Banana Fusarium Wilt (*Fusarium oxysporum* f. *sp. cubense*)

Control and Resistance, in the Context of Developing

Wilt-resistant Bananas Within Sustainable Production Systems

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**Abstract**

Banana (*Musa spp.*) is seriously threatened by the soil-borne fungus *Fusarium oxysporum* f. *sp. cubense* (Foc), also known as Panama disease. Attempts to control Fusarium wilt with fungicides damage soil health and have limited efficiency due to pathogenic variability. Elucidating the mechanism of infection and molecular basis of host defense through banana genome sequencing, genome editing and proteomic profile analysis will help formulate strategies to develop resistant cultivars. This will include research to better understand the functions of *Fusarium* wilt-resistance proteins. Transgenic approaches and protoplast fusion could be employed as tools for transferring resistance genes from wild relatives to commercial banana varieties, and may serve as a new strategy in solving the problems faced by banana breeding programmes. Evaluation of banana germplasm for resistance to *Fusarium* wilt using in vitro mutation and selection, along with somaclonal variation and somatic hybridization, could improve banana breeding efficiency for resistance against Foc. Plant hormones could also play an important role in regulating plant growth and defense by mediating developmental processes and signaling networks involved in banana responses to Foc. A complementary approach for managing *Fusarium* wilt, such as exclusion, surveillance and biological control as important components of integrated disease management programs must be considered to prevent and contain contagion. This includes studies on banana plant-microbe interactions, embracing both plant growth promoting rhizobacteria (PGPR) to induce Foc resistance, and exploring Foc-derived elicitors for inducing defense-related enzymes in bananas. The role of Silicon and crop and livestock integration must also be included in the Fusarium control toolbox. The current review also gathers knowledge of the biotechnological approaches along with biological control of Fusarium wilt of banana that will provide researchers insights and criteria to develop future studies.

**Keywords:** banana; *Fusarium* wilt; *Musa*; sustainable production system; Foc

1. Introduction

Banana (*Musa spp.*) is a key staple (sub) tropical food and fruit. Most cultivated bananas are seedless triploid varieties (*2n = 3x = 33*) derived from intra- or inter-specific crosses between two diploid wild species, *M. acuminata* (genome designated AA) and *M. balbisiana* (BB) (*Simmonds and Shepherd, 1955; Heslop-Harrison and Schwarzacher, 2007*). The most common varieties of dessert and East African Highland bananas are triploid AAA derived from crosses within *M. acuminata*, while common cooking triploid bananas (AAB or ABB) derive from crosses between *M. acuminata* and *M. balbisiana*. Wild diploid
banana produces seeds, whereas cultivated triploid banana is sterile, but develops parthenocarpic fruits (Li et al., 2013a). Global banana production is increasingly constrained by several pests and diseases, with Fusarium wilt being the most significant. Due to the perennial nature of bananas and the polycyclic nature of the disease, effective, long-term management of Fusarium wilt remains a challenge and requires the development of new/alternative management strategies (Chag et al., 2015). In 2006, Yi et al. (2007) reported that around 6 700 ha of banana plantations were infected by the Fusarium pathogen in the major banana growing regions, the Panyu district and Zhongshan city in Guangdong Province of China. Today, just over a decade later, Fusarium wilt disease is present throughout the entire banana growing regions of China and accounts for a loss of over 40 000 ha of banana plantations (Li et al., 2013a). Due to the ease with which the disease spreads, the global banana industry is under serious threat from this soil-borne fungal disease (Garcia-Bastidas et al., 2014; Ordonez et al., 2015; Zheng et al., 2018). Growing susceptible banana cultivars is unsuitable due to high survival of Fusarium chlamydosporae in the soil. Because of the negative effects of chemical fungicides on soil health, using novel strategies such as the biological control, hormonal application-induced defense responses to Fusarium wilt, developing resistant cultivars through mutation breeding, transgenic and cigenic approaches, protoplast culture and somatic hybridization to generate new types of materials, in vitro selection and somaclonal variation have all become attractive alternatives to using chemical fungicides and other conventional control methods (Subramaniam et al., 2006; Wu et al., 2010; Chen et al., 2011; Mohandas et al., 2013; D. Sun et al., 2013; Ghag et al., 2014; Swarupa et al., 2014; Wang et al., 2014; Raza et al., 2016; Dale et al., 2017). This paper reviews the context of banana Fusarium wilt, and the main aspects of its management, then considers how disease resistance may be elucidated, and how Foc resistance genes may be integrated into available germplasm.

