Transgenic Technologies for Bacterial Wilt Resistance

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Abstract

Banana production is severely affected by bacterial diseases jeopardizing the food security of millions of inhabitants in countries where farmers depend upon banana as staple food. Bacterial diseases like *Xanthomonas* wilt, Moko, blood, and Bugtok are the most important diseases threatening banana cultivation in several tropical and subtropical countries. Genetic improvement of banana through classical breeding is difficult due to the lack of resistant germplasm, sterile nature, and long generation time. Transgenic technology can complement classical breeding for developing bacterial disease-resistant varieties. Some success has been achieved for developing host plant resistance in order to control banana *Xanthomonas* wilt (BXW) disease. Currently, the transgenic bananas expressing either sweet pepper *Pflp* or *Hrap* gene are under evaluation for resistance to *Xanthomonas* wilt disease in field trials in Uganda. Management of bacterial diseases through cultural practices like removal of male buds and use of pathogen-free seed material and disinfected cutting tools can contain outbreak of diseases although these are not absolute solutions for control of bacterial diseases. In this chapter, we have discussed various management practices as well as existing transgenic technologies to control bacterial diseases of banana.

Keywords

Banana • *Xanthomonas campestris* pv. *musacearum* • Transgenic technology

13.1 Introduction

Bacterial diseases are production constraint for many crops throughout the world. The outbreaks of some of the bacterial diseases have shattered

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economies of several countries. The bacterial diseases may be systemic causing death of the entire plants, or it may be vascular causing yellowing and wilts or locally causing rot, spots, blights, and cankers or may be of a mixed nature. Bacterial diseases of bananas (Moko, blood, Bugtok, and *Xanthomonas* wilt) are very destructive, and they have devastated the banana production in various tropical and subtropical countries. Moko, Bugtok, and blood, caused by *Ralstonia solanacearum* species complex, are among the major biotic constraints for production of dessert and cooking bananas (Sequeira 1998). Moko and Bugtok diseases are caused by *Ralstonia solanacearum* phylotype II, whereas blood disease is caused by *Ralstonia solanacearum* phylotype IV (Fegan and Prior 2004). Moko is an important disease devastating banana and plantain production in Latin America, Caribbean, and the Philippines (Thwaites et al. 2000). However, Bugtok is an endemic disease affecting only cooking bananas in the Philippines (Thwaites et al. 2000). Blood disease has devastated banana production in Indonesian archipelago and there is risk of its spread to Australia and Papua New Guinea. Moko and blood diseases are systemic and their symptoms are similar: wilting of leaves, rotting of fruits, and vascular discoloration. However, symptoms of Bugtok disease differ from Moko and blood disease in that the symptoms are limited to the male buds and flowers of bananas; there is no wilting and vascular discoloration generally does not extend beyond peduncle (Thwaites et al. 2000). Banana *Xanthomonas* wilt (BXW) disease, caused by *Xanthomonas campestris* pv. *musacearum*, is only reported in Great Lakes region of East Africa (Tushemereirwe et al. 2004).

Generally, the plant diseases are managed through cultural practices, host plant resistance, chemicals, and regulations. The management practice varies from one disease to another depending upon the pathogen, the host, and the environmental factors. Once established, the bacterial diseases are difficult to control. Chemical control of bacterial disease is impossible, although several antibiotics have been tried. Antibiotics are not recommended for control of plant diseases because of the possibility of evolution of antibiotic-resistant strains of bacterial pathogens. Cultural practices including sanitation, crop rotation, host eradication, and improvement of crop environment can reduce pathogen levels or reduce the rate of disease development. The bacterial diseases can be managed cost-effectively through host plant resistance. Commercial growers and farmers favor resistant varieties if they are available. There is limited success in developing varieties resistant to bacterial diseases through conventional breeding due to lack of germplasm exhibiting resistance against most of the bacterial pathogens and also because of the appearance of new pathogen strains. Biotechnology has unraveled several new options to control plant bacterial diseases. Transgenic technologies to develop disease-resistant plants through improving host's regulatory mechanism or defense mechanism or expressing antimicrobial proteins are reported for several crops. This chapter reviews the attempts made for exploiting transgenic technologies for effective management of bacterial diseases and describes the progress on control measures of BXW disease.

