Original article

Banana: cultivars, biotechnological approaches and genetic transformation

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Summary Genetic modification of banana has been considered as a path towards increasing the value of this crop according to health and nutrition in developing countries. Banana as a crop is one of the most important and widely consumed fruits as a weaning food by children and as a starchy staple for all other consumers. As well as providing a low cost and easily produced source of energy, bananas are also rich in certain minerals and in vitamins A, C and B6. Growing urbanisation in many developing countries upgraded the crop importance as a source of revenue, occasionally providing the main source of income for rural communities. Genetically modified organism bananas have been advocated as carrier for vaccines and as a source of carotenoids that can counteract debilitating vitamin A deficiency. The rather high vulnerability of banana to pests and diseases triggered biotechnological applications in an attempt to produce new, more resistant banana cultivars. However, the potential biosafety of genetically modified banana and its applications should be taken into account prior to its extensive usage. The current survey summarises the most important biotechnological techniques (*in vitro* culture, DNA fingerprinting, somatic emrbyogenesis, DNA flow cytometry, etc.) and applications (micropropagation, *in vitro* selection, somaclonal variation, protoplast fusion, haploid production, etc.) in banana and emphasises on genetic transformation in conjunction with the expressed gene and modified trait aiming at a further improvement of this crop.

Keywords Banana, biosafety, biotechnology, genetic transformation, genetically modified organism.

Introduction

Banana is the common name used for the herbaceous plants of the genus *Musa* which is cultivated in more than 100 countries throughout the tropics and subtropics, with an annual world production of around 98 million tonnes, of which around a third is produced in each of the African, Asia-Pacific, and Latin American and Caribbean regions (Frison & Sharrock, 1999).

The oldest records of edible bananas come from India (600 BC), known only by hearsay in the Mediterranean region in the third century BC (Horry *et al*., 1997). It is believed that they were first introduced to Europe in the 10th century. Early in the 16th century, Portuguese mariners transported the plant from the West African coast to South America. The wild types found in cultivation in the Pacific have been traced to eastern Indonesia from where they spread to the Marquesas and gradually to Hawaii. Nowadays, bananas and plantains are grown in every humid tropical region and constitute the fourth largest fruit crop of the world (Morton, 1987).

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Description

Banana is a monoecious plant having male flowers at the tip of inflorescence and female flowers behind. The fruit of banana or plantain is a product of parthenocarpy and characterised as berry with a leathery outer peel that contains much collenchyma (Daniells *et al*., 2001). The fruits are formed in layers called combs or hands, consisting of 10–20 bananas, and there are 6–15 combs per stalk. The latter equals 40–50 kg per stalk or ten or more tons per acre. If commercially grown, the large terminal bud and bracts are removed to redirect sugars to the developing fruits. An unripened banana and the plantain have high starch and low sugar levels plus

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copious amounts of bitter-tasting latex. Starch is converted to sugar as the fruit ripens, so that bananas can eventually contain about 25% of total sugars. As the banana ripens, the latex is also decomposed. Plantain has the stinging, bitter latex, so the peel is removed with a knife and the pulp is soaked in salt water for 5–10 min prior to cooking (http://www.crfg.org). Bananas are harvested unripe and green, because they can ripen and spoil very rapidly. The fruits are cleaned of old floral parts, combs and divided into smaller bunches. Poorly formed fruits are removed, and bunches are thrown into a water bath, where latex is washed away. Then fruits are dried and usually placed in a ripening room for several days before their transfer to market, or exported after storing and packing with cushion (usually paper). Presence of naturally formed ethylene gas, produced by ripe fruits, hastens considerably the ripening of surrounding, greener fruits (http://www.botgard.ucla.edu).

Banana cultivars

The genus *Musa* is classified into four sections, the members of which include both seeded (wild) and non-seeded or parthenocarpic edible types (Ortiz, 1997). Two of the sections (*Callimusa* and *Australimusa*) contain species with chromosome number of $2n = 2x = 20$ while the species in the other two sections (*Eumusa* and *Rhodochlamys*) have as basic chromosome number ($n = 11$) (http://www.inibap.org). The centre of diversity of the species is believed to be either Malaysia (Simmonds, 1962) or Indonesia (Horry *et al*., 1997).

