Tomolo Papua New Guinea (PNG023) ITC1187
	AAA	14. Cavendish Grande Naine Guadeloupe ITC0180
		15. Cavendish Petite Naine ITC0654
		16. Cavendish Poyo Nigeria ITC0345
		17. Orotava Pisang Kayu, Indonesia (IDN098) ITC0420
		18. Ambon Pisang bakar, Indonesia (IDN106) ITC1064
		19. Gros Michel Gros Michel Guadeloupe ITC0484
		20. Rio Leite ITC0277
		21. Lujugira/Mutika Mbwazirume Burundi ITC0084
		22. Lujugira/Mutika Intokatoke Burundi ITC0082
		23. Ibota Yangambi km5 DR Congo ITC1123
	AB	34. Safet Velchi India ITC0245
		35. Kunnan India, Kerala ITC1034
	AAB	24. Nadan Lady Finger India ITC0582
		25. Pome/Prata Foconah DR Congo ITC0649
		26. Pome/Prata Prata Ana Brazil ITC0962
		27. Plantain Orishele Nigeria ITC1325
		28. Plantain Red Yade Cameroon ITC1140
		29. Silk/Figue Pomme
		30. Popoulu/Maia Popoulou Cameroon ITC0335
		32. Nendra Padaththi
		33. Mysore Pisang Ceylan Malaysia ITC1441
CbMa	AAB	31. Pisang raja Pisang Raja Bulu Indonesia (IDN093) ITC0843
CaMb	ABB	37. Pelipita Pelipita Philippines ITC0472
		38. Bluggoe Dole ITC0767
		39. Saba Saba Philippines ITC1138
		40. Monthan Monthan India ITC0046
		42. Ney Mannan Ice Cream ITC0020
CbMb	ABB	36. Klue teparod Kluai Tiparot Thailand (THA020) ITC0652
		41. Peyan Simili Radjah From India through DR Congo ITC0123
		43. P. Awak Namwa Khom Thailand (THA011) ITC0659
	Wild *M. balbisiana*	45. Pisang Klutuk Wulung Indonesia (IDN056) ITC1063
		46. Pisang Batu, Indonesia (IDN080) ITC1156
		47. Honduras Honduras (seeds) ITC0247
		48. Lal Velchi India NEU0051
		49. Tani ITC1120
		50. Cameroon Sri Lanka ITC0246
		51. Singapuri ITC0248
		52. Butuhan Philippines ITC0564

Abbreviations: C, cpDNA; M, mtDNA; a, originating from *M. acuminata*; b, originating from *M. balbisiana*.

The rarity of edible AB types raises the question as to whether the (AB) × AA route (parentheses indicate the source of female meiotic restitution) could have, in reality, made a contribution to the occurrence of the AAB types which predominate among African and Pacific plantains. The seeming absence of edible AB types outside of India makes the route rather implausible. The alternative, starting from a less-fertile edible AA and via the (AA) × BB cross, appears to be more realistic, since the AAB hybrid would have the CaMb cytotype, and its pollination by a male-fertile AA parent would generate an AAB with the CaMa cytotype. Such a scenario is more than feasible in the situation (as obtains in the lowlands and islands of south-east Asia) in which a small number of wild BB types is surrounded by many AA types. The 'wild' BB had probably been introduced in the remote past to this region by human intervention, and since become naturalized (De Langhe and de Maret, 1999).

AAB hybrids with CbMa cytotype are unusual

To date, only one accession ('Pisang Radjah') appears to possess the CbMa cytotype. A possible origin for this type

may have passed through a primary BA diploid formed by the cross (wild)BB × (edible)AA (CbMa), with the edibility and female restitution of the triploid BAA (CbMa) inherited from the AA pollen parent involved in the (BA) × AA cross.