13.2 Banana *Xanthomonas* Wilt (BXW) Disease

Among all the bacterial diseases damaging banana cultivation worldwide, BXW is considered as the major menace to banana production in Great Lakes region of East and Central Africa (Tripathi et al. 2009). The disease was first reported on *Ensete* (Yirgou and Bradbury 1968) and later on banana in Ethiopia (Yirgou and Bradbury 1974). Outside Ethiopia, BXW was first noticed in Uganda in 2001 (Tushemereirwe et al. 2004) and then in the Democratic Republic of the Congo (DR Congo) (Ndungo et al. 2006), Rwanda (Reeder et al. 2007), Kenya, Tanzania, and Burundi (Carter et al. 2009). The disease affects production of all types of banana including plantain, cooking, dessert, and juice-producing bananas (Ssekiwoko et al. 2006). BXW spreads very fast resulting in severe loses, unlike those of other diseases which gradually increase losses over years. The impact of BXW is due to absolute yield losses and death of the mother plant (Tripathi et al. 2009). The economic losses due to this disease were estimated at US$ 2–8 billion over a decade due to increase in price and production losses (Abele and Pillay 2007). The BXW disease causes yield losses through complete wilting of banana plant and rotting of fruits. Therefore, BXW disease jeopardizes the livelihood of millions of farmers growing bananas for food and cash.

13.2.1 Causal Agent

The causative organism of BXW disease is rod-shaped, gram-negative bacterium, named as *Xanthomonas campestris* (Yirgou et al. 1968, 1974). It has subsequently been retitled as *X. campestris* pv. *musacearum* (*Xcm*) (Young et al. 1991). The bacterium belongs to *Xanthomonadaceae* and produces yellow, circular, mucoid, slimy colonies (Tripathi et al. 2007). Previously, *X. campestris* pv. *musacearum* was considered to be a highly monomorphic pathogen with no genetic differences (Aritua et al. 2007). However, recent study on genome-wide sequencing reports the presence of two genetically distinct sub-lineages of the pathogen, one group including *X. campestris* pv. *musacearum* isolates from Burundi, Kenya, Tanzania, and Uganda and second group of isolates from DR Congo, Ethiopia, and Rwanda (Wasukira et al. 2012). This study indicates more than one introduction of BXW disease on banana in East Africa (Wasukira et al. 2012).

13.2.2 Symptoms of BXW

The main symptoms of BXW disease are yellowing and wilting of leaves, immature and uneven ripening, and rotting of fruits (Fig. 13.1a, b) with yellowish brown blotches and dark brown placental scars in the pulp (Tushemereirwe et al. 2004). Infection via insect transmission shows symptoms on different parts of flowers including shrinking and rotting of the male buds, wilting of bracts, and browning of peduncle first followed with further wilting of leaves occurring as the infection spreads to the complete plant. Cross sections of the pseudostem and rachis of infected plants show yellowish bacterial ooze. Ultimately, infected plants wilt completely and rot. Appearance of symptoms is very fast under favorable conditions and visible within 3–4 weeks in fields and 2–3 weeks after artificial inoculation of *X. campestris* pv. *musacearum* in glasshouse (Tripathi et al. 2008). Symptom development differs with cultivars, growth phase of plant, and mode of disease transmission (Tripathi et al. 2009).

