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Review on Biotechnological Approaches and Genetic Transformation in Banana (*Musa* spp)

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Abstract

Plant biotechnology has the potential to play a key role in the sustainable production of banana. Currently, no genetically transformed bananas are commercially available; however there is enormous potential for genetic manipulation of banana species for disease and pest resistance using the existing transformation systems. Banana crop faces numerous environmental challenges, particularly with fungal and bacterial pathogens as well as pests and abiotic stresses. The problem is aggravated by the limited diversity of cultivars. Conventional breeding methods have limited success in banana crop improvement due to low female fertility, sterility, ploidy levels and poor seed set; besides the process is time consuming. For many banana diseases like *Fusarium* wilt, BBTV, CMV and moko disease and no chemical treatment exists and destruction of the affected plants is the only method of control. For the others, the cost of chemical treatment is increasing as more virulent strains appear, or even prohibitive for small farmers in developing countries or else damaging to the environment. Genetically modified bananas would benefit both large commercial and small-scale farmers. Producers of export bananas spend millions of dollars annually to control phytopathogenic fungi and nematodes with chemicals. Bananas that are disease and pest resistant, therefore, would provide them with a source of planting material that is affordable and safe to use.

Keywords: Conventional breeding; Disease and pest resistant; Genetic manipulation; Phytopathogenic fungi

Introduction

Bananas and plantains (*Musa* sp.) are a major staple food, supplying up to 25% of the carbohydrates for approximately 70 million people in Africa's humid forest and mid-altitude region. World banana production is currently about 125 million tons annually, of which bananas cultivated for the export trade accounts for only 10%[1].

Bananas and plantain originated from South East Asia, a region considered as the primary centre of diversification of the crop and where the earliest domestication has occurred.

Hence, bananas and plantains are important for food security in the humid tropics and provide income to the farmers. About 24% of the agricultural households are engaged in banana production due to its many other attributes like carbohydrate stable, a source of animal feeds, production of alcoholic beverage, construction materials (thatch and binding ropes) and handicrafts (mats, baskets, hand bags, necklaces and decorations). The crop also provides soil surface cover, reduces soil erosion on steep slopes and a principal source of mulch for maintaining and improving soil fertility [2].

Wild bananas are diploids (2n=22 cromosomes), seed productive, generally allogamous and with fertile seeds upon which their dispersion and regeneration depend. Most commercial bananas are triploids (2n=3x=33), with the genome of AAA, AAB and ABB originating from polyploidization and interspecific hybridization of the two diploid species *M. acuminate* and *M. balbisiana*. However, not all triploids are important commercially.

Banana is a crop with dual propagation abilities, sexual through seeds and asexual through suckers. Banana is generally propagated vegetative through suckers. But the traditional method is laborious; time consuming and not very efficient as far as production of homogenous plant is concerned. Only 5 to 10 suckers can be obtained from a plant per year in conventional method. Sucker propagation is the only natural means of their perpetuation; artificial methods of propagation include macro propagation and micro propagation [3].

Banana suffers a range of production constraints including pests, especially banana weevils (*Cosmopolites sordidus* Germar) and root nematodes, diseases, particularly black sigatoka (*Mycosphaerella fijensis*), *Fusarium* wilt, bacterial wilt, frequent droughts and reduced soil fertility. These factors cause significant yield losses and shorten the plantation lifespan.

Breeding for disease-resistant banana cultivars using classical methods remains a tedious endeavor because of high sterility, polyploidy and long generation times of most of edible cultivars. Genetic transformation provides an opportunity for single genes of interest to be extracted from the genome of the source organism and transferred directly into the genome of the desired variety. This allows the candidate variety to retain all its original characteristics, with only simple addition of the desired trait.

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The genetic system of *Musa* is complicated owing to the inherent problems of sterility, heterozygosity and polyploidy in most of the clones. Asexual behavior is often an inseparable barrier in using cross breeding as a tool for genetic improvement. Mutation induction, using physical as well as chemical mutagens, is useful for the induction of genetic variability. DNA fingerprinting is a highly sensitive technique, useful for the detection and characterization of genetic variability and also for identification of various genotypes, at the molecular level. Notably, the polymorphism measured through this technique is free from any environmental interference and can be repeated with precision [4].