2. Fusarium wilt of banana and its distribution

Fusarium wilt is increasingly jeopardizing global banana production (Stover, 1962; Michielse and Rep, 2009; Ploetz and Churchill, 2011; Li et al., 2013a; Sasaki et al., 2013; Ploetz, 2015). The pathogen, probably originated in Southeast Asia and the disease was first reported in Australia in 1876. Fusarium oxysporum f. sp. cubense (Foc) exists as different pathogenic races such as Foc races 1, 2, 3 and 4, classified according to their ability to cause disease in a set of different banana cultivars. Race 1, which is responsible for the ‘Gros Michel’ epidemics, also affects ‘Silk’, ‘Pome’ AAB and ‘Pisang Awak’ ABB, race 2 affecting ABB cooking bananas, such as ‘Bluggoe’ (ABB), race 3, described as a pathogen of Heliconia spp. (Tropical American banana relatives), had a minor impact on ‘Gros Michel’ and seedlings of M. balbisiana have been recognized (Waite, 1963; Stover and Buddenhagen, 1986; Li et al., 2011). A relatively new variant of the pathogen, tropical race 4 (TR4) which affects Cavendish bananas as well as many other locally important types such as the plantains was confirmed for the first time outside Southeast Asia, spreading to the African continent (Garcia-Bastidas et al., 2014; Ordonez et al., 2015). Among the known races of Foc, Races 1 and 4 are of major international concern (Buddenhagen, 2009; Li et al., 2014; Chittaratth et al., 2017; Hung et al., 2017; Zheng et al., 2018). TR4 is highly virulent on current Cavendish banana cultivars. Its spores prefer to attach first to the banana root tip, before the mycelia directly penetrate the epidermal cell wall, and colonize and occlude the xylem vessels (Li et al., 2011; Ho et al., 2015; Zhang et al., 2017). Although TR4 is still restricted to Asia and Africa, it threatens global export of bananas, especially in America. Therefore, attempts to distinguish its spread in other areas should be started as soon as possible (Ploetz, 2006; Damodaran et al., 2009; Li et al., 2014; Ordonez et al., 2015; Ploetz, 2015; Zheng et al., 2018).

3. Management of Fusarium wilt

Some traditional methods such as chemical control (e.g. soil fumigants and fungicides) of TR4 have proved uneconomic, do not provide adequate control, and are environmentally unfriendly. Therefore, other more efficient and effective management strategies such as crop rotation, biological control (e.g. silicon amendments and companion planting with Allium spp.), and using arbuscular mycorrhizal fungi (AMF) and plant growth promoting rhizobacteria (PGPR) may be needed to overcome the disease (Akila et al., 2011). Some plant extracts also show antifungal activity and therefore are suitable to reduce mycelium growth under both greenhouse and field tests (Akila et al., 2011). In Foc TR4-infested areas, effective disease management is mostly restricted to using resistant cultivars, which have been developed with gene transfer (Dale et al., 2017). However, genetic improvement of banana must be put into practice for developing resistance to all races of Fusarium, including the most virulent race 4, therefore, more research is needed to elucidate the mechanism of genetic resistance in wild Foc-resistant bananas. An understanding of the mechanisms of disease progression/spread and plant signaling in response to Foc invasion in banana is also very useful for effective disease control. Identification of sources of resistance as a key step towards the generation or selection of new banana cultivars showing resistance to Foc-TR4 using some approaches, such as mutation induction and selecting of somaclonal variants also offer important management methods to control Fusarium wilt disease (Hwang and Ko, 2004; Li et al., 2014; Ghag et al., 2015; Ploetz, 2015; Xu et al., 2017). This review article highlights useful wilt management and identifies areas where more investigations are required in the search for more effective wilt control. Crop rotation, use of livestock and applications of silicon remain topical non-chemical/non-molecular approaches to controlling banana Fusarium wilt.

3.1. Rotation with other crops or integration with livestock

Uninterrupted cultivation of bananas in the field exacerbates Foc occurrence in the soil. Crop rotation as a management practice, in general, is highly effective and environmentally friendly means of control soil-borne diseases. In China, in Panyu, Guangzhou, an area heavily infested by Foc, banana is rotated with 2–3 years of commercially cultivated Chinese leek (Allium tuberosum) to control Fusarium wilt (Zhang et al., 2013). Therefore, Chinese leek has a potential to be developed into an environmentally friendly treatment to control Fusarium wilt of banana. Thus, exploring the use of other rotational crops that exhibit soil
borne-disease suppression is extremely important for maintaining the banana industry (Krupinsky et al., 2002; Huang et al., 2012; Wang et al., 2015). Recently, Wang et al. (2015) investigated the influence of two-year crop rotation systems of pineapple-banana and maize-banana on the population density of Foc. Their results showed that the pineapple-banana rotation was more effective than maize-banana in reducing Foc levels and suppressing Fusarium wilt disease incidence. The management of ground cover around the base of the banana plantations was also reported to be a significant factor in reducing the incidence and severity of Fusarium wilt in bananas (Pattison et al., 2014). Deltort et al. (2017) found that in soil with favorable abiotic properties, a good plant arrangement, can help to promote susceptible banana variety to Fusarium wilt suppression.