Bananas and plantains belong to the *Eumusa* section of the genus *Musa*, family Musaceae, and order Zingiberales (Gill, 1988). This section is the largest in the genus and the most geographically widespread, with species being found throughout South East Asia from India to the Pacific Islands. The genomic groups proposed by Simmonds & Shepherd (1955), to classify the edible clones are (AA), (BB), (AB), (AAA), (AAB), (ABB), (AAAA) and (ABBB) (Fig. 1).

Most cultivars are derived from two diploid wild species, *Musa acuminata* (AA genome) and *Musa balbisiana* (BB genome) (Osuji *et al*., 1997). Edible clones are classified as to the relative contribution of *M. acuminata* and *M. balbisiana* (Simmonds & Shepherd, 1955). *Musa acuminata* is the most widespread of the *Eumusa* species. Chromosome structural changes, either having occurred spontaneously or as a response to recombination events, resulted in the development of natural reproductive barriers within the species thereby causing subspecies divergence and genetic diversity in the species as a whole (Horry *et al*., 1997). In conjunction with chromosome restitution, this process gave rise to (i) autoploids and homogenomic hybrids which are essentially (AAA) dessert and beer bananas, and (ii) alloploids and heterogenomic hybrids comprising the plantains (AAB) and the cooking bananas (ABB) (Ortiz, 1997). The most important banana types, genomic groups and cultivars with their characteristic agronomical and quality traits, are presented in Table 1.

Triploid (AAA) cultivars arose from diploids, perhaps following crosses between edible diploids and wild *M. acuminata* subspecies (Ortiz, 1995). In most parts of South East Asia, the triploid cultivars, which are more vigorous and have larger fruit, replaced the original diploids (AA). The diploid and triploid *M. acuminata* cultivars were taken by humans to areas where *M. balbisiana* was native (India, Myanmar, Thailand, Philippines) and natural hybridisation resulted in the formation of hybrid progeny with the genomes (AB),

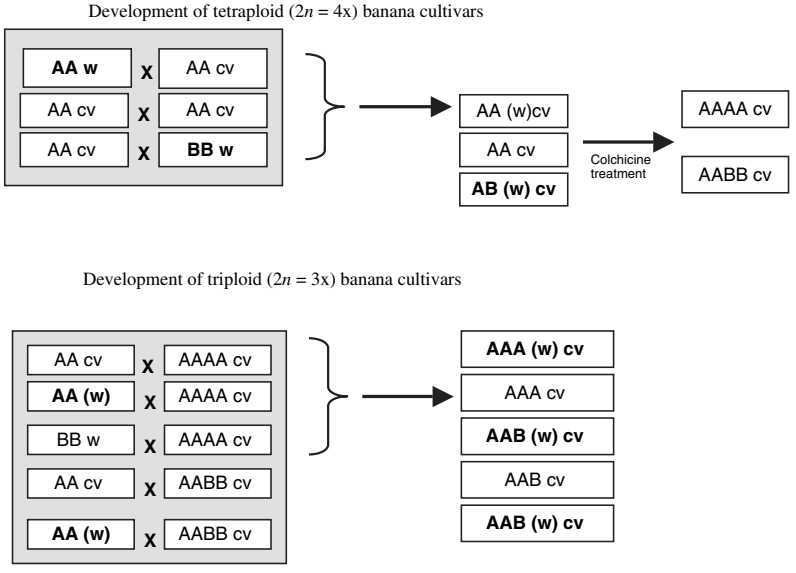

Figure 1 Types of banana according to genomic group and ploidy level. Possible crosses applied for the development of new triploid ($2n = 3x$) and tetraploid cultivars ($2n = 4x$). cv, Commercial cultivar; w, wild germplasm.

