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Bananas and Plantains (*Musa* spp.): Transgenics and Biotechnology

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ABSTRACT

Bananas and plantains (*Musa* spp.) are the 4th world's most important food crop after rice, wheat and maize in terms of gross value of production. They are major staple food and source of income for millions of people in tropical and sub-tropical regions. Most of the bananas grown worldwide are produced by small-scale farmers for home consumption or for sale in local and regional markets. Many pests and diseases have significantly affected *Musa* cultivation. Biotechnology and transgenic technology, together with conventional methods can assist in overcoming these problems in developing new banana cultivars. Some successes in genetic engineering of *Musa* have been achieved, enabling the transfer of foreign genes into the plant cells. The transgenic approach shows potential for the genetic improvement of bananas using a wide set of transgenes currently available which may confer resistance to pests and diseases. The use of appropriate constructs may allow the production of pest and disease resistant plants in a significantly shorter period of time than using conventional breeding, especially if several traits can be introduced at the same time. Tissue culture techniques like somatic embryogenesis, micropropagation and embryo culture are used for germplasm exchange, conservation and generation of hybrid materials. Biotechnology also provides great prospects for *Musa* improvement through genomics-based approaches for gene discovery, candidate gene validation, development of molecular markers and their utilization to assist classical breeding programs. This article discusses the application of transgenic technology and biotechnology for sustainable production of bananas and plantains.

Keywords: genetic transformation, genomics, micropropagation, molecular markers, tissue culture
Abbreviations: AFLP, Amplified Fragment Length Polymorphism; **AMPs**, antimicrobial peptides; **BBTV**, *Banana Bunchy Top Virus*; **BSV**, *Banana Streak Virus*; **Bt**, *Bacillus thuringiensis*; **BXW**, Banana *Xanthomonas* wilt; **ECS**, embryogenic cell suspension; **EST**, Expressed Sequence Tag; **MAS**, marker-assisted selection; **QTL**, Quantitative Trait Loci; **PTGS**, post transcriptional gene silencing; **RFLP**, Restriction Fragment Length Polymorphism; **RAPD**, Random Amplified Polymorphic DNA; **SSR**, Simple Sequence Repeats; **SNP**, Single Nucleotide Polymorphism

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Invited Review

INTRODUCTION

Bananas and plantains (*Musa* spp. L), are perennial monocotyledonous herbs (**Fig. 1A, 1B**) that grow well in humid tropical and subtropical regions. In terms of production, bananas are the 4th world's most important food crop, with vast majority of the crop grown and consumed in the tropical and sub-tropical zones. The crop is grown in more than 120 countries, with an annual world production of around 104 million tons, of which around a third is produced in each of the African, Asia-Pacific, Latin American and Caribbean regions (FAOSTAT 2004). Around 87% of all the bananas grown worldwide are produced by small-scale farmers for local consumption as a food security crop, and for local markets than for international trade. They provide a staple food for millions of people, particularly in Africa, an area where the green revolution has had little influence.

Approximately 13% of worldwide banana production is destined for the export market. Although, a small proportion of banana production enters the world market, the banana fruit is extremely important as an export commodity espe-

Fig. 1 Transgenics and biotechnology for improvement of banana. (**A**) mature banana plant with flower, (**B**) fruits initiation; (**C**) Black Sigatoka disease; (**D**) *Xanthomanas* wilt disease; (**E**) Yellow streaks on leaf due to *Banana streak virus* infection; (**F**): Toppling of plant due to nematode infection; (**G**) *In vitro* multiplication of banana shoots; (**H**) Tissue culture plants in soil; (**I**) PCR analysis of transgenic plants amplifying a 500 bp internal fragment of *gus*A gene; lane 1-8: transgenic plants, M: molecular marker, P: plasmid pCAMBIA2301 and C: control untransformed plants; (**J**) Expression of *gus*A gene in root and leaf segments; (**K**) Diversity of 24 wild diploid accessions of banana revealed by SSR primer 0588, *M. acuminata* accessions (1-12 and 14); *M. Schizocarpa* (13); *M. balbisiana* accessions (15-24), M is 100 bp molecular marker.

cially in Latin America and Caribbean which contribute over 83% of the total banana in the international market (FAO 2002). The value of banana exports greatly outranks that of other fruits, such as apples and oranges, as well as vegetables such as tomatoes and potatoes. The export banana industry is also the backbone of the economies of many Caribbean countries, and the crop plays a vital role in the social and political fabrics of the islands. In Africa, only five countries namely, Ivory Coast, Cameroon, Somalia, Ghana and Cape Verde export approximately 427,000 tons banana and plantain (FAO 2002). There are more than 500 banana varieties in the world, but the Cavendish is the most exported banana cultivars.

Bananas and plantains supply more than 25% of the carbohydrate requirements for over 70 million people in Africa (Robinson 1996). East Africa is the largest banana producing and consuming region in Africa with Uganda being the world's second leading producer after India, with the total production of about 10.5 million tons (FAOSTAT 2004). In some of the African countries such as Uganda the daily consumption of banana may exceed 1.6 kg per person (FAOSTAT 2001), highest in the world. Its ability to produce fruits all the year round makes it important food security crop and cash crop in the tropics. Bananas and plantains are consumed in a number of ways with each country that produces the crop having its own traditional dishes and methods of processing. For example, mature unripe bananas and plantains are eaten as a starchy food while ripe bananas are consumed raw as a dessert fruit. They can also be consumed boiled, roasted, or fried in ripe or unripe state. Nutritionally, fresh bananas contain 35% carbohydrates, 6-7% fiber, 1-2% protein and fat, besides the major elements such as potassium, magnesium, phosphorus, calcium, iron, and vitamins A, B6 and C (Robinson 1996). Bananas are also used in the manufacture of beer, wine and other products and form an important part of the cultural life of many people.

Many pests and diseases (**Fig. 1C-F**) have significantly affected *Musa* cultivation. Black Sigatoka (*Mycosphaerella fijiensis*), *Fusarium* wilt (*Fusarium oxysporum* f. sp. *Cubense*), bacterial wilt (*Xanthomonas campestris* pv. *musacearum*), viruses (*Banana bunchy-top virus*, *Banana streak virus*), nematodes (*Radopholus similes* and *Pratylenchus coffeae*) and weevils (*Cosmopolites sordidus*) cause significant crop losses worldwide. Bananas and plantains are predominantly smallholder crops, and most growers cannot afford costly chemicals to control pests and diseases. As diseases continue to spread, there is a growing demand for new improved varieties.

The improvement of bananas through conventional breeding remains difficult process due to long generation times, various levels of ploidy, sterility of most edible cultivars, and limited genetic variability. Despite these limitations, many improved hybrids combining genetic resistance to black Sigatoka with appropriate agronomic characteristics have been developed (Ortiz *et al*. 1998). There is great potential to improve sustainable banana yield by applying biotechnology, which could become an additional useful tool for plant breeders to ensure food security by stabilizing sustainable crop production and improving the socio-economic status of the growers. Large-scale multiplication of banana plants has been done by micropropagation, and is used by commercial companies for multiplication and transport of elite banana germplasm. The development of transgenic banana and plantain has been reported by several groups (May *et al*. 1995; Sagi *et al*. 1995; Becker *et al*. 2000; Ganapathi *et al*. 2001; Khanna *et al*. 2004; Tripathi *et al*. 2005a), although a commercial transgenic banana variety has yet to be released. This paper reviews the advances of application of tissue culture, transgenic technology and genomics for genetic improvement of bananas and plantains.

LIMITATIONS OF CONVENTIONAL BREEDING AND USE OF BIOTECHNOLOGY

Genetic improvement of bananas and plantains through conventional breeding remains difficult and lengthy process. Some important *Musa* subgroups (Cavendish, False Horn plantain) remain recalcitrant to conventional breeding. Most of the cultivated clones are triploids (3x) characterized by low male and female fertility. Seed set per bunch in many clones is less than one seed, and germination in soil is less than one 1%. Some banana cultivars can take more than a year to mature, which prolongs breeding efforts compared to annual crops. Consequently, breeders currently devolve much of their resources for obtaining, rather than evaluating progeny from crosses.

Low seed set and germination rates are the major hindrance for hybrid plant production in most triploid bananas. This makes it difficult to obtain adequate population sizes to select disease resistant cultivars through crossing. Application of aseptic embryo culture techniques has been observed to improve seed germination rates by a factor of 10 or more (Vuylsteke *et al*. 1990). Breeders are using other *in vitro* culture techniques such as shoot-tip culture for multiplication of newly bred genotypes. Micropropagated plants establish faster than conventional suckers and have almost uniform production cycle, which further facilitates establishment and evaluation of hybrids (Robinson 1996). *In vitro* culture also guarantees safe collection, exchange and conservation of germplasm required for identification of breeding traits and facilitates dissemination and propagation of newly selected cultivars or hybrids. The high *in vitro* multiplication rates also enable rapid production of clean or disease-free planting material for establishment of large pollination blocks.

In addition, the problems of fertility could be bypassed by using an array of available biotechnological techniques. Recombinant DNA technology for instance has been beneficial in the improvement of banana cultivars that are difficult to breed and remains the most promising solution for those varieties that are totally sterile. Additionally, the available germplasm may not harbor the desired genes for resistance to many of the diseases like the bacterial wilt caused by *Xanthomonas campestris* pv. *musacearum* that is threatening livelihoods in the Great Lakes region of eastern Africa. The genetic engineering is the best alternative option to control the bacterial wilt epidemics through host resistance.

Biotechnology makes it possible to incorporate genes coding for characters that are not available in the *Musa* gene pool (Tripathi *et al*. 2005b). Using molecular techniques, novel genes encoding agronomically important traits can be identified, isolated, characterized and introduced into cultivars through genetic transformation.

TISSUE CULTURE TECHNOLOGY FOR IMPROVEMENT OF BANANA AND PLANTAIN

Tissue culture has been used in crop improvement since the 1940s. It comprises a set of *in vitro* techniques and strategies that are part of the group of technologies called plant biotechnology. Tissue culture has been exploited to create genetic variability from which crop plants can be improved, to produce healthy planting material and to increase the number of desirable germplasms available to the plant breeder. Tissue culture techniques, in combination with molecular techniques, have been successfully used to incorporate specific traits through gene transfer. *In vitro* techniques for the culture of protoplasts, anthers, microspores, ovules and embryos have been used to create new genetic variation in the breeding lines, often via haploid production. Cell culture has also produced somaclonal variants with crop-improvement potential. The culture of single cells and meristems can be effectively used to eradicate pathogens from planting material and thereby dramatically improve the yield of established cultivars.