3.2. The effect of silicon (Si) in reducing the symptoms of Fusarium wilt of banana

The use of silicon (Si) can decrease the intensity of several diseases in some crops (Datnoff et al., 2007). Although Si is known to reduce the occurrence of pathogens on many plants, little information is available on the potential positive effects of Si on the susceptibility of banana to pathogens (Vermeire et al., 2011). In addition to the other methods for controlling Fusarium wilt of banana described in this review, silicon (Si) application shows promise as part of a new disease management strategy to avoid Foc infection and help ensure adequate banana production for the long-term (Fortunato et al., 2012). Si could have a positive effect on banana resistance against some pathogens and also provide an environmentally friendly alternative for the integrated control of crop disease (Vermeire et al., 2011). It has been also reported that Si application suppressed disease in Cucurbit species caused by foliar and soil-borne pathogens (Belanger et al., 2003). The obtained resistance of Si-amended plants against the fungal pathogen could be due to accumulation of Si in the leaves, thereby, interfering with the pathogen's penetration as a result of a mechanical barrier (Kim et al., 2002). Fortunato et al. (2012) evaluated the effect of Si in reducing the symptoms of Fusarium wilt on banana plants. Their results showed that supplying Si to banana plants, especially to a susceptible cultivar against Foc, had great potential in reducing the intensity of Fusarium wilt.

4. Induced disease resistance

4.1. Hormonal application induced defense responses to increase resistance against Fusarium wilt in banana

Plant hormones on plant-microbe interactions emerge as key regulators. The effects of the major plant hormones on the interaction between Fusarium wilt and host plants have also been reviewed. MeJA has been reported to activate host defense mechanisms against a broad range of pathogens and also regulate defense responses against biotic and abiotic stresses in various plant species (Penninckx et al., 1998; Di et al., 2016). Kozlowski et al. (1999) reported that MeJA could protect spruce seedlings against Pythium ultimum. The use of exogenous applications of MeJA to induce chemical defense responses and resistance among various horticultural crops is expanding (Darras et al., 2011). Reglinski et al. (2012) reported that application of MeJA to Pinus radiata seedlings resulted in induced resistance to subsequent wound inoculation with Diplodia pinea. Treating Medicago truncatula with MeJA induced minor resistance against the soil-borne charcoal rot pathogen (Gaige et al., 2010). Other research also indicated that MeJA had a significant impact on control of Monosporascus root rot and vine decline of melon (Aleandri et al., 2010). Treatment with exogenous MeJA reduced wound diameter of gray mold rot in tomato fruit infected by Botrytis cinerea (Zhu and Tian, 2012), enhanced defense enzyme activities and mitigated the disease severity caused by cylindrocarpon destructions infection of ginseng root (Sun et al., 2013). Sun et al. (2013) reported that the application of MeJA activated enzymes and reduced the level of H2O2 and malondialdehyde (MDA) in banana plantlets following inoculation with Foc TR4. The activation of resistance in banana plantlets to Foc TR4 by MeJA may also be ascribed to the operation of enzymes of secondary metabolite biosynthesis. Regarding the strong effect of MeJA on the interaction with Fusarium, understanding the mechanisms by which JA contributes to disease reduction or induction and which function is predominant among different plant species will require further research.

Salicylic acid (SA) has an important regulatory role in the plant immune response (An and Mou, 2011). Wang et al. (2014) asserted that salicylic acid (SA) has a key role in plant–microbe interactions. Their study showed the role of SA in conferring resistance to Foc TR4 in banana. A review of findings shows the age and condition dependent role of ethylene in plant interaction with Fusarium (Di et al., 2016).ABA either positively and negatively influences induction of resistance against Fusarium depending on the fungal strain involved (Ton et al., 2009; Robert-Sellamiantz et al., 2011). Whether or not there is an interaction of ABA with other hormones results in reduced Fusarium symptoms, should be examined in the future.

4.2. Plant-microbe interactions in Fusarium wilt of banana

Plants interact with a massive variety of soil microbes such as Foc. The adjusted interaction between Foc and its host results in disease expansion. However, the molecular mechanisms, arising from this equilibrium are insufficiently understood. Some studies on plant-pathogen revealed the importance of some genes for pathogen resistance. These genes include antimicrobial compounds that are toxic to pathogens (Brogden, 2005). Plant hormones play important roles in regulating plant growth and defense by mediating developmental processes and signaling networks involved in plant responses to a wide range of biotic interactions. Generally, salicylic acid (SA) plays an important regulatory role in the plant immune response and SA signaling reduces plant susceptibility, whereas methyl jasmonate (MeJA), ethylene (ET), abscisic acid (ABA) and auxin possess complex effects (Bai et al., 2013; Giron et al., 2013; Kazan and Lyons, 2014; Wang et al., 2014; Di et al., 2016).