Multiple origins of ABB hybrids

Boonruangrod *et al.* (2008) observed two cytotypes among ABB accessions: CaMb in 'Pelipita', 'Saba', 'Monthan', 'Ney Mannan' and 'Bluggoe' and CbMb in 'Pisang Awak', 'Peyan' and 'Klue Teparod' (Table 1). The Indian accessions 'Monthan', 'Ney Mannan' and 'Bluggoe' would have been generated from the cross (AB) × BB. However, for the Philippine cultivars 'Pelipita' and 'Saba', the (AB) × BB route is unlikely, since no edible AB types have been recorded in this region. Because edible AA types are endemic, the probable origin is [(AA) × BB] → (AAB) × BB → ABB.

This leaves the problem of the ABB (CbMb) types. The presence of Cb dictates that a BB type was the maternal parent. If the paternal parent of the primary hybrid was an AA type, then this BBA hybrid would have a CbMa cytotype, which has not to date been observed among ABB types. A theoretical route can be imagined, passing through a BA diploid derived from a cross (BB × AA), and its backcross to BB to produce BAB (CbMb) progeny. While this route is imaginable for the Indian ABB accession 'Peyan' and perhaps also for 'Klue Teparod', it does not provide an acceptable explanation of the origin for 'Pisang Awak', since no edible AB types are known in Thailand while the exceptional somaclonal diversity of 'Pisang Awak' indicates its possible origin as a triploid hybrid in this region.

In an alternative scheme, we would assume that edible BB types having female restitution do exist. Then the pedigree of the BBA (CbMb) types could have been via a [(BB) × AA] hybrid (BBA of cytotype CbMa), followed by its pollination with BB. The underlying assumption remains controversial, even though *balbisiana*-like plants bearing more-or-less seedless fruits have been described. Thus, Swangpol *et al.* (2007) have provided cpDNA sequence-based analysis to show that some ABB accessions must have *M. balbisiana* as a maternal ancestor. Furthermore, a DNA analysis, based on six discriminating nuclear gene fragments, of four *balbisiana*-like edible banana specimens from north Thailand (one of which was diploid and the others triploid) showed that no diagnostic A genome sequences were present (G. Volkaert, Kasetsart University, Thailand, pers. comm.).

BACKCROSSES AND THEIR EFFECT AT THE NUCLEAR LEVEL

Table 2 illustrates the potential importance of backcrosses in the creation of diploid and triploid clones.

Backcrosses involving primary AB or BA hybrids

Routes 1, 3, 4 and 6 all consider interspecific hybrids of probable Indian provenance, given that AB diploids are confined to India. Most of the AAB and ABB sub-groups probably also evolved in India. Historically, south-east Asia, including much of present-day Indonesia, was strongly influenced by Indian culture, and this is still reflected in the names given to a number of local banana accessions, such as 'Pisang Keling', 'Pisang Raja' (AAB) and 'Maduranga' (ABB). Farmer selection among AB hybrids in India would have probably concentrated on semi-fertile and semi-parthenocarpic types, thus allowing the formation of secondary AB hybrids and their subsequent pollination by edible AA types. As long as meiotic restitution did not occur in primary AB hybrids (Table 2, routes 1 and 4), random segregation of the A and B genome chromosomes during meiosis would have generated gametes carrying a variable proportion of A and B genome chromosomes. Ectopic pairing between homoeologous chromosomes could lead to reciprocal exchanges and gene conversions (Gaeta and Pires, 2010, Soltis and Soltis, 1999). Recombinant chromosomes A^b and B^a (the superscripts indicate B genome alleles in an A genome background, and vice versa) could then be transmitted to gametes and maintained in the population, leading to secondary diploids with genome constitutions such as A^bA, A^bB, AB^a and BB^a and eventually to triploids of constitution A^bAA, A^bAB, etc. In this way, a 33-chromosome triploid may not necessarily carry a full set of A or B alleles, and this may explain why the morphology of some AAB triploids departs from expectation.