13.2.3 Disease Transmission

The *X. campestris* pv. *musacearum* infects banana plant mainly through two ways. Farming tools (pangas, hoes, knives) and browsing animals that inflict damage to various parts of the plants (leaves, pseudostem, male bud, corm roots). When these tools inflict a wound to a diseased plant, they pick the bacteria and transmit them when they get to a clean plant. It is established that the bacteria survive for several days on the farming tools, therefore very effective in spreading the bacteria from infected plants to clean plants. In areas where there is excessive use of tools, this is a major means of disease transmission (e.g., Southeastern Uganda). In this case, the main agents spreading the disease are the farmers and the traders, but unfortunately they are unaware of this fact. The second main means of transmission is by insects that visit banana male buds to feed on sap that oozes out when the banana flowers fall off. As the insects feed, they mechanically bring in bacteria from the infected plants visited earlier. The insects and other agents like sapsucking birds and bats are able to spread the disease from field to field. This is the main means of disease transmission in all parts of Uganda where farmers do not intensively use farming tools in their plantations, e.g., in plantations of beer bananas (Pisang Awak commonly

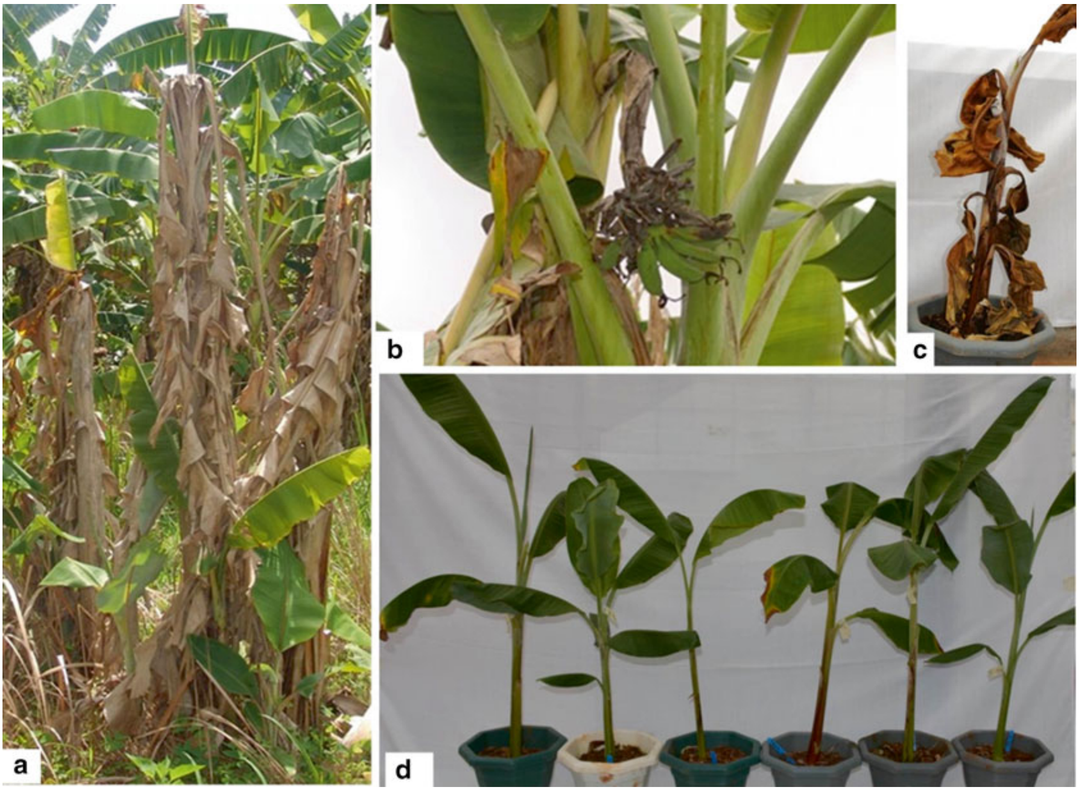

Fig. 13.1 (**a**) BXW-infected plants showing wilting of leaves; (**b**) diseased plants showing rotting of fruits; (**c**) completely wilted control non-transgenic plant in glasshouse; (**d**) transgenic plants showing no BXW symptom after artificial inoculation with pathogen under glasshouse conditions. Pictures (**c** and **d**) were taken 8 weeks post artificial inoculation

known as Kayinja) in Central and Western Uganda.

