VIEWPOINT

Did backcrossing contribute to the origin of hybrid edible bananas?

Edmond De Langhe1*, Eva Hřibová2, Sebastien Carpentier1, Jaroslav Doležel2 and Rony Swennen1

1Laboratory of Tropical Crop Improvement, Katholieke Universiteit Leuven, Kasteelpark Arenberg 13 bus 2455, 3001 Leuven, Belgium and 2Laboratory of Molecular Cytogenetics and Cytometry, Institute of Experimental Botany, Sokolovská 6, CZ-77200 Olomouc, Czech Republic

* For correspondence. E-mail edmond.delanghe@chello.be

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 mankind, 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, respectively (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 cytophyses 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 cytophype. ABB accessions have either a CaMb or a CbMb cytophype. 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 cytophype

To explain the absence of a B genome plastid and mitochondrion contribution to the cytophype of AB and AAB types, Carreel (1994) suggested that a fertile primary AB hybrid from a cross AAfemales × BBmales with CaMb cytophype may have been pollinated by an AA donor of cytophype 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 cytophype CaMa could then produce ABA (AAB) offspring of CaMa cytophype when pollinated by an AA type.
The rarity of edible AB types raises the question as to whether the \((AB) \times AA\) 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 \((AA) \times BB\) 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

---

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

| Cytotype | Accession |
|----------|-----------|
| CaMa | Wild M. acuminata |
| AA cultivars |  |
| 1. subspecies microcarpa | Borneo Malaysia, S/E Borneo IT0253 |
| 2. ssp. bumannoides | Calcutta 4 India, Calcutta IT0249 |
| 3. ssp. errans | Agutay Philippines IT0128 |
| 4. ssp. siamea | Khae (Phrae) Thailand IT0660 |
| 5. ssp. bumannica | Long Tavoy IT0283 |
| 6. ssp. banksi | Paliama Papua New Guinea (PNG067) IT0766 |
| 7. ssp. banksi | Banksii Papua New Guinea IT0623 |
| 8. ssp. zebrina | Zebrina Indonesia IT0177 |
| 9. ssp. zebrina | Maia Oa Hawaii IT0728 |
| 10. ssp. malaccensis | Malaccensis Peninsular Malaysia IT0250 |
| 11. Pisang jari buaya | Pisang Jari Buaya Malaysia IT0312 |
| 12. Sucrier | Pisang mas Malaysia IT0653 |
| 13. Cooking AA Tomolo | Papua New Guinea (PNG023) IT1187 |
| AAA |  |
| 14. Cavendish Grande Naine | Guadeloupe IT0180 |
| 15. Cavendish Petite Naine | IT0654 |
| 16. Cavendish Poyo | Nigeria IT0345 |
| 17. Orotava Pisang Kayu | Indonesia (IDN098) IT0420 |
| 18. Ambon Pisang bakar | Indonesia (IDN016) IT0106 |
| 19. Gros Michel | Gros Michel Guadeloupe IT0484 |
| 20. Rio Leite | IT0277 |
| 21. Lujugiria/Mutika | Mbwazirume Burundi IT0084 |
| 22. Lujugiria/Mutika | Intokateke Burundi IT0082 |
| 23. Ibota Yangambi km5 | DR Congo IT1123 |
| AB |  |
| 34. Salet Velchi India | IT0245 |
| 35. Kunnar India, Kerala | IT0134 |
| AAB |  |
| 24. Nandan Lady Finger | India IT0582 |
| 25. Pome/Prata Foconah | DR Congo IT0649 |
| 26. Pome/Prata Prata Ana | Brazil IT0962 |
| 27. Plantain Orishele | Nigeria IT1325 |
| 28. Plantain Red Yade | Cameroon IT1140 |
| 29. Silk/Figue Pomme |  |
| 30. Popoulou/Maia | Popoulou Cameroon IT0335 |
| 31. Nendra Padaththi |  |
| 32. Mysore Pisang Ceylan | Malaysia IT1441 |
| 33. Pisang jari buaya | Ratu Bulu Indonesia (IDN093) IT0843 |
| 34. Pisangan Pelita | Philippines IT0472 |
| 35. Bluggoe Dole | IT0767 |
| 36. Saba Saba | Philippines IT1138 |
| 37. Monthon | Monthon India IT0046 |
| 38. Ney Mannan Ice Cream | IT0020 |
| 39. Klue teparod | Klui Tiparot Thailand (THA020) IT0652 |
| 40. Peyan Simili Radjah | From India through DR Congo IT0123 |
| 41. P. Awak Namwa Khom | Thailand (THA011) IT0659 |
| 42. P. Awak Namwa Khom | Thailand (THA011) IT0659 |
| Wild M. balbisiana |  |
| 43. Pisang Klutuk Wulung | Indonesia (IDN056) IT0106 |
| 44. Pisang Batu, | Indonesia (IDN080) IT1156 |
| 45. P. Awak Namwa Khom | Thailand (THA011) IT0659 |
| 46. P. Awak Namwa Khom | Thailand (THA011) IT0659 |
| 47. Honduras (seeds) | IT0247 |
| 48. Lal Velchi | India NEU0051 |
| 49. Tani | IT1120 |
| 50. Cameroun | Sri Lanka IT0246 |
| 51. Singapuri | IT0248 |
| 52. Butuhan Philippines | IT0564 |

