

## Review

# Strategies for resistance to bacterial wilt disease of bananas through genetic engineering

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The livelihoods of millions of Ugandan farmers have been threatened by current outbreak of a banana bacterial wilt disease caused by *Xanthomonas campestris* pv. *musacearum*, which is very destructive and rapidly spreading in Uganda. Bananas are the highest value staple food and source of income for millions of people in this region. Economic impact of the disease is clear as a result of widespread destruction of banana, pre-harvest rotting of fruits, and a lack of farmers' ability to grow bananas in disease endemic areas. The disease attacks all varieties of banana, including East African Highland Bananas (EAHBs). No banana germplasm with bacterial wilt resistance has been identified. The transgenic approach shows potential for the genetic improvement of the crop using a wide set of transgenes currently available which may confer bacterial resistance. This article discusses the potential strategies to develop transgenic banana plants resistant to bacterial wilt disease.

**Key words:** Banana, bacterial wilt, genetic transformation, disease resistant.

## INTRODUCTION

Banana (*Musa* sp.) is the fourth most important global food crop after rice, wheat and maize in terms of gross value of production. Total world *Musa* production is currently about 97 million tones annually (FAOSTAT, 2003), of which bananas cultivated for the export trade accounts for only 10%. Hence, they are important for food security in the humid tropics and provide income to the farmers. It is a major staple food, supplying up to 25% of the carbohydrates for approximately 70 million people in Africa's humid forest and mid-altitude regions. The East and Central Africa sub-region alone produces nearly 20 million tons of bananas annually.

The livelihoods of millions of Ugandan farmers have been threatened by the current outbreak of bacterial wilt disease caused by the bacterium *Xanthomonas campestris*

pv. *musacearum* (Xcm, Tushemereirwe et al., 2002). Economic impact of the disease appears to be manifested as result of a lack of farmer's ability to propagate and grow out new planting sites of infection free banana, and also reduces yield of banana due to smaller bunch weights and fruit filling. The disease, which has been identified as a bacterial wilt was first reported in Ethiopia, where it caused only minor problems since banana production is small-scale and scattered. Xcm wilt was initially identified in the major banana-producing districts of Mukono and Kayunga in 2001, and as of early 2003, 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. Unlike in Ethiopia, in Uganda and other parts of eastern Africa, the spread of the disease is likely to be more rapid and difficult to control. Bananas serve as the highest value staple food and source of income for millions of inhabitants of the highlands and mid-altitude regions of Burundi, Kenya, Malawi, Rwanda, Tanzania, and Uganda.

X cm infection can result in severe losses in banana production and affects banana productivity to early ripening

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and rotting of fruits even in the absence of apparent external signs of the disease, and wilting and death of banana plants. Ratoon crops arising from infected mats are severely diseased and often wilt before producing bunches or produce bunches with rotted fruits. Xcm has spread from eastern districts of Uganda to northern and central districts where several farmers have abandoned banana cultivation. If unchecked, the disease would cause massive losses in the Uganda's western districts, an area of intensive banana cultivation, and to neighboring countries. The disease attacks all varieties of banana, including East African Highland Bananas (EAHBs).

The first symptoms include discoloration at the tip of the flower and withering of the flower bracts. Other symptoms include yellowing, wilting and premature ripening in young plants. When the banana is cut, a pink-purple coloration confirms presence of the disease. Even in some cases where these other symptoms fail to show, the coloration is always seen. The plant dies within a month from the first appearance of any of the symptoms.

Given the apparently rapidity of pathogen spread in Eastern Africa, and concomitant increase in inoculum load in regional locations, the threat of an epiphytotic requires some application of stopgap measures. Use of genetic transformation technologies for *Musa*, may provide a timely and cost-effective measure to address the dangers of the spread of this disease. Even if resistant germplasm sources are identified, which to date have not; a breeding cycle for banana germplasm development may be expected to require 6-20 years utilizing conventional breeding methodologies. The development of bacterial wilt resistance plants through conventional breeding suffers from the problems of long generation times, various levels of ploidy, sterility of most edible cultivars, and limited genetic variability.

More recently, transgenic plants have been produced that are resistant to a wide variety of bacterial diseases. These forms of resistance follow a number of chemical strategies, including the use of genes for bacterial toxin tolerance, antimicrobial peptides, and other defense-related proteins that tend to act as bactericidal compounds. This paper reviews the various potential strategies for developing bacterial wilt resistant banana plants through genetic engineering.