Salicylic acid (SA) and MeJA can play a key role in plant-microbe interactions to induce systemic defense against various pathogens (Sun et al., 2013; Wang et al., 2014). Infection by Foc R1 stimulated the accumulation of endogenous SA and activated the expression of genes involved in SA signaling in the Cavendish banana, which improved this cultivar's resistance to Foc TR4 (Wu et al., 2013). However, further confirmation is needed to
determine whether SA plays a promising role in Foc TR4 effects on banana (Wang et al., 2014). It has been reported that F. oxysporum requires components of auxin signaling and transport to colonize the plant more effectively (Kidd et al., 2011). More research is also needed to prove that whether cytokinins (CKs) can really be considered as key regulatory molecules acting universally in plant-biotic interactions (Giron et al., 2013).

ABA signaling not only regulates plant development and response to abiotic stress, but also affects the adjustment of inherent immunity. ABA signaling as defense response is widely investigated in Arabidopsis against most pathogens (Asselbergh et al., 2008) although information in terms of Fusarium infection is still inadequate. Negative functions of ABA was observed in some plant – pathogen interactions such as tomato and Erwinia chrysanthemi (Asselbergh et al., 2008) or Arabidopsis and Botrytis cinerea (Abuqamar et al., 2006). However, there is one report based on positive regulatory of ABA signaling and negative regulatory of JA response which resulted in an increased resistance to Foc (Anderson et al., 2004). Therefore, as a defense role, ABA has both negative and positive functions, although there is no report based on ABA signaling in Foc infection in banana.

Köberl et al. (2017) found members of Gammaproteobacteria as an indicator species of healthy banana plants on Fusarium wilt-infested and healthy fields in Nicaragua and Costa Rica. Spreading of soil-borne pathogens such as Foc using a single management strategy cannot be prevented efficiently. In the present review potential application of biological control includes application of plant growth promoting rhizobacteria (PGPR) and Fusarium-derived elicitors to induce resistance against Fusarium wilt, the effect of silicon (Si) in reducing the symptoms of Fusarium wilt of banana. Thus when we use compatible mixtures of botanical and biocontrol formulations, a significant reduction in the disease incidence besides growth promotion is achieved.

4.2.1. Biological control of Fusarium wilt in banana

Considering the urgency of Panama disease, biological control offers a complementary disease management approach (Favel et al., 2003; Lian et al., 2009; Gang et al., 2013; Raza et al., 2016). However, there has been little field research in which long-term biocontrol efficacy has been evaluated for Fusarium wilt of banana (Ploetz, 2004). The use of biocontrol agents (BCAs) has been proved to be an environmental friendly disease management strategy in recent years (Xue et al., 2015; Deltour et al., 2017; Fu et al., 2017). Further enquiry is still needed for the extension of effective and safe BCAs for commercializing their application (Tjamos et al., 2004; Gang et al., 2013; Raza et al., 2016). Some research indicates the suppressive effects of BCAs on Foc growth under in vitro conditions (Thangavelu et al., 2004; Mohammed et al., 2011; Gnanesekaran et al., 2015; Ho et al., 2015; Sekhar and Thomas, 2015) and in greenhouses (Sivamani and Gnananickam, 1988). Xue et al. (2015) selected one isolate from Bacillus spp. as a potential biocontrol agent which plays an important role in the management of banana disease. Considering that there is a lack of solid published scientific research work on biocontrol, particularly with practical field results, tactics that could be used to foreshadow biocontrol failures in the field will require better understandings of these interactions and pragmatic evaluations of their efficacy (Ploetz, 2015). The success of biological control depends not only on production methods, but also on the costs involved and effective antagonists. Also, these antagonists must be amenable to long-term storage as dry preparations (Jackson, 1997). Sivamani and Gnananickam (1988) reported that banana plants treated with native strains of Pseudomonas fluorescens exhibited less severe wilting and internal discoloration due to infections with isolates of races 1 and 4 of Fusarium oxysporum f. sp. cubense in greenhouse experiments. As a field trial, soil application of Trichoderma harzianum effectively controlled Fusarium wilt with an efficacy comparable to that of the fungicide carbendazim (Thangavelu et al., 2004). Currently, a variety of strains of Bacillus spp. have also been widely used as BCAs against soil-borne plant diseases such as Fusarium. The results demonstrated good performance with acceptable biocontrol efficacy (Sun et al., 2011). Previous reports have also demonstrated that siderophore-producing endophytic streptomycetes from banana roots are effective against the Fusarium wilt pathogen and could be developed as the BCAs against the banana Fusarium disease (Cao et al., 2005).