Backcrosses without AB

Routes 2, 5 and 7 deal with hybrids which probably arose outside India – the AAB African and Pacific plantains, the Philippines' ABB accessions 'Pelipita' and 'Saba', and the Thai AAB 'Pisang Awak' and its derivatives. In the whole of this vast region, and after almost a century of cultivar collection, not a single diploid accession has been firmly classified as AB, so that the intermediary AB formation towards these interspecific hybrids seems to be excluded there. The basis of routes 2 and 5 is the abundance of edible AA diploids

TABLE 2. *Seven schemes explaining the origin of the different cytotypes of banana cultivars**

Route	Initial cross	Backcross	End product	Examples
1	AA × BB → AB (CaMb)	AB × AA → AB	AB (CaMa)	Cultivars from India only
		(AB) × AA → ABA	AAB (CaMa)	Indian AABs
2	(AA) × BB → AAB (CaMb)	AAB × AA → AAB	AAB (CaMa)	Plantains, Maia Maoli
3	BB × AA → BA (CbMa)	(BA) × AA → BAA	AAB (CbMa)	Pisang Rajah
4	AA × BB → AB (CaMb)	(AB) × BB → ABB	ABB (CaMb)	Monthan, Ney Mannan, Bluggoe
5	(AA) × BB → AAB (CaMb)	AAB × BB → ABB	ABB (CaMb)	Saba, Pelipita
6	BB × AA → BA (CbMa)	(BA) × BB → BAB	ABB (CbMb)	Peyan, Klue Teparod
7	(BB) × AA → BBA (CbMa)	BBA × BB → BAB	ABB (CbMb)	Pisang Awak

* Genome formulae in parenthesis indicate the source of female restitution.

present in the region stretching from south Thailand to Papua New Guinea. Since the originally rare 'wild' BBs in this region have been naturalized (Simmonds, 1962), the relative rarity of genuine south-east Asian AAB/ABB sub-groups is not surprising.

Route 7 is an attempt to explain the origin of 'Pisang Awak' (ABB with the CbMb cytotype). As detailed earlier, this assumes the presence of edible BB types.

In these schemes, meiosis offers the opportunity for pairing between A and B chromosomes and formation of gametes not containing complete sets of A or B chromosomes (or their multiples), and the presence of recombinant chromosomes, resulting in a bias towards the A or B alleles in interspecific hybrids. A large spectrum of such hybrid triploids would thus have been generated with introgressed *acuminata* or *balbisiana* alleles, of which some could influence morphological and physiological characters. The schemes would provide the explanation why the morphology of several presumed AAB triploids is not scoring according to the strict (2A/1B) ratio.

In the light of the backcross hypothesis, some of the anomalies arising from the morphological scoring system can be revisited.

(*a*) The AAB types 'Mysore' and 'Pome' attract a higher aggregate score than expected for an AAB type. The presence of >11 B genome chromosomes and <22 A genome ones, and/or the presence of recombinant A^b chromosomes would result in a phenotype more like *balbisiana*, provided that additive gene action is involved in determining the diagnostic phenotypic traits

(*b*) The AAB accession 'Silk' has a rather low aggregate score for an AAB type. This could reflect an excess of A genome chromosomes and/or the presence of B^a recombinant chromosomes

(*c*) The accession 'Iholena' considered to be an AAA type on the basis of its morphology, clearly carries some B genome DNA (Lebot *et al.*, 1993). Thus it could be of the form AAB^a or A^bAA. (Fig. 1)

(*d*) As above, ABB types whose morphological score is >55 may represent mixed genomes or contain recombined chromosomes.

FIG. 1. 'Lele', a representative of the Hawaiian Iholena sub-group, classified as AAA by Stover and Simmonds (1987), but as AAB on the basis of isozyme analysis (Lebot *et al.*, 1993). Image courtesy Angela Kepler.

TOWARDS THE VERIFICATION OF THE BACKCROSS HYPOTHESIS

The backcross hypothesis requires the support of data emerging from experimental crosses along with analyses at nuclear, chromosomal DNA, and protein level. In the following, we consider a non-exhaustive set of experimental approaches that could be followed to clarify the evolution under domestication of cultivated bananas.

