

Genomics, Banana Breeding and Superdomestication

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Abstract

Bananas are important for food security, and even under poor conditions give a useful crop. Targets for banana breeding can be divided into five different groups: productivity characters, abiotic stress resistance, biotic stress resistance, post-harvest characters and those related to markets. Yield is a key characteristic selected by breeders and used by farmers, while biotic and abiotic stresses cause major yield losses and unstable production from year to year. *Musa* genomics has developed rapidly since 2000, with the Global *Musa* Genomics Consortium coordinating research on the genomics of banana, aiming to assure the sustainability of banana as a staple food crop by an integrated genetic and genomic understanding. In the 21st century, crop improvement has accelerated by the use of genetic maps and DNA markers to identify useful genes, combine desirable traits or resistance genes and accelerate selection, but the difficulty of working with the sterile, triploid banana crop means these approaches have not been used as extensively as in other crops. Now, knowledge of genomics and understanding of crop design allows superdomestication, involving interactions of breeders and genomic scientists to design the characteristics required from a banana cultivar and consider how to produce this ideal cultivar – how to find and evaluate the genes responsible for particular traits, and how to bring them together in a new cultivar. Superdomestication allows definition of a cultivar with a suite of ideal characters ranging from biotic and abiotic stress resistance, through yield, to post-harvest and nutritional quality – many being quantitative trait loci with changing patterns of expression depending on conditions. Collections of germplasm underpin the search for desirable traits, requiring measuring, finding and conserving biodiversity. The combination of expertise with exploitation of the genepool and use of genomic sciences opens a new range of opportunities for the future, requiring further development of *Musa* genomics.

INTRODUCTION

Bananas and plantains (*Musa* spp.) are a key crop across the humid subtropics with a global production of about 100 million tonnes, providing subsistence farmers and smallholders with both nutrition and a source of income. Even under poor conditions, bananas give a useful crop and contribute to food security. Most *Musa* cultivars are largely sterile with parthenocarpic fruits (fruit development occurs in the absence of seeds) and derived from inter- and intraspecific hybridisation between the species *Musa acuminata* and *Musa balbisiana*, designated with the diploid genomes AA or BB respectively (Heslop-Harrison and Schwarzacher, 2007). Most cultivars have arisen not through deliberate crossing by humans but through hybridisation events where the plants occur together, with farmers propagating parthenocarpic lines. Most, but not all, cultivars are triploid ($2n=3x=33$ chromosomes), typically with AAA, AAB or ABB genome constitutions. The word “banana” tends to describe fruits which are eaten fresh when ripe or used as sweet products; “plantains” and “cooking bananas” are usually cooked. Although there is no botanical distinction between the two, many cooking types are interspecific hybrids (AAB or ABB) while many sweet dessert types are AAA.

TARGETS FOR BANANA BREEDING

Targets for banana breeding, or indeed the breeding of any crop, can be divided into five interrelated groups: productivity characters, abiotic stress resistance, biotic stress resistance, post-harvest characters and those related to markets, environment and livelihoods.

Yield is a key characteristic selected by breeders and used by farmers. Since the beginning of agriculture, crop yields – the amount harvested per unit area in a time period – has been increasing. Increase of harvest index – the proportion of the plant in the harvested part (fruits for banana) – has been important in selection in many crops. Widespread use of dwarf cultivars is a genetic contribution to this in banana, while cultivars with rapid field establishment, with genetic and agronomic contributions, have led to shorter cropping cycles. It is critical that the plant can be propagated reliably at low cost; in banana, historically, this means that appropriate suckers are formed, but the ability to propagate efficiently by tissue culture is becoming increasingly important. For breeding new cultivars, some residual fertility is essential. ‘Highgate’ (AAA) – a mutant of ‘Gros Michel’ (AAA) – has been used as a female parent in the FHIA (*Fundación Hondureña de Investigación Agrícola*) breeding programme because after pollination it produces a few fruits with seeds. Cavendish (AAA), with no residual fertility, cannot be used as a parent. In the future, transformation may be used widely, and it is probable there will be genetic variation in transformability between lines, so cultivars where these procedures are reliable will be preferred.

Abiotic stresses – stress arising from suboptimal environmental conditions – reduce yield and yield stability from season to season, reducing food security. In banana, solutions to abiotic challenges, including water usage, salinity, temperature and wind damage, involve appropriate genetic material and improved agronomy. Abiotic conditions show short- and long-term changes; growing plants in new areas, under changing availability and salinity of water sources, with the adoption of new agronomic practices or new cultivars, and the challenges from a changing or less stable climate are all factors that mean abiotic stress tolerance must be considered as a breeding and selection objective.

