

Review

Banana *Xanthomonas* wilt: a review of the disease, management strategies and future research directions

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Banana production in Eastern Africa is threatened by the presence of a new devastating bacterial disease caused by *Xanthomonas vasicola* pv. *musacearum* (formerly *Xanthomonas campestris* pv. *musacearum*). The disease has been identified in Uganda, Eastern Democratic Republic of Congo, Rwanda and Tanzania. Disease symptoms include wilting and yellowing of leaves, excretion of a yellowish bacterial ooze, premature ripening of the bunch, rotting of fruit and internal yellow discoloration of the vascular bundles. Plants are infected either by insects through the inflorescence or by soil-borne bacterial inoculum through the lower parts of the plant. Short- and long-distance transmission of the disease mainly occurs via contaminated tools and insects, though other organisms such as birds may also be involved. Although no banana cultivar with resistance to the disease has been identified as yet, it appears that certain cultivars have mechanisms to 'escape' the disease. Management and control of the disease involve methods that reduce the inoculum's density and spread of the pathogen. Removal of the male bud (de-budding) has proven to be very effective in preventing the disease incidence since the male bud appears to be the primary infection site. The economic impact of banana *Xanthomonas* wilt is not fully understood but its impact on food security in the region is very significant. While germplasm screening for the disease is ongoing, efforts to genetically engineer resistance in some banana cultivars are also making good progress. This paper presents a review of the disease and management strategies that have been successful in curtailing its spread.

Key words: Banana, *Xanthomonas* wilt, disease management, future strategies.

INTRODUCTION

Banana and plantain, hereafter referred to as bananas, are the most important staple food crop in Uganda and a number of other countries in the East African Great Lakes region. Cooking bananas account for one third of the calorie intake from starchy staples in Uganda (FAO, 2004). It is estimated that about 35 to 50% of household food budget expenditures are allocated to banana consumption (Kiiza et al., 2004). Bananas are also an impor-

tant income source for about 30 percent of the Ugandan farmers, being marketed at a rate between 25 and 50 % of production, particularly in Western Uganda (Okech et al., 2004). The production of bananas is affected by diseases of fungal, bacterial and viral origins. Banana *Xanthomonas* wilt (BXW) also known as banana bacterial wilt (BBW) caused by *Xanthomonas vasicola* pv. *musacearum* (*Xvm*) (formerly *Xanthomonas campestris* pv. *musacearum*) (Valentine et al., 2006) is an emerging disease of bananas in East Africa. It is a vascular disease that results in permanent wilting and eventual death of the plant. Initially identified in Ethiopia in 1960's on a close relative of banana *Ensete ventricosum* (Yirgou and

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Bradbury, 1968, 1974), the disease was first reported from Uganda in 2001 on both dessert, brewing and East African highland bananas (Tushemereirwe et al., 2003, 2004). The disease has also been reported from the northern Rwanda (M. Pillay, personal observation), the eastern Democratic Republic of Congo (Ndungo et al., 2006) and Tanzania (Mgenzi et al., 2006). All banana cultivars and genome groups are susceptible to the disease although field observations suggest that the disease appears to be more prevalent on 'Pisang awak' (ABB), a cultivar that also has a greater frequency of insect visitors compared to others (M. Pillay personal observation). Once established in an area, the disease spreads rapidly and results in total yield loss. Ratoon crops arising from infected mats often wilt before even producing bunches or produce bunches with rotten fruits (Eden-Green, 2004). *Xanthomonas* wilt poses one of the greatest threats to banana production in Eastern Africa where banana is a staple food and it has the potential of destabilizing food security in the region. In Uganda, the disease is reported to occur in epidemic proportions destroying entire banana fields in some area, especially those dominated by 'Pisang awak' in some areas. Although no detailed studies on the assessment of crop losses due to this disease have been presented, field observations indicate that the disease reduces yields to varying levels, depending on the growth stage of the crop, degree of cultivar susceptibility and prevailing climatic conditions. For example, observations in central Uganda (Mukono district) show that disease symptoms are more pronounced and spread faster during high rainfall seasons (M. Pillay, personal observation). Leaf symptoms are hardly visible in the dry seasons although susceptible plants harbor the pathogen which at this time is generally concentrated in the lower parts of the plant and in the corms. BXW is a vascular disease that results in yellowing and wilting of leaves and yellowing of immature and mature fruits. Symptoms are somewhat cultivar-specific and determined by the route and stage of infection (Brandt et al., 1997). Foliar symptoms, yellowing, wilting (often associated with loss of turgor and collapse of the petiole) quite often resemble those of *Fusarium* wilt but the excretion of a yellowish bacterial ooze from cut tissues is characteristic of banana bacterial wilt (Thwaites et al., 2000; Tushemereirwe et al., 2003, 2004). Depending on the route of infection, leaves of flowered plants may show yellowing and wilt symptoms but the bunch may appear green and normal outwardly, although internally fruits often exhibit a reddish brown discoloration and are inedible. In contrast, the first symptoms may be blackening and shriveling of the male bud, extending into the lower part of the immature fruit bunch, often followed by premature ripening of some or all of the fruits and internal fruit discoloration. Internally, yellowing and/or brown discoloration of vascular bundles can be seen throughout the plant when the plant is sectioned, but this discoloration is often much more app-