Genetic engineering has been successfully employed to incorporate virus resistance into existing desirable plant cultivars. Both pathogen-derived resistance approach (involving coding sequences like coat protein and replication initiation protein Rep or non-coding regions) and non-pathogen derived strategies (use of plant bodies against viral coat protein and Rep) have been applied with differing efficiencies.

Materials and Methods

Breeding objectives in *Musa*

According to Pillay et al., the most important objectives of *Musa* breeding include:

- Increased bunch size and yield.
- Host plant resistance against the major pathogens including those causing Sigatoka, *Fusarium* and *Xanthomonas* wilts and viruses.
- Host plant resistance against nematodes and insect pests.
- Fruit quality traits, e.g., increased vitamin A, iron and zinc levels.
- Better adaptation to abiotic stresses such as drought, heat and other stresses that may be enforced by predictions in climate change.

Breeding for yield is a major target followed by breeding for host plant resistance to pathogens and pests that impact on yield.

Applications of earlier biotechnology in banana

Micro propagation of banana has been achieved using shoot tip and from male floral apices. There are also reports of somatic embryogenesis and regeneration in liquid medium. The important factor affecting the efficiency of micro propagation system is the rate of multiplication. It has been observed that banana multiplication rate is genotypic dependent as well as variable behavior has been observed among cultures initiated from same banana genotypes cultured *in vitro*. The present work was planned to study the multiplication rates of banana shoot tips derived from different suckers under *in vitro* conditions during successive sub-culture [5].

In vitro propagation of banana through shoot tip cultures is useful in the rapid multiplication of desirable disease free clones. In addition, careful selection and updating of mother plants results in improved crop yield. New and effective means of propagating bananas would be advantageous over the conventional use of sucker material, for germplasm maintenance, exchange and transportation.

A high number of applied biotechnological techniques are increasingly being used worldwide towards improving the handling and properties of plantain and banana germplasm (Table 1). 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 plants. Ioannis et al., has reviewed the application biotechnological techniques (*in vitro* cuture, protoplast fusion, somatic embryogenesis, *in vitro* mutagenesis, anther culture, DNA flow cytometery, DNA finger printing and molecular techniques in banana improvement programmers (Figure 1).

Techniques	Application
In vitro culture	Micro propagation, germplasm conservation, embryo culture, virus free meristem culture
Protoplast fusion	Development of new hybrids
Somatic embryogenesis	Somaclonal variation
In vitro mutagenesis	Development of new cultivars
Anther culture	Haploid production, development of new diploid cultivars
DNA flow cytometry	Ploidy level estimation, study of somaclonal variation
DNA fingerprinting	Cultivar identification, genetic analysis
Molecular markers	Breeding (early selection)/MAB, genome mapping, detection of somaclonal variation

Table 1: Biotechnological techniques and applications in banana.

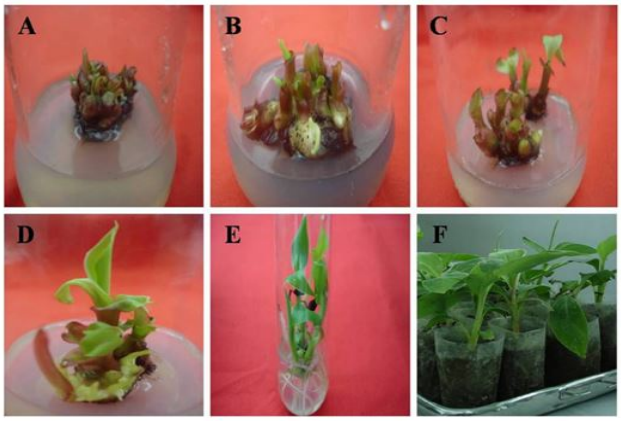

Figure 1: *In vitro* shoot tip culture of banana cv. Agnishwar.