Biotic stresses to the banana crop are widely considered elsewhere in these proceedings. The impact of pathogens can be lessened by agronomy, biosecurity and use of chemicals to control pathogens or vectors, but these measures are costly (agrochemical costs represent a third of the production costs of commercial bananas), environmentally undesirable (whether from sprays or agronomic practices such as maintaining bare soil or burning) and ultimately short term. Pathogens evolve rapidly in crop systems because of selective pressure, but there is genetic resistance to most pathogens in germplasm for most species. New mechanisms of disease which are constraining production are being revealed by research; in banana, this includes the integration of the pararetroviruses banana streak viruses, BSVs, in the genome, first found late in the 20th century (Harper et al., 1999).

Breeding objectives related to consumers are important to consider. The year 2008 marked the first time in human history where more than half of the human population was urban, a milestone in a process of continuous change since the dawn of agriculture. This trend is accelerating, and it is extremely unlikely that individuals, or most governments, will wish to reverse the trend away from employment of much of the population as agricultural labourers. In the developed world, less than 2% of the population is now engaged in farming. The requirements of a fruit that is to be eaten by a subsistence farmer or locally are notably different from one which must be harvested on a larger scale, graded, packed, transported, stored, marketed and sold to consumers. Altered ripening characters are needed without, for example, finger loss, and small bruises or blemishes from insect damage or harvesting not only have a cosmetic effect but can lead to ethylene production, rapid ripening and decay. Thus, a major challenge of breeders in partnership with farmers is to reduce the 50% post-harvest wastage of many crops sold in the market – the amount estimated to be harvested but never eaten by a consumer, after all the inputs of labour, water, fertilisers and crop protection have been made. Stability and

sustainability of production are also requirements for marketing. Finally, related to consumer application, new uses of crops, whether through processing (Aurore et al., 2009) or use of what has previously been wasted or regarded as crop byproducts but is now more appropriately named a coproduct, must be considered by breeders as a way to increase utilisation of crops and farmer income. Crop and food processing waste can be a pollutant and potentially expensive to dispose of, so plant breeders must consider ways to minimise its production and alternative uses.

Related to the changes in population, breeding for the next generation of banana cultivars must consider how the crops that will be produced fit into social systems, as now recognised by Bioversity's "Commodities for Livelihoods" programme. Increasing affluence across the world is also changing the nature of people's diets: meat, fruit and oil consumption is rising, a trend which is global. A better quality of food is an important aspect of increasing the standard of living in developing countries. Making yields stable from season to season also helps increase living standards, not only by giving access to a source of food, but by reducing price fluctuations and giving a stable income. Reduction of income stability has an effect of reducing access to capital loans for farmers, used to purchase equipment and machinery or fertiliser and agrochemicals, since the ability to repay becomes less certain.

Are there any features of these which are particularly related to Asian Challenges? While there are specific breeding requirements with temporal or regional constraints, the high-level challenges for the banana crop highlighted above are hardly restricted. Similar demands are made by farmers in temperate and tropical, wet or arid regions, in developing and industrial countries, and whether small holders, cash croppers or commercial growers. While diseases may be restricted to regions, their spread or new mutations of pathogens are likely to mean resistance is eventually required throughout the cropping area.

It is also useful to ask whether any of the demands on our crop germplasm are particularly novel or modern. Insufficient food and poor food security, combined with overexploitation of resources must have combined to drive the change from a hunter-gatherer to the farming society, whether by replacement of populations or transfer of technologies. Over the subsequent millennia, selection of appropriate plants overcame the challenges, such as environmentally destructive practices like slash-and-burn, huge changes in climate or global spread of diseases. In general terms, it has been estimated in other crops that improved agronomy, including fertilisers, cultural practices and crop protection chemicals, has been responsible for about half the increase in yield.