arent in the central tissues of the pseudostem than in the outer leaf sheaths. A cream or yellow-colored ooze, typical of many bacterial infections, exudes within a few minutes of cutting tissue and copious quantities may be produced over a period of several hours. Eventually, the affected pseudostem wilts and dies but in some cultivars the disease is partially systemic and daughter suckers can appear unaffected (Tushemereirwe et al., 2003).

Although the mechanisms of pathogenicity in BXW have not been investigated, symptom manifestation in other bacterial wilts such as those caused by *Ralstonia solanacearum* appears to result from multiple virulence factors working synergistically within the plant. Some of the factors may include toxins, extracellular polysaccharides (EPS) and a consortium of extracellular enzymes that break down plant cell walls. The enzymes seem to facilitate bacterial invasion and spread by digesting cortical cell walls and the pit membranes that separate adjacent xylem vessels. Enzymatic cell wall degradation is probably responsible for creating the gels and tylosis typically found in vessels of wilting plants (Beckman, 1987).

THE PATHOGEN: DIAGNOSTIC TOOLS

The pathogen *X. vasicola* pv. *musacearum* belongs to a group of bacteria that are found only in association with plants or plant materials (Thwaites et al., 2000). In media or environments that are rich in glucose content, the pathogen produces copious amounts of extracellular polysaccharide, called xanthan gum, which can contribute to significant blockage of vessels in infected plant tissues. As with other Xanthomonads, *Xvm* grows much slower than other bacterial species such as *Pseudomonas*, *Burkholderia* and *Ralstonia* (Tripathi, pers. comm.). Thus, outside of the host, *Xvm* does not compete well with other bacteria and it is thought that this slow growth trait has implications for survival of the pathogen in the soil when it is released from infected plant. Studies have been initiated to understand pathogen survival in the soil. However, the initial progress was hindered due to the absence of a selective medium specific to *Xvm*. Preliminary evidence showed that the bacteria can survive in chopped plant debris in the soil for over six months. Understanding pathogen survival is important because it is interlinked to recommendations on disease management measures based on crop rotation or fallowing.

Recent advances in developing semi-selective media are contributing to progress in studies on pathogen survival and epidemiology, and determination of the insect species involved in pathogen transmission. Progress is being made to develop serological tests of the pathogen, with polyclonal antibodies already successfully generated and tested and plans are underway to develop monoclonal antibodies. Initiatives are on course for development of PCR-based protocols that would be useful for studies

on many aspects especially understanding the population structure of the pathogen (Eden-Green, pers. comm.).

Epidemiology: sources of inoculum, mode of infection and transmission of the pathogen

Many bacterial plant pathogens propagate and survive on floral parts, stems and leaves as epiphytic populations that play a significant role in disease epidemiology. This is true with fireblight pathogen of apple and pear caused by *Erwinia amylovora* and blossom blights caused by pathovars of *Pseudomonas syringae*, and some leaf spot diseases caused by *Xanthomonas* sp. (Agrios, 2005). Epiphytic populations of *Xvm* have not been described and they are not known to play any role in the epidemiology of banana bacterial wilt. Eden-Green (2004) noted that, plant residues, contaminated soils and water, infected mats and traded products including fruits, leaves and planting materials are thought to be the major sources of inoculum of *Xanthomonas* wilt. Although Brandt et al. (1997) reported that the pathogen is easily spread by any object that comes in contact with contaminated plant parts, Eden-Green (2004) noted that the contribution these sources make to the spread of the disease depends on the survival of the bacterium and its mode of transmission. Agrios (2005) also noted that all diseases in which the pathogen is carried internally or externally by one or a few specific vectors, dissemination of the pathogen depends to a large extent or entirely on that vector. Although the relative importance of many of these factors is not fully known, tentative conclusions can be drawn on the basis of field observations and knowledge from other banana bacterial diseases.