Table 1 Common types and commercial cultivars of banana characterised by genomic group, agronomical and quality traits

Type or species (genomic group)	Cultivar	Agronomical and quality traits	Cavendish (AAA)
Dwarf Cavendish		Accepted sensory & physicochemical traits	Mediate fruit size
Robusta (AAA)	Valery	Large bunches of high quality fruit	
Lacatan (AAA)	Pisang	High aromatic	Sweet pulp
Cavendish Nana		Good quality traits, high yielded	Sensitivity to choke throat, sensitivity to *Fusarium oxysporum*
Giant Cavendish	Williams	Very good quality traits	Sweet taste, long size fruits
	Grand Naine	High yielded, very good quality traits	Sensitivity to *Mycosphaerella fijiensis*
Plantain (AAB)	French	Cooking bananas	Horn
Musa balbisiana (ABB)	Bluggoe	Drought tolerance	Long size fruits
Dwarf Prata (*AAB*) × SH 3142 *(AA)* (AAAB)	Gold finger	Tolerance to low temperatures, drought tolerance, resistance to *Fusarium oxysporum*	Fruit acidity, small fruit size

(AAB) and (ABB) (Daniells & Smith, 1991). The Indian subcontinent is thought to have been the major centre for hybridisation of *acuminata* types with the indigenous *M. balbisiana* and the region is noted for the wide variation of (AAB) and (ABB) cultivars (Ortiz, 1997). *Musa balbisiana* is considered to be more resistant to diseases and tolerant to abiotic stress, than *M. acuminata*. Such characteristics often occurred in cultivars containing a 'B' genome. Hybridisation would have given rise to a wide range of edible types of banana, some of which would have survived and been multiplied under domestication (Simmonds, 1962). Consequently, a diverse selection of banana cultivars is thought to have arisen in South East Asia along with the earliest developments of agriculture many thousands of years ago (Price, 1995). The subsequent dispersal of edible bananas outside of Asia was most likely brought about solely by humans (Simmonds, 1962). This early dispersal of banana cultivars resulted in the development of distinct sub-groups of cultivars. Secondary diversification within the major sub-groups of cultivated bananas is thought to have been the result of somatic mutations rather than sexual reproduction. Mutations affecting traits of economic or horticultural interest were selected by farmers over the years and multiplied by vegetative propagation to produce morphotypes (http://www.inibap.org).

Although the precise number of *Musa* cultivars is uncertain, Samson (1982) has estimated that they must be over 300 (Valmayor *et al.*, 2000). A collection of most *Musa* accessions is maintained by the Plantain and Banana Improvement Programme of the International Institute of Tropical Agriculture in a field gene bank located at Onne near Port Harcourt, Nigeria (Swennen & Vuylsteke, 1991).

Applications of biotechnology in banana

A high number of applied biotechnological techniques are increasingly being used worldwide towards improving the handling and properties of plantain and banana germplasm (Table 2). *Tissue culture* is used for germplasm exchange, conservation and rapid multiplication, while *in vitro* seed germination (based on embryo culture or rescue) plays a critical role in generating hybrid

Table 2 Biotechnological techniques and applications in banana

Technique	Application	References
In vitro culture	Micropropagation, germplasm conservation, embryo culture, virus free meristem culture	Arias (1992), Crouch *et al.* (1998), Vuylsteke (1989), Israeli *et al.* (1995), Creste *et al.* (2004)
Protoplast fusion	Development of new hybrids	Sagi *et al.* (1995), Assani *et al.* (2005)
Somatic embryogenesis	Somaclonal variation	Gimenez *et al.* (2001), Damasco *et al.* (1997)
In vitro mutagenesis	Development of new cultivars	Damasco *et al.* (1997), Bhagwat & Duncan (1998)
Anther culture	Haploid production, development of new dilpoid cultivars	Assani *et al.* (2003)
DNA flow cytometry	Ploidy level estimation, study of somaclonal variation	Roux *et al.* (2003)
Genetic transformation	Development of new genetically modified organism cultivars	Côte *et al.* (1997), Ganapathi *et al.* (2002), Becker *et al.* (2000)
DNA fingerprinting	Cultivar identification, genetic analysis	Crouch *et al.* (2000)
Molecular markers	Breeding (early selection)/MAB, genome mapping, detection of somaclonal variation	Ferreira *et al.* (2004), Crouch *et al.* (2000), Ramage *et al.* (2004)