The planting material from infected field can also spread disease. Sometimes, the infected plants do not show symptoms during incubation period, which can be up to 3 or more months.

13.3 Management of BXW Disease

The bacterial wilt diseases are systemic, and once pathogens have established in banana plantation, their control becomes very difficult (Eden-Green 2004). The infected banana plants should be removed immediately in order to limit spread of the disease. At present there are no chemicals, biocontrol tools, or resistant cultivars available to control BXW (Tripathi et al. 2009). Therefore, the disease management should involve combination of approaches like exclusion, eradication, cultivation of disease-tolerant banana varieties, and protection. Once BXW disease appears in a plantation, there is no option other than removal of all diseased plants and leaving the field unplanted or to follow crop rotation. Researchers at NARL, Kawanda, established that the bacteria survive no more than 45 days in soil rotting banana residues (Mwebaze et al. 2006). Conditions favoring rotting of plants also speed up natural destruction of the bacteria. There are only two known alternate hosts, *Canna* sp. and *Zebrina* sp., which are relatives of the banana plants (belong to the same plant family), of *X. campestris* pv. *musacearum* pathogen.

Currently, control of BXW is centered on protecting uninfected plants once the disease gets to an area as there is no effective curative measure for affected plants. The control measures target and block the bacteria from getting transmitted to uninfected plants. The male bud of banana plants presents the extreme risk for *X. campestris* pv. *musacearum* infection. Therefore, early removal of the male bud is an effective means of preventing the spread of bacterial pathogen through insects (Tinzaara et al. 2006). The removal of male buds is recommended for both affected and unaffected banana plants for limiting spread of the disease. The male buds should be removed by twisting the peduncle to break it with a forked wooden stick, instead of using cutting tools. BXW can be contained in banana fields if debudding is applied effectively. However, removal of male buds has not been adopted well by farmers (Kagezi et al. 2006), particularly in areas where farmers believe that the quality of the banana fruits especially for juice cultivars is affected by removal of male buds (Bagamba et al. 2006).

Previously, rouging the complete diseased banana mat was recommended to farmers (Tushemereirwe et al. 2004). Recently, it was demonstrated that it is feasible to only remove plants showing disease symptoms from the affected banana mat and the remaining uninfected plants on the same mat can be saved from BXW infection. This is feasible as insect-transmitted BXW infection starts from the flowers of the banana plant and takes some time before reaching to the corm of the plant, which is connected to the other plants on the mat (Sekiwoko et al. 2006). The technique of single-stem removal has been confirmed to be effective in control of BXW disease in Kenya, Democratic Republic of the Congo, and Uganda (Kubiriba and Tushemereirwe 2014). A 6-month fallow/unplanted period is recommended to minimize the risk of reinfection of newly planted banana plants in previously diseased fields (Tripathi et al. 2009). The mechanical spread of *X. campestris* pv. *musacearum* can be limited by disinfecting the cutting tools used for pruning or removing of diseased plants. Suspending the use of cutting tools in infected banana fields is crucial for control of BXW (Tushemereirwe et al. 2006). The *X. campestris* pv. *musacearum* bacterium can survive up to 22 days on cutting tools stored at room temperature (Buregyeya 2010).

Effective control of BXW disease is only possible if the cultural practices are advocated along with institutional approaches that effectively mobilize stakeholders using the technologies (Kubiriba et al. 2012). Other stakeholders from government and nongovernment organizations involved in rural development also contributed to the planning, generation, and promotion of these technologies. It also reported that sometimes farmers could change recommended practices in order to suit their conditions (Bagamba et al. 2006). The cultural practices are proved to be effective for managing the BXW disease in those areas where they have been deployed carefully and consistently (Tushemereirwe et al. 2006).