In vitro regeneration is the technique of growing plant

Table 1 Regeneration of bananas and plantains.

Cultivars	Type of regeneration	Explants	Medium		Suspension cultures	Embryo germination	Reference
			Shoot initiation	Callus induction			
Dessert banana cv. 'Philippine' 'Lacatan' and 'Grand Nain'; Plantain cv. 'Pelipita' and 'Saba'	Dir. Org.	Shoot tips	MS + BAP	-	No	-	Cronauer and Krikorian 1984
Dessert bananas cv. 'dwarf Cavendish', 'Robusta'; cooking banana cv 'Bluggoe'; Plantain cv. 'Asamiensa', 'Agbagba', 'Pisang Ntanga', 'Prata' and 'Silk'	Dir. Org.	Shoot tips	MS + BAP + IAA	-	No	-	Banerjee and de Langhe 1985
Dipoid bananas *Musa acuminate* and *Musa balbisiana*	SE	Zygotic embryos		MS + Picloram	No	MS + NAA	Escalant and Teisson 1989
Dipoid banana *Musa acuminata;* Dessert banana cv. 'Grand Nain'; Cooking banana cv. 'Cardaba' and 'Bluggoe'	ECS	Leaf sheaths, Corm sections		MS + Dicamba	Yes	½ MS + Zeatin	Novak *et al*. 1989
Cooking banana cv. 'Bluggoe'	ECS	Shoot tips		½ MS + 2,4-D + Zeatin	Yes	½ MS + BAP or Zeatin	Dheda *et al*. 1991
Dessert banana cv. 'Grand Nain'	ECS	Male flowers		MS + 2,4-D + IAA + NAA	Yes	MS + BAP + IAA	Escalant *et al*. 1994
Banana cv. 'Rasthali'	ECS	Shoot tips		MS + 2,4-D + zeatin	Yes	½ MS + BAP + GA_3	Ganapathi *et al*. 2001
Hybrid banana 'FHIA-21'	ECS	Male flowers		MS + 2,4-D + IAA + NAA	Yes	MS + BAP + IAA	Daniels *et al*. 2002
Diploid banana cv 'Calcutta 4' and *Musa balbisiana*; Dessert banana cv. 'Williams' ; Plantain cv. 'Agbagba' ; Hybrid banana '5295-1' and '548-9'	Dir. Org.	Apical meristems	MS + BAP	-	No	-	Tripathi *et al.* 2003

BAP, 6 benzylamino purine; 2,4-D, 2,4-dichlorophenoxyacetic acid; Dir. Org., direct organogenesis; ECS, embryogenic cell suspension; GA_3, gibberellic acid; IAA, indole-3-acetic acid; MS, Murashige and Skoog medium; NAA, α-naphthaleneacetic acid; SE, somatic embryogenesis.

cells, tissues or organs isolated from the mother plant, on artificial media under laboratory conditions. Depending on different physical and physiological factors, in combination with various growth regulators, regeneration occurs via micropropagation or embryogenesis. These techniques have been used in a number of ways in crop propagation and improvement.

Micropropagation

Micropropagation is the production of multiple copies of a single plant using tissue culture techniques. Often, the tissue used is the meristem, where new leaves and stems are produced. Each growing tip on a plant can be excised and grown into a complete new plant. In many cases, viruses that infect a plant are not present in the meristematic tissues like growing tips, so viruses that have infected the parent plant are eliminated from plants produced by micropropagation whereas conventional method of propagation allows viruses to be transmitted to the new crop plants, resulting in diseased plants and reduced yields. Micropropagation of banana in the laboratory can eliminate virus diseases and ensure that each new crop is planted with virus-free materials, greatly increasing yields.

Traditionally, bananas are propagated vegetatively using suckers or corms. Unlike seeded commercial cultivars of other crop species, production of planting materials through suckers take a long time and very few quantity of planting materials are produced per mother plant. Realizing the potential of *in vitro* propagation, shoot-tip cultures have gained world-wide popularity for rapid mass multiplication of planting materials. Since the first report of banana *in vitro* clonal propagation in 1960s, the tissue culture technology in banana has undergone significant improvement and now used widely in banana production worldwide.

Tissue culture systems allow propagating plant material with high multiplication rates in an aseptic environment for example an *in situ* banana plant can produce about 10 shoots after 1 year and a shoot meristem *in vitro* can produce about 125-144 shoots within 8 weeks (**Fig. 1G, 1H**). Through repeated subculturing of proliferating shoots, an open-ended system can be maintained (Tripathi *et al*. 2003).

The other advantage of propagation through tissue culture is also production of disease-free planting materials. Several major diseases of bananas are known to be transmitted through vegetative planting materials, like suckers and corms. In addition, some pathogens like nematodes and those causing *Fusarium* wilt, moko and bacterial wilt are also transmitted through the soil and root tissues. There is success in many countries of using the disease-free tissue culture planting materials in rehabilitating the banana industry that is ravaged by a disease (Molina 2002). In Taiwan, the epidemic of Panama wilt, *Fusarium* race 4, on the popular export variety Cavendish had been successfully managed with massive use of tissue culture, coupled with selection and use of somaclonal-variation-derived resistant varieties. In India and China, the use of tissue culture planting materials is a very effective control tactic in reducing the incidence of *banana bunchy top virus* (BBTV) epidemic. In the commercial export banana production in the Philippines, tissue culture planted farms are known to have less problems of nematodes. Less nematicide treatments are required than those of the traditional sucker-planted farms and those perennially maintained plantations.

The micropropagation technique for cultivated banana and plantain is now well established (**Table 1**; Cronauer and Krikorian 1984; Banerjee and De Langhe 1985). Use of this technique may be limited by the risk of somaclonal variation, a widespread occurrence in some *in vitro* cultures (Vuylsteke *et al*. 1991). Shoot tip culture is simply easy and applicable to wide range of *Musa* genotypes. An efficient regeneration protocol, which seems to be independent of ploidy level and genomic background, was developed for *Musa* species using apical meristems (Tripathi *et al*. 2003). The selected species represent major groups of banana including fertile diploid bananas (AA and BB genomes), the sterile triploid plantains (AAB), Cavendish bananas (AAA), and tetraploid hybrids (AAAA and AAAB). Micropropagated plants establish faster, grow more vigorously, are taller, have a shorter and more uniform production cycle and yield higher than conventional propagules (Vuylsteke and Ortiz 1996).

Somatic embryogenesis or suspension culture

Somatic embryogenesis is a process where a group of somatic cells/tissues leads to the formation of somatic embryos, which resemble the zygotic embryos of intact seeds and can grow into seedlings on suitable medium. Recently, much progress has been made in the establishment of embryogenic cell culture from banana explant sources and from a variety of cultivars (**Table 1**). Novak *et al*. (1989) established embryogenic cell suspensions (ECS) from somatic tissues such as leaf sheaths and corm sections of cv. 'Grand Nain'. Dheda *et al*. (1991) cultured the sections of highly proliferated shoot tip cultures of cv. 'Bluggoe' to produce ECS cultures. Embryogenic suspensions have also been established from immature zygotic embryos (Escalant and Teisson 1989). Young male flowers are the most responsive starting materials for initiating embryogenic cultures of 'Grand Nain' (Escalant *et al*. 1994), cv. 'Rasthali' (Ganapathi *et al*. 2001) and hybrid cv. 'FHIA-21' (Daniels *et al*. 2002). These suspension cultures can be regenerated into plantlets through somatic embryogenesis at high frequencies and grown in the field (Novak *et al*. 1989; Dheda *et al*. 1991). The plant recovery frequencies were as high as 81.5% (Daniels *et al*. 2002).

Plant regeneration from cell suspension cultures was investigated for its potential in mass propagation and as a tool in genetic transformation using recombinant DNA technology. However, most of the procedures are still laborious and genotype-dependent.

Embryo culture

Embryo culture has been used to rescue hybrid plants from wide crosses, which often fail to produce mature viable seeds. In these cases the immature embryo tissue can be removed from the developing seeds and cultured in the laboratory to produce the hybrid plants. Immature embryos represent the most regenerable tissue for many species. Embryo culture enables the breeder to successfully make wide crosses with a greater number of related species of wild plants and have access to a much wider range of genes that can be used for genetic improvement of crop plants. Wide crosses and embryo culture have been valuable tools, especially for the transfer of disease resistance genes from wild relatives into crop plants.

Embryo culture was the first application of a tissue culture technique in banana nearly 46 years ago (Cox *et al*. 1960). The principal applications of embryo culture are rescuing embryos after interspecific hybridization, clonal propagation of families, which contain recalcitrant species, and overcoming seed dormancy and seed sterility. Interspecific crosses are often attempted to transfer desired traits such as disease resistance, stress tolerance or high yield from wild species into important crop species. Incompatibility after these crosses normally results in embryo abortion and this is often caused by breakdown of the endosperm or embryo-endosperm incompatibility. *In vitro* culture of hybrid embryos often successfully bypasses post zygotic incompatibilities.

Seed set in triploid *Musa* spp. is very low due to high levels of sterility (Ortiz and Vuylsteke 1995). This makes cross-breeding of plantain and banana difficult. Nonetheless, several triploid plantain and banana cultivars produce seeds after hand pollination with diploid parents. Axenic *in vitro* germination of hybrid seed has been a cornerstone of banana breeding programs. Indeed, embryo culture increases rates of seed germination by a factor of 10 or more (Vuylsteke *et al*. 1990). Currently, researchers at International Institute of Tropical Agriculture (IITA) are using *in vitro* embryo culture to create interspecific mapping populations and to rescue hybrid materials for breeding programs.

