

**9th ICABR International Conference on  
Agricultural Biotechnology: Ten years later**

Ravello (Italy), July 6 to July 10, 2005

**BIOTECHNOLOGY FOR IMPROVEMENT OF BANANA  
PRODUCTION IN AFRICA**

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**Abstract**

Bananas (*Musa* sp.) are the developing world's fourth most important global food crop after rice, wheat and maize in terms of gross value of production. Many pests and diseases have significantly affected banana 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 and weevils cause significant crop losses worldwide. Bananas 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. Development of disease-resistant banana by conventional breeding remains a difficult endeavor because of the long generation times, various levels of ploidy, sterility of most edible cultivars, and limited genetic variability. Genetic engineering offers an alternative method for crop enhancement.

Relative success in genetic engineering of bananas and plantains has been achieved recently, enabling the transfer of foreign genes into the plant cells. Genetic transformation using microprojectile bombardment of embryogenic cell suspensions is now routine. The protocol has also been developed for *Agrobacterium*-mediated transformation of embryogenic cell suspensions and apical shoot meristem of various cultivars of banana. The transgenic approach shows potential for the genetic improvement of the crop using a wide set of transgenes currently available which may confer resistance pests and diseases. The use of appropriate constructs may allow the production of nematode, fungus, bacterial and virus-resistant plants.

**Key Words:** *Musa*, genetic engineering, crop improvement, Africa

## **Introduction**

Bananas and plantains (*Musa* sp.) are a major staple food, supplying upto 25% of the carbohydrates for approximately 70 million people in Africa's humid forest and mid-altitude region (IITA, 1998). World banana production is currently about 97 million tonnes annually (FAOSTAT, 2003), of which bananas cultivated for the export trade accounts for only 10%. Hence, bananas and plantains are important for food security in the humid tropics and provide income to the farmers. Many pests and diseases have significantly affected banana *Musa* cultivation. Black Sigatoka (*Mycosphaerella fijiensis*), wilt (*Fusarium oxysporum* f. sp.

*cubense*), bacterial wilts (*Xanthomonas campestris* pv. *Musacearum*), viruses (*Banana bunchy-top virus*, *Banana streak virus*) and nematodes cause significant crop losses worldwide (Carlier et al., 2000; de Waele, 2000; Ploetz and Pegg, 2000; Thwaites et al., 2000, Tushemereirwe et al., 2002). Development of disease-resistant banana by conventional breeding remains a difficult endeavor because of the long generation times, various levels of ploidy, sterility of most edible cultivars, and limited genetic variability. Genetic engineering may offer an alternative method for crop enhancement. This paper reviews the strategies to address the major constraints for banana & plantain production through genetic transformation.

## **POTENTIAL STRATEGIES FOR DEVELOPING DISEASE RESISTANT BANANA**

### **VARIETIES**

#### **Resistance to Fungal Diseases**

Fusarium wilt caused by *Fusarium oxysporum* f. sp. *cubense*, has been reported to infect highland bananas in Uganda, but symptoms are atypical and severity is not well known. Black sigatoka caused by the fungus *Mycosphaerella fijiensis*, is the most devastating disease of *Musa* in Africa. 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, such as the removal of affected leaves, and adequate spacing of plants and efficient drainage within plantation and these are very expensive practices.

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 (Lorito et al., 1998), genes encoding

elicitors of defense response (Keller et al., 1999) and antimicrobial peptides (Cary et al., 2000; Li et al., 2001). 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; Zasloff, 1987), cecropins from the giant silk moth (Boman and Hultmark, 1987), mammalian (Ganz and Lehrer, 1994) and plant defensins (Broekaert et al., 1995). The cecropin (Alan and Earle, 2002; De Lucca et al., 1997) and its derivatives (D4E1: Cary et al., 2000; Rajasekaran et al., 2001) as well as its hybrids peptides with melittin (Osusky et al., 2000) 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 (Osusky et al., 2000).

Similarly, magainin is effective against the plant pathogenic fungi (Kristyanne et al., 1997; Zasloff, 1987). Li et al. (2001) 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 phytopathogenic bacteria and fungi (De Gray et al., 2001). Recently, Chakrabarti et al. (2003) 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 (Broekaert et al., 1997; Yun et al., 1997). The AMPs of plant origin may be the potent candidates for fungal resistance in banana as they have high in vitro activity to *Mycosphaerella 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 (Remy, 2000).