## STRATEGIES FOR DEVELOPING BACTERIAL DISEASE RESISTANT PLANTS

One approach to control bacterial disease is to improve a plants' defense against a particular pathogen. This has been made possible by genetic engineering by using genes found in fungi, insects, animals and other plants. Antimicrobial proteins, peptides, and lysozymes 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.

### Expression of antimicrobial proteins

Antimicrobial peptides (AMPs) with  $\alpha$ -helical structures are ubiquitous and found in many organisms. AMPs have been isolated from frogs, insects, and mammalian phagocytic vacuoles (Biggins and Sansom, 1999; Tossi et al., 2000). AMPs are selective for prokaryotic membranes over eukaryotic membrane due to the predominantly negatively charged phospholipids in the outer leaflet of the prokaryotic membrane (Biggin and Sansom, 1999; Tossi et al., 2000). Such preference is considered a regulatory function in target selectivity.

### Magainins

Magainin is a defense peptide secreted from the skin of the African clawed frog (*Xenopus laevis*), first discovered by Zasloff (1987). Magainins and their analogs have been studied as a broad-spectrum topical agent, a systemic antibiotic, a wound-healing stimulant, and an anticancer agent (Jacob and Zasloff, 1994). However, only magainin analogs (MSI-99 and Myp30) have recently been transferred into plants for use against bacteria. Li et al. (2001) have reported disease resistance, to both a fungal and a bacterial pathogen, conferred by expression of a magainin analog, Myp30, in transgenic tobacco (*Nicotiana tabacum* var. Petit Havana). Another analog MSI-99, when expressed in tobacco via chloroplast transformation conferred both *in vitro* and *in planta* resistance to plant pathogenic bacteria and fungi (De Gray et al., 2001).

### Cecropins

Cecropins are antibacterial lytic peptides native to the hemolymph of *Hyalophora cecropia*, the giant silk moth. These peptides interact with the outer phospholipid membranes of both Gram-negative and Gram-positive bacteria and modify them by forming a large number of transient ion channels (Durell et al., 1992). 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 are considered as potential candidates to protect plants against bacterial pathogens. Transgenic tobacco plants expressing cecropins have

increased resistance to *P. syringae* pv. *tabaci*, the cause of tobacco wildfire (Huang et al., 1997). Synthetic lytic peptide analogs, Shiva-1 and SB-37, produced from transgenes in potato plants reduce bacterial infection caused by *Erwinia carotovora* subsp. *atroseptica* in transgenic potato plants (Arce et al., 1999). Transgenic apple expressing the SB-37 lytic peptide analog showed increased resistance to *E. amylovora*, pathogen for fire blight, in field tests (Norelli et al., 1998). More recently, the expression of the D4E1 in poplar has resulted resistance to *Agrobacterium tumefaciens* and *Xanthomonas populi* (Mentag et al., 2003).

### Attacins

Attacins are another group of antibacterial proteins produced by *Hyalophora cecropia* pupae (Hultmark et al., 1983). The mechanisms of antibacterial activity of this protein are to inhibit the synthesis of the outer membrane protein in gram negative bacteria (Carlsson et al., 1998). Attacin expressed in transgenic potato enhanced its resistance to bacterial infection by *E. carotovora* subsp. *atroseptica* (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; Reynold 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).

### Lysozymes

Lysozymes are a ubiquitous family of enzymes that occur in many tissues and secretions of humans, animals, as well as in plants, bacteria and phage. The lysozyme attacks the murein layer of bacterial peptidoglycan resulting in cell wall weakening and eventually leading to lysis of both Gram-negative and Gram-positive bacteria. Hen egg-white lysozyme (HEWL), T4 lysozyme (T4L), T7 lysozyme (Huang et al., 1994), human and bovine lysozyme genes have been cloned and transferred to enhance plant bacterial or fungal resistance. The lysozyme genes have been used to confer resistance against plant pathogenic bacteria in transgenic tobacco plants (Trudel et al., 1995). T4L, from the T4-bacteriophage, also has been reported to enhance resistance of 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 is evidence of efficacy of bovine lysozyme isozyme c2 (BVLZ) transgene against a variety of *Xanthomonas campestris* strains in both monocotyledon and dicotyledon crops including tomato, tobacco, rice and potato (Mirkov and Fitzmaurice, 1995). Since this bactericidal transgene has been shown to function in monocot and has clear efficacy against at least several strains of *X. campestris*, its usefulness as a transgene for resistance to *X. campestris* in *Musa* has a high probability of success.