Anther culture

Anther culture is a specific application of tissue culture used in the production of F_1 hybrid varieties. The first step is to develop inbred parent lines by repeated self-pollination. This can be a very slow process in some crops, such as banana, which normally require more than a year to flower. However, by culturing pollen grains in the laboratory, haploid plants that contain only one copy of each chromosome can be produced. These plants can then be induced to double their chromosome number by a chemical treatment with colchicine, quickly resulting in plants that have two identical sets of chromosomes, or are completely inbred (homozygous). This procedure can dramatically reduce the time required to develop inbred parents for breeding of F_1 hybrid varieties and facilitates the selection of recessive traits. Assani *et al*. (2003) reported the production of haploid plants of *Musa balbisiana* (BB). Callus was induced from anthers in which the majority of the microspores were at the uninucleate stage. The frequency of callus induction was 77%. About 8% of the anthers developed androgenic embryos, of the 147 plantlets obtained, 41 were haploids (n=x=11). But the frequency of regeneration was low (1.1%). This technique is yet to be optimized in other cultivars of *Musa* for the production of improved varieties. The haploid banana plants can be important material for the improvement of banana through breeding programmes.

Germplasm conservation and exchange

Tissue culture is commonly used in germplasm conservation because of seedlessness of many banana accessions. While tissue culture has a drawback in germplasm conservation due to its propensity to somaclonal variation as a result of *in vitro* culture and repeated sub-culturing, it is still the most common method of *in vitro* germplasm conservation, pending the full implementation of cryopreservation in *Musa* genebank.

Most banana cultivars produce no seeds and plants are preserved in live collections in fields at high cost, labour and space. These plants are exposed to pests and diseases and can be lost. The propagation of various cultivars of banana and plantain by conventional methods is laborious and time consuming as far as production of a large number of homogeneous plants is concerned. *In vitro* collections offer a safer and cheaper alternative. And also, *in vitro* plantlets are the materials of choice for the international exchange of germplasm simplifying quarantine procedures as they are pest and disease-free, safer and easier to handle than bulky suckers. *In vitro* culture is playing an important role in propagating, maintaining, and exchanging *Musa* germplasm. Shoot-tip culture and disease-indexed cultures for germplasm exchange have been widely adopted for conserving and distributing important banana and plantain collections.

The International *Musa* germplasm collection of INI BAP (International Network for the Improvement of Banana and Plantain) Transit Centre at K.U. Leuven, Belgium uses an *in vitro* culture. The collection also contains improved material that has been acquired from breeding programmes under the terms and conditions of a special germplasm acquisition agreement. At the International Institute of Tropical Agriculture (IITA), natural *Musa* germplasm and hybrids produced by the breeding programme are maintained *in vitro* for germplasm conservation, exchange and for using in breeding activities.

The *in vitro* maintenance of *Musa* germplasm is constrained by the occurrence of somaclonal variation (Vuylsteke *et al*. 1991). As the frequency of somaclonal variation could be linked to multiplication and growth rates among other factors, *Musa* germplasm is now stored under slow growth conditions as well as at ultra low temperatures. Shoot-tip cultures maintained at low temperatures (15-18°C) have been used for the slow-growth storage of *Musa* germplasm (Banerjee and de Langhe 1985).

Cryopreservation is considered to be the only valid al-

ternative for long-term preservation of *Musa* germplasm, because at ultra-low temperatures, physical and chemical reactions are arrested and time-related changes are eliminated. Cryopreservation techniques have been developed for more than 80 different plant species cultivated under various forms including cell suspensions, calluses, apices, somatic and zygotic embryos (Engelmann 1997). Cryopreservation of *Musa* has been reported using somatic embryos and embryogenic cell suspensions (Panis *et al*. 1990). Cryopreservation is not feasible in all cultivars since the production of somatic embryos and cell suspensions is cultivar dependent. In case of banana, slow freezing and vitrification were both totally ineffective, while the encapsulation-dehydration method resulted in a survival rate of 8.1%. A simple technique was recently developed for cryopreservation of meristem cultures, which involves preculture on high-sucrose medium followed by rapid freezing (Panis *et al*. 1996). This cryopreservation method was tested on seven banana cultivars belonging to different genomic groups and resulted in viability rates between 12 and 72% depending on the cultivar. This method can be used as routine cryopreservation method for banana genebanks.

Somaclonal variation

Somaclonal variation, that is, genetic variation among plants regenerated from *in vitro* cultures, has been widespread (Larkin and Scowcroft 1981). Examples of phenotypic variants in populations of banana derived from culture have been widely documented (Vuylsteke *et al*. 1991). Larkin and Scowcroft (1981), defined somaclonal variation as a general term for all variability generated in plants derived from cell culture. In addition to chromosomal instability, somaclonal variation can result from structural changes within chromosomes, point mutations (both spontaneous and induced), gene amplification, mitotic crossing-over, transposable elements and DNA methylation. In general, all forms of somaclonal variation can be minimized, but probably not eliminated, through the culture of organized meristems initiated from terminal or axillary buds or growing points.

Somaclonal variation appears to be ubiquitous in *Musa*. Rates of somaclonal variation in plants derived from shoot-tip culture vary from 0 to 70% according to genotype (Vuylsteke *et al*. 1991). This genetic instability may be a risk associated with the application of *in vitro* culture techniques for germplasm handling and storage. To solve this problem, DNA markers are being identified for a range of serious mutant genotypes to enable them to be removed from micropropagated populations at an early stage. The most somaclonal variants of banana recovered have been found to exhibit natural variation, the occurrence of which was enhanced *in vitro* (Vuylsteke *et al*. 1991).

Somaclonal variation may provide another source of novel and useful variability. Dwarfism in 'Cavendish' bananas or inflorescence variations in plantains are often observed after micropropagation of respective mother genotypes (Vuylsteke *et al*. 1991). Banana cv. 'Cavendish' resistant to *Fusarium* wilt was acquired through somaclonal variation in Taiwan (Hwang and Ko 2004). Somaclonal variation has so far had a limited direct contribution to the genetic enhancement of banana. Thus, the full potential of somaclonal variation for banana improvement should be explored. Ultimately, the value of somaclonal variation in breeding lies is the capacity to obtain at high frequency genetic variants with the desired characteristics. Screening at the whole-plant level for somaclonal variants with disease resistance requires considerable space and labor. If screening could be performed at the cellular level, with selection pressure applied *in vitro*, this technique could be useful.

Somaclonal variations include useful variations (as mentioned above), as well as unwanted mutations. In cases of undesirable mutations *in vitro*, the clonal conformity of the germplasm is in jeopardy. The importance of this issue in a vegetatively propagated crop like *Musa* lies in the fact that the germplasm is distributed worldwide mostly through disease-free *in vitro* plantlets that must be genetically identical to the original mother plant. Therefore, a good knowledge of somaclonal variation is also of great importance for the certification of germplasm integrity. Somaclonal variations are due to genomic instability of genetic and epigenetic origins (Kaepler *et al*. 2000; Leroy *et al*. 2000). Somaclonal variations detectable at the level of phenotypes have been estimated to vary between 1 and 50% in micropropagated bananas (Israeli *et al*. 1995). The use of biotechnology tools facilitates the characterization of such changes in the genome. Genetic changes (changes in DNA sequence) and epigenetic changes due for instance to DNA methylation *in vitro* can be identified by molecular marker techniques, a number of which have been applied for the detection of somaclonal variations in *Musa*. This includes a modified method of the amplified fragment length polymorphism (AFLP) technique (Xu *et al*. 2000; Peraza-Echeverria *et al*. 2001; Gimenez *et al*. 2005), and variants of microsatellite markers (Leroy *et al*. 2000). The design of appropriate molecular probes from such DNA markers will facilitate the tagging of somaclonal variations and the relations to plant phenotypes.

In vitro induced mutagenesis

In the past few decades, induced mutagenesis has been applied successfully in crop improvement, and mutants of agronomic and economic significance were generated in a number of major crops as previously reviewed (Chopra 2005). The most important cultivars of banana and plantain are triploid (2n=3x=33) with AAA genome constitution for dessert bananas and African highland bananas, AAB for plantains and ABB for cooking bananas. Two wild species, *Musa acuminata* (AA genome) and *Musa balbisiana* (BB genome) are known to be the wild diploid ancestors and donors of the A and B sub-genomes of the cultivars. From the original interspecific events that generated the triploids, domestication and selection have relied on spontaneous mutations for thousand of years, while genetic improvement in *Musa* (relatively recent) uses mainly female triploid by male-fertile diploid to introgress desirable agronomic traits from the diploid into the triploid backgrounds. Due to complexities such as partial or total sterility, parthenocarpy, transmission of non-reduced gametes during bi-parental crosses, breeding in *Musa* is a daunting and time-consuming task. In fact, using a parent that is almost sterile to raise sexual progenies in sufficient number to combine desirable traits is difficult. There is a great need of new methodologies to complement the existing breeding methods in *Musa*. Such new approaches include induced *in vitro* mutations capable of creating genetic variability directly in *Musa* cultivars and hybrids, therefore broadening the genetic base of a crop in which there is limited sexual reproduction. Mutagenic agents are classified into chemical (e.g. ethyl methane sulphonate, dimethyl sulphate, ethylene imine, sodium azide), physical (e.g. gamma rays, X-rays, neutrons) and transposable elements for gene knockout (e.g. transposons, retrotransposons, T-DNA insertions). Also, with the *Musa* genome sequencing initiative, and at a time when there is an increased interest in understanding gene structure and function through the analysis of mutants, induced mutations are expected to play a key role in *Musa* functional genomics, especially for the elucidation of biochemical and plant developmental pathways.

TRANSGENIC TECHNOLOGY FOR IMPROVEMENT OF BANANA AND PLANTAIN

Transgenic technology has become an important tool for crop improvement. Genetic engineering i.e. the introduction and stable integration of genes into the nuclear genome and their expression in a transgenic plant offers a better alternative for the genetic improvement of cultivars not amenable

to conventional cross breeding such as Cavendish bananas and False Horn plantains. Due to lack of cross-fertile wild relatives in many banana-producing areas, as well as the male and female sterility of most edible cultivars and clonal mode of propagation, gene flow is not an issue for this crop, making a transgenic approach even more attractive.

The successful genetic transformation in plants requires the production of normal, fertile plants expressing the newly inserted gene(s). The process of genetic transformation involves several distinct steps, namely identification of useful gene, the cloning of the gene into a suitable plasmid vector, delivery of the vector into plant cell followed by integration, expression (**Fig. 1I, 1J**) and inheritance of the foreign DNA encoding a polypeptide. With the advent of plant biotechnology and the rapid development of gene transfer techniques, the potential to introduce desirable character traits is no longer restricted to those occurring in close relatives.

Development of stable and reproducible transformation and regeneration technologies opened new horizons in banana breeding. Despite technical difficulties of transforming a monocot species, transformation protocols are available for most banana cultivars. Several transformation strategies have been published over the years by different banana biotechnologists (**Table 2**).

