

Field Resistance of Cavendish Somaclonal Variants and Local Banana Cultivars to Tropical Race 4 of *Fusarium* Wilt in the Philippines

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Abstract

Recent epidemics of *Fusarium* wilt, caused by the virulent strain Tropical Race 4 (TR4) of *Fusarium oxysporum* f. sp. *ubense* (Foc), pose a serious threat to the banana industry. Four Cavendish (AAA genome) somaclonal variants selections from Taiwan ('GCTCV-105', 'GCTCV-119', 'GCTCV-218' and 'GCTCV-219') and three important Philippine local cultivars ['Latundan' (AAB genome, Silk subgroup), 'Lakatan' (AAA genome, Lakatan subgroup) and 'Saba' (ABB genome, Saba subgroup)], were compared to the commercial Cavendish 'Grand Naine' (AAA genome, Cavendish subgroup) in a heavily Foc-infested soil in the Southern Philippines. Experimental plots were planted with 10 tissue culture-derived seedlings, replicated 10 times, and arranged in a completely randomized design. Disease incidence was assessed weekly by monitoring early symptoms of yellowing of older leaves and/or other symptoms such as pseudostem splitting. Onset of disease symptoms was recorded. The infected plants were allowed to develop severe typical symptoms of *Fusarium* wilt (leaves showing clear yellowing, and marginal necroses appear on older leaves) and then checked for vascular necroses as confirmation. The commercially-grown cultivars 'Grand Naine' and 'Lakatan' showed susceptibility with a disease incidence of 64 and 76% respectively in the primary crop; and 79 and 92% respectively in the ratoon crop. Disease incidence in the GCTCV cultivars ranged from 0-6% in the primary crop and 0-8% in the ratoon crop. 'Saba' showed 0% incidence in the primary as well as in the ratoon crop. Foc VCG 01213/16, the Foc strain associated with TR4, was confirmed in the infected plants using a Polymerase Chain Reaction (PCR)-based diagnostic tool. Results confirm the stability of resistant somaclones to Foc TR4, even in agro-ecosystems differing from those where these were first developed. They also show that selecting favorable somaclonal variants is a feasible approach in cultivar improvement, and they have a potential role in integrated Foc TR4 management. The study identifies the TR4-resistant cultivar 'Saba', showing that TR4 does not severely affect all cultivars.

INTRODUCTION

Fusarium wilt disease, caused by *Fusarium oxysporum* f. sp. *ubense* (Foc), is a major concern of the global banana industry. The disease wiped out 'Gros Michel' (AAA

genome) plantations in Central America in the 1950s, which consequently caused the costly shift to Foc-resistant Cavendish cultivars. Recently, a virulent strain that also attacks Cavendish, Tropical Race 4 (TR4) has been found, causing an epidemic in Asia (Ploetz, 2005; Molina et al., 2009). The reported epidemic of Fusarium wilt in Cavendish plantations in the Philippines (Molina et al., 2008) - the top banana exporting country in Asia (FAOSTAT, 2013) - poses a serious threat to the sustainability of the Philippines' multi-million dollar Cavendish-based export industry and to the local cultivars also grown by small-scale farmers. At immediate risk is the 80,000 hectare monoculture Cavendish plantations in southern Philippines, an industry providing annual export earnings of more than US\$70 million, and direct employment to more than 350,000 people (PBGEA, 2013).

The use of resistant cultivars for a long-term management strategy of Foc has long been sought. But breeding programs, both conventional and genetic engineering approaches, have so far failed to produce cultivars of equal or better productivity and quality, which satisfy the discriminating standards of the global Cavendish market. The Taiwan Banana Research Institute (TBRI) has achieved some successes in the selection and use of disease-resistant somaclonal variants of Cavendish (Giant Cavendish Tissue Culture Variant - GCTCV) as part of the integrated approach to sustain the banana export industry in that country (Hwang, 2004; Lee et al., 2011). Forty two of these cultivars were shared with the International Transit Center (ITC) of Bioversity for inclusion in the National Repository, Multiplication, Distribution Centers (NRMDCs) created by the Banana Asia Pacific Network (BAPNET) for national evaluation programs (Molina, 2004). Through a public-private partnership, preliminary trials were carried out by Bioversity and the Lapanday Fruits and Development Corp. 'GCTCV-119' proved to be highly resistant to Foc TR4 compared to the commercial cultivar 'Grand Naine' (Molina et al., 2011). Subsequent replicated field trials were then carried out including more resistant somaclones as well as local cultivars.

This paper reports the results of a field trial (under commercial plantation setting) of introduced somaclonal Cavendish selections from TBRI, comparing them with regular Cavendish cultivars and some local non-Cavendish cultivars. Results of the study will be of great value in the attempt to mitigate the threat of Foc epidemics in the country.

