A Greenhouse Bioassay for the *Fusarium oxysporum* f. sp. *cubense* x 'Grand Naine' (*Musa*, AAA, Cavendish Subgroup) Interaction

M.A. Dita[1,2], C. Waalwijk[2], L.V. Paiva[3], M.T. Souza Jr.[2,4] and G.H.J. Kema[2]
[1]Embrapa Cassava & Tropical Fruits, Cruz das Almas, 44380-000, Bahia, Brazil
[2]Plant Research International, 6708 PB, Wageningen, The Netherlands
[3]Universidade Federal de Lavras, Caixa Postal 3037, Lavras- MG, Brazil
[4]Embrapa LABEX Europe, 6708 PB, Wageningen, The Netherlands

Keywords: Fusarium wilt, Panama disease, tissue culture

Abstract

Several disease resistance screening protocols for Fusarium wilt of banana (causal agent *Fusarium oxysporum* f. sp. *cubense* - Foc) under greenhouse conditions have been reported. Here, we report a standardised rapid and reliable greenhouse bioassay for this pathosystem. This is indispensable for banana phenotyping, particularly since the occurrence of tropical race 4 (TR4), which is a significant threat for the global Cavendish-based banana export industry. Using a double-pot system, hardened 3-months-old tissue-culture plants of 'Grand Naine' (AAA, Cavendish subgroup) were individually inoculated with three TR4 isolates and one race 1 isolate with known pathogenicity on 'Silk' (AAB) bananas. All TR4 isolates caused similar symptoms and no differences regarding incubation period or severity were observed. In addition, all TR4 isolates were successfully recovered from the symptomatic rhizomes on Komada medium.

INTRODUCTION

Host resistance is the most efficient and desirable measure to manage Fusarium wilt of banana, caused by *Fusarium oxysporum* f. sp. *cubense* (Foc), because no known chemical or cultural methods are available to effectively control the pathogen. This is clearly exemplified by genotypes of the Cavendish subgroup (AAA) that were massively introduced since the middle of the past century to control Foc race 1, which devastated the 'Gros Michel' (AAA)-based banana industry. However, a new race known as tropical race 4 (TR4), which is highly virulent to Cavendish clones, was identified in Southeast Asia in the early 1990s. Although this race is still restricted to Southeast Asia, it is currently considered a major threat to global banana production (Molina et al., 2008; Ploetz, 2006). Development of resistant cultivars requires a reliable phenotyping assay during the breeding process. However, a standard and worldwide accepted methodology is still undefined (Smith et al., 2008). Currently, selection for Foc resistance in banana breeding programmes, such as the one at Embrapa, is field based and can take up to 3 years. In addition, field-based phenotyping procedures render variable results due to heterogeneous inoculum distribution and interactions with other soil microorganisms. Greenhouse phenotyping protocols circumvent these disadvantages as well as quarantine restrictions, enabling rapid and safe collection of disease data outside banana-growing areas. Here, we report a reliable and rapid greenhouse bioassay for the Foc-*Musa* interaction.

MATERIALS AND METHODS

Hardened 3-month-old tissue-culture plants of 'Grand Naine' (AAA, Cavendish subgroup) were used as plant material. For inoculum production, 500-ml Erlenmeyer flasks containing 250 ml of mungbean liquid medium were inoculated with three mycelial plugs of Foc from 5-day-old colonies grown in Potato-Dextrose Agar (PDA). Inoculated flasks were incubated during 6 days (25°C, 200 rpm). In addition, Foc was grown in 250-ml flasks containing 50 g of sterilised maize kernels (25°C, 10 days).

Prior to inoculation, plants were removed from pots and soil was carefully removed. In order to enable efficient plant handling, roots were trimmed to a length of

~40 cm. Plants were individually inoculated with three Foc TR4 isolates (NRRL36114, Foc-II5 and BPS3.4) belonging to vegetative compatibility group (VCG) 01213, and one race 1 isolate, CNPMF08-R1, that is pathogenic on 'Silk' (AAB) banana. Inoculation was performed by root dipping (30 min, 10^5 conidia/ml) and transfer to 8-L pots partially filled with sterile water-river sand supplemented with 20 Foc precolonised maize kernels. Pots were then arranged according to the double-pot system (Mohamed et al., 2000) and were daily watered with tap water and weekly supplemented with 50 ml of Hoagland's solution. Uninoculated plants were used as control. The experiment was repeated three times using pots as experimental units with five replications according to a completely randomised block design. Disease assessments were performed from 7 to 40 days after inoculation (dai). During each evaluation, one plant was removed for the evaluation of internal symptoms and rhizome discolouration. All the experiments were performed at 28°C and 80% relativity humidity.