Transformation procedures

Gene insertion in plants can be achieved by direct gene transfer through particle bombardment or through biological vectors like a disarmed tumour inducing (Ti)-plasmid of *Agrobacterium tumefaciens*. Particle bombardment uses accelerated metal (gold or tungsten) microprojectiles coated with DNA to penetrate and deliver foreign genes into plant cells, which are then selected and regenerated into plants. On the other hand, *A. tumefaciens*, a soil bacterium, transforms its hosts by integrating a segment (called T-DNA) of its tumor-inducing plasmid into the nuclear genome. The transfer of this T-DNA is regulated by a complex process with the involvement of numerous bacterial genes (majorities are called virulence genes) that are located outside the T-DNA. This interesting feature allows for the use of T-DNA as a vehicle to introduce virtually any gene into plant cells. Whichever technique is used, the precise transformation conditions for a specific cell type require optimization for each species, and possibly even, each variety.

Genetic transformation offers an attractive means for introduction of agronomically important traits into banana cultivars. Some success in genetic engineering of banana and plantain has been achieved recently to enable the transfer of foreign genes into plant cells. Genetic transformation using microprojectile bombardment of embryogenic cell suspension is now routine (Sagi *et al*. 1995; Becker *et al*. 2000). An efficient method for direct gene transfer via particle bombardment of embryogenic cell suspension has been reported in cooking banana cv. 'Bluggoe' and plantain cv. 'Three Hand Planty' (Sagi *et al*. 1995). While Becker *et al*. (2000) reported the genetic transformation of Cavendish banana cv. 'Grand Nain'.

Agrobacterium-mediated transformation offers several advantages over direct gene transfer methodologies (particle bombardment, electroporation, etc), such as the possibility to transfer only one or few copies of DNA fragments carrying the genes of interest at higher efficiencies with lower cost and the transfer of very large DNA fragments with minimal rearrangement (Shibata and Liu 2000). Transformation of plants by *Agrobacterium* mediated DNA transfer is currently the most commonly used phenomenon in accomplishing plant gene transfer. Protocols have been developed for efficient *Agrobacterium*-mediated transformation in both dicot and monocot plants, including a large number of crop species. For quite some time monocotyledons were considered to be insensitive to *Agrobacterium* infection, however, in recent years several reports on *Agrobacterium* mediated transformation in a number of monocot plant species including rice (Hiei *et al*. 1994), barley (Tingay *et al*. 1997), maize (Ishida *et al*. 1996) and wheat (Cheng *et al*. 1997) have successfully been reported. Strains containing supervirulent plasmids have facilitated transformation of some recalcitrant monocot plants. It is believed that the factor that limits transformation success in monocot plants is not transfer and integration of T-DNA into the plant genome but the plant regeneration. Often the regeneration rates are poor with monocot plants and this is further reduced under selection during transformation.

Banana was generally regarded as recalcitrant for *Agrobacterium*-mediated transformation. Hernandez (1999) has reported that *A. tumefaciens* are compatible with banana indicating the potential for genetic transformation. The recovery of transgenic plants of banana obtained by means of *A. tumefaciens*-mediated transformation has been reported. The protocol has been developed for *Agrobacterium*-mediated transformation of embryogenic cell suspensions of the banana cvs. 'Rasthali', 'Cavendish' and 'Lady finger' (Ganapathi *et al*. 2001; Khanna *et al*. 2004). Ganapathi *et al*. (2001) reported *Agrobacterium*-mediated transformation of shoot apex derived embryogenic cell suspensions (ECS) of cv. 'Rasthali' (AAB) recording production of up to 40-plants/0.5 ml packed cell volume of cells in this cultivar. Banana functional genomics and plant improvement initiatives demand higher transformation frequencies and a standard protocol that can be used to transform all banana genomic groups. Khanna *et al*. (2004) described centrifugation-assisted *Agrobacterium*-mediated transformation protocol developed using banana cultivars from two economically important genomic groups (AAA and AAB) of cultivated banana.

At present most of the transformation protocols use cell suspension, however establishing cell suspension is lengthy process and cultivars dependent. The protocols have also been established using shoot tips from various cultivars of

Table 2 Summary of genetic transformation reports in banana and plantain.

Banana/Plantain cultivar	Method	Explant	Transgene/Trait	Selection marker	Reporter gene	References
Cavendish banana cv. 'Grand Nain'	Agro	AM		*npt*II	*gus*A	May *et al*.1995
Plantain cv. 'Three Hand Planty' and cooking banana cv. Bluggoe	MPB	ECS		*hpt*	*gus*A	Sagi *et al*. 1995
Cavendish banana cv. 'Grand Nain'	MPB	ECS	BBTV	*npt*II		Becker *et al*. 2000
Banana cv. 'Rasthali'	Agro	ECS		*als*	*gus*A	Ganapathi *et al*. 2001
Cavendish banana cv. 'Grand Nain' and 'Lady Finger'	Agro	ECS		*hpt*	*gus*A	Khanna *et al*. 2004
				*npt*II	m*gfp*	
Banana cv. 'Rasthali'	Agro	ECS	MSI-99	*npt*II		Chakrabarti *et al*. 2003
Plantain cv. 'Agbagba'	Agro	AM		*hpt*	*gus*A	Tripathi *et al*. 2005a
Bananan cv. 'Rasthali'	Agro	ECS	HBsAg			Kumar *et al*. 2005
Plantain cv. 'Agbagba'	Agro	AM	BSV	*hpt*	*gus*A	Author's lab
East African Highland Banana cv. 'Nakitembe' and beer banana cv. 'Pisang Awak'	Agro	AM	*pflp*	*hpt*	*gus*A	Author's lab

Agro, *Agrobacterium*; *als*, acetolactate synthase gene; AM, apical meristem; BBTV, banana bunchy top virus sequence; BSV, banana streak virus sequence; ECS, embryogenic cell suspension; *gus*A, β-glucuronidase; HbsAg, hepatitis B surface antigen; *hpt*, hygromycin phosphotransferase; MPB, microprojectile bombardment; MSI-99, magainin analogue; *npt*II, neomycin phosphotransferase; *pflp*, plant ferredoxin like protein.

banana (May *et al*. 1995; Tripathi *et al*. 2005a). This technique is applicable to a wide range of banana cultivars irrespective of ploidy or genotype (Tripathi *et al*. 2003, 2005a). This process does not incorporate steps using disorganized cell cultures but uses micropropagation, which has the important advantage that it allows regeneration of homogeneous populations of plants in a short period of time. This procedure offers several potential advantages over the use of embryogenic cell suspensions as it allows for rapid transformation of banana species and meristematic tissues have potential to regenerate plants from many different cultivars, unlike somatic embryogenesis which is restricted to only a few cultivars. The transformation of meristematic cells may result in chimeric plants when only one or a few cells receive T-DNA. To obtain uniformly transformed plants, two-steps of selection and regeneration was performed to avoid regeneration of any non-transformed cells. The transformation of East African Highland bananas using meristematic tissues has also been established in the author's lab.

Fungal and bacterial disease resistance

Many fungal and bacterial diseases have significantly affected banana cultivation. Two fungal diseases are of overriding importance. Panama disease, a vascular wilt caused by *Fusarium oxysporum* f. sp. cubense, was responsible for the destruction of the 'Gros Michel' export industry in many countries and for it being replaced by Cavendish varieties and the spread of new races of this pathogen now threatens its successors, the various forms of Cavendish. The second disease Black sigatoka, caused by the fungus *Mycosphaerella fijiensis* f. sp. *cubense*, is the most devastating disease of banana worldwide. It causes significant reductions in leaf area, yield losses of 50% or more, premature ripening, and has a wider host range that includes the plantains, dessert and cooking bananas. Black Sigatoka is controlled with frequent applications of fungicides and cultural practices. These control methods are either require high levels of expensive inputs or have a high labor requirement, which adds to the cost burden to the grower and harmful to the environment.

There is outbreak of banana *Xanthomonas* wilt (BXW) disease caused by the bacterium *Xanthomonas campestris* pv. *musacearum* (*Xcm*), which is devastating banana plantation in east Africa. The economic impact of the disease is potentially disastrous, as there is absolute loss in infected fields, and the farmers do not have the option of relocating to new planting sites that are infection free. Farmers are struggling because they do not have resistant varieties. Experience with bacterial wilt diseases in other crops shows that resistant varieties, if available, are often the most sustainable and cost-effective method of disease management since they are preferred by commercial growers and small scale farmers.

The most attractive strategy for disease control in crops is to improve a plants' defense against a particular pathogen. Recent advances in genetic engineering offer ways to transfer a resistance gene found in other plants, microbes, insects and animals into crop varieties without changing other favorable traits. Plant defense genes from other plants and antimicrobial proteins are now a potential source of plant resistance.

The most attractive strategy to control highly destructive fungal disease like Black Sigatoka in banana is probably the production of disease resistant plants through the transgenic approach including the expression of genes encoding antimicrobial peptides. Antimicrobial peptides (AMPs) have a broad-spectrum antimicrobial activity against fungi as well as bacteria and most are non-toxic to plant and mammalian cells. Examples of AMPs are magainin from the African clawed frog (Bevins and Zasloff 1990), cecropins from the giant silk moth (Boman and Hultmark 1987) and plant defensins (Broekaert *et al*. 1997). The cecropin (Alan and Earle 2002) and its derivatives (D4E1: Rajasekaran *et al*. 2001) have been found to inhibit the *in vitro* growth of several important bacterial and fungal pathogens. Transgenic tobacco plants expressing cecropins have increased resistance to *Pseudomonas syringae* pv. *tabaci*, the cause of tobacco wildfire (Huang *et al*. 1997).

Similarly, magainin is effective against the plant pathogenic fungi (Kristyanne *et al*. 1997). Li *et al*. (2001) reported enhanced disease resistance in transgenic tobacco expressing Myp30, a magainin analogue. Chakrabarti *et al*. (2003) reported successful expression of this synthetic peptide and enhanced disease resistance in transgenic tobacco and banana. On the basis of the broad-spectrum activity against fungal pathogens, individual or combined expression of cecropin, magainin and their derivatives in banana may result in increased resistance to several pathogens.