plants (Crouch *et al*., 1998). A reproducible *anther culture method* for producing haploid plants of banana would be useful in conventional breeding programs, as well as in somatic protoplast fusion procedures, to obtain directly triploid cells starting from diploid and haploid cells. Assani *et al*. (2003) reported successful regeneration of haploid plants ($2n = x = 11$) in four genotypes of the species *M. balbisiana* (BB), which is known to carry resistant genes against economically important banana diseases. A *protoplast fusion technique* to obtain somatic hybrids between triploid and diploid bananas, was put forward by Matsumoto *et al*. (2002). The technique is particularly useful in introducing disease resistance from wild relatives or other species into a cultivated variety. Assani *et al*. (2005) compared the most frequently used fusion method (electrofusion technique) with the chemical procedure using polyethylene glycol (PEG). According to their observations with regard to frequency of binary fusion, protoplast fusion with the fusogen polyethylene glycol was the best. Conversely, electric fusion was found to be better with respect to mitotic activities, somatic embryogenesis and plantlet regeneration rate. *Mutation breeding* could also contribute to genetic improvement of banana plants. However, there has been no practical implementation of a mutation breeding program for banana. Bhagwat & Duncan (1998) reported the use of gamma irradiation on explants of *in vitro* grown cultures of banana (AAA group) to evaluate the effectiveness of inducing mutations and also with the aim of producing variants tolerant to the fungus *Fusarium oxysporum* f. sp. *Cubense* well known as Panama disease.

Somatic embryogenesis techniques in the genus *Musa* aimed at developing new, high performance micropropagation techniques and cell regeneration systems useful for genetic transformation and cultivar improvement (Kosky *et al*., 2002). Moreover, the cell culture could be associated with mutation induction, *genetic transformation* processes (biolistic or *Agrobacterium* systems) or selection methods based on *somaclonal variation*. The use of biotechnological approaches, such as *in vitro* mutagenesis and genetic transformation, are seriously impeded since the treatment of multicellular meristems results in a high degree of chimerism (Roux *et al*., 2003). The high incidence of 'off-types' produced by banana meristem culture is a major concern to commercial growers. At the same time, somaclonal variation is an important tool for the improvement of banana germplasm (Khayat *et al*., 2004). Vidal & Garcia (2000) managed to obtain a somaclonal variant (CIEN BTA-03) resistant to Yellow Sigatoka from a susceptible banana clone (Williams clone), by increasing the production of adventious buds using 6-benzylaminopurine, at high concentrations. Molecular techniques have been applied to detect and partially characterise the somaclonal variants in banana. Ramage *et al*. (2004) used random amplified polymorphic molecular markers (RAPD) to detect dwarf off-types in micropropagated Cavendish bananas (AAA group). Aneuploidy, which involves an under- or over-representation of one or more chromosomes, is a frequent type of mutation in *Musa*. *Flow cytometry* is a convenient and rapid method for the detection of such aneuploidy (Roux *et al*., 2003). A substantial amount of research was carried out to distinguish and classify *Musa* accessions on the basis of morphological characteristics. However, the classification of certain accessions on this basis has been disputed (Crouch *et al*., 1998). *Molecular markers* were used to study diversity in *Musa*, as well as in *Musa* genotyping and mapping (Kaemmer *et al*., 1997). Ferreira *et al*. (2004) employed RAPD molecular markers for the characterisation of banana diploids (AA) with contrasting levels of Black Sigatoka (*Mycosphaerella fijiensis*) and Yellow Sigatoka (*Mycosphaerella musicola*) resistance. Creste *et al*. (2004) estimated genetic diversity of *Musa* diploid and triploid accessions by microsatellite markers. Genetic variability was investigated among forty genotypes of banana using B-genome derived SSRs molecular markers (Oreiro *et al*., 2006). In a comparative analysis of phenotypic and genotypic diversity among plantain landraces (*Musa* spp. AAB group), a rather poor correlation was found between RAPD-based estimates of genetic diversity and a phenotypic index based on agronomic characters. These results suggested that the traditional designations of plantain landraces based on morphotype do not provide a true reflection of overall genetic divergence (Crouch *et al*., 2000). Furthermore, this study was conducted to compare different PCR-based marker systems [RAPD, variable number tandem repeats (VNTR) and amplified fragment length polymorphism (AFLP)] for the analysis of *Musa* breeding populations. AFLP assays had by far the highest multiplex ratio while VNTR analysis detected the highest levels of polymorphism. In general, there was a poor correlation between estimates of genetic similarity based on different types of marker (Crouch *et al*., 2000).