The farmers' knowledge and their decision to control plant diseases depend upon suitability of improved technologies (Sherwood 1997). More than 80 % of the farmers growing bananas in Uganda understand BXW disease and its diagnosis, spread, and control, due to massive awareness campaigns (Muhangi et al. 2006). Even though most of the banana farmers know control of BXW, only 30 % of them applied the knowledge to manage BXW (Tushemereirwe et al. 2006). Therefore, participatory approaches were used, which has resulted in control of BXW disease to below 5 % for over 3 years in some of the major banana-growing areas in Uganda (Kubiriba et al. 2012). Majority of the farmers participating in farmer's field school managed the BXW disease in their banana plantations.

The most striking strategy for controlling BXW is to use disease-resistant plants. Yet, no banana cultivars are known showing high resistance against *X. campestris* pv. *musacearum*. A few banana cultivars may escape insect-transmitted infection as they have male flowers with persistent bracts that protect the inflorescence against insects contaminated with *X. campestris* pv. *musacearum*. These include East African highland cooking banana cultivars such as “Nakitembe” and “Mbwazirume”. The lack of natural resistance in banana against *X. campestris* pv. *musacearum*, along with the difficulties associated with conventional breeding of banana and labor demanding cultural practices, favors a transgenic technology to control BXW.

13.4 Transgenic Technologies for Resistance to Bacterial Diseases

Plants have well-developed defense mechanism to fight against pathogens. Transgenic technologies have potential to enhance host plant resistance against pathogens by either expressing resistance genes (Keen 1999) or overexpressing defense genes (Bent and Yu 1999; Rommens and Kishore 2000).

Resistance (R) genes have been identified and cloned from different plants for use against several pathogens (Bent 1996). These R-genes facilitate resistance against pathogens including bacteria, fungi, viruses, and pathogenic nematodes. Many R-gene products share structural motifs, which specify that resistance against various pathogens may be through similar molecular or chemical pathways. The R-gene *Pto* from tomato (*Lycopersicon esculentum* Miller) demonstrated resistance against several strains of *Pseudomonas syringae* pv. *tomato* (Martin et al. 1993; Kim et al. 2002). Transgenic tomato expressing *Pto* gene also showed resistance against bacterial pathogen *Xanthomonas campestris* pv. *vesicatoria* and the fungal pathogen *Cladosporium fulvum* (Mysore et al. 2003).

Several R-genes (*Bs1*, *Bs2*, and *Bs3*) conferring resistance in a “gene-for-gene” fashion have been cloned from pepper (Hibberd et al. 1987). Their corresponding avirulence genes (*avrBs1*, *avrBs2*, and *avrBs3*) have also been identified in *X. campestris* pv. *vesicatoria* and shown to be fundamental for resistance (Minsavage et al. 1990; Swanson et al. 1988). The pepper *Bs2* gene confers resistance to bacterial spot disease of tomato caused by *X. campestris* pv. *vesicatoria*, which has the corresponding bacterial avirulence gene, *avrBs2* (Tai et al. 1999). The *RPS4* gene from *Arabidopsis* provides resistance against *P. syringae* pv. *tomato* containing *avrRps4* (Gassmann et al. 1999).

Broad-spectrum durable disease resistance is very important for control of bacterial diseases. The maize *Rxo1* gene provides resistance against *Xanthomonas oryzae* pv. *oryzicola* causing bacterial streak disease in rice and *Burkholderia andropogonis*, which causes bacterial disease in the sorghum and the maize (Zhao et al. 2005). The *Arabidopsis RRS1* gene provides broad-spectrum resistance to various strains of *R. solanacearum*, which also cause Moko disease in banana (Deslandes et al. 1998). Similarly, overexpression of *Arabidopsis NPR1* gene provides resistance to bacterial pathogens (Cao et al. 1998). Overexpression of *Arabidopsis NPR1* or the rice *NH1* gene enhanced resistance to the rice bacterial blight pathogen *X. oryzae* pv. *oryzae* and the blast pathogen *Magnaporthe grisea* Herbert (Chern et al. 2005; Yuan et al. 2007; Quilis et al. 2008; Feng et al. 2011). The rice *Xa21* gene also conferred resistance against *X. oryzae* pv. *oryzae*, causing bacterial leaf blight disease (Song et al. 1995; Wang et al. 1996; Yoshimura et al. 1998).