RESULTS AND DISCUSSION

The developed phenotyping assay resulted in consistent Fusarium wilt symptom expression in 'Grand Naine' with all TR4 isolates. Wilting already occurred at 7 dai, and the typical external (yellowing) and internal (rhizome discolouration) symptoms appeared at 14 dai (Figs. 1 and 2). Additional typical symptoms of Fusarium wilt, such as splitting of the pseudostem and shortening of the emerging leaves, were also observed (Fig. 1). In contrast to other reported bioassays (Smith et al., 2008; Mohamed et al., 2000; Ribeiro et al., 2011), the incubation period of TR4 was short (7 dai), even in older plants. This suggests an augmented virulence of Foc TR4 on 'Grand Naine' compared to compatible interactions involving Foc race 1.

All the TR4 isolates caused similar symptoms, and no differences regarding incubation period or severity were observed (data not shown). In addition, all TR4 isolates were successfully recovered from the symptomatic rhizomes on Komada medium and the identity was confirmed by a TR4 molecular diagnostic (Waalwijk et al., 2011). At 40 dai, TR4-inoculated plants showed severe wilting and internal necrosis, even in the pseudostem (Figs. 1 and 2). No symptoms were observed in plants inoculated with Foc race 1. These results confirm the compatible and incompatible interactions of 'Grand Naine' with Foc TR4 and race 1, respectively. The latter strain was compatible on 'Silk' plants (Ribeiro et al., 2011).

CONCLUSIONS

The described assay demonstrates that rapid and reliable phenotyping for Foc host reaction is possible under greenhouse conditions. The high inoculum pressure and the stress during the inoculation process did not interfere with the resistance response as incompatibility of race 1 with 'Grand Naine' was confirmed. Hence, the bioassay is an excellent tool to perform high-throughput phenotyping screens and also enables detailed plant-pathogen interaction studies.

ACKNOWLEDGEMENTS

We thank Kerry O'Donnell (National Center for Agricultural Utilization Research, USA), Ivan Buddenhagen and Corby Kistler (ARS-USDA, USA) for providing isolates used in this study. Dr. Miguel A. Dita gratefully acknowledges a CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) post-doctoral fellowship. This research is partially funded by the Dutch Dioraphte Foundation and the EU Endure program.

Literature Cited

Mohamed, A.A., Mak, C., Liew, K.W. and Ho, Y.W. 2000. Early evaluation of banana plants at nursery stage for Fusarium wilt tolerance. p.174-185. In: A.B. Molina, N.K. Masdek and K.W. Liew (eds.), Banana Fusarium Wilt Management: towards Sustainable Cultivation, Proc. Int. Workshop on the Banana Fusarium Wilt Disease, Genting Highlands Resort, Malaysia, 18-20 October 1999.

Molina, A.B., Fabregar, E.G., Sinohin, V., Fourie, G. and Viljoen, A. 2008. Tropical race 4 of *Fusarium oxysporum* f. sp *cubense* causing new Panama wilt epidemics in Cavendish varieties in the Philippines. Phytopathology 98:S108-S108.

Ploetz, R.C. 2006. Fusarium wilt of banana is caused by several pathogens referred to as *Fusarium oxysporum* f. sp *cubense*. Phytopathology 96:653-656.

Ribeiro, L.R., Amorim, E.P., Cordeiro, Z.M., Silva, S. de Oliveira e and Dita, M.A. 2011. Discrimination of banana genotypes for Fusarium wilt resistance in greenhouse. Acta Hort. 897:381-385.

Smith, L.J., Smith, M.K., Tree, D., O'Keefe, D. and Galea, V.J. 2008. Development of a small-plant bioassay to assess banana grown from tissue culture for consistent infection by *Fusarium oxysporum* f. sp. *cubense*. Australas. Plant Path. 37:171-179.

Waalwijk, C., Dita, M.A., Buddenhagen, I., Paiva, L.V., Souza Jr., M.T. and Kema, G.H.J. 2011. A detection method for tropical race 4 of the banana pathogen *Fusarium oxysporum* f. sp. *cubense* Acta Hort. 897:339-343.