Another source of antimicrobial proteins has been lysozyme, either from bacteriophage, hen eggs or bovine. The lysozyme attacks the murein layer of bacterial peptidoglycan resulting in cell wall weakening and eventually leading to lysis of both Gram-negative and Gram-positive bacteria. The lysozyme genes have been used to confer disease resistance against plant pathogenic bacteria in transgenic tobacco (Trudel *et al*. 1995), potato (Düring *et al*. 1993), apple (Ko 1999). Human lysozyme transgenes have conferred disease resistance in tobacco through inhibition of fungal and bacterial growth, suggesting the possible use of the human lysozyme gene for controlling plant diseases (Nakajima *et al*. 1997).

There are many reports on the application of plant proteins with distinct antimicrobial activities (Broekaert *et al*. 1997). Thionins are cystein-rich low molecular weight proteins (about 5 kDa) and have been identified in various organs of a number of plant species. They show antimicrobial activity when tested *in vitro* against various bacteria and fungi (Florack and Stiekema 1994). Expression of α-thionin gene from barley in tobacco confers enhanced resistance to bacterial pathogens (Carmona *et al*. 1993). Epple *et al*. (1997) observed that constitutive over expression of thionin in transgenic *Arabidopsis* resulted in enhanced resistance against *F. oxysporum* f. sp. *matthiolae*. The expression of thionin, viscotoxin A3, in transgenic *Arabidopsis thaliana* showed increased resistance to infection of clubroot pathogen *Plasmodiophora brassicae* (Holtorf *et al*. 1998). Unfortunately, most thionins can be toxic to animal and plant cells and thus may not be ideal for developing transgenic plants.

There are a number of known plant defensins, which are known to protect against plant pathogens. The radish defensin Rs-AFP2 conferred partial resistance to the tobacco pathogen *Alternaria longipes* (Terras *et al*. 1995), whereas a defensin from alfalfa (alfAFP) has been expressed in potato in the greenhouse as well as in the field (Gao *et al*. 2000). Kanzaki *et al*. (2002) reported the over expression of the WTI defensin from wasabi conferring enhanced resistance to blast fungus in transgenic rice.

The AMPs of plant origin may be the potent candidates for fungal resistance in banana as they have high *in vitro* activity to *Mycospaerella fijiensis* and *Fusarium oxysporum* f. sp. *cubense*, two major fungal pathogens of banana and also they are non-toxic to human or banana cells. Several defensins isolated from radish and dahlias have been found toxic to both fungal pathogens. Large number of transgenic lines of plantain expressing defensin type AMPs have been developed at KULeuven (Remy *et al.* 1998). Many hundreds of transformed lines have been generated and screened under screen house conditions in Belgium for disease resistance and the most promising lines of transgenic bananas and plantains are currently being evaluated in the greenhouse and field in Cuba and Costa Rica.

Plants have their own networks of defense against plant pathogens that include a vast array of proteins and other organic molecules produced prior to infection or during pathogen attack. Pathosystem-specific plant resistance (*R*) genes have been cloned from several plant species against many different pathogens (Bent 1996). These include *R*

genes that mediate resistance to bacterial, fungal, viral, and nematode pathogens. Many of these *R* gene products share structural motifs, which indicate that disease resistance to diverse pathogens may operate through similar pathways. In tomato, the *R* gene *Pto* confers resistance against strains of *Pseudomonas syringae* pv. *tomato* (Kim *et al*. 2002). *Pto*-overexpressing plants show resistance not only to *P. syringae* pv. *tomato* but also to *Xanthomonas campestris* pv. *vesicatoria* and to the fungal pathogen *Cladosporium fulvum* (Mysore *et al*. 2003). Similarly, the Arabidopsis *RPS4* gene specifies disease resistance to *Pseudomonas syringae* pv. *tomato* strain DC3000 expressing *avrRps4* (Gassmann *et al*. 1999). The *Bs2* resistance gene of pepper specifically recognizes and confers resistance to strains of *Xanthomonas campestris* pv. v*esicatoria* (Tai *et al*. 1999). Transgenic tomato plants expressing the pepper *Bs2* gene suppress the growth of *Xcv*.

The *Xa21* gene isolated from rice has been shown to confer resistance against many isolates of *X. oryzae* pv. *oryzae* (Wang *et al*. 1996). Transgenic plants expressing *Xa*21 under the control of the native promoter of the genomic fragment of the *Xa*21 gene showed enhanced resistance to bacterial leaf blight caused by most *Xoo* races. The *Xa1* gene also isolated from rice confers resistance to Japanese race 1 of *Xanthomonas oryzae* pv. *oryzae*, the causal pathogen of bacterial blight (Yoshimura *et al*. 1998).

A series of resistance gene analogues have been isolated from banana, using degenerate PCR primers targeting highly conserved regions in proven plant resistance genes (e.g. kinase or transmembrane-encoding domains, or leucine-rich repeat sequences). Plant disease resistance genes involved in signal transduction contain domains that are conserved throughout mono- and dicotyledons. Primers have been designed to those domains in the *RPS2* gene of *Arabidopsis thaliana* and the N gene of tobacco. Using these primers for PCR, candidate resistance genes have already been cloned from soybean, potato, rice, barley and *Arabidopsis*. A similar strategy has been applied to clone candidate resistance genes from banana (Wiame *et al*. 2000). A series of disease resistant genes were isolated from the somaclonal mutant CIEN-BTA-03 (resistant to both *M. fijiensis* and *M. musicola*) and the parent 'Williams' that fall into two classes: nucleotide-binding site-leucine-rich repeat-containing kinases, and serine-threonine protein kinases of the *pto* type (Kahl 2004). All the resistance genes were fully sequenced, and eight of them are also transcribed in the mutant, its parental genotype, 'Pisang Mas' and a tetraploid *M. acuminata*. The researchers at Queensland University of Technology, Australia, have isolated the complete gene sequence of R gene candidate (RGC-2) from *Musa acuminata* sp. *malaccensis*, a wild diploid banana segregating for resistance to *Fusarium oxysporum* f. sp. *cubense* (FOC) Race 4. These genes have been characterized and used for development of *Fusarium* wilt resistant transgenic banana (Dale *et al*. 2004). The transgenic plants resistant for *Fusarium* wilt were developed with banana cultivars 'Grand Nain' and 'Lady finger' and ready for glass house trails.

Plants also employ a wide array of defense mechanisms against pathogen attack. Among those, hypersensitive response (HR) is an induced resistance mechanism, characterized by rapid, localized cell death upon their encounter with a microbial pathogen. Several defense genes have been shown to delay the hypersensitive response induced by bacterial pathogen in non-host plants through the release of the proteinaceous elicitor. Elicitor-induced resistance is not specific against particular pathogens. Hence, manipulation of such defense genes may be more ideal.

The ferredoxin-like amphipathic protein (*pflp*, formerly called AP1) and hypersensitive response-assisting protein (*hrap*), isolated from the sweet pepper, *Capsicum annuum*, are novel plant proteins that can intensify the harpinPSS-mediated hypersensitive response (Chen *et al*. 2000; Huang *et al*. 2004). These proteins have dual function; iron depletion antibiotic action and harpin triggered HR enhancing. The transgenes have been shown to delay the hypersensitive response induced by various pathogens like *Erwinia*, *Pseudomonas*, *Ralstonia* and *Xanthomonas* spp. in non-host plants through the release of the proteinaceous elicitor, hairpinPss in various crops including dicots like tobacco, potato, tomato, broccoli, orchids and monocots like rice (Tang *et al*. 2001; Lu *et al*. 2003; Huang *et al*. 2004). Also elicitor-induced resistance is not specific against particular pathogens, so it could be very useful strategy (Wei and Beer 1996). The research is in progress at IITA for producing bacterial wilt disease resistant banana varieties using the *pflp* gene.

Viral disease resistance

Three virus diseases are of major importance. Banana bunchy top, caused by *Banana bunchy top virus* (BBTV), genus *Nanavirus* is one of the most threatening diseases in the world, as infected plants do not produce fruit. *Banana bract mosaic* (BBM), *Potyvirus* cause locally serious losses in several Asian countries. *Banana streak virus* (BSV), genus *Badnavirus* has however had a major impact on banana production in Africa. BSV infection induces yield losses and restricts movement of improved germplasm because some forms of this highly variable virus become fully integrated into the 'B genome' of certain banana and plantain and may subsequently be expressed under stress circumstances. Recent reports indicate that BSV infection may arise from the activation of viral sequences that are integrated into the *Musa* genome (Harper *et al*. 1999). Tissue culture and hybridization through conventional breeding may be triggers for the activation of the integrant to produce BSV infection (Delanoy *et al*. 2003). This problem of virus activation suggests that traditional techniques for virus eradication, such as meristem tip culture, are not appropriate because these treatments would merely activate the integrated BSV sequences. Recently, Helliot *et al*. (2003) have reported that the anti-retroviral and anti-hepadnavirus molecules, adefovir, tenofovir and 9-(2-phosphonomethoxyethyl)-2,6-diaminopurine (PMEDAP), efficiently eradicate the episomal form of BSV from banana plants.

The most promising transgenic strategies for the ssDNA viruses like BBTV is expressing a defective gene which encodes an essential virus lifecycle activity, for instance, the replication of the virus is encoded in the replication gene or genes (Rep). Resultant Rep protein may retain the ability to bind to its target viral DNA but lack the functions of the Rep (Brunetti *et al*. 2001). The defective Rep protein binds to the invading viral DNA and is thought to out-compete the native viral Rep protein, thus reducing or eliminating virus DNA replication. Lucioli *et al*. (2003) expressed the first 630 nucleotides of the Rep gene of *Tomato yellow leaf curl Sardinia virus* to generate resistance. The duration of the resistance was related to the ability of the invading virus to switch off transgene expression through post-transcriptional gene silencing (PTGS). Many researchers are trying to develop transgenic plants of *Musa* resistant to BBTV targetting the PTGS mechanism using mutated or antisense Rep genes.

The RNAi technology is very promising for developing virus resistant transgenic crops (Waterhouse *et al*. 1998; Noris *et al*. 2004). Efforts to control ssDNA viruses, specifically the geminiviruses, by RNAi have been reported. The non-coding intergenic region of the gemini-virus *Mungbean yellow mosaic India virus* (MYMIV) was expressed under the control of the CaMV35S promoter and used to biolistically inoculate MYMIV infected black gram (*Vigna mungo*) plants (Pooggin *et al*. 2003). Plants treated with the construct showed a complete recovery from infection that lasted until senescence. This work showed that phytopathogenic ssDNA viruses can potentially be controlled by RNAi.