Note: (A) Initiation of proliferations of shoot tip (explants) in banana cv. Agnishwar cultured in MS medium fortified with 4 mg/l BAP. (B) The best response for shoot formation was observed in MS medium fortified with 4 mg/l BAP. (C) The best length of multiple shoot from shoot tip explants in banana cv. Agnishwar culture was found in MS medium supplemented with 4 mg/l BAP. (D) Production of multiple shoot in banana cv. Agnishwar cultured in MS medium fortified with 4 mg/l BAP. (E) Rooting of *in vitro* regenerated shoots was higher in banana cv. Agnishwar cultured in MS medium supplemented with 1.0 mg/l IBA. (F) Acclimatized plantlets in polythene bag containing normal soil, sand and compost [6].

Applications of genetic engineering in banana

All technologies required for genetic engineering of bananas have become available in the last ten years. Male buds were used to generate cell suspensions. Cryopreservation of these cells has reduced losses by contamination. It has also made the cells readily available, reducing the need to repeatedly go through the complicated procedure to generate new ones. Several genetic transformation systems have been used for bananas. These include electroporation of protoplast, particle bombardment (Biolistic gun) and agro bacterium-mediated gene transfer.

Among the available DNA delivery techniques, agro bacterium-mediated transformation is preferred due to:

- The simplicity and low cost of the technique.
- Low copy numbers of the transgene.
- Ability to transfer large DNA segments with minimal rearrangement (Table 2).

Expressed gene	Method	Modified trait
MSI-99, a magainin analogue	Agrobacterium	Resistance to *Fusarium oxysporumf.* sp. cubense and *Mycosphaerella musicola*
Antimicrobial peptides	Particle bombardment	Resistance to *Fusarium oxysporumf.* sp. cubense and *Mycosphaerella musicola*
Antimicrobial peptides	Particle bombardment	Resistance against pre-harvest and postharvest diseases *Verticillium theobromae* or *Trachysphaera fructigena*
Protein engineered rice (OcIdeltaD86) Cystatin	Agrobacterium	Resistance to nematode *Radopholussimilis*
Cysteine proteinase inhibitor (oryzacystatin-I)	Agrobacterium	Resistance to banana weevil (*Cosmopolites sordidus*)
Hepatitis B antigen, HBsAg (pHBS, pHER, pEFEHBS)	Agrobacterium	Edible vaccine against hepatitis B
Synthetic cercosporins	Agrobacterium	Resistance to bacterial wilt (*Xanthomonas* spp.)
Antiretroviral genes (adefovir, tenofovir)	Agrobacterium	Resistance to banana streak virus
Human lysozyme gene	Agrobacterium	Resistance to *Fusarium oxysporum*
Geneencoding ferredoxin-like amphipathic protein (pflp)	?	Resistance to bacterial wilt (*Xanthomonas* spp.)

Table 2: Genetic modification in banana (methodology used, expressed gene and modified traits).

Results and Discussion

Resistance to fungal diseases

The most attractive strategy for black sigatoka control in banana is probably the production of disease resistant plants through the transgenic approach including the expression of genes encoding plant, fungal or bacterial hydrolytic enzymes, genes encoding elicitors of defense response and 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, cecropins from the giant silk moth, mammalian and plant defensins. The cecropin and its derivatives as well as its hybrids peptides with melittin have been found to inhibit the *in vitro* growth of several important fungal pathogens. The synthetic cecropin-melittin chimeric peptide provided field-level resistance against *Verticillium dahliae* in potato [7].

Similarly, magainin is effective against the plant pathogenic fungi. Li et al., reported enhanced disease resistance in transgenic tobacco expressing Myp30, a magainin analogue. Another substitution

analogue MSI-99 when expressed in tobacco *via* chloroplast transformation conferred both *in vitro* and in planta resistance to phyto pathogenic bacteria and fungi. Recently, Chakrabarti et al., reported successful expression of this synthetic peptide and enhanced disease resistance in transgenic tobacco and banana. On the basis of their 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.

There are many reports on the application of plant proteins with distinct antimicrobial activities. 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 and also they are non-toxic to human or banana cells. Several hundreds of transgenic lines of *Musa* especially plantains expressing AMPs have been developed at KULeuven.