### **Resistance to Bacterial Diseases**

The livelihoods of millions of Ugandan farmers have been threatened by the current outbreak of bacterial wilt disease produced by infection with *Xanthomonas campestris* pv. *musacearum* (Xcm, Tushemereirwe *et al.*, 2002). Xcm infection can result in heavy banana crop production losses and affect banana productivity by not only causing wilting and death of young banana propagules, but also by severe crop yield reductions in mother crop and subsequent ratoon plant production cycles. The symptoms are yellowing and wilting of leaves, finally all leaves wither and the plant rots. The fruit ripens unevenly and prematurely with sections showing unique yellowish blotches in the flesh fingers and dark brown placental scars. Xcm wilt was initially identified in the major banana-producing districts of Mukono and Kayunga in 2001, and as of early 2005, has subsequently spread throughout at least of the major banana producing district in Uganda, and appears to be manifesting itself as a disease threat of potential epiphytotic proportions.

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

(Jaynes *et al.* 1987), plants (Broekaert *et al.* 1997), animals (Vunnam *et al.* 1997), and humans (Mitra and Zhang 1994, Nakajima *et al.* 1997) are now a potential source of plant resistance.

Pathosystem-specific plant resistance (R) genes have been cloned from several plant species (Bent, 1996). 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 (Rommens *et al.* 1995).

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 (Martin *et al.*, 1993; 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).

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* (Tai *et al.*, 1999). Transgenic tomato plants expressing the pepper *Bs2* gene suppress the growth of *Xcv*. The *Bs2* gene is a member of the nucleotide binding site–leucine-rich 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* (Xoo, Song *et al.*, 1995; Wang *et al.*, 1996). 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 *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. Bioassays showed that some *Xoo* races were not affected by the *Xa 21* 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 (Yoshimura *et al.*, 1998). *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.

Plants 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 (Dangl *et al.*, 1996). The HR cell death forms a physical barrier to prevent further pathogen infection. In addition, a local HR is often associated with activation of plant defense responses in the surrounding and even distal uninfected parts of the plants leading to the development of systemic acquired resistance (SAR) (Xie and Chen, 2000).

Hypersensitive response-assisting protein (HRAP) is a novel plant protein that can intensify the harpinPSS-mediated hypersensitive response (HR) in harpinPSS-insensitive plants (Chen *et al.*, 2000). Recently, a ferredoxin-like amphipathic protein (*pflp*, formerly called AP1) was isolated from the sweet pepper, *Capsicum annuum* (Lin *et al.*, 1997). *pflp* has been shown to delay the

hypersensitive response induced by *Pseudomonas syringae* pv. *syringae* in non-host plants through the release of the proteinaceous elicitor, harpinPss. The plants carrying the *pflp* gene showed enhanced resistance to *Xanthomonas oryzae* pv. *oryzae* (Xoo) race 6 at various levels (Tang *et al.*, 2001). This suggests the *pflp* gene could be a useful candidate for genetic engineering strategies in rice to provide bacterial blight resistance. *pflp* has also been shown to enhance resistance in transgenic orchids against *E. carotovora*, causing soft rot disease (Liau *et al.*, 2003).

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*, *Pseudomonas*, *Ralstonia* and *Xanthomonas spp* through transgenic technology (Feng, TY, per comm.). Elicitor-induced resistance is not specific against particular pathogens (Wei and Beer, 1996).

The other strategy for developing bacterial disease resistant varieties is the use of antimicrobial peptides (AMPs) isolated from frogs, insects, and mammalian phagocytic vacuoles (Tossi *et al.*, 2000). 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 *Xanthomonas campestris* whereas they exert no toxicity at bactericidal concentration to cultured cells or protoplasts of several plant species (Kaduno-Okuda *et al.*, 1995; Nordeen *et al.*, 1992; Rajasekaran *et al.*, 2001). 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 (Huang *et al.*, 1997).