Unfortunately, there appear to be no strategies that have been developed that generate high-level resistance to the plant dsDNA or pararetroviruses, including the badnaviruses like BSV. Researchers at the IITA in collaboration with

the John Innes Centre (JIC), UK, are attempting to generate transgenic resistance to BSV based upon PTGS. This approach involves the specific silencing of a viral gene or genes known to be involved with replication or pathogenesis.

Pest resistance

Nematodes are recognized as severe production constraints to bananas and plantains, with losses due to nematodes estimated at about 20% worldwide (Gowen and Queneherve 1990). Locally however, losses of 40% or greater can frequently occur, particularly in areas prone to tropical storms due to toppling as a result of wind damage on affected plants. The banana weevil, *Cosmopolites sordidus*, is also an important pest of banana. Pest management in bananas is mainly based on crop rotation and chemical control (Gowen and Queneherve 1990). However, crop rotation is not often practiced and use of nematicides is often not practical or affordable to subsistence farmers or is environmentally unacceptable. There is evidence that nematode resistance and tolerance sources, though limited, are present in the *Musa* gene pool (Pinochet 1996). Some resistance has been identified against the most damaging nematode species, the burrowing nematode (*Radopholus similis*), but this needs to be combined with consumer acceptable traits. However, *Pratylenchus* sp. causes more losses than *R. similis*. Furthermore, several species of nematodes are often present together, necessitating a broad spectrum resistance in order to reduce these losses significantly.

There are several possible approaches for developing transgenic plants with improved weevil and nematode resistance. A variety of genes are available for genetic engineering for pest resistance (Sharma *et al*. 2000). Among these are proteinase inhibitors, *Bacillus thuringiensis* (Bt) toxins, plant lectins, vegetative insecticidal proteins (VIPs), and α-amylase inhibitors (AI). Proteinase inhibitors contribute to host plant resistance against pests and pathogens (Green and Ryan 1972). They operate by disrupting protein digestion in the insect mid-gut via inhibition of proteinases. The two major proteinase classes in the digestive systems of phytophagus insects are the serine and cysteine proteinases. Coleopteran insects, including the banana weevil, mainly use cysteine proteinases (Murdock *et al*. 1987) and studies indicate a combination of both serine and cysteine proteinases is useful for insect control (Gerald *et al*. 1997). These inhibitors have already been used for insect control in transgenic plants (Leple *et al*. 1995). Presently, cysteine proteinase activity has been identified in the mid-gut of the banana weevil and *in vitro* studies have shown that cysteine proteinases are strongly inhibited by both a purified recombinant rice (oryzacystatin-I [OC-I]) and papaya cystatin (Abe *et al*. 1987; Kiggundu *et al*. 2003).

The use of proteinase inhibitors (PIs), as nematode antifeedants is an important element of natural plant defence strategies (Ryan 1990). This approach offers prospects for novel plant resistance against nematodes and reduces use of nematicides. The potential of PIs for transgenic crop protection is enhanced by a lack of harmful effects when humans consume them in seeds such as rice and cowpea. Cysteine PIs (cystatins) are inhibitors of cysteine proteinases and have been isolated from seeds of a wide range of crop plants including those of sunflower, cowpea, soybean, maize and rice (Atkinson *et al*. 1995). Transgenic expression of PIs provides effective control of both cyst and root-knot nematodes. The cystatins were shown to mediate nematode resistance when expressed in tomato hairy root (Urwin *et al*. 1995), rice (Vain *et al*. 1998), pineapple (Urwin *et al*. 2000) and potato (Urwin *et al*. 2001). The partial resistance was conferred in a small-scale potato field trial on a susceptible cultivar by expressing cystatins under control of the CaMV35S promoter (Urwin *et al*. 2001). The enhanced transgenic plant resistance to nematodes has been demonstrated by using dual proteinase inhibitor transgenes (Urwin *et al*. 1998). Since cystatins have been shown to function in rice, which like *Musa* is a monocottyledon, and also have clear efficacy against a wide range of nematode species, their usefulness as transgenes for development of transgenic *Musa* for resistance to nematodes can be evaluated as having a high probability of success. Recently, Cavendish banana ('Grand Nain') has been transformed using *A. tumefaciens* to express a protein engineered rice cystatin and tested in screen house for nematode resistance (Atkinson *et al*. 2004).

RNAi, first characterized in *Caenorhabditis elegans* (Fire *et al*. 1998), has evolved into a powerful gene silencing tool for analysis of gene function in a wide variety of organisms (Hannon 2002). In plants and nematodes, introducing or expressing dsRNA triggers the target gene-specific RNAi pathway (Novina and Sharp 2004), including RNAi of target genes at sites distal to the location of dsRNA that is ingested by nematodes (Timmons and Fire 1998). The use of RNAi for functional analysis of plant parasitic nematode genes has been established (Urwin *et al*. 2002; Bakhetia *et al*. 2005). The advantage of the RNAi approach is that no novel protein production is required to achieve nematode resistance. This offers considerable biosafety advantage given that RNA molecules represent no food risk and there is little likelihood of non-target effects (Bakhetia *et al*. 2005).

The expression and biological activity of the Bt toxins has been investigated in GM plants for insect control. Bt gene technology is currently the most widely used system for lepidopteran control in commercial GM crops (Sharma *et al*. 2000). The expression of a selected *Bt* gene for weevil resistance may be a rather long-term strategy since no potential *Bt* gene with high toxic effects against the banana weevil has been identified as yet (Kiggundu *et al*. 2003). Some Bt proteins are also effective against saprophagous nematodes (Borgonie *et al*. 1996). The Cry5B protein is toxic to wild type *Caenorhabditis elegans*, other *C. elegans* mutants are resistant to Cry5B but susceptible to the Cry6A toxin (Marroquin *et al*. 2000). The approach using cry genes has potential for plant nematode control (Wei *et al*. 2003).

α-Amylase inhibitors (AI) and chitinase enzymes might also have a future potential for weevil control. α-Amylase inhibitors operate by inhibiting the enzyme α-amylase, which breaks down starch to glucose in the insect gut (Morton *et al*. 2000). Transgenic azuki beans are produced with enhanced resistance to bean bruchids, which are Coleopteran insects like weevils (Ishimoto *et al*. 1996). The α-amylase inhibitors may be of interest for weevil control in GM banana. Chitinase enzymes are produced as a result of invasion either by fungal pathogens or insects. Transgenic expression of chitinase has shown improved resistance to Lepidopteran insect pests in tobacco (Ding *et al*. 1998).

Edible vaccine

Researchers are working to develop a banana that is an "edible vaccine" to fend off hepatitis, one of the world's most widespread and devastating diseases. Dr. Arntzen's group at Boyce Thompson Institute for Plant Research, USA, had successfully developed tobacco plants producing a vaccine against hepatitis B. It found that the vaccine produced in plants is similar in form and function to that from human serum or recombinant yeast and provoked a strong immune response when injected into mice, while B and T-cell epitopes were preserved. Successful expression of antigens in plants was also achieved for Rabies virus G-protein in tomato (McGarvey *et al*. 1995), Norwalk virus capsid protein in tobacco and potato (Mason *et al*. 1996), Hepatitis B virus surface antigen in tobacco and potato (Thanavala *et al*. 1995), *E. coli* heat-labile enterotoxin B subunit (LT-B) in tobacco and potato (Hirst and Holmgren 1987), Cholera toxin B subunit (CT-B) in potato (Arakawa 1997). Foods under study include potatoes, bananas, lettuce, rice, wheat, soybean, corn and legumes. Banana is a good candidate for edible vaccines since they were eaten raw, appealing to

children, inexpensive to produce, native to many developing countries. But the only limitation is the time from transformation to evaluation of fruit is 2 years or more.

Embryogenic cells of bananan cv. 'Rasthali' (AAB) have been transformed with the 's' gene of hepatitis B surface antigen (HBsAg, Kumar *et al*. 2005). Higher monoclonal antibody binding of 67.87% of the antigen was observed when it was expressed with a C-terminal ER retention signal. HBsAg obtained from transgenic banana plants is similar to human serum derived one in buoyant density properties. The transgenic plants were grown up to maturity in the green house and the expression of HBsAg in the fruits was confirmed by RT-PCR. Attempts were also made to enhance the expression of HBsAg in the leaves of transgenic banana plants by wounding and/or treatment with plant growth regulators. This is the only report on the expression of HBsAg in transgenic banana fruits.

Transgenic plants that produce medicinal compounds such as subunit oral vaccines and antibodies have already been developed, but experts concede that application of this technology is at least a decade away. There are several technical and logistical problems which need to be addressed before edible vaccines through plants become a reality in practice. Most inserted genes are expressed in very low levels in plants. To enhance expression, focus is on the development of efficient promoters especially to target the production of proteins into edible parts of the plants, and on factors such as enhancers, signal sequences and optimized codon usage. Arntzen *et al*. (2005) recently reported that a synthetic cholera vaccine gene that was more 'plant'-like in its sequence is four times more productive than the original gene. The stability of vaccine proteins when transgenic fruits or leaves are stored at ambient conditions is another concern. Antibodies in leaves have to be extracted immediately, or they will decay with the leaves themselves. There is also concern about oral tolerance in case increased levels of vaccines become ineffective when consumed orally by suppressing systemic immunity. Further research may identify useful adjuvants that enhance oral immunogenicity. The dosage is a major problem as vaccine content in plants may vary depending on where and when they are grown. Therefore, a delivery scheme should be developed to ensure the required dosage level, and that edible vaccine producing plants are taken as a routine food source. Only further collaborative research between plant and medical scientists may resolve these and other issues. In the near-term, the edible-vaccine technology might be better targeted at animals. In fact, such an approach may benefit agriculture and livestock as billions of dollars are spent presently on vaccinating farm animals and poultry. Transgenic plants supplying feedstock containing edible vaccines may represent the first commercial application of this intriguing technology.

Enhanced micronutrients

Nutritionally enhanced crops could make a significant contribution to the reduction of micronutrient malnutrition in developing countries. Biofortification (the development of nutritionally enhanced foods) can be advanced through the application of several biotechnologies in combination. Genomic analysis and genetic linkage mapping are needed to identify the genes responsible for natural variation in nutrient levels of common foods. These genes can then be transferred into familiar cultivars through conventional breeding and MAS or, if sufficient natural variation does not occur within a single species, through genetic engineering.