Transformation of banana and plantains for fungal diseases started in the 1990's. Various transformation techniques have been used to produce transgenic bananas with antifungal peptides which are highly active *in vitro* against major pathogenic fungi such as black Sigatoka and *Fusarium wilt* of bananas. The transgenics showed resistance to black Sigatoka under laboratory conditions. Several hundreds of transgenic lines of *Musa* especially plantains expressing AMPs have been developed at KULeuven [8].

Improved resistance to Sigatoka was obtained when banana was transformed with the endochitinase gene ThEn-42 from *Trichoderma harzianum* and the grape stilbene synthase (StSy) gene. The superoxide dismutase gene Cu, Zn-SOD from tomato, under control of the ubiquitin promoter, was also added to this cassette to improve scavenging of free radicals generated during fungal attack. Since the genes conferring Sigatoka tolerance may have a wide range of antifungal activities the regenerated banana plants were also inoculated with the fungus *Botrytiscinerea*. The best transgenic lines exhibiting Sigatoka tolerance were also found to have tolerance to *B. cinerea* in laboratory assays.

Resistance to bacterial diseases

To date, no source of any banana germplasm exhibiting resistance to the disease has been identified. Use of genetic transformation technologies, may provide a timely and cost-effective measure to address the dangers of the spread of this disease. Resistance genes have been exploited to develop bacterial disease resistant plants in many crops like rice, tobacco, tomato and apple. One approach to control bacterial disease is to improve a plants' defense against a particular pathogen. Plant defense genes and antimicrobial proteins that naturally occur in insects, plants, animals and humans are now a potential source of plant resistance.

Pathosystem-specific plant resistance (R) genes have been cloned from several plant species. R genes cloned from resistant varieties can be transferred to susceptible cultivars of same plant species making them resistant to pathogens. It is also possible to transfer R genes from one plant species to another species [9].

Many of these R gene products share structural motifs, which indicate that disease resistance to diverse pathogens may operate through similar pathways. In tomato (*Lycopersicon esculentum*), the R gene *Pto* encodes a Ser/Thr kinase and confers resistance against strains of *Pseudomonas syringae* pv tomato that express the effector proteins *AvrPto* or *AvrPtoB*. 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*. The *Bs2* resistance gene of pepper specifically recognizes and confers resistance to strains of *Xanthomonas campestris* pv. vesicatoria that contain the corresponding bacterial avirulence gene, *avrBs2*. Transgenic tomato plants expressing the pepper *Bs2* gene suppress the growth of Xcv. The *Bs2* gene is a member of the Nucleotide Binding Site-Leucinerich Repeat (NBS-LRR) class of R genes. *Xa21* gene isolated from rice has been shown to confer resistance against many isolates of *X. oryzae* pv. oryzae. This gene is a member of a large multigene family, and encodes a receptor kinase-like protein with an extracellular leucine rich repeat motif. Transgenic plants expressing *Xa21* under the control of the native promoter of the genomic fragment of the *Xa21* gene showed enhanced resistance to bacterial leaf blight caused by most Xoo races. Bioassays showed that some Xoo races were not affected by the *Xa21* gene and there is always the danger that susceptible pathogens will evolve resistance to the transgenes used against them.

The *Xa1* gene also isolated from rice confers resistance to Japanese race 1 of *Xanthomonas oryzae* pv. oryzae, the causal pathogen of bacterial blight. *Xa1* is a member of the NBS-LRR class of plant disease resistance genes, but quite different from *Xa21*, another disease resistance gene isolated from rice. Interestingly, *Xa1* gene expression was induced on inoculation with a bacterial pathogen and wound, unlike other isolated resistance genes in plants, which show constitutive expression. The induced expression may be involved in enhancement of resistance against the pathogen.