Mode of infection and transmission

Successful infection of a host plant by a bacterium involves the movement of the bacterium towards the host, contact between the two, penetration of the host by the bacterium and proliferation of the bacterium inside the host immediately following ingress (Gnanamanickam et al., 1999). Field observations in Uganda suggest that *Xvm* infects banana plants either through the lower parts of the plant (roots, mats and cut leaf petioles) possibly from soil-borne inoculum and/or through the inflorescence from inoculum dispersed by insects and perhaps aerosols. Transmission from plant to plant within a field is thought to be principally accomplished by flying insects and mechanically by contaminated tools used in pruning operations. Brandt et al. (1997) claimed that mole rats can also transmit the disease as they tunnel from one plant to another. The role that other organisms such as moles, as well as other larger animals such as cattle and goats that move through the infected fields, could also contribute to the spread of bacterial wilt is not yet esta-

blished. Nectar collecting birds and bats have been observed moving from plant to plant especially in fields of 'Pisang awak'. These organisms cannot be discounted as vectors of the disease.

The entry of pathogens into plants is thought to be facilitated by mechanical injuries or injuries caused by soil-borne organisms such as nematodes and insects. Removal of excess suckers from contaminated plants creates numerous open wounds through which the bacteria exude. This 'pool' of bacteria might act as a source of inoculum that could be spread by insects and other vectors. Injured or decaying infected tissues can provide inoculum that is released into the soil and spread through soil water. In Moko disease caused by *R. solanacearum*, soil and mechanical transmission though reported are considered to be much less important than insect transmission (Molina, 1999).

Although some bacterial pathogens such as *E. amylovora*, some *Pseudomonads* and soft rot *Erwinias* are reported to be disseminated by wind-blown rain, there is no evidence yet of the dissemination of banana bacterial wilt disease via aerosols. Dispersal of *Xvm* by rain splash has not been reported although it is likely that rain splash and high wind can move bacterial ooze from exposed surfaces of infected plants to healthy plants. In other bacterial pathogens, Anon (2002) noted that water droplets can carry and disperse bacterial cells over long distances under cyclonic conditions. Goto (1992) on the other hand reported that dispersal of bacterial inoculum under storm conditions that can result in subsequent infection apparently does not occur over long distances. Nevertheless, this aspect needs further investigation in order to ascertain the role played by aerosols in disease transmission under different cultivation conditions, especially as related to plant density.

Several investigators (Buddenhagen and Elsasser, 1962; Stover, 1972; Soguilon et al., 1995) have reported on the significant role played by various insect species in the transmission of bacterial diseases in bananas. In Uganda, field observations suggest that insect vectors are important in spreading the disease within and between fields. The most common insects visiting banana flowers are stingless bees, fruit flies and grass flies (Tinzara et al., 2006). *Xanthomonas* was isolated from these insects as well as honey bees visiting male flowers of both symptomatic and asymptomatic plants. Transmission appears to occur mainly, if not exclusively, through the male or neuter part of the floral raceme, probably through freshly exposed tissues (scars) left by flowers and bracts that, in many varieties, are shed daily. This explains why cultivars with persistent neutral flowers such as some Cavendish varieties and clones belonging to the 'Nakitembe' clone set often escape the disease even under high disease pressure. In contrast, cultivars such as 'Pisang awak' and 'Bluggoe' which attract many insects are more prone to infection than other cultivars. Addis et al. (2004) reported that Cavendish varieties with persis-

tent bracts that are widely grown in Western Ethiopia are not infected. Similarly, Bakelana and Ndungo (2004) reported that Cavendish varieties without persistent bracts do not get infected readily in the eastern Democratic Republic of Congo (DRC) and nor have persistent bracts. However, the Cavendish varieties grown in Uganda do not have persistent bracts and the absence of infection may be due to other factors such as altitude and insect vectors. However, it should be noted that these cultivars are not resistant per se but only escape the disease due to their inflorescence morphology. These cultivars do show disease symptoms when they are artificially inoculated. Buddenhagen and Elsasser (1962) and Stover (1972) reported that inflorescence to inflorescence transmission occurs through 'wounds' of the male flower scars (cushions) when male flowers are shed. Wilting of bracts of the male bud, premature yellowing and rotting of fruits coupled with bacterial exudation from the peduncle are common symptoms of infection through the inflorescence as a result of insect transmission. Anon (2002) observed that symptom manifestation of Moko disease in case of insect-transmission is first seen in the flower buds and peduncles, which become blackened and shrivelled. A similar scenario with regard to symptom development is observed in case of plants infected with *Xvm*. Disease transmission over very long distances (>120 km) has been observed in Eastern Africa. Long distance transmission of the disease is probably due to other vectors such as birds or bats since the latter are considered to be the pollinators of banana. Another possible source of contamination is the tools used by banana merchants who travel from infected areas to non-infected zones and harvest bananas and leaves for sale. In the latter case, the disease may suddenly appear in a single farm.