Once infected, plants employ different defense-related approaches in order to survive from pathogen attack. These approaches include induction of pathogenesis-related (PR) genes, strengthening of cell walls, accumulation of phytoalexins, and induction of salicylic acid (SA) pathway. A hypersensitive response (HR) is the initial defense response, which limits spread of pathogens from the infection site. HR leads to systemic acquired resistance (SAR), which induces expression of many PR genes, resulting in resistance against various pathogens (Durrant and Dong 2004).

Plant disease resistance can also be achieved by inserting antimicrobial peptides (AMPs) (Cary et al. 2000; Li et al. 2001) and enhancing the plant defense (Keller et al. 1999). AMPs have a broad-spectrum antimicrobial activity against bacterial and fungal pathogens and are safe to plants and mammals. Several AMPs like lysozymes (Düring et al. 1993), magainins (Zasloff 1987), cecropins (Boman and Hultmark 1987), attacins (Hultmark et al. 1983), thionins (Molina et al. 1993), and defensins (Broekaert et al. 1995) are well documented. Lysozyme, which can be obtained from bacteriophages, hen eggs, or cows, is a hydrolytic enzyme and part of the immune system of animals. Lysozyme attacks the murein layer of bacterial peptidoglycan, which results to death of both gram-negative and gram-positive bacteria by weakening their cell wall. Expression of the lysozyme gene showed resistance against plant pathogenic bacteria in transgenic potato (Düring et al. 1993), tobacco (Trudel et al. 1995), and apple (Ko 1999).

The maginins are antimicrobial proteins secreted from the skin of the African clawed frog (*Xenopus laevis*) (Zasloff 1987). Transgenic tobacco expressing magainin analog MSI-99 or Myp-30 showed resistance against bacterial and fungal pathogens (Li et al. 2001; De Gray et al. 2001; Chakrabarti et al. 2003). The cecropins isolated from hemolymph of *Hyalophora cecropia* are antibacterial lytic peptides, which kill gram-negative and gram-positive bacteria by creating transient ion channels in the outer phospholipid membranes (Durell et al. 1992). Different types of cecropins including native (cecropin B), synthetic (Shiva-1, D4E1), and mutant (SB-37, MB39) showed antimicrobial activity against bacterial pathogens like *Erwinia carotovora*, *E. amylovora*, *P. syringae*, *R. solanacearum*, *and X. campestris* (Nordeen et al. 1992; Kaduno-Okuda et al. 1995; Rajasekaran et al. 2001). Cecropin expressing transgenic tobacco plants conferred resistance against *P. syringae* pv. *tabaci* causing wildfire disease (Huang et al. 1997). Transgenic potato expressing Shiva-1 and SB-37 showed resistance against *E. carotovora* (Arce et al. 1999). The expression of SB-37 in transgenic apple also showed increased resistance to fire blight disease caused by *E. amylovora* (Norelli et al. 1998). Poplar plants expressing D4E1 conferred resistance to *Agrobacterium tumefaciens* and *X. populi* (Mentag et al. 2003).

The attacins are antibacterial proteins produced by *Hyalophora cecropia* pupae (Hultmark et al. 1983). Attacin expressed by transgenic pear and apple demonstrated enhanced resistance to fire blight disease caused by *E. amylovora*, under glasshouse and field conditions (Norelli et al. 1999; Reynoird et al. 1999). Transgenic apple expressing attacin in the intercellular space has conferred resistance to fire blight disease (Ko et al. 2000). The thionins is another group of plant antimicrobial proteins, which inhibit bacterial pathogens (Molina et al. 1993). Transgenic tobacco expressing alpha-thionin gene from barley conferred resistance against *P. syringae* (Carmona et al. 1993). Defensins isolated from mollusks, acari, arachnids, insects, mammals, and plants are also source of antimicrobial proteins. Plant defensins from *B. oleracea* and *B. campestris* demonstrated resistance to bacterial leaf blight disease of transgenic rice (Kawata et al. 2003).

