

# Fusarium Wilt Disease of Banana: Current Development of Fusarium Resistant Banana

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## Mini Review

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## Abstract

Fusarium wilt of banana constantly causing considerable losses on banana industry of both local and global markets. Global exportation of banana was greatly affected by this disease since 1950s. After the emergence of a hypervirulent Tropical Race 4 strain of *Fusarium oxysporum* f. sp. *cubense*, many genetic improvements aiming to create resistant cultivars were attempted, mainly through transgenic approach. This review provides the recent finding of potential resistant genes and various efforts in creating resistant cultivar from year 2008 to year 2018.

**Keywords:** Banana; Fusarium wilt; Resistance

## Introduction

Banana is a very popular fruit crops. According to FAO, banana is ranked eight on the list of most important food crop globally and it's the fourth most important crop for the least developed countries. Globally, nearly 85% of the annual banana production was consumed locally and only less than 15% were exported (FAOSTAT, 2015). Exportation of banana is frequently being hampered by the fact that banana is highly susceptible to diseases caused by various pathogens (fungi, bacteria, viruses and nematodes). Disease is the major biotic constraints facing by banana export trade where it first obvious setback traced back to the loss of Gros Michel to *Fusarium oxysporum* f. sp. *cubense* race 1 in 1950s [1]. Currently, almost all bananas traded internationally and a considerably part of locally consumed bananas is of Cavendish and other cultivars with no resistance towards *F. oxysporum* f. sp. *cubense* Tropical Race 4 (*FocTR4*). Many studies have been conducted to screen potential

resistance gene candidates in banana germplasm. Genetic improvement programs are aiming at creating new resistant varieties. The source of resistance gene comes from two directions, the resistance gene analogues from wild relatives of banana which can be identified with a NBS-LRR domain, and other broad-spectrum resistance genes which response after a pathogen invasion.

## Impacts of Fusarium Wilt on Banana Industry

In early 1990s, the occurrence of *FocTR4* epidemic in Malaysia collapsed a then newly established Cavendish exportation effort which targeted the expanding East Asia and Middle East market in merely 2 years' time [2]. Since the infection of the *FocTR4* is irreversible and untreatable for decades [3], its economy impact is profound where not restricted to the losses related to yields but also the gradual losses of arable lands for banana. *FocTR4* is known to infect non-host species such as weeds and these

asymptomatic species will act as an reservoir of inoculum which in turn spread to nearby uninfected lands and water sources [4,5].

Two decades ago, *Foc*TR4 first occurred and was restricted in Asian banana producing countries such as Malaysia and Indonesia, as well as Northern Australia. But since late 2013, *Foc*TR4 was reportedly found outside of South East Asia and Pacific Areas for the first time. Incidences caused by *Foc*TR4 were spotted from Middle East [6,7] and then crept into Africa [8]. In Malaysia, a country-wide survey carried out in year 2008-2009 showed that around 883 hectares of banana plantation lands were infected with Fusarium wilt. This costed the country's banana industry about USD14.1 million losses per annual. Constraint the disease incurred additional cost to producers and causing small holders to cease operation due to insustainability [1]. Also, diseased plants with lower yields and quality significantly reduce the incomes of growers and adversely impact the banana industry on both local and international levels.

### Transgenic Efforts in Fusarium Wilt Resistance

The establishment of resistant banana cultivar is usually performed through transgenic studies since

conventional breeding approaches are not applicable to banana because most of the commercial cultivars are sterile and seedless. The resistance gene candidates consist of those have potential to offer broad-spectrum resistance and those provide the banana with specific resistant against *Foc*. Efforts are also been made on studying the potential of manipulating plant defense pathway such as salicylic acid pathway to heighten the response of banana towards broad range pathogen particularly *Foc* invasion [9-11].

Generally, the transgenes transformed into banana plant for *Foc* resistance were coding for broad-spectrum antimicrobial or antifungal peptide (Table 1). The transgenic bananas generated were all specifically tested for Fusarium wilt resistant trait but they might potentially carrying a broad-range pathogen resistance. Dale et al. (2017) [12] reported a transgenic Cavendish banana carrying *RGA2* transgene showing promising Fusarium wilt resistance after a 3 year field trial. In the Cavendish banana, 3 endogenous *RGA2* homologues were present in addition of the transgenic cassette. But it was found that the native expression of *RGA2*s were 10 folds lower than the *RGA2* transgene driven by a maize polyubiquitin promoter (Ubi-P). The over expression of *RGA2* have no detrimental effect on the banana growth and bunch size.