Genetically modified bananas

Genetic transformation is of great interest in banana because (i) the cultivated varieties are triploid and sterile, (ii) some resistance sources are not available among genetic resources (i.e. virus resistance) and (iii) the foreign gene within the genetically modified plant cannot be transferred to another plant because the triploid plants will not produce fertile pollen. Therefore, the risk of direct gene contamination is minimised both for other plants and for the environment.

A sexual gene transfer methods such as transformation may be required for characteristics lacking in *Musa* genebanks, or for the genetic improvement of cultivars

not amenable to conventional cross breeding such as Cavendish bananas or Horn plantains. Relative success in genetic engineering of bananas and plantains has been achieved recently to enable the transfer of foreign genes into plant cells (Table 3). Protocols for *electroporation of protoplasts* derived from embryogenic cell suspensions (Sagi *et al*., 1998), *particle bombardment* of embryogenic cells (Sagi *et al*., 1995; Côte *et al*., 1997; Becker *et al*., 2000), and co-cultivation of wounded meristems with *Agrobacterium* (May *et al*., 1995) are available for bananas and plantains. The *Agrobacterium*-mediated transformation method may be more widely applicable as it is based on the use of differentiated tissue that can be routinely regenerated into whole plants. In addition, it has been applied to a wide range of plantain and banana cultivars and synthetic hybrids (Bosque-Pérez *et al*., 1998). Moreover, *Agrobacterium*-mediated transformation offers several advantages over direct gene transfer methodologies like particle bombardment and electroporation (Gheysen *et al*., 1998; Hansen & Martha, 1999; Shibata & Liu, 2000).

Various diseases caused by fungi and viruses have seriously endangered the production of banana and plantains. Through genetic transformation technologies, disease resistant varieties may be produced. Transgenic plants have been produced for the cultivars Williams, Gros Michel, Bluggoe and Three Hand planty, using gene constructs encoding for various antifungal peptides which have previously proved to be highly active *in vitro* against major pathogenic fungi of bananas (Remy *et al*., 2000). Fusarium wilt caused by *Fusarium oxysporum* f. sp. c*ubense* and Black sigatoka, by the fungus *Mycosphaerella fijiensis* f. sp. *cubense*, which are the most devastating fungal diseases of *Musa*. The most attractive strategy for black sigatoka control in *Musa* is probably the production of disease resistant plants through the transgenic approach. These approaches included the expression of genes encoding plant, fungal or bacterial hydrolytic enzymes (Lorito *et al*., 1998), genes encoding elicitors of defence response (Keller *et al*., 1999) and antimicrobial peptides (Cary *et al*., 2000). Efforts are presently focused on the recently described antimicrobial proteins (AMPs) which are stable, cysteine-rich small peptides isolated from seeds of diverse plant species (Sági *et al*., 1998). AMPs have a broad-spectrum antimicrobial activity against fungi as well as bacteria and most are non-toxic to plant and mammalian cells. On the basis of their broad-spectrum activity against fungal pathogens, individual or combined expression of cecropin (identified from the cecropia moth and is very active against Gram-negative bacteria), magainin and their derivatives (acts on both Gram-positive and Gram-negative bacteria, fungi and protozoa) in *Musa* may result in enhanced resistance to several pathogens (Tripathi, 2003; Tushemereirwe *et al*., 2002). The AMPs of plant origin may be the potent candidates for fungal resistance in *Musa* as they have high *in vitro* activity to *Mycospaerella fijiensis* and *Fusarium oxysporum* f. sp. *cubense* and they are non-toxic to human or banana cells as well. Several hundreds of transgenic lines of *Musa*, especially plantains, expressing AMPs were developed using particle bombardment of embryogenic cell suspensions (Remy, 2000), but none of these transgenic plants was used in field trials due to the lack of biosafety guidelines in most tropical countries. Further research is anticipated to demonstrate whether these transgenic plants can express the functional antimicrobial peptides at levels high enough to control fungal leaf or root diseases in the field. It was shown that one AMP gene is also expressed in the fruit, thereby providing the