Vitamin and mineral deficiencies are one of the major cause of mortality and morbidity of millions of children every year in developing world, which can be easily prevented by adding just a few key nutrients to staple food. Genetic modification of banana has also been considered as a path towards increasing the value of this crop to health and nutrition in developing countries. Although much of the necessary technology is now available, these applications have yet to advance to the stage of practical evaluation. However, recent works in engineering rice with genes for β-carotene biosynthesis and the development of golden rice (Ye *et al*. 2000) have shown the feasibility of enriching foods with vitamin A through biotechnology. Recently, iron has been also enhanced in rice by the introduction of the ferritin gene driven by endosperm-specific promoter (Vasconcelos *et al*. 2003). The *Musa* germpalsm is particularly diverse at the diploid level. Some wild species and hybrids of banana and plantain display significant variation of pulp color that may indicate different levels of pigments, including β-carotene. This together with contents in minerals remains however to be assessed, introgressed and/or pyramided in the most preferred cultivars. Such research activities are ongoing on banana and plantain in a number of research organizations including IITA.

GENOMICS FOR BANANA AND PLANTAIN IMPROVEMENT

Genomics, the whole repertoire of techniques to study the genomes of organisms aims to understand the structure of the genome and to study genes and their functions. Recent breakthroughs of genomics in plants include the whole genomes sequencing of *Arabidopsis thaliana* and rice. The application of genomics in banana and plantain improvement is still in its infancy due to the limited amount of information available yet on the genome content and organization. The *Musa* genome is among the smallest of crop species. It comprises 11 chromosomes with a total haploid genome size of 500 to 600 Mb (Dolezel *et al*. 1993). This is 25% larger than rice and 75% larger than the genome of *Arabidopsis*. *Musa* genome exhibits features that make it attractive to comparative, functional and evolutionary genomics. This includes the combination of parthenocarpy, sterility, vegetative propagation, and the presence of various levels of ploidy such as diploid ancestral species *M. acuminata* (AA) and *M. balbisiana* (BB), diploid interspecific hybrids (AB), and various autopolyploids of A genomes and allopolyploid of A and B genomes. The two main species targeted for sequencing are *M. acuminata* and *M. balbisiana* (the progenitors of current cultivars). Both species contain a range of traits including resistance to biotic and abiotic stresses that are of great interest for banana and plantain improvement (Simmonds 1962). Among the methods available for genome sequencing, complete bacterial artificial chromosome (BAC) sequencing, BAC end sequencing, expressed sequence tag (EST) sequencing, and reduced representation sequencing methods such as low-cot and repeat portion sequencing are being applied to *Musa* genome. At present, the Genebank database hosts around 10,000 *Musa* ESTs, the database maintained by EMBRAPA (Brazil) has 19,000 ESTs, while 33,000 ESTs are registered in the sputnik database, and BAC libraries are maintained at the Institute of Experimental Botany in the Czech Republic. However, to make this EST resource useful in banana and plantain improvement, one needs yet to order and assemble them into contigs and unigenes. Also, the depth of *Musa* cDNA libraries need to be known, and full-length cDNA libraries, normalized and representative would be needed from different plant organs and treatments in case vs. control materials. Other genomics activities such as T-DNA tagging to isolate and characterize novel promoters and genes in *Musa* are being carried out (Remy *et al*. 2007). There is no doubt that these numbers will increase dramatically in the coming years through the ongoing efforts of the banana research community including the Global *Musa* Genomics Consortium. The exploitation of this sequence resource coupled with bioinformatics will lead to fundamental insights into the structural and functional organization of the *Musa* genome.

Genomic analysis of plant pathogens is of key importance for quarantine, epidemiology research, diagnosis, and to understanding the development and suppression of plant

diseases. A pathogen of primary interest to the banana and plantain research community is *Mycosphaerella fijiensis* the causative agent of the most devastating disease (Black Sigatoka). *Mycosphaerella* is one of the largest genera of plant pathogenic fungi with 1,800 names and at least 40 anamorph genera (Goodwin *et al*. 2001). An international project funded by the U.S. Department Of Energy-Joint Genome Institute was initiated to sequence the genomes of *M. graminicola* (from wheat) and *M. fijiensis* (from banana and plantain). So far, more than 30,000 ESTs from *M. fijiensis* genome were produced. The genome sequencing is currently at approximately 2.5x, and the genome size estimate was revised to approximately 68 Mb (Kema *et al*. 2007). In addition of key application areas mentioned above, unraveling *M. fijiensis* genome will also be complementary to the sequencing of the *Musa* genome for understanding plant-microbe interactions. In the future, the same pathogen genomics strategy would be well justified for important pests and pathogens such as nematodes and *Fusarium* wilt that constitute also major threats for sustainable banana and plantain production.

Viruses cause destructive and economically important diseases on plantains and bananas worldwide. *Banana streak virus* (BSV) is integrated in the B genome and can be activated by various stresses, making its management and its control challenging. The genome of various isolates of BSV has been sequenced and shown to be organized in a manner characteristic of *badnaviruses*. Comparison of this sequence with those of other *badnaviruses* showed that BSV is a distinct virus (Harper and Hull 1998). Recently, sensitive PCR-based diagnostics tools were developed for the detection of BSV (Delanoy *et al*. 2003).

Usefulness of comparative genomics for banana and plantain

Comparative genomics in diverging species and interspecific hybrids presents a powerful approach to study genetic differentiation, genome evolution and reproductive isolation. Two wild species, *Musa acuminata* (A genome) and *M. balbisiana* (B genome) gave rise to current edible bananas and plantains (Simmonds 1962). There is a large consensus on the fact that edibility of mature fruits arose from mutations causing parthenocarpy and female sterility in diploid *M. acuminata* (Simmonds 1962), while hardiness is contributed by the B genome since *M. balbisiana* accessions strive abundantly in areas with pronounced dry seasons alternating with monsoons. Also fruit characteristics such as starchiness and acid taste are attributed to the B genome (Simmonds 1962). Comparative analyses of the A and B genomes would shed lights on the genetic of these key agronomic traits and their transfer (or introgression) into the sterile triploid cultivars. To date, there is little information about the relationships between the A and B genomes. The current sequencing activities will shed lights on theses relationships once enough genome regions are harvested and compared between the A and B genomes. Previous genetic mapping efforts were focused on the A genome (Faure *et al*. 1993). This preliminary map within the A genome has shown a high level of segregation distortion (36%), indicated that the *Musa* genome may be rich in translocations. Genomic *in situ* hybridization was used to differentiate the A and B chromosomes (Osuji *et al*. 1997), and Random Amplified Polymorphic DNA (RAPD) was also used to identify DNA markers specific to the A and B genomes (Pillay *et al*. 2000). However, the analysis of the synteny between the A and B genome is confronted to the fact that it is difficult to recover a sufficient number of progenies from interspecific crosses between the A and B genomes. At IITA, we have recently conducted extensive interspecific hybridization works assisted by seed rescue through *in vitro* culture (Vroh-Bi personal communication). We are now in possession of more than 200 hundred progenies from a single bi-parental A x B cross. This mapping population is a major source for comparative mapping in *Musa*.

With the availability of sequence resources, particularly from *Arabidopsis thaliana*, rice, and major species of the grass family, genomes of crop species are being linked through comparative genetic maps (Paterson *et al*. 2000). On an evolutionary point of view, most plant genes belongs to multigene families because plant genomes underwent extensive duplication events that make the distinction between paralogs and orthologs difficult (Jensen 2001). However, conserved genes, particularly single to low-copy regions that have conserved most of their sequence composition during plant evolution exist within and among plant families. These so-called conserved orthologs (Fulton *et al*. 2002), constitute a powerful tool for linking plant genomes. For instance, bananas are monocotyledons belonging to the genus *Musa* (family *Musaceae*, order *Zingiberales*) which of course, clusters phylogenetically with other monocotyledons including *Gramineae* (e.g. wheat, maize, rice), and *Liliaceae* (e.g. onion). Fortunately, rice genome has been sequenced, and an increasing number of sequences are available from wheat and maize. This offers a great opportunity to *Musa* geneticists to link the *Musa* genome to that of rice, wheat, and maize, therefore profiting by the enormous genomic resource already available in these monocottyledons. Such a strategy is being applied already by the Generation Challenge Programme (GCP) to link the *Musa* and the rice genome, although conducted currently with a very limited number of conserved orthologs markers for a showcase.

Comparative genomics has also the potential to play a critical role in the elucidation of biochemical pathways in *Musa*. The knowledge gained from the *Arabidopsis* and rice genomes in the dissection of important biochemical pathways, allows genomic analyses of many aspects of plant biology in other crops. For instance, notable progress has been made in identifying genetic pathways and molecular components associated with the control of flowering time (earliness) and the function of the circadian clock in *Arabidopsis* (Piñeiro and Coupland 1998). One of the bottlenecks to banana production is the long crop cycle. Earliness is therefore one of the major interests in banana and plantain genetics and breeding. A number of genes of the photoperiod pathways have been extensively studied and characterized in *Arabidopsis* (Koornneef *et al*. 1998). The use of the sequences of these genes may allow the isolation and characterization of their homologues in *Musa*. Another pathway of interest in *Musa* genomics may be the gibberellin signaling pathway. Gibberellins are a large family of plant growth hormones that participates in many aspects of plant growth, development and environment response (Olszewski *et al*. 2002). Edible bananas and plantains are natural parthenocarpic fruits (developed in absence of fertilization, and seedless). Parthenocarpy has a genetic base, and one of the bottlenecks of *Musa* breeding, especially for introgressing useful genes from wild seeded diploids into improved varieties is the presence of unwanted seeds in some hybrids of high agronomic value. In a number of plant species, parthenocarpy can be induced by the application of gibberellin as demonstrated for instance in tomato (Fos *et al*. 2000). Recently, the gibberellin receptors were identified in *Arabidopsis* and found to be orthologs of the rice gibberellin receptors (Nakajima 2006). Similar comparative approaches can be used to analyze and utilize gibberellin genes in bananas and plantains. It is also worth noting that gibberellin genes are involved in flowering time and could be used as candidates in the search of early maturing varieties of bananas and plantains.