Some plant extracts show antifungal activity and potentially reduce mycelium growth under both greenhouse and field conditions (Akila et al., 2011). The possibility of in vitro biological control of Fusarium oxysporum f. sp. cubense by using some Indian medicinal plants such as Calotropis gigantea L., Centella asiatica L., Ocimum sanctum L., Piper betle L. and Vitis negundo L. was investigated by Gnanesekaran et al. (2015). Among the five plants tested, P. betle L. plant extracts exhibited maximum antifungal activity against the tested plant pathogen Foc followed by V. negundo, C. gigantea, C. asiatica and O. sanctum plant extracts. As mentioned above, regarding the application of potential BCAs which exhibited excellent suppression of Fusarium wilt in vitro and in vivo, newly isolated and characterized biocontrol strains should be also evaluated under field conditions to assess their biocontrol efficacy.

Successful inoculation of tissue cultured banana plants with fungal endophytes has been reported by Paparu et al. (2004). Pre-inoculation with beneficial endophytes through banana plantlets might be an effective strategy for either biological control or growth promotion of banana (Ho et al., 2015). Addition of artificial inoculation to tissue cultured banana plantlets resulted in a substantial reduction in the infection and severity of Fusarium wilt disease, as well as increased in plant growth parameters (Jie et al., 2009). Ali et al. (2015) applied biosynthesized silver nanoparticles for the control of some plant pathogenic fungi. These researches could open a new avenue of research for using organisms as bioindicators of environmental pollution.

4.2.2. Application of plant growth promoting rhizobacteria (PGPR) to induce resistance against Fusarium wilt of banana plants

Plant growth-promoting rhizobacteria (PGPR) have been considered as the most promising agents for cash crop production in managing soil-borne disease (Raguchander et al., 1997). The successful colonization of host rhizophores is very important for effective control of soil-borne pathogens, including Foc (Ploetz, 2005). Evaluating the effectiveness of biological control for Foc infected banana, Thangavelu and Gopi (2015) demonstrated that the combined application of endophytic and rhizospheric bacterial strains resulted in effective suppression of Fusarium wilt of banana, both in pot trials and in the field. Several substances produced by PGPR such as antibiotics have been related to pathogen control and indirect promotion of growth development.

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in many plants (Beneduzi et al., 2012). Bacillus amyloliquefaciens strain NJN-6 is an important PGPR which can produce secondary metabolites antagonistic to several soil-borne pathogens. Thus, application of PGPR strain NJN-6 significantly suppressed the incidence of Fusarium wilt disease and promoted the growth of banana plants. Some active compounds produced by NJN-6 also showed the initial protection of the plants against soil-borne pathogens (Yuan et al., 2013; Xue et al., 2015; Fu et al., 2016). Resistance-inducing and antagonistic PGPR might be useful in formulating new inoculants with combinations of different mechanisms of action, leading to a more efficient use for biocontrol strategies to improve cropping systems in the future (Beneduzi et al., 2012).

4.2.3. Application of Fusarium-derived elicitors for inducing defense-related enzymes in bananas

Elicitors as molecules which are involved in plant defense responses are directly derived from microbes. Introduction of elicitors as a sustainable agricultural practice minimizes the need for chemical control (Thakur and Sohal, 2012). An elicitor prepared from Foc isolated from an infected banana rhizosphere induced the accumulation of resistance-associated enzymes in leaves of susceptible and resistant variety of banana. Therefore, it could be concluded that Fusarium-derived elicitor effectively induced defense in a susceptible variety (Thakker et al., 2011). To further study the infection process and pathogenesis of Fusarium wilt, developing protoplasts transformation and a gene knockout system for Fusarium oxysporum will lay the foundation for the study of pathogenic gene function validation, plant-pathogen interaction and early monitoring and screening resistant materials for banana disease resistant breeding (Zhang et al., 2017). Considering that the employment of elicitors in crop protection is still in the very early stages of use as a new control method, further research in this area is needed to demonstrate elicitors’ effectiveness in banana disease control.

5. Significance of molecular implications in host defense

5.1. Understanding the functions of Fusarium wilt resistance proteins

Use of new technologies such as genomic and proteomic studies helps researchers discover many disease resistance proteins such as plant pectin methyltransferases (PMEs) which are directly involved in the disease resistance of plants during the infection process (Martin et al., 2003; Raiola et al., 2011; Wojtasik et al., 2011; Bai et al., 2013; Ma et al., 2013). Before pathogen treatment, the resistant cultivar displayed higher PME activity than the susceptible cultivar. Thus, Ma et al. (2013) concluded that increased PMEs and afterwards decreased degrees of ethylesterification (DMs) accompanied by increased low methylesterified homogalacturonan (HGs) in the root vascular cylinder appear to play a key role in specifying of banana susceptibility to Fusarium. HGs are the major group of cell-wall pectins, which has a key role in creating a barrier against environmental stresses, including pathogen attacks (Vorwerk et al., 2004; Mohnen, 2008). Overexpression of the PME inhibitor (PMEI) protein reduced the activity or expression level of PME, resulting in higher resistance of plants against pathogens (Lionetti et al., 2007; An et al., 2008; Volpi et al., 2011). Understanding the molecular mechanisms of these proteins in banana–Fusarium interaction will reveal how these proteins relate to the induction of resistance against Fusarium oxysporum f. sp. cubense (Foc).