Attacins are another group of antibacterial proteins produced by *Hyalophora cecropia* pupae (Hultmark *et al.*, 1983). Attacin expressed in transgenic potato enhanced its resistance to bacterial infection by *E. carotovora* subsp. *atropetica* (Arce *et al.*, 1999). Transgenic pear and apple expressing attacin genes have significantly enhanced resistance to *E. amylovora* in *in vitro* and greenhouse (Norelli *et al.*, 1994; Reynoird *et al.*, 1999; Ko *et al.*, 2000). In field tests, reduction of fire blight disease has been observed in transgenic apples expressing attacin genes (Norelli *et al.*, 1999). Transgenic apple expressing attacin targeted to the intercellular space, where *E. amylovora* multiplies before infection, has significantly reduced fire blight, even in apple plants with low attacin production levels (Ko *et al.*, 2000).

Another source of antibacterial proteins has been lysozyme, either from bacteriophage, hen eggs or bovine. The lysozyme genes have been used to confer disease resistance against plant pathogenic bacteria in transgenic tobacco plants (Trudel *et al.*, 1995). T4L, from T4-bacteriophage, also has been reported to enhance resistance in transgenic potato against *E. carotovora*, which causes bacterial soft rot (Düring *et al.*, 1993). Transgenic apple plants with the T4L gene showed significant resistance to fire blight infection (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 disease (Nakajima *et al.*, 1997). There are evidences of efficacy of bovine lysozyme isozyme c2 (BVLZ) enzyme against a variety of *Xanthomonas campestris* strains, as a transgene, in both monocot and dicot crops such as tomato, tobacco, rice and potato (Mirkov and Fitzmaurice, 1995).

## 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. Where as, *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 (Geering et al., 2001; Harper et al., 1999; Ndowora et al., 1999). 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. (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 *Banana streak virus* (BSV) from banana plants.

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. Researchers at International Institute of Tropical Agriculture (IITA), Nigeria in collaboration with John Innes Centre (JIC), UK, are attempting to generate transgenic resistant to BSV based on the novel approach of gene silencing. This approach involves the gene silencing of transgenic sequences for virus resistance in transgenic plants by preprogramming plant cells to specifically degrade viral sequences that are homologous to the expressed transgene.

## Resistance to Nematodes

Nematodes are recognized as severe production constraints to bananas and plantains (Gowen and Queneherve, 1990), with losses due to nematodes estimated at about 20% worldwide (Sasser and Freckman, 1987). Locally however, losses of 40% or greater can frequently occur, particularly in areas prone to tropical storms. Nematode management in bananas and plantains is mainly based on crop rotation and chemical control (Gowen and Queneherve, 1990). However, in continuously grown banana and plantain situations crop rotation is not practiced. The use of chemical nematicides is often not practical to subsistence farmers or is environmentally unacceptable. There are evidences 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.* is more dangerous than *R. similis*. 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 defence strategies (Ryan, 1990). The transgenic delivery of PIs can affect the sexual fate and growth of the cyst and root knot nematodes (Urwin et al.,1997). 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 (1996) 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 (Atkinson, 1996). There is no evidence that expression of cystatins impairs plant growth or yield in the trial (Urwin et al., 2001).

The other strategies for nematode resistance include the use of natural resistance genes (R-genes), 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* (Cai et al., 1997). The Mi-1.2 gene of tomato confers resistance against *Meloidogyne* species (Milligan et al., 1998). 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 (Burrows et al., 1998). 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 (Marroquin et al., 2000). The approach using cry genes has potential for plant nematode control (Wei et al., 2003) .

### **Genetic Transformation of Banana**

Genetic transformation has become an important tool for crop improvement. Relative success in genetic engineering of bananas and plantains 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 (Becker et al., 2000; Cote et al, 1997; Sagi et al., 1995). However, *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 (Gheysen et al., 1998; Hansen and Wright, 1999; Shibata and Liu, 2000).

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 protocol has been developed for *Agrobacterium* mediated transformation of embryogenic cell suspensions of the banana cultivars Rasthali (Ganapathi et al., 2001). At present most of the transformation protocol use cell suspension, however establishing cell suspension is lengthy process and cultivars dependent. The protocol has also been established using shoot tips from various cultivars of banana (May et al., 1995; Tripathi et al., 2002). This technique is applicable to a wide range of banana cultivars irrespective of ploidy or genotype (Tripathi et al., 2002, 2003). 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 (ECS) as it allows for rapid transformation

## **Conclusions**

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 Africa.

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