Abbreviations: C, cpDNA; M, mtDNA; a, originating from M. acuminata; b, originating from M. balbisiana.
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 ChMb 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 (ChMb) 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 ChMa 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 (ChMb) 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 this (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.

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 (ChMa) | (BA) × AA → BAA | AAB (ChMa) | Pisang Rajah |
| 4     | AA × BB → AB (CaMb) | (AB) × BB → ABB | ABB (CaMb) | Monthan, Ney Mannan, Bluggoe |
| 5     | (AA) × BB → BA (ChMb) | AAB × BB → ABB | ABB (ChMb) | Saba, Pelipita |
| 6     | BB × AA → BA (ChMb) | (BA) × BB → BAB | ABB (ChMb) | Peyan, Klue Teparod |
| 7     | (BB) × AA → BBA (ChMb) | BBA × BB → BAB | ABB (ChMb) | 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" 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" 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" or A"BB. (Fig. 1)

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

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.

\[ AAB/ABB \times AA/BB. \]  \hspace{1cm} (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.

\[ AB \times AA/BB. \] (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 Faure 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 F1 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 (Ozkán 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 intergeneric 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. textils (Carrel, 1994). Similarly, ‘Tonton Kepa’ (ITC0822) reported as a hybrid between M. acuminata and M. schizocarpa (Carrel 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 meiooses 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. balbisiana 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 abscisic 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 Klui 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 F1 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; Botley 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 identified. 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 Némcová 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).

LITERATURE CITED

Adams KL, Wendel JF. 2005. Novel patterns of gene expression in polyploid plants. Trends in Genetics 21: 539–543.

Adams KL, Cronn R, Perloff R, Wendel JF. 2003. Genes duplicated by polyploidy show unequal contributions to the transcriptome and organ-specific reciprocal silencing. Proceedings of the National Academy of Sciences, USA 100: 4649–4654.

Bartoš J, Alkhimova O, Doleželová M, De Langhe E, Doležel J. 2005. Nuclear genome size and genomic distribution of ribosomal DNA in Musa and Ensete (Musaceae): taxonomic implications. Cytogenetic and Genome Research 109: 50–57.

Boonruangrod R, Desai D, Fluch S, Berenyi M, Burg K. 2008. Identification of cytoplasmic ancestor gene-pools of Musa acuminate Colla and Musa balbisiana Colla and their hybrids by chloroplast and mitochondrial haplotyping. Theoretical and Applied Genetics 118: 43–55.