Field experiments

At least two crossing schemes could supply informative data to support (or exclude) the backcross hypothesis.

$$AAB/ABB \times AA/BB. \quad (1)$$

According to Shepherd (1999), some weakly female fertile triploids AAB/ABB, when pollinated with AA or BB, can produce viable AA, BB and AB diploids. If in such cases the meiosis invariably produces gametes with pure A and/or B genomes (complete sets of A or B chromosomes without A– translocations), the application of the Shepherd–Simmonds scoring method on diploid progeny should reveal distinct groups corresponding with the pure AA, AB and BB morphotypes. However, if the scoring results in a less clear pattern with a number of diploids showing morphotypes between the 'pure' AA, AB and BB, then the gametes produced by triploids either contain a mixture of A and B chromosomes, recombinant chromosomes with A and B alleles, or both.

To distinguish between these two alternatives, several obstacles need first to be overcome. First, the number of extant diploid hybrids is low, because banana improvement focuses on material with agronomic potential and most diploid progeny from triploid × diploid crosses are discarded. Secondly, triploids which are able to generate appreciable numbers of diploid progeny are rare. Some possible AAB candidates have been described (Swennen and Vuylsteke, 1993; Shepherd, 1999), while among ABB types, 'Champa Madras' and some clones within the 'Bluggoe' sub-group have been identified (Shepherd, 1999). A further problem is that many presumed diploid progenies are in fact aneuploid. Thus, diploidy needs to be confirmed by chromosome counting. Finally, it is possible that certain A or B genome alleles are not fully expressed in the hybrid context, complicating the phenotype-based identification of the genomic constitution of the diploid. In a large-scale experiment involving crosses

between several female fertile plantains (AAB) and 'Calcutta 4' (AA), many progeny were found to be either AA or BA diploids (Vuylsteke *et al.*, 1993). All these progeny had, however, an AA phenotype, with the exception of the coloration of the pseudostem. The conclusion drawn was that the accepted AAB designation for plantain could have overestimated the expected 33 % contribution of the B genome.

$$AB \times AA/BB. \quad (2)$$

The edible AB banana 'Ney Poovan' is both female and male sterile (Simmonds, 1962). Until recently, synthetic AB hybrids were not valued for the genetic improvement of commercial AAA cultivars. Systematic efforts to obtain them were initiated during the 1980s in Brazil, in an attempt to replace the disease-susceptible AAB subgroups 'Prata' (= 'Pome') and 'Maça' (= 'Silk'). Backcrosses of these AB types with AA or BB produced many diploid progeny (Shepherd, 1999). Of particular relevance here are the products of the cross 'Bluggoe' (ABB) × 'Calcutta 4' (AA). These were morphologically rather variable (Shepherd, 1999), leading to the suggestion that the megagametophytes, while mostly consisting of recombinants between two possibly differentiated B genome chromosome sets, 'may perhaps have included segments of one or more 'A' chromosomes . . . In some cases, an evident possibility exists for the transfer of specific genes from BB to AA'. Thus the AB × AA/BB route does have potential for allele exchange between A and B genome chromosomes during meiosis.

Analysis at the nuclear, chromosomal, DNA and protein levels

Cytoplasmic inheritance in Musa. In the light of the importance of cytoplasmic markers in tracing the evolutionary history of banana hybrid clones, it is important to confirm the observation of Fauré *et al.* (1994). While paternal inheritance of the chloroplast DNA is now accepted as common in angiosperms, the recorded exceptions point to more complex cytoplasmic inheritance patterns, e.g. in wheat (Tsukamoto *et al.*, 2000). A detailed analysis of interspecific F_1 hybrids between *M. acuminata* subspecies and *M. balbisiana* is called for, to confirm that indeed the chloroplast DNA and the mitochondrial DNA genomes are inherited exclusively from the maternal and paternal parent, respectively, at least in the case of these two *Musa* species.