GENOMICS AND ITS APPLICATIONS

"Genomics" describes a set of approaches to understand and study the genome of an organism – the DNA in the nucleus and the organelles, the nature and control of the genes it carries, and the evolution and diversity of those DNA sequences. Genomics involves the sequencing of DNA, including the determination of the complete DNA sequence of the nuclei of organisms, chloroplasts and mitochondria, and viruses. The study of genomics leads to an understanding of the organisation of the genome and the interaction of the different elements within it. The results from genomics can be used to measure DNA diversity in diverse germplasm of a crop and its wild relatives, to determine the relationships between these accessions, to map the locations of genes along the chromosomes and DNA sequence, to determine large-scale genomic or chromosomal rearrangements (which also requires cytogenetic approaches; e.g. Osuji et al., 1997, 1998) and to characterise the interactions between gene loci and different genomes in the nucleus. As noted by the US Environmental Protection Agency, "Genomics is the study of all the genes of a cell, or tissue, at the DNA (genotype), mRNA (transcriptome) or protein (proteomic) levels".

Musa genomics has developed rapidly since 2000 (Roux et al., 2008). The Global *Musa* Genomics Consortium aims to assure the sustainability of banana as a staple food crop by developing an integrated genetic and genomic understanding of the crop,

allowing targeted breeding, transformation and more efficient use of *Musa* biodiversity. Many resources are available: large-insert genomic BAC libraries made from DNA of *Musa acuminata* ‘Calcutta 4’ and *Musa balbisiana* ‘Pisang Klutuk Wulung’ (Pifanelli et al., 2008), and DNA sequence information, with annotation and curation on the *Musa* genomics website. Cheung and Town (2007) have analysed 3 million bases of *Musa* DNA sequence from BAC ends and shown many features of its organisation and genetic structure, while Hřibová et al. (2007), and Hřibová and Doležel (2011) have characterised the repetitive DNA component of the *Musa* genome, representing a substantial part of the 550 Mbp of DNA (Lysak et al., 1999). Expressed sequence tags (ESTs) have been published, including by Santos et al. (2005). Genome expression profiling has been carried out by Coemans et al. (2005), along with transcriptional profiling (e.g. Choudhury et al., 2008). Microarrays can be used to examine changes in gene expression (Davey et al., 2009), and advances are being made in generating protein profiles from banana (Carpentier et al., 2008). Banana genomics researchers can exploit similarities between banana and other species to identify gene functions.

The complete DNA sequence of the banana genome will soon be available (Horry, 2011), and several genotypes and species will be sequenced within a short timescale. The small number of markers on publically available genetic maps of banana (Fauré et al., 1993) has been a limitation to *Musa* genetics, but the complete DNA sequence will give a physical map of all the genes. DNA-based markers now can be used to evaluate the diversity of banana germplasm and its precise properties, characterise stress-related and other genes, and in the future can be expected to enable the measurement and mapping of quantitative trait loci. The diversity and phylogenies of banana are now being explored using various DNA markers (e.g. Boonruangrod et al., 2009; Nair et al., 2005; Teo et al., 2005; Heslop-Harrison and Schwarzacher, 2007; and references therein).

As well as diversity within the existing gene pool of *Musa* which can be discovered with genomic tools, new mutations have been found or induced. Variants have been found either in fields or following tissue culture, and these have been exploited in commercial plantations – particularly well known is the Dwarf Cavendish variant. Induced mutations following gamma radiation have also been exploited and may be useful sources of novel agronomic characters or disease resistance (Roux, 2004). The cultivar ‘Novaria’ (AAA), derived from ‘Grand Naine’ (AAA), shows early flowering (Roux, 2004). The AAB cultivar ‘Pisang Mutiara’, tolerant to black leaf streak, was selected in Malaysia as a somaclonal variant of ‘Pisang Rastali’, and there may be differences in some genes related to disease resistance (Azhar and Heslop-Harrison, 2008).

BREEDING AND SUPERDOMESTICATION OF BANANAS

For the first several thousand years of cropping of banana, farmers propagated, grew and cropped the best plants they saw, a process that worked exceptionally well for banana, where key features of parthenocarpy, high yield (large bunches) and resistance to diseases are very evident. Where cultivars failed, they were replaced by others, for example in the replacement of Gros Michel with Cavendish in the 1950s following devastation of Gros Michel by *Fusarium* wilt, caused by race 1 of *Fusarium oxysporum* f. sp. *cubense*. A similar strategy was used a century ago for all crop species. However, during the 20th century, breeding strategies in most crop species have involved crossing of lines and selection of optimum progeny in field trials of huge numbers of progeny. In most crops, this has led to the almost complete replacement of landraces with improved varieties, with a life of individual varieties being less than a decade, or a little longer in tree crops such as *Citrus*. The crossing programmes have followed the paradigm of intercrossing pairs of optimum varieties and then selecting progeny following inbreeding for several generations which performed better than either parent, summed up in the mantra of “cross the best with the best and hope for the best”. In the 21st century, crop improvement has accelerated by the use of genetic maps and DNA markers to identify useful variant alleles of genes, to plan recombination between desirable traits, to combine different resistance genes and accelerate selection. Improved quantitative methods have

enabled the location of quantitative traits and better measurement of yield components.