MATERIALS AND METHODS

Trials were established in January 2011 in a field, which had previously a high level of incidence of TR4 within the plantation of the Lapanday Foods Corporation, Callawa, Davao City, Philippines. Eight banana cultivars (Table 1) were evaluated. Experimental units consisted of 10 tissue-culture derived plantlets, and were replicated 10 times, arranged in a completely randomized design. A total of 100 plants per cultivar were evaluated.

Disease incidence was assessed and recorded weekly by determining the number of infected plants, showing typical external symptoms of Fusarium wilt, like yellowing of older leaves and/or pseudostem splitting. To confirm infection, the pseudostems of suspected plants were cut transversely to examine the confirmatory reddish to dark brown internal vascular tissue discoloration. To confirm the identity of the pathogen, strands of infected pseudostem tissues were identified using the primer developed by Dita et al (2010).

RESULTS AND DISCUSSION

Results confirm the high susceptibility of commercial Cavendish cultivars (Table 2). ‘Grand Naine’ succumbed to the disease with an average 65% and 79% incidence in the primary and ratoon crop respectively. The GCTCVs suffered very low levels of disease incidence ranging from 0-6% in the primary crop. Incidence did not increase into the ratoon crop indicating stability of resistance of the GCTCVs (Table 2). Among the local cultivars, the popular cooking cultivar ‘Cardava’ (BBB genome, Saba subgroup) showed high resistance as it remained free from *Fusarium* wilt incidence, even in the ratoon crop. On the other hand, ‘Lakatan’ (AAA genome, Lakatan subgroup) and ‘Latundan’ (AAB genome, Silk subgroup), two popular local dessert cultivars, showed high levels of susceptibility with 76-92% and 60-70% disease incidence in the primary and the ratoon crop respectively. The identity of Foc infections was confirmed to be VCG1213/16, the strain associated with Foc TR4.

All the GCTCVs were significantly more resistant to Foc TR4 than ‘Grand Naine’. Agronomic and yield data are yet being consolidated and analyzed at the time of this report. However, in previous studies (Molina et al., 2011), ‘GCTCV-119’ had a relatively lower yield than ‘Grand Naine’ but acceptable fruit quality. Moreover, ‘GCTCV-119’ was taller than ‘Grand Naine’ and its harvest maturity was a bit later. A similar trend was observed among the GCTCVs in the current study. The GCTCVs were sweeter compared to ‘Grand Naine’ as shown by their higher total soluble solids (TSS) and lower titratable acidity (TA) (Molina et al., 2011), indicating potential acceptance in the export market. These cultivars present great potential as components for an integrated management approach in managing TR4 for the export banana industry, especially for the small-scale Cavendish growers whose farms are severely damaged by epidemics of Foc TR4.

CONCLUSIONS

Results of this study confirm the stability of resistant somaclones to Foc TR4, even in agro-ecosystems differing from those where these were first developed. It is also a proof that selection of favorable somaclonal variants is a practical and feasible approach in cultivar improvement, especially for the traded commercial Cavendish cultivar, where conventional and genetic engineering approaches have so far failed. Another important result of this study is the fact that ‘Cardava’ (synonym of Saba), a very important local cooking cultivar, proved to be highly resistant to TR4. This proves that there are TR4-resistant cultivars, contrary to the earlier belief that TR4 is so virulent that it severely affects all cultivars. The GCTCVs have strong potential as an important component of an integrated Foc TR4 management. The reduced incidence of infection would result in preventing damage and yield loss, and reducing inoculum production thus limiting the rate of epidemic spread and build up.

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Tables

Table 1. List of cultivars evaluated for resistance to *Fusarium oxysporum* f. sp. *ubense* Tropical race 4.

Cultivars	Genome	Subgroup	Description
Grand Naine	AAA	Cavendish	Popular export cultivar
GCTCV-105	AAA	Cavendish	Somaclone selection from Taiwan
GCTCV-119	AAA	Cavendish	Somaclone selection from Taiwan
GCTCV-219	AAA	Cavendish	Selection from GCTCV 119
GCTCV-218	AAA	Cavendish	Somaclone selection from Taiwan

Lakatan	AAA	Lakatan	Popular local dessert type banana
Latundan	AAB	Silk	Popular local dessert type banana
Cardava	BBB	Saba	Popular local cooking type banana

Table 2. Fusarium wilt incidence in selected banana cultivars in Callawa Davao City from 2011 to 2013.

Cultivars	Fusarium wilt incidence in Callawa (%)	
	Plant crop	Ratoon crop
Grand Naine	64	79
GCTCV-105	3	8
GCTCV-119	0	0
GCTCV-219	1	1
GCTCV-218	6	6
Lakatan	76	92
Latundan	60	70
Cardava	0	0