Nuclear DNA amount. Musa species vary in genome size; those of *M. acuminata* and *M. balbisiana* differ from one another by approx. 10 % (Doležel *et al.*, 1994; Lysák *et al.*, 1999; Bartoš *et al.*, 2005). However, the assumption that the genome size of AB hybrids is the sum of those of its parental genomes does not stand up and, furthermore, genome size can vary between AAB accessions (Lysák *et al.*, 1999). These observations could be explained by intraspecific variation for genome size in both *M. acuminata* and *M. balbisiana* (Lysák *et al.*, 1999), and the existence of genome rearrangements in hybrids and allopolyploids, including sequence deletion, transposon activation and chromosomal rearrangements (Ozkan *et al.*, 2001; Feldman and Levy, 2005; Ma and Gustafson, 2008; Buggs *et al.*, 2009; Parisod *et al.*, 2009). This implies that individual hybrids may carry different recombinant chromosomes and hence different proportions of A and B genomes, as discussed earlier. Thus genome size of hybrids is known to be non-additive and cannot be used as a criterion for assigning genome content of unknown hybrids.

Genomic in situ *hybridization (GISH).* GISH was developed to identify the genomic origin of chromosomes in hybrids and polyploids (Schwarzacher *et al.*, 1989). So far in *Musa*, it has only been possible to recognize the origin of centromeric chromosome regions (Osuji *et al.*, 1997). Thus, D'Hont *et al.* (2000) were unable to exclude unequal representation of one of the two genomes present in AB hybrids. However, GISH was able to show that the ABB cultivar 'Pelipita' carries eight A and 25 B genome chromosomes (rather than 11A + 22B). This latter observation provides one of a few pieces of clear evidence available to date that backcrossing and/or chromosome irregularities underline the origin of banana hybrids. The utility of GISH in *Musa* will depend critically on possibilities to improve the resolution of parental chromosomes.

Chromosome pairing at meiosis. The analysis of meiotic behaviour of AB hybrids led Shepherd (1999) to conclude that the homology between the *Musa* A and B genomes was weak. In a similar study on triploids, he observed low homology between the A and B genomes, depending on genotype and environment to the extent that pairing configurations could not be used to distinguish between auto- and allotriploids (Shepherd, 1999). Although the meiotic behaviour ranged from no restitution to a total restitution, it was unrelated to genome constitution. It is known that the extent of bivalent formation during meiotic prophase in polyploid plants rarely depends on chromosome homology alone, and therefore meiotic configuration may be a weak criterion on which to base genomic relationships (de Wet and Harlan, 1972; Jauhar and Joppa, 1996; Kopecký *et al.*, 2008). This implies that the analysis of meiotic behaviour in *Musa* hybrids may not provide unambiguous data on the homology of their genomes and chromosomes. On the other hand, detailed studies may throw light on the extent of meiotic restitution either during the first or the second division, detect intergenomic recombination (provided the parental chromosomes can be distinguished by GISH), and the range of gamete chromosome numbers.

Analysis at DNA level. DNA technology offers a number of options to determine the genomic content of *Musa* hybrids. The spacer regions (ITS, IGS and ETS) associated with the ribosomal RNA (rDNA) locus have been exploited widely as a diagnostic taxonomic and evolutionary tool (Rauscher *et al.*, 2004; Boonruangrod *et al.*, 2009; Peterson *et al.*, 2009), although it has been recognized that rDNA genes are subject to concerted evolution, which can result in the complete loss of one of the homoeologues (Soltis *et al.*, 2008). Nothing is known concerning the mode of rDNA evolution in *Musa*, but the IGS has been shown to be highly informative between *M. acuminata* and *M. balbisiana* (Lanaud *et al.*, 1992), while Nwakanma *et al.* (2003) were able to use the ITS to confirm the presence of both A and B rDNA copies in several triploid and tetraploid accessions. Boonruangrod *et al.* (2009) were unable to detect acuminata-type ETS loci

in cultivars 'Kluai Tiparot' (ABB or ABBB, ITC0652) and 'Simili Radjah' (ABB, ITC0123). The authors conclude that this resulted from the presence of an incomplete A genome in these hybrids.