Resistance genes enhance the hypersensitive response (HR) in nonhost plants through the release of the elicitor from bacterial pathogens. The HR is characterized by robust and localized cell death at the point of pathogen infection forming a physical barrier, which prevents spread of pathogen (Mehdy 1994). In addition, HR also induces plant defense responses in the surrounding and even distal parts of the plants leading to systemic acquired resistance (SAR) (Xie and Chen 2000). Elicitor-induced resistance can provide broad-spectrum resistance. Therefore, transgenic technologies using defense genes may be more ideal and it might provide resistance for bacterial as well as fungal pathogen.

The ferredoxin-like amphipathic protein (PFLP) and hypersensitive response-assisting protein (HRAP) isolated from the sweet pepper (*Capsicum annuum* L.) are plant proteins, which intensify HR (Lin et al. 1997; Chen et al. 2000). The expression of *Pflp* gene in transgenic tobacco, tomato, orchids, calla lily, and rice has demonstrated resistance against various bacterial pathogens such as *Erwinia*, *Pseudomonas*, *Ralstonia*, and *Xanthomonas* spp. (Huang et al. 2004, 2007; Liau et al. 2003; Tang et al. 2001; Yip et al. 2007). The transgenic plants overexpressing *Pflp* gene showed disease resistance through hypersensitive response and enhanced production of active oxygen species (AOS) (Dayakar et al. 2003; Haung et al. 2004).

The *Hrap* gene can protect plants from bacterial pathogen infection and belongs to hypersensitive cell death (HCD)-associated genes (Chen et al. 2000). The HRAP protein dissociates the multimeric forms of harpin$_{PSS}$ into dimers and monomers triggering hypersensitive cell death (Chen et al. 2000). The HRAP protein has a signal peptide, which leads its secretion into extracellular space and its transcription is activated early during incompatible interaction of sweet pepper with *P. syringae* (Chen et al. 1998, 2000). The expression of the *Hrap* gene in tobacco and *Arabidopsis* plants conferred resistance against virulent bacterial pathogens (Ger et al. 2002; Pandey et al. 2005).

13.5 Development of Transgenic Bananas Resistant to BXW Disease

Transgenic technologies facilitating transfer of useful genes across species have been shown to offer numerous advantages to circumvent the natural bottlenecks to breed banana for its improvement. It provides a cost-effective alternative to develop banana varieties resistant to BXW disease, due to lack of high resistance among banana genotypes. In order to stably integrate transgene into plant genome, availability of efficient and reproducible transformation and regeneration protocols is most important. Even though transforming a monocot species is difficult, genetic transformation of banana is routine in few labs (Becker et al. 2000; Sagi et al. 1995; Ganapathi et al. 2001; Khanna et al. 2004; Tripathi et al. 2012, 2015).

Researchers at IITA and NARL developed transgenic bananas expressing sweet pepper *Hrap* or *Pflp*, in order to determine if these genes can confer resistance to BXW disease. Both *Hrap* and *Pflp* genes have been demonstrated efficacy for control of several bacterial diseases. These genes have been acquired from the patent holder Academia Sinica through the African Agricultural Technology Foundation (AATF).