Figures

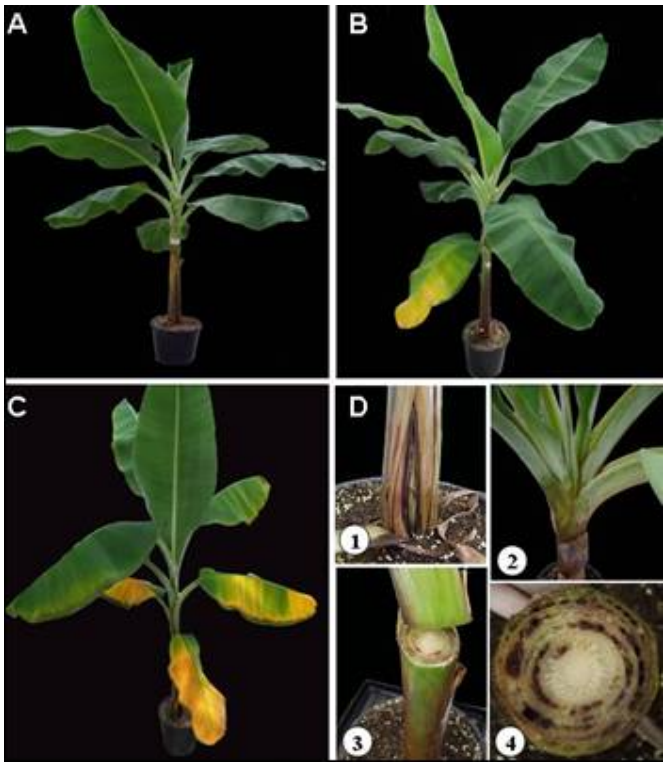

Fig. 1. 'Grand Naine' (AAA, Cavendish) banana plants inoculated with *Fusarium oxysporum* f. sp. *cubense* tropical race 4 (TR4). A. Non-inoculated (control) plant; B. Plant showing initial yellowing in the older leaf at 14 days after inoculation (dai); C. Plant showing intense yellowing at 21 dai; D. Panel showing different symptoms observed in TR4-inoculated plants (1, splitting at the pseudostem base; 2, shortening of the emerging leaves; 3 and 4, internal necrosis in the pseudostem).

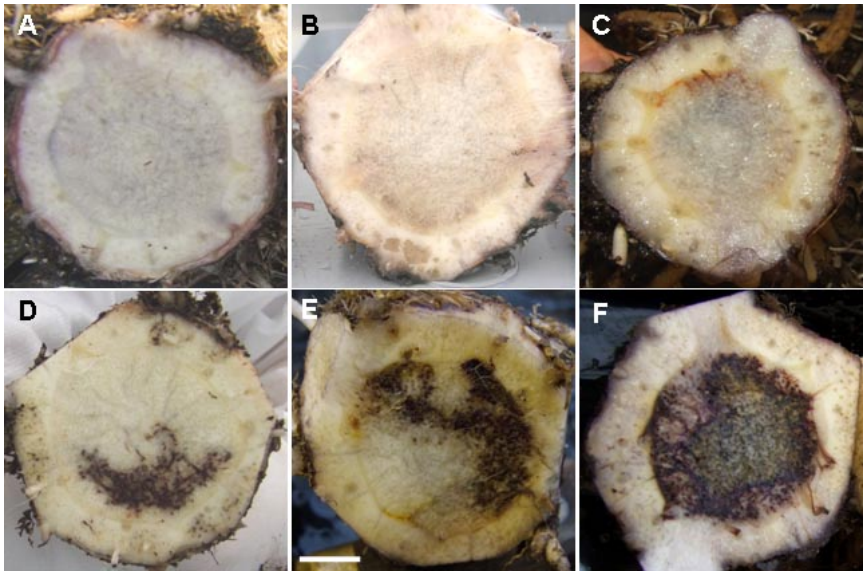

Fig. 2. Rhizome discoloration caused by *Fusarium oxysporum* f. sp. *cubense* on 'Grand Naine' (AAA, Cavendish) banana plants. A. Non-inoculated (control) plant; B, Plant inoculated with race 1 isolate; C-F. Rhizome discolouration caused by tropical race 4 at 7, 14, 21 and 40 days after inoculation, respectively. Bar = 2 cm.