In a study of the ITS region, banana hybrid clones have been identified in which ITS of one of the presumed parents was missing (E. Hřibová *et al.*, unpubl. res.). For example, hybrid cultivars 'Maritú' (AAB, ITC0639) and '3 Hands Planty' (AAB, ITC 1132) did not contain the ITS sequence corresponding to the B genome. The absence of the B-genome ITS was also observed in 'Cachaco' (ABB, ITC0643). ITS analysis casts some doubt on genome constitution in other presumably hybrid *Musa* clones such as a wild diploid accession under the name 'Butuhan' (ITC1074), which contained only one type of ITS sequence, although it has been reported as a hybrid between *M. balbisiana* and *M. textilis* (Carreel, 1994). Similarly, 'Tonton Kepa' (ITC0822) reported as a hybrid between *M. acuminata* and *M. schizocarpa* (Carreel *et al.*, 1993) contained only S-genome ITS.

It is not obvious how a concerted evolution of ITS could be completed during vegetative propagation and without meiosis. Consequently, these observations may indicate that the primary hybrids went through additional cycle(s) of sexual reproduction during which the chromosome(s) bearing ITS from the second parent was replaced by random segregation. Alternatively, the number of meioses was sufficiently high to allow for completion of concerted evolution. In any case, our observation on ITS in hybrids warrants further study to confirm their hybrid origin and unravel processes leading to evolution of their genomes. One approach would be to use an array of genes distributed across the parental genomes to avoid the limitation of studying only one locus.

An ideal system to characterize genomic constitution of hybrids would involve many genome-specific markers covering uniformly the parental genomes. To date, relatively few markers have been developed in *Musa*, although a set of approx. 200 microsatellite (SSR) loci has recently been detailed (Hippolyte *et al.*, 2010). The DArT (Diversity Array Technology) platform, recently applied to *Musa* (Kilian, 2007), may be highly effective for genome analysis, thanks to its capacity to detect large numbers of loci in parallel. One of the most attractive approaches would be to generate large numbers of genome-specific single nucleotide polymorphism (SNP) markers. The progress in mass parallel sequencing methods (Mardis, 2008) allows generation of tags from a majority of genes via the RNA-seq approach (Wang *et al.*, 2009) and identify genome-specific SNPs for detailed characterization of genome constitution. The ongoing project on sequencing the A genome *Musa* will further facilitate development of markers in a high-throughput manner (http://www.genoscope.cns.fr/spip/September-8th-2009-Banana-genome.html).

Analysis at the proteome level. *Musa acuminata* and *M. balbisiana* differ in phenotype and as proteins are one of the main drivers of the phenotype, one may assume that proteins specific for *M. acuminata* and *M. balbisina* evolved during evolution. Large sets of proteins can be analysed in a high-throughput manner using the methods of proteomics (Carpentier *et al.*, 2008). One of them, two-dimensional electrophoresis, separates proteins according to their isoelectric point and molecular mass. If isoforms specific for *M. acuminata* and *M. balbisiana* differ in amino acid composition, they can be easily distinguished using two-dimensional electrophoresis. A hybrid between *M. acuminata* and *M. balbisiana* should produce both A-isoforms and B-isoforms of the same protein proportional to the number of A and B chromosomes.

Recently, via proteomics analysis of AA, BB, AAA, AAB, ABB and BBB cultivars, a number of A- and B-specific protein isoforms have been detected and it has been concluded that the proteome phenotype does not necessarily correspond to the expected genome formulas (S. Carpentier *et al.*, unpubl. res.). For example, the A-specific isoforms of phosphoglycerate kinase and abscissic acid ripening protein 1 could not be detected in Cachaco (ABB, ITC0643) and Cacambou (ABB, ITC0058). Moreover, A-specific isoforms have been identified in cultivar Kluai Lep Chang Kut (ITC0647) with assumed BBB genome (Valmayor *et al.*, 2000). The observations at protein level provide additional evidence for a more complex genome structure in some banana cultivars. However, as protein synthesis is controlled by gene regulation, the absence of a protein does not prove the absence of a gene. Thus, the absence of the abscisic acid ripening A-specific isoform in Cachaco was confirmed both at RNA and DNA level (Henry *et al.*, unpubl. res.).