Boonruangrod R, Fluch S, Burg K. 2009. Elucidation of origin of the present day hybrid banana cultivars using the 5ETS rDNA sequence information. Molecular Breeding 24: 77–91.

Bottley A, Xia GM, Koehner RMD. 2006. Homoeologous gene silencing in hexaploid wheat. The Plant Journal 47: 897–906.

Buggs RJ, Doust AN, Tate JA, et al. 2009. Gene loss and silencing in Tragopogon miscellus (Asteraceae): comparison of natural and synthetic allotetraploids. Hereditas 103: 73–81.

Cailleux A, Quero-Garcia J, Lescure J-P, Lebot V. 2006. Nature of taro (Colocasia esculenta (L.) Schott) genetic diversity prevalent in a Pacific Ocean Island, Vanua Lava, Vanuatu. Genetic Resources and Crop Evolution 53: 1273–1289.

Carpentier S, Panis B, Vertommen A, et al. 2008. Proteome analysis of non-model plants: a challenging but powerful approach. Mass Spectrometry Reviews 27: 354–377.

Carrell F. 1994. Etude de la diversité génétique des bananiers (genre Musa) à l’aide des marqueurs RFLP. These de Doctorat, Institut National Agronomique Paris-Grignon, Paris, France.

Carrell F, Fauré S, Gonzalez de Leon D, et al. 1993. Evaluation de la diversité génétique chez les bananiers diploïdes à l’IRAF-CIRAD. Fruits (numero spécial): 25–40.

Carrell F, Gonzalez de Leon D, Lagoda P, et al. 2002. Ascertaining maternal and paternal lineage within Musa by chloroplast and mitochondrial DNA RFLP analyses. Genome 45: 679–692.

Cheesman EE. 1948. Classification of the bananas. IIIc. Musa paradisiaca L. sp., Musa sapientum L. syst. Kew Bulletin 2:145–153.

Chen ZJ, Ni ZF. 2006. Mechanisms of genomic rearrangements and gene expression changes in plant polyploids. Bioessays 28: 240–252.

Comai L, Tyagi AP, Winter K, et al. 2000. Phenotypic instability and rapid gene silencing in newly formed arabidopsis allotetraploids. The Plant Cell 12: 1551–1567.

De Langhe E, de Maret P. 1999. Tracking the banana: its significance in early agriculture. In: Gosden C, Hather J. eds. The prehistory of food. London: Routledge, 277–296.

De Langhe E, Vrydaghs L, de Maret P, Perrier X, Denham T. 2009. Why bananas matter: an introduction to the history of banana domestication. Ethnobotany Research and Applications 7: 165–177.

Denham TP, Haberer SG, Lentfer C, et al. 2003. Origins of agriculture at Kuk Swamp in the Highlands of New Guinea. Science 301: 189–193.

D’Hont A, Paget-Goy A, Escoute J, Carreel F. 2000. The interspecific genome structure of cultivated banana, Musa spp. revealed by genomic DNA in situ hybridization. Theoretical and Applied Genetics 100: 177–183.

Doležel J, Doleželová M, Novák FJ. 1994. Flow cytometric estimation of nuclear DNA amount in diploid bananas (Musa acuminate and M. balbisiana). Biologia Plantarum 36: 351–357.

Elias M, Penet L, Vindry P, McKee D, Panaud O, Robert T. 2001. Unmanaged sexual reproduction and the dynamics of genetic diversity of a vegetatively propagated crop plant, cassava (Manihot esculenta Crantz), in a traditional farming system. Molecular Ecology 10: 1895–1907.

Fauré S, Noyel JL, Carrel F, Horry JP, Bakry F, Lanaud C. 1994. Maternal inheritance of chloroplast genome and paternal inheritance of mitochondrial genome in bananas (Musa acuminate). Current Genetics 25: 265–269.
Allopolyploidy: a shaping force in the evolution of wheat genomes. *Cytogenetic and Genome Research* **109**: 250–258.