Xanthomonas wilt transmission appears to be affected by altitude, debudding and the presence of 'Pisang awak' in the area (Addis et al., 2004; Ndungu et al., 2004). For example, no inflorescence symptoms were observed in the Kambata region of the southern Ethiopia (1850 masl) where 'Pisang awak' is widely grown and debudding is not practiced. On the contrary, floral infections were common in the Kaffa region of Western Ethiopia (1600 masl) an area with large numbers of 'Pisang awak' and where debudding is unknown. In Uganda, the disease is known to have spread rapidly where 'Pisang awak' is widely grown and where de-budding is not practiced at elevations ranging from 1050 - 1400 masl. Disease spread was very slow in Rwanda (1500 - 1600 masl) where both cultivation of 'Pisang awak' and de-budding were uncommon. Slow disease spread was also observed in the Masisi region, North Kivu in the Democratic Republic of Congo where 'Pisang awak' is the dominant cultivar but the altitude is above 1700 masl. The effect of altitude is perhaps linked to the presence or absence of insect vectors and needs to be investigated further.

MANAGEMENT OPTIONS

In general, bacterial diseases of plants once established are difficult to control owing to the lack of an effective chemical or other curative treatment. Early detection and destruction of the diseased plants is a key step in preventing disease spread (Karamura et al., 2005). In the case of banana bacterial wilt, the situation is complicated since all banana cultivars examined, to date, are apparently susceptible and no single control measure has been found to be effective (M. Pillay pers comm.; Welde et al., 2006). Consequently, management must focus on methods that reduce the initial inoculum and subsequent spread of the pathogen between host plants. Since disease transmission through the male bud is an important natural mode of spread, timely removal of the male buds is being advocated and has been taken up by farmers to interrupt the transmission cycle and prevent the spread of the disease.

De-budding has to be done as soon as the last hand of the bunch is formed. This would prevent flower infection and result in bigger more evenly filled fruits (Blomme et al., 2005a). Other methods of disease management practices include immediate rouging and burying of diseased plants. Early removal of a pseudostem with floral infection prevents the disease from moving down the plant and infecting young developing suckers (Blomme et al., 2005b). In this case, intensive surveillance and reporting of the disease are necessary. It is therefore mandatory to sensitize farmers about the disease in awareness campaigns. There should be strict controls on the movement of banana planting materials. Similarly, Brandt et al. (1997) reported that the only recommended control measures for the bacterial wilt of *Ensete* are cultural practices which include the use of healthy, disease-free suckers for planting material, destruction and controlled movement of diseased plants, cleaning of equipment that has come in contact with diseased plant material and rotation of crops. On the other hand, Eden-Green (2004) emphasized the use of control measures that reduce or prevent further spread of the disease to new areas or areas that are not yet infected that is., disease inhibition since experience with related diseases show that once they become established in smallholder banana cropping systems, control is very difficult and eradication becomes almost impossible. Disease restraint depends on two key actions viz. prompt removal of sources of inoculum and reducing or eliminating opportunities for further spread. In fact, these actions are mutually reinforcing but the greatest degree of control will be obtained when infection sources are promptly eliminated and the risks of transmission are reduced. While information on *Xvm* is still lacking, adoption of known preventive measures should be encouraged among farmers. Although the phytosanitary approaches currently being recommended are labor intensive and not easily adopted by farmers, they are presently the only known means of preventing

further spread of the epidemic until more sustainable management options are available. In many countries removal of the male bud is an adopted cultural practice in the management of banana stands, especially in commercial plantations. However, this is not practiced widely by small-holder farmers who predominate in Eastern Africa. Male bud removal has to be practiced at the precise stage since very early removal causes the lower hands of the bunch to curve upwards. This upward curvature of the bunch appears to be more pronounced in some cultivars and seems to affect bunch size adversely. Herbicides can be used successfully to destroy infected plants. Mature plants can be injected with 1.2 ml of 2, 4-Dichlorophenoxyacetic acid (2, 4-D). At least 1.6 ml of the original concentration of 2, 4-D (irrespective of the dilution) needs to be applied per mature plant. The effect of 2, 4-D is visible two weeks after injection when the pseudostems of injected plants break at the base and fall off. By this time the 2, 4-D has reached the underground corm that begins to rot (G. Blomme, pers comm.). This method is much easier than manually rouging and burying infected plants.