Table 3 Genetic modification in banana (methodology used, expressed gene and modified traits)

Expressed gene	Method	Modified trait	References
MSI-99, a magainin analogue	*Agrobacterium*	Resistance to *Fusarium oxysporum* f.sp. cubense and *Mycosphaerella musicola*	Chakrabarti *et al.* (2003)
Antimicrobial peptides	Particle bombardment	Resistance to *Fusarium oxysporum* f. sp. cubense and *Mycospaerella fijiensis*	Remy *et al.* (2000), Sági *et al.* (1998), Tripathi (2003)
Antimicrobial peptides	Particle bombardment	Resistance against preharvest and postharvest diseases *Verticillium theobromae* or *Trachysphaera fructigena*	Cary *et al.* (2000), Sági *et al.* (1998)
Protein engineered rice Cystatin (OcIdeltaD86)	*Agrobacterium*	Resistance to nematode *Radopholus similis*	Atkinson *et al.* (2004)
Cysteine proteinase inhibitor (oryzacystatin-I)	*Agrobacterium*	Resistance to banana weevil (*Cosmopolites sordidus*)	Kiggundu *et al.* (2002)
Hepatitis B antigen, HBsAg (pHBS, pHER, pEFEHBS)	*Agrobacterium*	Edible vaccine against hepatitis B	Ganapathi *et al.* (2002), Kumar *et al.* (2005)
Synthetic cercosporins	*Agrobacterium*	Resistance to bacterial wilt (*Xanthomonas* spp.)	Rajasekaran *et al.* (2001)
Antiretroviral genes (adefovir, tenofovir)	*Agrobacterium*	Resistance to Banana Streak Virus	Helliot *et al.* (2003)
Human lysozyme gene	*Agrobacterium*	Resistance to *Fusarium oxysporum*	Pei *et al.* (2005)

opportunity to enhance the resistance against preharvest and postharvest diseases such as cigar-end rot and crown rot (Sági *et al*., 1998).

Magainin is one of the earliest reported antimicrobial peptides isolated from skin secretions of the African clawed frog *Xenopus laevis*. Chakrabarti *et al*. (2003) have recently reported successful expression of a synthetic substitution analogue of magainin, MSI-99 and enhanced disease resistance. MSI-99 was subcloned into plant expression vectors pMSI164 and pMSI168. Transgenic banana plants were obtained for both pMSI164 and pMSI168 transformations and displayed considerable resistance to *F. oxysporum* f. sp. *cubense* and *Mycosphaerella musicola*. The obtained results suggest that MSI-99 can be successfully applied in imparting enhanced disease resistance in transgenic banana plants (Chakrabarti *et al*., 2003). *Xanthomonas campestris* pv. *musacearum* is gradually spreading in East Africa and if uncontrolled could result in massive losses. To date, no banana germplasm exhibiting resistance to the disease was identified. Use of genetic transformation technology with bactericidal transgenes encoding for peptides such as cecropins and lysozyme, may offer an alternative and potential solution to these problems (Tripathi, 2003).