Feldman M, Liu B, Segal G, Abbo S, Levy AA, Vega JM. 1997. Rapid elimination of low-copy DNA sequences in polyploid wheat: A possible mechanism for differentiation of homoeologous chromosomes. *Genetics* **147**: 1381–1387.

Gaeta RT, Pires JC. 2010. Homoeologous recombination in allopolyploids: the polyploid ratchet. *New Phytologist* **186**: 18–28.

Gaeta RT, Pires JC, Iniguez-Luy F, Leon E, Osborn TC. 2007. Genomic changes in resynthesized *Brassica napus* and their effect on gene expression and phenotype. *The Plant Cell* **19**: 3403–3417.

Groube L. 1989. The taming of the rainforest: a model for late Pleistocene forest exploitation in New Guinea. In: Harris D, Hillman GC, eds. *Foraging and farming: the evolution of plant exploitation*. London: Unwin Hyman, 292–304.

Heslop-Harrison JS, Schwarzer T. 2007. Domestication, genomics and the future for banana. *Annals of Botany* **100**: 1073–1084.

Hippolyte I, Bakry F, Seguin M, et al. 2010. A saturated SSR/DArT linkage map of *Musa acuminata* addressing genome rearrangements among bananas. *BMC Plant Biology* **10**: 65. doi:10.1186/1471-2229-10-65.

Jauhar PP, Joppa LR. 1996. Chromosome pairing as a tool in genome analysis: merits and limitations. In: Jauhar PP, ed. *Methods of chromosome analysis in plants*. Boca Raton, FL: CRC Press, 9–37.

Johns T, Keen SL. 1986. On-going evolution of the potato on the Altiplano of Western Bolivia. *Economic Botany* **40**: 409–424.

Kilian A. 2007. Towards effective deployment of diversity arrays technology (dart) in banana genomics and sequencing. In: *Abstracts of the International Conference ‘Plant and Animal Genome XV’*. San Diego, CA: Sherago International, 169.

Kopecký D, Lukaszewski AJ, Doležel J. 2008. Meiotic behaviour of individual chromosomes of *Festuca pratensis* in tetraploid *Lolium multiflorum*. *Chromosome Research* **16**: 987–998.

Lanaud C, Dumontcel HT, Jolivot MP, Glaszmann JC, Deleon DG. 1992. Cytometric analysis of nuclear DNA content in bananas. *Euphytica* **65**: 1344–1350.

Ma XF, Gustafson J. 2008. Allopolyploidization-accommodated genomic sequence changes in triploids. *Annals of Botany* **101**: 825–832.

Mardis ER. 2008. The impact of next-generation sequencing technology on genetics. *Trends in Genetics* **24**: 133–141.

McKay D, Elias M, Pujol B, Duputie A. 2010. Homoeologous recombination in allopolyploids: the polyploid ratchet. *New Phytologist* **186**: 18–28.

Noyer JL, Causse S, Tomekpe K, Bouet A, Baurens FC. 2005. *Cytogenetic and Genome Research*. 318–332.

Osuji JO, Harrison G, Crouch J, Heslop-Harrison JS. 1997. Breeding black sigatoka resistant plantains with a wild banana. *Tropical Agriculture* **70**: 74–77.

Tsukamoto N, Asakura N, Hattori N, Takumi S, Mori N, Nakamura C. 2000. Identification of paternal mitochondrial DNA sequences in the nucleus-cytoplasm hybrids of tetraploid and hexaploid wheat D and D2 plasmons from *Aegilops* species. *Current Genetics* **38**: 208–217.

Wang Z, Gerstein M, Snyder M. 2006. RNA-Seq: a revolutionary tool for transcriptomics. *Nature Reviews Genetics* **10**: 57–63.

Zohary D. 2004. *Unconscious selection and the evolution of domesticated plants*. Economic Botany **58**: 5–10.