Changes in (allo)polyploid genomes

Although we have stressed the likelihood that backcrossing was involved in evolution by domestication of interspecific hybrid banana varieties to explain departure of (and variation in) phenotype from expectation, a non-exclusive explanation can be based on the modification of gene expression in F_1 hybrids and polyploids. There is a growing body of evidence showing that gene expression in (allo)polyploids such as *Arabidopsis*, *Gossypium*, *Brassica*, *Spartina*, *Tragopogon*, *Triticale* and *Triticum* is non-additive caused by a combination of genome restructuring, the activation of retroelements, elimination of genes and deletion of particular genome regions (Feldman *et al.*, 1997; Comai *et al.*, 2000; Adams *et al.*, 2003; Bottley *et al.*, 2006; Gaeta *et al.*, 2007; Ma and Gustafson, 2008; Buggs *et al.*, 2009; Parisod *et al.*, 2010).

Gene expression in natural and newly synthesized (allo)polyploids can be altered without changing parental genomic sequences after epigenetic modifications, including DNA methylation, histone modification and RNA interference as has been documented in *Arabidopsis*, *Brassica*, *Spartina* and *Triticum* (Shaked *et al.*, 2001; Chen and Ni, 2006; Gaeta *et al.*, 2007; Parisod *et al.*, 2009). When considering epigenetic modifications, it is important to mention the results of Noyer *et al.* (2005) who analysed 30 plantains representing phenotypic diversity of this group of cultivars. The results confirmed a very narrow genetic base of plantains, which may have originated from one seed. However, heritable differences were observed for cytosine methylation. Despite this, no correlation was observed between the phenotypic classification and methylation diversity. Thus, in addition to characterizing the genomic constitution of hybrid banana clones, it is also necessary to analyse patterns of homoeologous gene expression at the

whole genome level. This has become possible recently thanks to the advances in DNA array and next generation technologies (Mardis, 2008). In any case, the assessment of the role of epigenetic modifications on phenotypic diversity of banana hybrid clones should consider the effect of reciprocal crosses as gene expression may depend on the direction of the cross due to genomic imprinting.

CONCLUSIONS

The aim of this paper is to refocus the debate over the evolution of edible banana interspecific hybrid clones. We suggest that not all AB, AAB and ABB cultivars are simple allopolyploids derived from a small number of wide crosses, which left the parental A and B genomes largely unscathed. We hypothesize instead that most, if not all, cultivars have genomes consisting of different proportions of A- and B-genome chromosomes and/or recombinant chromosomes. Results obtained so far at chromosomal, nuclear and cytoplasmic DNA as well as protein levels seem to support this idea. If more experimental data confirm our hypothesis, then the hybrid banana cultivars must have evolved via backcrossing interspecific hybrids to parental species, a process which eventually led to formation of a complex spectrum of genotypes. Out of this varied germplasm, basic cultivars were selected, and their subsequent variation through somaclonal variation led to the so-called AB, AAB and ABB sub-groups. Similar processes might underline the evolution of the edible AA and AAA types by hybridization between subspecies of *M. acuminata*. A possible multiple backcross origin of cultivated bananas has implications for the design of strategies aiming to improve the crop. The presence of unbalanced numbers of A and B genome alleles clearly complicates the elaboration of an effective breeding scheme, which at present mostly aims at substituting an A genome allele by an alternative derived from a AA diploid source of resistance or tolerance to biotic and abiotic stresses Therefore, a rigorous validation of the present hypothesis is needed and we suggest possible experimental approaches. The study would not only throw more light on the origin of one of the most important crops, but provide data of general relevance for the evolution under domestication of many other important clonal crops.

ACKNOWLEDGEMENTS

We thank Ines van den Houwe (International Transit Center, ITC) for supplying various *Musa* accessions. We are grateful to our colleague Pavla Němcová for providing unpublished results, critical reading and useful comments. We thank Pat Heslop-Harrison and two anonymous reviewers for helpful comments and www.smartenglish.co.uk for linguistic advice in the preparation of this manuscript. J.D. has been supported by the Grant Agency of the Czech Republic Academy of Sciences (Contract No. IAA600380703). S.C. has been supported by the Grant Agency of the Flanders Research Foundation (FWO).

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