The *pflp* and *hrap*, isolated from sweet pepper would be good candidate genes for many crops to convert susceptible to resistant plant against many bacterial pathogens, such as, *Erwinia, Pseudomona, Ralstonia* and *Xanthomonas* spp through transgenic technology. Elicitor-induced resistance is not specific against particular pathogens. The other strategy for developing bacterial disease resistant varieties is the use of Antimicrobial Peptides (AMPs) isolated from frogs, insects and mammalian phagocytic vacuoles. Cecropins are antibacterial lytic peptides native to the hemolymph of *Hyalophora cecropia*, the giant silk moth. Native (Cecropin B), mutant (SB37, MB39) and synthetic (Shiva-1, D4E1) cecropins are active *in vitro* against a wide range of plant pathogenic bacteria including *Erwinia. carotovora, E. amylovora, Pseudomonas syringae, Ralstonia solanacearum* and *Anthomonas campestris* whereas they exert no toxicity at bactericidal concentration to cultured cells or protoplasts of several plant species. Therefore, cecropins have been considered as potential candidates to protect plants against bacterial pathogens. Transgenic tobacco plants expressing cecropins have increased resistance to *Pseudomonas syringae* pv. tabaci, the cause of tobacco wildfire.

Resistance to viral diseases

Banana bunchy top is one of the most threatening diseases in the world as infected plants do not produce fruit but so far, only few areas are affected in Africa. Whereas, Banana Streak Virus (BSV), genus *Badnavirus* has a major impact on banana and plantain production. BSV infection induces yield losses and restricts movement of improved germplasm (due to quarantine restrictions), particularly in sub-Saharan Africa. Recent reports indicate that BSV infection may arise from the activation of viral sequences that are integrated into the *Musa* genome. Tissue culture and the hybridization might be triggers for the activation of the integrant to produce BSV infection. 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., have reported that the anti-retroviral and antihepadna virus molecules, adefovir, tenofovir and 9-(2-Phosphonomethoxyethyl)-2,6- Diaminopurine (PMEDAP), efficiently eradicate the episomal form of Banana Streak Virus (BSV) from banana plants.

Resistance to nematodes

There are evidences that nematode resistance and tolerance sources, though limited, are present in the *Musa* gene pool. 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. is more dangerous than *R. similes*. Furthermore, a number of species of nematode are often present together, necessitating a wide spectrum resistance.

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 defense strategies. The transgenic delivery of PIs can affect the sexual fate and growth of the cyst and root knot nematodes. This approach offers prospects for novel plant resistance against nematodes and reduces reliance on nematicides. The potential of PIs for transgenic crop protection is enhanced by a lack of harmful effects when humans in seeds such as rice and cowpea consume them. This transgenic approach to incorporate a gene coding for the production of cystein proteinase inhibitor against root nematodes has been reported by Atkinson and appears to be successful for a number of crops including rice, potato, pineapple and cooking banana. Cystatins have been shown to be effective against a wide range of nematode species and therefore offer a solution to protecting banana against a combination of pest species. In field trials, transgenic potato lines demonstrated up to 70% resistance against nematodes. There is no evidence that expression of cystatins impairs plant growth or yield in the trial.

The other strategies for nematode resistance include the use of natural resistance genes (Rgenes), lectins and *Bacillus thuringiensis* (Bt) genes. Several R-genes are targeted against nematodes. The *Hs1pro-1* from a wild species of beet confers resistance to the cyst nematode *Heterodera schachlii*. The Mi-1.2 gene of tomato confers resistance against *Meloidogyne* species. To date there has been no reports of Mi-1.2 being functional after transfer to a plant other than tomato.

Some lectins like snowdrop lectins (GNA) do have biological activity against nematodes. But many lectins have toxic effects on insects and mammals. Concerns regarding toxicological safety may prove a substantial additional limitation to the future commercial development of lectins. Some BT proteins have effects against saprophagous nematodes. The Cry5B protein is toxic to wild type *C. elegans* whereas some mutants of *C. elegans* are resistant to it but susceptible to Cry6A toxin. The approach using cry genes has potential for plant nematode control.

Resistance to insect pests

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: PIs, *Bacillus thuringiensis* (Bt) toxins, plant lectins, vegetative insecticidal proteins and alpha-amylase inhibitors. There is no information on the utilization of either these or any other options that use utilized genetic engineering for banana resistance against weevil attack.