Status and prospects for molecular markers in banana and plantain

To date, many types of molecular markers are available for diversity analysis, marker-assisted breeding and diagnostics in crop species and related pathogens. Despite the limited knowledge of the *Musa* genome, a number of commonly

used molecular markers such as RFLP, SSR, RAPD, and AFLP are applied in banana and plantain mostly for diversity analysis and genetic mapping (Faure *et al*. 1993; Ude *et al*. 2002). In *Musa* breeding, many agronomic traits need to be tagged yet by molecular markers and introgressed in improved varieties. In sub-Saharan Africa for instance, traits of priority for molecular breeding in *Musa* include parthenocarpy, apical dominance, earliness, plant height, and nutritional qualities. Despite the slight increase of the number of microsatellite markers in *Musa*, the single most important and recent development of molecular markers in bananas and plantains remains the Diversity Arrays Technology or DArT markers, a microarray technology that can detect and type DNA variation at several hundred of genomic loci in parallel without prior knowledge of sequence information (Jaccoud *et al*. 2001). This technique is particularly adapted to bananas and plantains currently considered as "orphan" crops in genomics due to the relatively limited information available on the genome. Using complexity reduction method with the restriction enzymes *Pst*I as primary cutter and TaqI or *Bst*NI as a secondary cutter, the DArT system was established for *Musa* through two arrays of 6,000 clones, each array including 700 to 800 polymorphic markers capable of differentiating within and among the A and B genomes of *Musa* (Huttner *et al*. 2007).

Other high throughput DNA markers such as single nucleotide polymorphisms (SNPs) and insertion-deletions (Indels) are absent yet due to the fact that the available *Musa* sequences are not assembled yet into contigs and unigenes. As the sequencing activities progress, SNPs and Indels are expected to play also a major role in *Musa* genomics via high throughput genotyping platforms.

Association mapping in banana and plantain

A major area of application of molecular markers, not yet explored in *Musa*, is association mapping that depends on linkage disequilibrium (LD), the non-random association of alleles at different loci (Terwilliger and Weiss 1998). The effectiveness of marker-assisted selection and fine mapping of quantitative trait loci (QTL) depends on the extent of LD. The LD decay depends on the number of meioses elapsed before the generation of the mapping population under study. Bi-parental mapping populations (even in advanced intercrosses) have limited number of meioses and therefore give a coarse resolution of marker-QTL association. Association mapping can be applied directly in natural populations and appear therefore as an attractive approach for species that are intractable for breeding such as *Musa*.

Mapping in natural populations has the advantage to use the many meiotic events that occurred during hundred of years of crop evolution, thus allowing for much finer mapping than in standard bi-parental crosses. The lack of elaborated linkage maps in *Musa* complicates marker-assisted breeding. Although the major cultivars are sterile triploids, *Musa* genus is diverse, including a number of fertile wild diploid species that may constitute models for association mapping. To date, the extent of linkage disequilibrium in *Musa* is unknown both at genome-wide and candidate gene levels. At IITA, we have recently conducted an investigation using SSR markers in diploid species and in triploid landraces (**Fig. 1K**). These studies showed the feasibility of association mapping in *Musa* (Vroh-Bi and Tenkouano 2007). The increase of *Musa* genome resource and further analyses of LD will establish the efficiency of genome scans versus candidate gene-based association approaches in bananas and plantains. Like in other major crop species, association mapping in *Musa* has the potential to identify candidate genes for agronomic traits. The validation of these candidates would be provided through physiological analyses, genetic transformation, and/or gene-assisted breeding.

FUTURE SCOPE OF TRANSGENICS AND BIOTECHNOLOGY FOR GENETIC IMPROVEMENT OF BANANA AND PLANTAIN

Bananas and plantains are seriously threatened by pests and numerous viral, bacterial and fungal diseases. Thus resistance to biotic stresses is an important part of regional or national efforts. Since the major cultivated varieties of banana are sterile and therefore do not set seed, traditional breeding is more difficult than genetic transformation using molecular techniques. Although attempts to produce transgenic bananas and plantains are still proceeding too slowly, public acceptance of these novel plants and their products should already be prepared for through sound information and risk assessment, although the chances of transfer of transgenes from transgenic field material to wild species (the major public concern) are expected to be negligible in view of the sterility of many cultivars. The scope for further improvement of banana and plantain is large; along with other methods of crop improvements transgenic technology should provide fast and effective methods.

Currently, no transgenic bananas and plantains are commercially available. However, many research institutes/organization and universities are concentrating on the development of pest or disease resistance varieties and improving the nutritional contents of banana and plantain. The number of transgenic bananas is continuously increasing. Transformation protocols, including tissue culture techniques, suitable transformation constructs with modified promoters driving one or more transgenes, appropriate transformation techniques such as particle bombardment and *Agrobacterium*-mediated gene transfer, the detection of the transgenes and characterization of their insertion sites, are well developed. Transgenes have been used exclusively from heterologous sources rather than specifically from bananas.

Initially, it is likely that transgenic banana varieties brought to the market will focus on diseases (bacterial and fungal) and pest (nematode) resistance as the technologies are in advance stage in many labs. However, the long-term commercial potential of plant biotechnology is considered to be in the development of value-added traits that will address a wide range of specific needs or market niches.

Genetic modification of banana has also been considered as a path towards increasing the value of this crop to health and nutrition in developing countries. As a crop that is widely consumed as a weaning food by children and as a starchy staple by all sectors of the community in some countries, banana has been advocated as a carrier for vaccines and as a source of carotenoids that can counteract debilitating Vitamin A deficiency. However, although much of the necessary technology is now available, these applications have yet to advance to the stage of practical evaluation.

The next future product can be banana varieties with longer shelf life. Banana is the most widely consumed fruit worldwide. Fruits are picked before they are allowed to ripen. They are then transported to their final destination under controlled atmosphere conditions where they are gassed with a plant hormone, ethylene, to induce ripening. Once ripening has been artificially triggered, the fruit has to be eaten or sold immediately before they spoil. First round of banana field trials by Senesco Technologies, Inc. and Rahan Meristem show that using Senesco's delayed ripening technology significantly extends the shelf-life of banana. Senesco claims that banana fruit lasted twice as long as the control (non-enhanced) fruit (Crop Biotech Update 2003). The Senesco bananas ripened normally, but the onset of spoilage and blackening that follows ripening was significantly delayed. The banana field trials indicate that the delayed ripening technology slows the process of cell death once ripening has occurred, without affecting normal growth of the plant and its fruit. This ensures that bananas are the same size, shape, weight and color as non-enhanced bananas, with the same taste and nutritional characteristics.

Global Agricultural production has been seriously

threatened with the continuing deterioration of arable land, scarcity of water and increasing environmental stress. Drought and salinity are the two most prevalent abiotic constraints limiting crop and forage productivity in desertification-prone agro-ecosystems globally. Over the recent decades, rainfall has seemingly become less reliable, which is putting pressure on the food security and livelihood status of smallholder farmers in sub-Saharan Africa. Drought does not only affect typical dry land annual crops such as millet, but increasingly affects the more semi-humid areas where semi-perennial food crops such as cassava and banana dominate. Conventional plant breeding and crop physiology have had limited success in building and deploying tolerance to climatic stresses in the developing world. Fortunately, it is now possible to use transgenic approaches to improve drought tolerance in agriculturally important crops. Recent advances in transgenic approaches to provide enhanced drought tolerance hold promise to move forward. Drought resistant banana varieties will extend the geographic spread of banana production in Africa.

Musa genomics can open up new avenues for more efficient breeding of the crop. It is important to investigate the possibilities via which the primary production and other uses of banana can be promoted for the benefit of the growing world's population. Strategies for future genomics research in banana and plantain include the development of molecular markers, construction of genetic and physical maps, identification of genes and gene expression and whole genome sequencing. Sequencing of other plant genomes such as *A. thaliana* and *O. sativa* has provided an enormous amount of data that could reveal unknown features of their genomes. Such data could also be generated for *Musa*. These include sequence composition of various genomic regions, an inventory of the various genic and non-genic sequences (genes and repetitive DNA such as satellites, mini- and macrosatellites, pseudogenes, retropseudogenes, retrotransposons, DNA transposons and many others), the distribution of various elements along the chromosomes, potential duplications, translocations, inversions, macro- and microsynteny, structure of centromeres and telomeres, the exact genome size, and number of open reading frames (Kahl 2004). Together with genome sequencing, the handling of such data must be considered since bioinformatics for banana genomics has not been developed. *Musa* is not included in international genome analysis initiatives. A Global *Musa* Genomics Consortium was established in 2001 with the goal of assuring the sustainability of banana as a staple food crop by developing an integrated genetic and genomic understanding, allowing targeted breeding, transformation and more efficient use of *Musa* biodiversity. Basically, the Consortium aims to apply genomics to the sustainable improvement of banana and plantain. The consortium believes that genomic technologies such as analysis and sequencing of the banana genome, identification of its genes and their expression, recombination and diversity can be applied for the genetic improvement of the crop (Frison *et al*. 2004).

Although a number of marker techniques are now available for genomic research, they have not been as widely used in banana and plantain as in some of the other crop plants. Markers that co-segregate with a trait can be exploited to accelerate the selection of that trait. This will be especially useful in bananas because of its long life cycle. New markers such as SNPs have not yet been applied to banana research and promise to have an impact on protein function. Genetic and physical mapping of the *Musa* genome will make it possible to isolate genes that can be used in genetic transformation. Although a map of a diploid banana is available a much greater effort in developing high density maps to identify QTLs is necessary for *Musa*. The development of ESTs and cDNA libraries are crucial areas of research in *Musa* that needs greater emphasis. In addition, no attempt has been made to design expression chips. Expression chips are being used in many laboratories for other crops.

There is enormous potential for genetic manipulation of bananas and plantains for disease and pest resistance using the existing transformation systems and the genes isolated from *Musa* genome. The use of appropriate gene constructs may allow the production of nematode, fungus, bacterial and virus-resistant plants in a significantly shorter period of time than using conventional breeding, especially if several traits can be introduced at the same time. It may also be possible to incorporate other characteristics such as drought tolerance, thus extending the geographic spread of banana and plantain production, and thus contributing significantly to food security and poverty alleviation in developing countries. Long-term and multiple disease resistance can be achieved by integrating several genes with different targets or modes of action into the plant genome. Technically, this can be done either in several consecutive steps or simultaneously. In conclusion, transgenics and biotechnology has enormous potential for genetic improvement of bananas and plantains.

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