Embryogenic cell suspensions (ECS) of banana cultivars “Sukali Ndiizi” and “Nakinyika” were transformed through *Agrobacterium*-mediated procedure, and hundreds of independent transgenic lines expressing *Hrap* or *Pflp* gene were generated (Tripathi et al. 2010; Namakwaya et al. 2012). The generated transgenic events were validated for presence of gene by PCR analysis. The integration and low copy number of transgene was confirmed by Southern blot analysis. All the PCR-positive transgenic lines were evaluated for resistance against *X. campestris* pv. *musacearum* by artificial inoculation of in vitro plantlets in the laboratory as described by Tripathi et al. (2008). About 50–60 % of transgenic lines tested did not develop any disease symptoms, confirming that the *Hrap* or *Pflp* gene can enhance resistance against *X. campestris* pv. *musacearum*. The transgenic lines showing enhanced resistance were further evaluated in glasshouse using potted plants. The majority of transgenic lines showing enhanced resistance with in vitro assays also did not develop any symptoms of BXW disease after artificial inoculation of potted plants in the glasshouse, whereas control non-transgenic plants developed symptoms and wilted completely (Fig. 13.1c, d). The results from laboratory and glasshouse confirmed that transgenic bananas expressing *Hrap* or *Pflp* gene provide high resistance to BXW (Tripathi et al. 2010; Namukwaya et al. 2012). The transgenic lines exhibiting strong resistance to BXW were further characterized by molecular analysis to check the expression of transgenes.

The best 65 resistant lines showing high resistance against *X. campestris* pv. *musacearum* under screenhouse were further evaluated in a confined field trial at NARL, Kawanda, Uganda. The majority of transgenic lines showed significantly higher resistance in comparison to non-transgenic control plants (Tripathi et al. 2014b). Both mother and ratoon crops of 11 transgenic lines (7 *Hrap* lines and 4 *Pflp* lines) were found to be highly resistant showing 100 % disease resistance, whereas non-transgenic control plants wilted completely. The field trial results also confirmed the successful transfer of the disease resistance trait from mother to progeny in several lines. The flowering and yield (bunch weight and fruit size) of BXW-resistant transgenic banana lines was comparable to un-inoculated control non-transgenic plants of same varieties (Tripathi et al. 2014b). The best ten lines were further planted in second confined trial to test durability of disease resistance trait and agronomic performance. As bacterial pathogens evolve fast, there is risk of breaking down of resistance with single gene-based resistance. To minimize this, we are developing transgenic banana varieties using stacked genes (*Hrap-Pflp*) for durable high resistance to BXW disease.

We have also identified additional transgenes providing resistance to BXW. These genes can be stacked in order to develop enhanced and durable disease resistance. We tested the potential of rice pattern recognition receptor (PRR), XA21, for providing resistance against *X. campestris pv. musacearum* as it has been reported to confer resistance to the rice pathogen *X. oryzae* pv. *oryzae* (Ronald et al. 1992; Wang et al. 1996). Transgenic banana cultivar "Gonja manjaya" (AAB) expressing rice *Xa21* was generated and tested for resistance against *X. campestris* pv. *musacearum* through rapid bioassay and in glasshouse using potted plants. In both assays, about 50 % of the *Xa*21 transgenic lines tested showed complete resistance to BXW, whereas nontransgenic control plants develop severe symptoms and wilted completely. Our results confirmed that the expression of the rice *Xa21* gene in transgenic banana provides enhanced resistance to BXW disease (Tripathi et al. 2014a).

13.6 Conclusion

Banana *Xanthomonas* wilt (BXW) is one of the most significant diseases and is considered as the biggest menace to banana production in the East and Central Africa, which is the largest banana-producing region and where most of the bananas are grown by smallholder farmers for local consumption. The use of disease-resistant varieties is considered the timely, economically, and ecologically friendly approaches to control bacterial diseases. However, the development of banana varieties resistant to bacterial diseases through classical breeding is restricted, as sources of germplasm displaying resistance against bacterial pathogens has not been identified yet. As BXW is spreading rapidly and devastating banana production across East Africa, genetic engineering can provide an efficient, safe, and viable way to develop BXW-resistant banana varieties. Transgenic bananas expressing sweet pepper *Hrap* or *Pflp* gene demonstrated enhanced resistance to BXW under glasshouse and field conditions. These transgenic lines will be further tested in multi-location trials in order to test them in different environmental and climate conditions. These BXW-resistant varieties will enhance the resources available to control the disease and save livelihoods of millions of people who depend upon banana as staple food. The transgenic technology using *Hrap* and *Pflp* may also control other bacterial diseases of banana like Moko, Bugtok, or blood disease effecting banana production.

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