Fruit ripening

Bananas are climacteric fruits, which are characterized by low rates of respiration and ethylene production at the pre-climacteric stage during ripening, followed by a sudden upsurge at the climacteric and a shape decline at the post-climacteric stage.

Ripening in fruits is a natural phenomenon by which they taste sweeter and gain palatability. Biochemically, it beg with concomitant increase in acidity till a level (brix-acid ratio) when fruits taste/smell tarter. Being a climacteric fruit, ripening in banana is an important phenological event. Edible cultivated bananas are triploids with AAA, AAB and ABB genomic composition of which the A-genome (*M. balbisiana*) has been associated with banana pre-harvest development and post-harvest ripening. Cultivars possessing more of A-genome exhibit high yield, long fingers, and long-term storage an attribute of best fruit quality. Post-genomic research has strongly indicated role of the bZIP transcription factors in fruit development process in many crops.

Bananas are highly perishable, with a short shelf life after harvest. Several common practices have been employed in attempts to prolong the shelf life of the fruit. In recent years, major efforts have been devoted to elucidating the underlying mechanism of ripening at the molecular level. In banana, several ripening-related genes have also been identified by differential expression of cDNA libraries in ripening fruits.

Both enzymes are encoded by multigene families, and ACC synthase has been shown to be encoded by at least nine genes. In banana cv. Grand Naine, the level of ACC synthase transcripts was low or undetectable in flesh tissue of pre-climacteric fruit, but it surged transiently in climacteric fruit, followed by a rapid decline in post-climacteric fruit.

Delay the ripening and senescence process by interfering with the expression of one or more of these genes

- PG breaks down pectin fraction of plant cell walls (softening).
- PG is absent from green fruit and synthesized during ripening.
- **GM strategy:** Creating transgenic plants with antisense RNA producing versions of the gene.
- Insertion of the anti-sense RNA producing gene for polygalacturonase reduced both gene polygalacturonase mRNA and enzyme activity.
- Antisense ethylene technology is applicable to all climacteric fruits and to other systems triggered by ethylene.
- Inhibited fruit ripening in banana, permitting them to ripen on the plant instead of harvesting green.
- Long shelf life with flavor (flavor saver).
- Reduced its post-harvest loss.

Nutritional enhancement

The genetic enhancement of micronutrient content (*i.e.*, biofortification) of banana by conventional breeding combined with the use of biotechnological tools has the potential to increase the concentrations of micronutrients (Fe, Zn) and vitamin A in new cultivars. Pillay et al., citing Dale and Tushemeirewe reported that researchers in Australia are transforming bananas for increased vitamin A, vitamin E or iron and its increasing pulp of banana color and its increasing beta-carotene content.

Molecular markers and breeding

The rapid development of molecular techniques and their application to plant breeding has resulted in significant genetic gains in agricultural crops, some of which have already entered the market. Biotechnological tools may offer an alternative method for crop enhancement for solving or reducing some of the constraints of conventional banana breeding.

Molecular markers assist selection of traits that are expressed late in the plant cycle. Several DNA markers for bananas have been reported. Research at IITA identified PCR-based molecular markers Amplified Fragment Length Polymorphism (AFLP) and Randomly Amplified Polymorphic DNA (RAPD) that discriminate between the A and B genomes and together with flow cytometry for ploidy analysis, facilitate early selection of hybrids with putative banana or plantain labels.

Application of molecular markers in *Musa* has primarily concentrated on the analysis of diverse germplasm. RFLP analysis may not detect sufficient polymorphism between closely related genotypes and it is not readily amenable to the high throughput demands of molecular breeding applications.

Simple Sequence Repeats (SSR) are ideal genetic markers for detecting differences between and within species of genes of all eukaryotes. Some SSR makers have been validated for genotyping *Musa* accessions for resistance to *Fusarium wilt*, massr 18a (F), massr 18b (R), massr 20a (F), massr 20b (R), massr 24a (F), massr 24b (R). Venkatachalam et al. showed in their genetic study of Indian bananas that there is a strong SSR markers have been used successfully in banana genotyping and several maps have been developed which will help to identify markers for use in breeding programmes. Hippolyte et al., developed a map for SSR basing on structural heterozygosity of related diploids. Mbanjo developed an SSR map for populations segregating for nematode resistance (*Radopholus similis*). SSRs from these maps have been used in different studies like diversity in bananas basing on agro-morphological classification as well as the ploidy.