### Thionins

Thionins are plant antimicrobial proteins which are able to inhibit a broad range of pathogenic bacteria *in vitro* (Molina et al., 1993). Carmona et al. (1993) reported the expression of alpha-thionin gene from barley in transgenic tobacco confers enhanced resistance to two pathovars of *P. syringae*. Unfortunately, most thionins can be toxic to animal and plant cells and thus may not be ideal for developing transgenic plants (Reimann-Philipp et al., 1989).

### Expression of plant defense genes

Plants have their own networks of defense against plant pathogens that include a vast array of proteins and other organic molecules produced prior to infection or during pathogen attack. Recombinant DNA technology allows the enhancement of inherent plant responses against a pathogen by either using single dominant resistance genes not normally present in the susceptible plant (Keen, 1999) or by choosing plant genes that intensify or trigger the expressions of existing defense mechanisms (Bent and Yu, 1999; Rommens and Kishore, 2000).

### Plant resistance (R) genes

Pathosystem-specific plant resistance (*R*) genes have been cloned from several plant species (Bent, 1996). These include *R* genes that mediate resistance to bacterial, fungal, viral, and nematode pathogens. Many of these *R* gene products share structural motifs, which indicate that disease resistance to diverse pathogens may operate through similar pathways. The *Bs2* resistance gene of pepper specifically recognizes and confers resistance to strains of *X. 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. The *Xa1* gene in rice confers resistance to Japanese race 1 of *X. 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. The rice *Xa21* gene confers resistance to *X. oryzae* pv. *oryzae* race 6 (Song et al., 1995). Fifty transgenic rice plants carrying the cloned *Xa21* gene display high levels of resistance to the pathogen. The sequence of the predicted protein carries both a leucine-rich repeat motif and a serine-threonine kinase-like domain.

The *Pto* gene is another class of R genes, encoding a serine/threonine protein kinase that confers resistance in tomato to *P. syringae* pv *tomato* strains that express the type III effector protein AvrPto. (Martin et al., 1993; Kim et al., 2002). Overexpression of *Pto* in tomato under control of the cauliflower mosaic virus (CaMV) 35S promoter has been shown to activate defense responses in the absence of pathogen inoculation. *Pto*-overexpressing plants show resistance not only to *P. syringae* pv *tomato* but also to *X. campestris* pv *vesicatoria* and to the fungal pathogen *Cladosporium fulvum* (Mysore et al. 2003). Therefore, *Pto* genes are considered as potential candidates to protect plants against pathogens.

## CURRENT STATUS OF MUSA TRANSFORMATION

Genetic transformation using microprojectile bombardment of embryogenic cell suspension is now routine (Becker et al., 2000; Sagi et al., 1995). An efficient method for direct gene transfer via particle bombardment of embryogenic cell suspension has been reported in cooking banana cultivar Bluggoe and plantain Three Hand Plant (Sagi et al., 1995). While Becker et al. (2000) reported the genetic transformation of Cavendish banana cv. Grand Nain.

The recovery of transgenic plants of banana obtained by means of *Agrobacterium tumefaciens* mediated transformation has been reported. 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 *Musa* (May et al., 1995; Tripathi et al., 2002). This technique is applicable to a wide range of *Musa* cultivars irrespective of ploidy or genotype (Tripathi et al., 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 of *Musa* species.

Till date there is no report on the genetic transformation of East African Highland Bananas (EAHBs). Therefore, researchers of International Institute of Tropical Agriculture (IITA), Uganda in collaboration with National Agriculture Research Organisation (NARO), Uganda are attempting to establish the genetic transformation of EAHBs using the shoot tips, at Kawanda Agriculture Research Institute (KARI).

## CONCLUSIONS

The range of potential strategies for genetically engineered resistance in crops has expanded dramatically during the past few years. The transgenic banana plants resistant for bacterial wilt disease can be developed by using genes for antimicrobial peptides, and other plant defense-related proteins that tend to act as bactericidal compounds.

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