5.2. Transgenic approaches for development of Fusarium wilt resistance in banana

Banana cultivars resistant to Fusarium wilt exist and several have also been conventionally bred via different breeding programmes (Ploetz, 2006). Genetic transformation of elite banana cultivars for resistance to Fusarium seems to be a more promising approach to improve disease resistance or tolerance, and it has been accomplished by using techniques such as particle bombardment or sonication assisted vacuum infiltration of apical meristem and/or by using multiple bud clumps followed by Agrobacterium-mediated gene transformation (Khanna et al., 2004; Tripathi et al., 2005; Maziah et al., 2007; Subramanyam et al., 2011; Yip et al., 2011; Ghag et al., 2014). One of the major steps in genetic engineering of banana for disease resistance is to test the effectiveness of the new proteins against the target pathogen which involves the exposure of the plants to the pathogen in a suitable infective unit such as spores (Mert and Karakaya, 2003; Subramaniam et al., 2006). There are several reports based on the successful development of transgenic plants through cell suspension using Agrobacterium (Becker et al., 2000; Huang et al., 2000; Ganapathi et al., 2001; Chakrabarti et al., 2003; Khanna et al., 2004; Kumar et al., 2011; Shekharwat et al., 2011). Some defense-related genes have been also used for transformation of banana for Fusarium resistance, but only short-term results from greenhouse or incubator experiments are usually reported, but field results for race 1 and TR4 tolerance are often absent (Chakrabarti et al., 2003; Maziah et al., 2007; Paul et al., 2011; Ghag et al., 2012; Mahdavi et al., 2012; Ploetz, 2015). However, Pei et al. (2005) reported that two of 51 transgenic banana “Cavendish” fruited in a TR4 field trial. In spite of all progress in transgenic programs of banana, there is still no known commercially available Fusarium wilt-resistant cultivar. Therefore, long-term field results for creating transformed bananas are needed to demonstrate promise that they might offer when combating this disease. Based on the antifungal activities of thaumatin-like proteins, these latter have been reported as a good candidate for genetic engineering toward production of disease-resistant banana plants (Mahdavi et al., 2012). To select the necessary genes that can confer resistance in both glasshouse and field conditions, one should know the plant defense responses and stepwise spread of the disease after infection of Foc. Arinaitwe (2008) asserted that the gene encoding antimicrobial peptides (AMPs) were strong candidate for fungal resistance in Musa as they are highly inhibitory in vitro to Foc. Mohandas et al. (2013) reported that higher AMP protein content in the transgenic banana cultivar “Rashtali” with AMP gene through Agrobacterium-mediated transformation of embryogenic cell suspension was negatively correlated with Foc disease symptoms. Overexpression of this gene in banana could lead to the development of stable resistance against fungal pathogens (Ghag et al., 2014). More recently, transgenic bananas with additional RGA2 and Ced9 inserts show promising resistance against Fusarium wilt tropical race 4 after 3-year field trial (Dale et al., 2017).
Furthermore, the discovery that Cavendish encodes an RGA2 homolog provides the exciting prospect of using gene editing technology to generate TR4 resistance by enhancing the expression of these endogenous genes (Hu et al., 2017). However, even if transformed bananas were accepted in the marketplace, there are still significant technical challenges in creating cultivars that resist TR4 or other races of this pathogen in the field (Li et al., 2014).

Recently, microarray technology has also played an important role in deciphering the underlying networks of gene regulation in plants that lead to a wide variety of defense responses. Microarray is an important tool to quantify and profile the expression of thousands of genes simultaneously, with two main aims, including gene discovery and global expression profiling. Several microarray technologies are currently in use; most include a glass slide platform with spotted cDNA or oligonucleotides. Till date, microarray technology has been used in the identification of regulatory genes to understand the signal transduction processes underlying disease resistance and its intimate links to other physiological pathways (Lodha and Basak, 2012). A thorough knowledge of plant disease resistance using a successful combination of microarray and other high throughput techniques, as well as biochemical, genetic, and cell biological experiments is needed for practical application to secure and stabilize yield of many crop plants (Lodha and Basak, 2012). Microarray studies also showed that cell wall-strengthening genes may be important for banana resistance to Fusarium wilt (Van den Berg et al., 2007). Microarray approaches have been also used to identify the candidate genes significantly involved in the early stages of the banana–Foc TR4. The results demonstrated that anti-oxidation, cell wall modification and synthesis of anti-fungal proteins are the most important aspects to study for understanding the Foc resistance mechanism in commercial banana plants (Li et al., 2015).