A few banana improvement programmes involved in making hybrids and selection have generated improved lines [examples are FHIA in Costa Rica, EMBRAPA (*Empresa Brasileira de Pesquisa Agropecuária*) in Brazil and IITA (International Institute of Tropical Agriculture) in Africa]. Most of today's banana production is however not from the products of deliberate crossing and improvement programmes, but follow the older approach of selection among a few thousand old forms. Hybridisation and the generation of segregating populations for key traits is very difficult in banana, because crossing of a mostly triploid crop is not straightforward, so as pointed out above, there are few genetic maps which now facilitate breeding in many other crops by showing the locations in the genome of particular genes of agronomic interest. Secondly, *Musa* can be regarded as an 'orphan crop' where there has been limited research work to underpin the genetics, with a few notable exceptions in research institutes. Therefore, it can be suggested that banana breeding has largely missed out on the opportunities afforded by directed plant breeding.

Nevertheless, there is a large amount of diversity in the banana gene pool, available both within national collections and from the International Transit Centre, Belgium. New projects are increasing the range of diversity known (e.g. Häkkinen, 2011), although there may be restrictions on its use in tropical countries for biosecurity or possession of germplasm reasons.

It can be argued that the application of genomic tools will enable banana breeding to advance rapidly. This process can be called superdomestication (Vaughan et al., 2007). Superdomestication involves interactions of breeders and genomic scientists to design the characteristics required from a banana cultivar, and then considers how to produce this ideal cultivar – how to find and evaluate the genes responsible for particular traits and how to bring them together in a new cultivar. Superdomestication requires definition of a cultivar with a suite of ideal characters ranging from biotic and abiotic stress resistance, through yield, to post-harvest ripening and packaging qualities – a few involving single genes, but many being quantitative trait loci with many genes involved and with changing patterns of expression depending on conditions. The diverse collections of *Musa* germplasm and the genes within the accessions underpin the search for desirable traits, now requiring work targeted to measuring, as well as finding and conserving, the biodiversity; novel approaches to understanding, conserving and using banana genetic diversity are now required urgently.

The combination of expertise from breeders and farmers, complemented with exploitation of the gene pool and use of genomic sciences for characterisation and breeding, opens up a new range of opportunities for the future of the banana crop, although undoubtedly requires further development of *Musa* genomics. The finding of desirable genes and characterisation of their effects will be a major challenge, although already a number of publications have shown the nature and diversity of genes related to biotic stress resistance in *Musa* (Miller et al., 2008; Azhar and Heslop-Harrison, 2008; Peraza-Echeverria et al., 2008).

After a superdomesticated cultivar has been designed, then the approaches to produce it can be considered, whether resynthesising hybrids from desirable wild relatives, hybridising elite material with residual fertility, generating and rescuing embryos (Bakry, 2008) or transgenic approaches (see e.g. Pérez-Hernández et al., 2006 and earlier papers back to 1994; Swennen et al., 2011). Increased understanding of the physiological basis of parthenocarpy, based on genetic knowledge (Ortíz and Vuylsteke, 1995) may even allow the character to be introduced into lines which previously were seeded. Exploitation of the reproduction of banana through seeds and the manipulation of gamete ploidy (see Ortíz et al., 2009; Ssebuliba et al., 2008) may allow new cultivars to be made with the planned combination of desirable characters. In many cases, the breeder will be most involved in generating the elite plants, but the known value of the outcome, and the possibility of using DNA based molecular tools to assist the selection, can make a desirable elite cultivar much more likely to be found.

CONCLUSIONS

The limited knowledge of the genetics of banana and the nature of the crop as a parthenocarpic, mostly triploid, sterile plant means that many aspects of breeding and selection that have been possible in other crops could not be applied in banana. The germplasm pool, as well as new mutations, includes a large amount of variability which new technologies will allow to be accessed and introduced into new and improved varieties in a designed manner. Superdomestication underpinned by genomic research with collection and assessment of the biodiversity in the genus are likely to ensure the future of the *Musa* crop.

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