Economic impact of BXW and implications for management strategies

Compared to pre-infection levels, the total banana yield loss due to BXW infection is estimated at 30-52 % between 2001 and 2004 (Karamura et al., 2006). This has caused a reduction in the amount of banana harvested by households which has impacted livelihoods negatively. Many households have switched to other crops while others have abandoned banana cultivation.

Although an economic analysis of BXW has to be based on findings from Central Uganda, where the disease has occurred first and is presently most common, it is possible to forecast the economic impact of a BXW pandemic in Uganda by extrapolating the observations made in this region. BXW has now been reported in 34 districts in Uganda, apparently spreading from Central Uganda, where banana production is less intensive and mainly subsistence oriented to the high-production areas in Western Uganda. However, whereas in Central Uganda infestation rates reach levels of 18 - 27%, the major banana producing areas in the South-West of Uganda still show little or no infection (Tushemereirwe and Opolot, 2005). This may be due to the fact that in Western Uganda, mainly cooking banana (AAA) is cultivated, which is less susceptible to insect-borne BXW than the exotic varieties such as 'Pisang awak' that are primarily planted in Central Uganda. Kayoby et al. (2005) reported that if uncontrolled BXW spreads at an infection rate of 8% per annum in cooking bananas, the total production loss of bananas is expected to be about 56% over a ten-year period, translating into a reduction from 4.5 million tons to eventually 2.1 million tons per year. Such a

spread over the whole of Uganda would induce economic losses of 2 billion dollars over a decade, arising from price increases and significant reductions in production. However, producers would benefit either in the first few years of the pandemic, or during a whole decade if the infestation rates are lower than 8% (Abele and Pillay, 2007). This is due to the fact that in a normal market development, with increasing income, demand for cooking bananas will decline, and so will prices, because of the typical characteristics of a starchy staple food, that is, the perceived inferiority of the good (Henze, 1994). This means that at moderate production losses due to BXW, farmers over-compensate these quantity losses through the price increases, which are at present occurring in Uganda (FOODNET/MIS 2006). While initially producers are benefiting, consumers are losing from the first outbreak of the disease, due to reduced quantities and increasing prices.

The above described scenario has significant implications for the management of the disease. Generally, management measures in particular curative measures are taken when a certain disease damage threshold is reached, normally when or shortly before the economic losses are greater than the costs of pest management (Peterson and Hunt, 2003). BXW management in this respect has two problems: as the producers have to decide when to commence management, they will probably begin too late with the management from an overall economic perspective, as they do not take into account the much higher and much earlier occurring consumer losses. This problem is aggravated by the fact that there are no curative but only preventive measures to control the disease. This shifts the timeline for management backward, implying that the producers are even less willing to engage in management long before the disease affects their fields. A third additional factor is that the disease affects the respective local plant population quickly and effectively, so that by the time the farmer becomes aware, it will be too late to respond.

The above factors explain the presently low awareness of farmers in areas that are not yet heavily affected by the disease, and the even lower rate of farmers that know or apply preventive BXW control measures (Tushemereirwe and Opolot, 2005). Prices for Ugandan cooking bananas are considerably high at the moment, so that farmers, especially in the unaffected high producing areas of western Uganda have no incentive to adopt control measures since they benefit from higher prices. Solutions to this dilemma are the provision of public goods, especially publicly financed measures to prevent the spread of the disease. At the same time, these measures have to conform to the market, that is, not affect market prices and quantities. The most prominent measure is breeding for resistance, and the publicly financed multiplication of resistant cultivars (e.g. through tissue culture or other controlled multiplication methods) and dissemination through public extension services. To be in

conformity with the market, the new varieties should be introduced whenever obsolete plants have to be replaced on farmers' fields, according to farmers' decisions on how to most profitably introduce new cultivars to replace the old ones.