5.3. Proteomic analyses of Fusarium wilt responses in bananas

With the completion of banana genome sequencing, proteomic profile analysis can provide a powerful tool to investigate the complex defense mechanism in banana (Li et al., 2014). By comparing the protein profiles of resistant and susceptible banana cultivars via a proteomic approach, one can find out how susceptible and resistant varieties of banana respond to TR4 during an infestation (Li et al., 2013b). Currently, several proteomic studies have been successfully applied to evaluate the effect of cold tolerance and osmotic stresses on banana growth and development as well as proteomic profiling of banana roots in response to F. oxysporum (Li et al., 2013b; Ghas et al., 2014; Li et al., 2014). The usage of proteomic profiling to study the molecular mechanism of banana roots infected with Foc4 has been also reported by Li et al. (2013b). Proteomic analysis of Fusarium wilt responses in banana revealed that thirty-eight differentially expressed proteins were identified to function in cell metabolism. Most of these proteins were positively regulated after Foc4 inoculation (Li et al., 2013b).

6. Developing and evaluation of banana germplasm for resistance to Fusarium wilt

Wild banana germplasm provides a promising source of potential Foc-TR4 resistance genes as a valuable genetic resource for banana breeding programmes aiming to produce cultivars resistant to Fusarium wilt (D’Hont et al., 2012; Li et al., 2014). Finding ways to develop bioassays which can differentiate between resistant and susceptible cultivars will efficiently accelerate breeding and selection for disease resistance against Fusarium wilt (Wu et al., 2010; Xu et al., 2017). Thus, the identification of sources of resistance is a key step towards the generation or selection of new banana cultivars showing resistance to Foc-TR4. Earlier studies have shown the existence of resistance alleles to Foc race 1 in subspecies of M. acuminate such as subsp. malaccensis, subsp. burmannica, subsp. microcarpa and subsp. siamea (Vakili, 1965). In addition, M. acuminate subsp. malaccensis has been shown to be resistant to Foc-TR4 (Smith and Hamill, 1999). Li et al. (2014) characterized eight Musa species for resistance to Foc-TR4 under both greenhouse and field conditions, aiming to generate data to guide breeding strategies for developing banana cultivars resistant against Fusarium wilt. Although these studies confirmed some wild bananas to be important pools of Foc-resistance genes, a more systematic evaluation of wild banana species is necessary to generate the information regarding traits and characters on their Foc resistance. It is worth noting that there is a large variation in degree of resistance found in B genome types, and wild BB diploids that are resistant to Foc-TR4 could be identified eventually. The rich genetic diversity within the wild banana species may be responsible for this variation. In the future, seedlings from different populations should be screened (Li et al., 2014).

6.1. Screening as the most critical aspect of breeding to develop resistance against Fusarium wilt

It is widely accepted that the breeding and selection for disease tolerance or resistance is the most effective and sustainable Foc-management option (Buddenhagen, 2009). Currently, screening for Foc-TR4 resistance has been performed largely on cultivated bananas, but little information is available on the wild Musa species (Zhang et al., 2018). In a study implemented by Li et al. (2014), genotypes with high levels of resistance to Foc-TR4 were identified, including two wild species (M. basjoo and M. itinerans) that were unaffected by Foc-TR4, even under high inoculum pressure. Several protocols to infect banana with Foc in vitro (Wu et al., 2010) or under greenhouse conditions (Sun and Su, 1984; Smith et al., 2008; Dita et al., 2011; Ribeiro et al., 2011) have been also reported. Li et al. (2014) used a two-step process comprising a combination of greenhouse and field studies that provided comprehensive and reliable information regarding disease reaction on the evaluated genotypes of banana (Li et al., 2014). To develop a global screening and evaluation protocol is critical to select reliable resistant materials.

6.2. Somaclonal variations of bananas for resistance to Fusarium wilt

Promising Foc-resistant or tolerant clones acquired through nonconventional breeding techniques have been proposed as an aid in banana breeding programmes (Novak, 1992; Wu et al., 2010). To assess correlation between in vivo and in vitro behavior, shoot tip cultures from banana clones susceptible and resistant to Foc races 1 and 4 were grown in vitro in the presence of fusaric acid and fungal crude filtrates. In this study, peroxidase activity...
was used as a parameter to discriminate between susceptibility and tolerance which correlates well with field response of host plant against pathogens (Morpurga et al., 1994). At present, attempts to develop new banana genotypes resistant to Fusarium wilt using conventional breeding techniques face significant obstacles mainly because most cultivars of Musa AAA Cavendish subgroup are totally sterile and seedless. Thus, non-conventional approaches such as in vitro selection (Matsumoto et al., 1995; Bhagwat and Duncan, 1998a, 1998b) have been taken into consideration. Whilst, several resistant clones has been also acquired through somaclonal variation (Hwang and Ko, 2004). Wu et al. (2010) investigated the utility of in vitro inoculation of rooted banana plantlets grown on modified medium as a reliable and rapid bioassay for resistance to Foc. Their studies resulted in evaluation methods developed for rapid screening of Musa species for resistance against Foc. Besides conventional breeding, somaclonal variation is also an effective strategy which has been used to improve various horticultural traits. Some level of resistance to Fusarium wilt has been obtained by using somaclonal variation techniques from the Taiwan Banana Research Institute (Hwang and Ko, 2004). Other somaclonal variants of Cavendish namely GCTCV-53 and GCTCV-119 also identified, which showed resistance to Fusarium wilt (Hwang and Ko, 2004).