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. Engineering resistance to BBTV is an obvious objective for banana transformation, since no natural resistance to this virus has so far been identified in the *Musa* genepool. One of the strategies currently being tested in several laboratories is the expression of various BBTV genes in transgenic banana plants in order to interfere with the normal replication, encapsidation or movement of the virus. Another approach is the expression of heterologous antiviral proteins that are known to act by the inhibition of viral replication or translation in bananas (Sági *et al*., 1998). Similar strategies can also be considered against another recently emerged DNA virus, the *Banana streak virus* (BSV), whose molecular research in laboratories worldwide revealed a number of interesting features including its integration into the banana genome (Sági *et al*., 1998). The BSV, genus *Badnavirus*, has a major impact on banana and plantain production in Africa (Swennen & Vuylsteke, 2001). Researchers at International Institute of Tropical Agriculture (Nigeria) in collaboration with John Innes Centre (UK) are experimenting with transgenic plants resistant to BSV (including expression of integrated sequences) based on the novel approach of gene silencing (http://www.inibap.org).

Nematodes are recognised as severe production constraints to bananas and plantains (Gowen & Queneherve, 1990). There are several possible approaches for developing transgenic plants with improved nematode resistance. The use of proteinase inhibitors (PIs), as nematode antifeedants, is an important element of natural plant defence strategies (Ryan, 1990). Cysteine PIs (cystatins) are inhibitors of cysteine proteinases and were isolated from seeds of a wide range of crop plants consumed by humans including those of sunflower, cowpea, soybean, maize and rice (Atkinson *et al*., 1995).

Atkinson *et al*. (2004) were the first to report transgenic resistance against the nematode *Radopholus similis*, a major pest of banana. Cavendish banana was effectively transformed by means of *Agrobacterium tumefaciens* to express a protein engineered rice cystatin (OcIdeltaD86) of value for control of plant parasitic nematodes.

The banana weevil (*Cosmopolites sordidus*) is a pest of substantial importance in Africa and greatly affects banana and plantain production. Although remarkable progress was made in banana transformation, the identification and introduction of useful genes into banana to reduce losses caused by the banana weevil is still a major challenge. Among the various genes available for genetic engineering for pest resistance are (Carozi & Koziel, 1997; Sharma *et al*., 2000): PIs, *Bacillus thuringiensis* (Bt) toxins, plant lectins, vegetative insecticidal proteins and alpha-amylase inhibitors.

The PIs if expressed in bananas, could greatly improve their resistance to the banana weevil. However, any strategy targeting to the use of a gene coding for an inhibitor should ideally include a design program for optimising the inhibitor action against the target enzyme. Cysteine proteinase activity was recently identified in the mid-gut of the banana weevil. *In vitro* studies revealed that these cysteine proteinases are strongly inhibited by both a purified recombinant rice (oryzacystatin-I) and papaya cystatin (Kiggundu *et al*., 2002). Nevertheless, the incorporation of genes on several occasions is random in plant genome thereby resulting in either non-anticipated expression or lower expression level. In view of the dynamic of plant genome, the insertion gene may interact with the other genes thus potentially having unexpected or disastrous effect upon the plant itself.

Over the last years there has been a growing interest in 'molecular farming' for the production of added value compounds of pharmaceutical, cosmetic and industrial importance. Out of these, edible vaccines are of prime importance for human health care (Ganapathi *et al*., 2002). The production of antigens in genetically engineered plants is anticipated to provide an inexpensive source of edible vaccines and antibodies in the fight against infectious diseases such as hepatitis B (Prakash, 1996). In this regard, banana can serve as an ideal system for the production and delivery of edible vaccines. Ganapathi *et al*. (2002)

sub-cloned hepatitis B surface antigen (HBsAg) and transformed embryogenic banana cells using *Agrobacterium*-mediated transformation in order to produce transgenic banana plants for edible vaccines against hepatitis B. Embryogenic cells of banana cv. Rasthali (AAB) were transformed with the 's' gene of HBsAg using *Agrobacterium* mediated transformation. Four different expression cassettes (pHBS, pHER, pEFEHBS and pEFEHER) were utilised to optimise the expression of HBsAg in banana. The transgenic nature of the plants and expression of the antigen were confirmed by PCR, Southern hybridisation and reverse transcription (RT)-PCR. HBsAg obtained from transgenic banana plants was similar to human serum derived one in analogous density properties (Kumar *et al*., 2005).