Current status and management challenges of banana *Xanthomonas* wilt

The impact of BXW on banana production in eastern Africa is not yet fully determined. Although the economic loss of BXW on the welfare of the farmers and the economy of the countries is not well documented, the impact of banana bacterial wilt on food security is very significant. As the disease emerged in the region, significant progress in research on various aspects of the disease such as biology of the pathogen, epidemiology and management of the disease has been initiated. While significant advances have been made in understanding the pathogen and its management practices, some major challenges still exist. One of the major challenges is the identification of resistant germplasm and development of resistant cultivars through conventional breeding. Screening of both local and elite banana germplasm for resistance is a major ongoing activity. Differential responses of the cultivars to the pathogen have been observed in the field. The mechanisms leading to differential responses by the host plants need to be investigated. Germ-plasm screening trials and field observations in Uganda have shown that some cultivars are able to escape the disease because of their inflorescence morphology. Such an observation was also made by Buddenhagen et al. (1987).

Very little is known about the life cycle of *Xvm*. A clear understanding of the pathogen's life cycle and its significance to the epidemic development in bananas is critical to the wilt research agenda. Information on pathogen population structure, pathogen diversity and phylogeny is still lacking and yet important in determining the best strategy for deployment of resistance. The duration of survival of the bacteria in the soils is not well documented and the relative importance of different routes of infection remains a major challenge. Although prevention of airborne dispersal of the bacteria between inflorescences may be the most important means of controlling the primary spread of the disease, especially between farms and villages, other modes of infection undoubtedly occur and are critical to containment. For example, even in areas of greatest infestation, farmers continue with the habit of borrowing farming tools (Brandt et al., 1997). The majority of the farmers who control the disease by rouging infected plants do not sanitize their tools with bleach or heat as recommended. Infected plants are not buried but left to decompose in the farm. These diseased plants might serve as sources of inoculum for new infections. Control options such as destruction of infected mats and routine de-budding to reduce the rapid spread of the dis-

ease are difficult to organize with sufficient rigor to eradicate the problem in developing countries where farmers lack the structured organizations required to apply eradication programs throughout affected zones or districts and funds are lacking to enforce them. De-budding is also labor intensive and some farmers are unable to cope with this additional task because of old age or infirmity; in other cases plantings (especially of 'Pisang awak') are owned by "absentee" farmers who are difficult to engage in community control campaigns.

Alternative food sources such as sweet potato and cassava have been provided to farmers in badly affected areas by NGOs. While some farmers have adopted alternative crops, others still prefer banana as the staple food. One of the most susceptible cultivars in Uganda 'Pisang awak', locally as 'kayinja', is widely grown for the production of "banana beer" that is source of an income for many farmers. Farmers are reluctant to destroy 'kayinja' and other ABB cultivars such as 'Bluggoe' (*Kivuvu*) even if the plants are infected. It is difficult to persuade farmers to destroy diseased mats since occasionally a diseased mat may still produce a normal bunch. Many farmers also obtain cash income from selling 'kayinja' leaves that are used in preparation of food. 'Kayinja' produces numerous suckers, which are able to proliferate well in large mats, needs very little attention and produces large laminas that are not shredded easily by wind. Therefore farmers tend to preserve stands of these plants even if they are infected with *Xvm*.

FUTURE DIRECTIONS

Much of the research on *Xanthomonas* wilt has focused on germplasm screening, management options and control. While significant advances in understanding the biology and management of the disease have been gained, there is need for more research in a number of areas. The development of disease resistant banana cultivars remains a high priority since farmers are reluctant to employ labor-intensive disease control measures. This however requires a clear understanding of the molecular basis of interaction between the bacterium and the host plant, and an analysis of the intermediate products produced by both the pathogen and plant following infection. Additionally, determination of the population structure of the pathogen from a wider geographic area is required in order to develop a database on *Xvm* isolates and consequently determine the best strategy for deployment of resistance and or to incorporate the non-matching resistance genes to the existing pathogen. Use of biotechnological approaches may be one of the best strategies in managing this disease.