6.3. Mutation induction and screening for Fusarium wilt tolerance in mutated banana

Application of in vitro mutagenesis especially among vegetatively propagated banana has significantly improved the efficiency of mutation techniques in breeding programs (Kulkarni et al., 2007). Shirani Bidabadi et al. (2012) evaluated the possibility of induced variations in banana cultivars through in vitro mutagenesis by treating the shoot tips with ethyl methanesulfonate (EMS). In vitro mutagenesis has also been applied to isolate useful variants in banana by Suprasanna et al. (2008). Bhagwat and Duncan (1998b) treated shoot apices of in vitro-grown cultures of banana with some mutagens like sodium azide, diethyl sulphate, and ethyl methanesulphonate to evaluate their effectiveness in inducing mutations and also with the aim of producing Foc-tolerant variants. Regenerated plants were screened for tolerance to the fungus under greenhouse conditions and were considered to be tolerant. EMS-induced mutation of banana using a micro-cross-section cultural system has been also reported to be potentially useful for banana improvement (Chen et al., 2013). A better knowledge of mutation induction would help us to understand host defenses against Fusarium oxysporum and to formulate strategies to develop tolerant banana cultivars in the future.

6.4. Somatic hybridization of banana by protoplast fusion to increase the resistance against Fusarium wilt

Transferring specific genes by genetic transformation from any sources into cultivated crop species is a potent tool to enrich the gene pool of commercial cultivars. However, many characters of agricultural interests are multigenic thus, many resistance genes in banana have not been identified. Hence, somatic hybridization by protoplast fusion could be employed as a promising alternative strategy to improve banana (Xiao et al., 2009). The use of cell fusion techniques in banana breeding could be feasible, although very limited successes of somatic hybridization have been reported for banana (Matsumoto et al., 2002; Xiao et al., 2009). Somatic hybridization between triploid and diploid bananas has been reported by using protoplast fusion techniques (Matsumoto et al., 2002). Using the asymmetric protoplast fusion approach, Xiao et al. (2009) also obtained somatic hybrids between recipient protoplasts of Musa silk cv. Guoshanxiang (AAB) and donor protoplasts of Musa acuminate cv. Mas (AA). Protoplast fusion could be employed as a promising tool for transferring resistance gene between banana varieties and could be a new strategy to solve the problems in the banana breeding programs. To accomplish this goal, further study is needed to widen combination between banana varieties and then assess the agronomic characters of those obtained hybrids.

7. Conclusion

Elucidating the mechanism of infection and molecular basis of host defense through banana genome sequencing, genome editing and proteomic profile analysis will help formulate strategies to develop resistant cultivars. Over the last decade breeding programmes have successfully created genotypes that show resistance to Fusarium wilt in banana. However, perception of host-pathogen interaction has been necessary because of the possibility of engendering newly recognized variants of the pathogen in the future. Breeding for resistant cultivars is one of the best tools in affected areas, but tools are scarce, time-consuming, or commercially not easily acceptable. Better resistance to this disease will be implemented through identifying and revealing the mechanism of the supplementary molecular tools such as gene editing in the host. Hence, the use of modern plant breeding methods, together with traditional methods, will help researchers to improve banana breeding efficiency. Transgenic approaches and protoplast fusion could be employed as tools for transferring resistance genes from wild relatives to commercial banana varieties, and may serve as a new strategy in solving the problems faced by banana breeding programmes. Evaluation of banana germplasm for resistance to Fusarium wilt using in vitro mutation and selection, along with somaclonal variation and somatic hybridization, could improve banana breeding efficiency for resistance against Foc. A complementary approach for managing Fusarium wilt, such as exclusion, surveillance and biological control as important components of integrated disease management programs must be considered to prevent and contain contagion. This includes studies on banana plant-microbe interactions, embracing both plant growth promoting rhizobacteria (PGPR) to induce Foc resistance, and exploring Foc-derived elicitors for inducing defense-related enzymes in bananas. This review gathers knowledge of the biotechnological approaches along with biological control of Fusarium wilt of banana that will provide researchers insights and criteria to develop future studies.

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