Transgene	Source	Function	Banana cultivar	Resistance assessment stage	References
Plant ferredoxin-like protein ( <i>pflp</i> )	<i>Capsicum annuum</i>	antimicrobial peptide	cv. Pei Chiao (AAA)	Greenhouse bioassay	[13]
<i>PR5</i> or Thaumatin-like proteins ( <i>TLPs</i> )	<i>Oryzae sativa</i>	Antifungal activity	Pisang Nangka (AAB)	Greenhouse bioassay	[14]
Petunia floral defensins	<i>Petunia hybrida</i>	Antimicrobial peptide	cv. Rasthali (AAB)	Greenhouse bioassay	[15]
Synthetic dsRNAmolecules	-	dsRNA targeting adenylate cyclase	-	<i>In vitro Foc</i> inhibition assay	[16]
<i>Ace-AMP1</i>	<i>Allium cepae</i>	Antimicrobial peptide	cv. Rasthali (AAB)	Greenhouse bioassay	[17] [18]
Endochitinase gene ( <i>chit42</i> )	<i>Trichoderma harzianum</i>	Antifungal activity	cv. Furenzhi (AA)	Greenhouse bioassay	[19]
Small interfering RNAs (siRNAs)	-	Silence vital fungal genes	cv. Rasthali (AAB)	Greenhouse bioassay	[20]
(ihpRNA-VEL and ihpRNA-FTF1)					
Cell-death genes ( <i>MusaDAD1</i> , <i>MusaBAG1</i> and <i>MusaBI1</i> )	<i>Musa acuminata</i>	cell-death-related and highly induced by <i>Foc</i> infection	cv. Rasthali (AAB)	Greenhouse bioassay	[21]

Defensin gene ( <i>Sm-AMP-D1</i> )	<i>Stellaria media</i>	Antimicrobial peptide	cv. Rasthali (AAB)	Greenhouse bioassay	[22]
<i>Ced9</i>	<i>Caenorhabditis elegans</i>	antiapoptosis	cv. Grand Nain	Field trial	[23]
Resistance gene analogues	<i>Musa acuminata</i> ssp. <i>malaccensis</i>	nucleotide-binding and leucine-rich repeat (NB-LRR)-type resistance (R) gene	cv. Grand Nain	Field trial	[24]
( <i>RGA2</i> )					

Table 1: Transgenes used in generate Fusarium wilt resistant transgenic banana.

A more targeted approach is the utilization of RNA-mediated gene silencing construct to interrupt vital fungal genes. Two intron hairpin-mediated expression of small interfering RNA (siRNAs) were constructed by Ghag et al. (2014a) [20] and transformed into embryogenic cell suspensions of banana cv. Rasthali via *Agrobacterium*-mediated transformation. Transformants were selected *in vitro*, acclimatized and subject to *Foc* bioassay to confirm the resistance. *Foc* vital genes *velvet* and *Fusarium transcription factor 1* were silenced by the siRNAs expressed from the transgenic host and the siRNAs was able to translocate into the cytosol of *Foc*. This sequence-targeted approach has advantage over the broad-spectrum antimicrobial genes transgenic approach as the construct is strictly targeting vital *Foc* genes and will have no risk in wipe out other beneficial symbionts of banana.

While the other utilized transgene to confer Fusarium wilt resistant to existing cultivars, Chen, et al. (2013) [25] utilized ethyl methane sulphonate (EMS) to induce mutations in plantlets of banana, cv. Brazil. The induced micro-cross-sections of pseudostem were regenerated and 5 Fusarium resistant lines were identified from early screening technique followed by a bioassay. However, all resistant lines generated still produce certain amount of regenerants which showing disease symptoms, ranging from 7% to 34% of disease incidences. The major drawbacks of induced mutations are the untraceable random mutation in the genome thus the effects on the plant other than Fusarium-resistant are difficult to be predicted.

Apart from antimicrobial peptide-encoding genes, miRNAs are promising candidates since they potentially regulating the plant response toward biotic stresses. But overexpression of a common miRNA, *Musa miRNA156* resulted in transgenic plant with abnormal leaf anatomy and stunted growth [26]. This inferred the importance of careful assessments on the potential detrimental effects of transgenic plants with desired wilt resistance traits before disseminate for cultivation.

## Future Works

Fusarium wilt will continuously causing losses in banana industry unless a resistant cultivar can be generated and mass cultivated successfully in a reliable way. Although most of the transgenic banana improvement projects were carried out up to the greenhouse trials, specifically the screening for *Foc* resistance. However, a more thorough assessments are needed to assess the possible deleterious effects of transgenes on banana quality and yield, their impacts on banana natural microbiomes or rhizospheres and the environments, and lastly the biosafety of transgenic bananas.

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