Strategies for development of bacterial wilt resistant banana cultivars through genetic engineering

Genetic engineering has become an important tool for crop improvement. It offers numerous important opportu-

nities for the improvement of existing elite varieties and development of new cultivars. A major advantage of genetic engineering is that it allows breeders to rapidly develop new varieties by the introduction of cloned genes into commercial varieties. The approach combines both the traditional breeding and the transgenic approach for deriving elite cultivars with multiple resistances to pathogens. Several methods exist for transformation of bananas and plantain. 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 the cooking banana cultivar Bluggoe and plantain Three Hand Plantain (Sagi et al., 1995) while Becker et al. (2000) reported the genetic transformation of Cavendish banana cv. Grand Nain. The protocol has also been developed for *Agrobacterium* mediated transformation of embryogenic cell suspensions of the banana (Ganapathi et al., 2001; Khanna et al., 2004). At present most of the transformation protocols use cell suspensions, however establishing cell suspensions is a lengthy process and is also cultivar-dependent. A transformation system has also been established using shoot tips from various cultivars of *Musa* (May et al., 1995; Tripathi et al., 2005a). This technique is applicable to a wide range of *Musa* cultivars irrespective of ploidy or genotype (Tripathi et al., 2003, 2005a). This process does not incorporate steps involving disorganized cell cultures but uses micro-propagation, which has the important advantage of allowing regeneration of homogeneous populations of plants in a short period of time and offers several potential advantages over the use of embryogenic cell suspensions (ECS) as it allows for rapid transformation of *Musa* species. Recently, researchers at the International Institute of Tropical Agriculture (IITA), Uganda in collaboration with National Agriculture Research Organization (NARO), Uganda have established a genetic transformation system for East African Highland Bananas (EAHBs) using the shoot tips. The genetic transformation system developed can be used for the production of bacterial wilt resistant varieties of banana, using transgenes already demonstrated to confer resistance against bacterial wilt in other crops.

The most attractive strategy for bacterial disease control in crops is to improve plant defense mechanisms against a particular pathogen. Recent advances in genetic engineering offer ways to transfer resistance genes found in other plants, microbes, insects and animals into crop varieties without changing other favorable traits. Plant defense genes from other plants and antimicrobial proteins are now a potential source of plant resistance (Tripathi et al., 2005b). The state of genetic engineering of banana for disease resistance and future possibilities have been extensively reviewed (Sagi et al., 1998; Tripathi, 2003; Tripathi et al., 2004; Tripathi, 2005). The range of potential strategies for genetic engineering agai-

nst banana bacterial wilt in bananas has been reviewed by Tripathi et al. (2004).

Plants have their own network of defenses 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 expression of existing defense mechanisms (Bent and Yu, 1999; Rommens and Kishmore, 2000). The molecular recognition of pathogens by plants is often characterized by a gene-for-gene relationship that requires a specific plant resistance (*R*) gene and a corresponding pathogen avirulence (*avr*) gene (Flor, 1971). Genetic evidence from a wide diversity of plant pathosystems suggests that when an appropriate *R-avr* gene pair is present, the result is host resistance, whereas absence or inactivation of either member of the gene pair results in susceptibility of the host to the pathogen.

Pathosystem-specific plant resistance (*R*) genes have been cloned from several plant species (Bent, 1996). Resistance genes have been isolated from many crops and bacterial-resistant transgenics are being produced by incorporating the *R* genes in susceptible plants within a genus or a family or even outside the family. *R* gene-mediated resistance has several attractive features for disease control. When induced in a timely manner, the concerted responses can efficiently halt pathogen growth with minimal collateral damage to the plant. No input is required from the farmer and there are no adverse environmental effects. Unfortunately, *R* genes are often quickly defeated by co-evolving pathogens. Many *R* genes recognize only a limited number of pathogen strains and therefore do not provide a broad-spectrum resistance. Also efforts to transfer *R* genes from model species to crops, or between distantly related crops, could be hampered due to restricted taxonomic functionality.

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. Several defense genes have been shown to delay the hypersensitive response induced by bacterial pathogens in non-host plants through the release of the proteinaceous elicitor. Elicitor-induced resistance is not specific against particular pathogens. Hence, manipulation of such defense genes may be more ideal.

Research is in progress at IITA in collaboration with NARO-Uganda, Academia Sinica (Taiwan) and the African Agricultural Technology Forum (AATF) in Kenya for producing BXW resistant banana varieties using transgenes encoding for plant ferredoxin-like protein (pflp) and hypersensitive response assisting protein (hrap) isolated from sweet pepper. Hypersensitive res-