Biosafety

The potential biosafety risks of transgenic varieties have generated controversy in international community. According to the U.S. National Research Council (2002), these risks include: (i) the flow of transgenes, (ii) evolution of resistance in the targeted pest population, (iii) plant escape and establishment of self-reproducing populations, (iv) effects on non-target species, and (v) health hazards (Smale *et al*., 2006). Biosafety frameworks, laws, and regulations in developing countries have been developed in response to the implementation of the Cartagena Protocol on Biosafety. The Cartagena Protocol, a supplement to the Convention on Biological Diversity, addresses the safe transfer, handling, and use of living modified organisms (LMOs), especially those that may have an adverse effect on biodiversity, taking into account risks to human and animal health.

Bananas are being considered as the vehicle of choice for phytoceuticals, particularly for applications in the developing countries, because of their worldwide popularity, abundance and baby-friendliness. On the contrary, bananas contain low protein levels and are unlikely to produce large amounts of recombinant proteins (i.e. vaccines). Banana trees also take a few years to mature and the fruit spoils fairly rapidly after ripening thereby making transportation and storage difficult (http://www.biosafety-info.net). It should be pointed out that clonal propagation and the system for disseminating plant material are responsible for engendering the risk of resistance evolution for transgenic bananas rather than gene flow. The risk of resistance evolution in the targeted pest population may be great with the soil and root borne problems of banana, since mats move slowly with new roots in a given location and farmer propagation reproduces the same trait. Large-scale multiplication schemes, such as those envisaged for tissue culture systems, would contribute to genetic uniformity in the trait (Smale *et al*., 2006). Gene flow is possible among tetraploid bananas, such as recently developed hybrids, although normally these are reproduced through self – propagation. However, no flow of transgenes is possible among East African highland bananas because they are sterile triploids (http://photoscience.la.asu.edu).

Extensive research over the past two decades has shown that a wide range of valuable proteins can be expressed efficiently in plants. Examples include human serum proteins and growth regulators, antibodies, vaccines, industrial enzymes, biopolymers and molecular biology reagents (Smale *et al*., 2006). One of the most important advantages of edible vaccines is the potential to drastically reduce or eliminate transport costs (http://www.biosafety-info.net). Furthermore, edible vaccines would not require the purification, strict refrigeration, and injections that make conventional vaccines expensive to use. The edible vaccines would encourage preventive medicine in the Third World. There are numerous regulatory and safety (dosage) issues associated with 'being vaccinated via a banana', and current research is directed more toward oral vaccination with more or less pure compounds that could be produced inexpensively (http://photoscience.la.asu.edu). However, the most important objections to edible vaccines arise from: (i) their inadequate absorbance from the gut and being eventually broken down by the gut enzymes, (ii) the fact that elimination of transport cost is not feasible and (iii) contradictory environmental repercussions (http://www.biosafety-info.net).

Conclusions

Plant biotechnology has the potential to play a key role in the sustainable production of *Musa*. However, there is enormous potential for genetic manipulation of *Musa* species in order to improve their disease and pest resistance. The use of appropriate constructs may allow the production of nematode, fungal, bacterial and virus-resistant plants in a significantly shorter period of time than using conventional breeding, especially if several traits can be introduced simultaneously. It may also be possible to incorporate other characteristics such as drought tolerance, thereby extending the geographic spread of banana and plantain production, and thus contributing substantially to enhanced food security and poverty alleviation.

Banana seems like a model plant for genetic modification due to its advantage of clonal propagation and lack of pollen fertility thereby ensuring optimal conditions for minimal cross-contamination. However, high caution is required for biosafety experiments and potential risk assessment bearing in mind that it is consumed by most humans and mainly by children.

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