Among all these markers, microsatellites have an advantage over other molecular markers because of their co-dominance and being powerful in their resolving power and therefore are becoming more popularly used. Some of the prominent features of these markers are that they are dominant fingerprinting markers and co-dominant Sequence Tagged Microsatellites (STMS) markers. Therefore SSR markers for weevil resistance will be a tool that is very useful for accelerating rapid assay breeding that allows the differentiation between resistant and susceptible genotypes in a reasonably short period of time.

Dungu suggested the need to have a super variety that is resistant to both weevils and nematodes. Mbanjo developed SSR markers for resistance to nematodes using the same resistant parent used for weevil screening. This therefore suggests a need to find out if SSR markers for nematodes resistance have any association with weevil resistance traits in *Musa* spp. Having markers that select for both nematode and banana weevil resistance is important in selecting cultivars that have resistance to both weevils and nematodes at once which saves time and resources.

Genetic analysis of resistance to black Sigatoka and other important pests and diseases in diverse germplasm could enable the identification of markers for distinct sources of resistance genes. MAS could then be used for directing the pyramiding of resistance genes as a strategy for generating potential durable resistance to specific pest and diseases.

The cloning of genes underlying important agronomic characters offers to revolutionize progress in plant research and breeding, particularly in the area of pest and disease resistance. A significant number of diverse disease resistance genes have now been cloned which function against viruses, bacteria and fungi. This work has facilitated the testing of strategies for the development of transgenic plants resistant to insect pests, viral, bacterial and fungal diseases and nematodes. The cloning of several plant disease resistance genes in model plant species was facilitated by chromosome walking from closely associated markers on dense linkage maps. The study and utilization of these genes from model plant systems is likely to influence the genetic improvement of a large range of crops. Parthenocarpy is as important for *Musa* as apomixes may be for field crops. An SSRLP marker for parthenocarpy has already been identified in *Musa*. It is proposed that dense linkage mapping of this genomic region could be carried out using AFLP analysis followed by map based cloning. A similar strategy could also be followed for the map-based cloning of genes contributing to dwarfism and albinism in plantains and bananas. The cloning of these genes would be of great value to both fundamental and applied researchers of many crops.

Conclusion

Plant biotechnology has the potential to play a key role in the sustainable production of banana. Currently, no genetically transformed bananas are commercially available; however there is enormous potential for genetic manipulation of banana species for disease and pest resistance using the existing transformation systems. The use of appropriate 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 world.

Banana crop faces numerous environmental challenges, particularly with fungal and bacterial pathogens as well as pests and abiotic stresses. The problem is aggravated by the limited diversity of cultivars. Conventional breeding methods have limited success in banana crop improvement due to low female fertility, sterility, ploidy levels and poor seed set; besides the process is time consuming.

For many banana diseases like *Fusarium wilt*, BBTV, CMV and moko disease and no chemical treatment exists and destruction of the affected plants is the only method of control. For the others, the cost of chemical treatment is increasing as more virulent strains appear, or

even prohibitive for small farmers in developing countries or else damaging to the environment. These problems point to the necessity of developing alternate strategies for banana improvement. Biotechnological approaches such as tissue culture and genetic transformation has the potential to overcome this important disease. Genetically modified bananas would benefit both large commercial and small-scale farmers. Producers of export bananas spend millions of dollars annually to control phytopathogenic fungi and nematodes with chemicals. These chemicals are not only expensive, but are harmful to the environment and can lead to selection of resistance in pests and pathogens. Small-scale farmers are further unable to afford fungicides, nematicides and insecticides to control pests and diseases in the poorer countries of the world. Bananas that are disease and pest resistant, therefore, would provide them with a source of planting material that is affordable and safe to use.

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