ponse-assisting protein (HRAP) and a ferredoxin-like amphipathic protein (PFLP), isolated from sweet pepper (*Capsicum annum*) are novel plant proteins that can intensify the harpinPSS-mediated hypersensitive response (Ger et al., 2002; You et al., 2003). These proteins have dual function; iron depletion antibiotic action and harpin triggered HR enhancing. The transgenes have been shown to delay the hypersensitive response induced by various pathogens like *Erwinia*, *Pseudomonas*, *Ralstonia* and *Xanthomonas* sp. in non-host plants through the release of the proteinaceous elicitor, harpinPss in various crops including dicotyledons such as tobacco, potato, tomato, broccoli, orchids and monocotyledons like rice (Huang et al., 2004). Also elicitor-induced resistance is not specific against particular pathogens, so it could be very useful strategy. Since the two transgenes *pflp* and *hrap*, isolated from sweet pepper, have been shown to function in a monocot (rice) with demonstrated efficacy against bacterial pathogens including *Xanthomonas*, its usefulness as a transgene for resistance to *Xvm* in banana has a high probability of success. The integration of transgenic approaches with classical resistance breeding offers an environmentally friendly option of controlling bacterial diseases.

Antagonistic bacteria

Among micro-organisms, there are forms that inhibit the growth of other microbes. They are usually called antagonists. Bacterial antagonists have been used in many studies to suppress bacteria of the same or similar genus. Establishment of bacterial antagonists on plant surfaces is a critical phase in disease control. Populations of antagonists established on plant surfaces are necessary for competition with pathogens for sites and nutrients (Wilson and Lindow, 1993). Biological control of *Xvm* by using antagonistic bacteria has not been discussed and remains an option for controlling the disease. Biological control of *Xvm* can be affected both aerially, to control the predominant aerial infection and at the root level to counteract soil borne infection. Bacterial antagonists of *E. amylovora* have been used to control the fire blight effectively in pear and apple blossoms (Stockwell et al., 1998). When applied to the blossoms at early to mid-bloom, the biological control agents *Pseudomonas fluorescens* A506 and *Erwinia herbicola* C9-1 proliferates on pear and apple stigmas and exclude the pathogen from the infection sites. The incidence of fire blight was reduced by about 60% with two applications of bacterial antagonists (Johnson et al., 1993; Nucló et al., 1998). It is possible to develop such a scheme for *Xvm*. Many root associated bacteria have been extensively examined for their roles in natural and induced suppression of soil borne diseases (Klopper et al., 1999).

Introduction of antagonistic bacteria into the roots can bring about biological control by producing metabolites that directly inhibit the pathogen, such as antibiotics, hydrogen cyanide, and cell wall degrading enzymes.

Recently it was shown that eleven bacterial strains (2 of *Pseudomonas putida*, 3 of *Pseudomonas fluorescens*, 4 of *Burkholderia cepacia* and 2 of *Burkholderia glathei*) reduced wilt incidence in tomato by 80-100%. The antagonistic endophyte strain of *P. putida* reduced bacterial wilt of potato by 71% (Priou et al., 2006). Combined with other control components, the use of microbial antagonists may prove a successful and ecologically safe strategy to reduce the incidence and yield loss due to banana *Xanthomonas* wilt.

CONCLUSIONS

Xanthomonas wilt of banana is a relatively new disease and much remains to be done before the disease can be controlled or eradicated. Bacterial wilt disease is not unique to banana but has been reported in 44 families of plants including economically important species in the families *Solanaceae*, *Leguminosae*, and *Zingiberaceae*. Despite the volume of international research on bacterial wilts, they remain a major limiting factor in the production of food and industrial crops. Some of the research on *Xanthomonas* wilt of banana has been highlighted at the 4th International Bacterial Wilt Symposium held in the United Kingdom from 17 - 20 July 2006. The various topics on wilts in relation to economically important crops discussed at this conference give an idea of the research that is lacking in *Musa*. We highlight a few of these aspects while the interested reader can find more information from the abstracts. While screening for sources of resistance among banana, varieties has been a priority, more emphasis should be placed on sources of resistance from wild bananas. In our germplasm screening trials we have found that varieties vary with regards to earliness and intensity of symptom expression (Mwangi et al. 2006a). Understanding the mechanisms of resistance to *Xanthomonas* wilt should remain a high priority area of research. The persistence of the pathogen in the soil and technologies to support replanting of banana in infected fields could contribute to reduced wilting incidence and rehabilitation of devastated fields (Mwangi et al., 2006b). The genetic variability and the evolution of the pathogen have not been studied in detail. Understanding the diversity can lead to the development of targeted diagnostic tests and the genetic basis of resistance. Other areas of research include biocontrol, the use of herbicides in the destruction of diseased plants, molecular markers to identify the pathogen, socio-economic aspects and integrated disease management.

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