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Systemic acquired resistance in Cavendish banana induced by infection with an incompatible strain of *Fusarium oxysporum* f. sp. *cubense*

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ABSTRACT

Fusarium wilt of banana is caused by the soil-borne fungus *Fusarium oxysporum* f. sp. *cubense* (*Foc*). The fact that there are no economically viable biological, chemical, or cultural measures of controlling the disease in an infected field leads to search for alternative strategies involving activation of the plant's innate defense system. The mechanisms underlying systemic acquired resistance (SAR) are much less understood in monocots than in dicots. Since systemic protection of plants by attenuated or avirulent pathogens is a typical SAR response, the establishment of a biologically induced SAR model in banana is helpful to investigate the mechanism of SAR to Fusarium wilt. This paper described one such model using incompatible *Foc* race 1 to induce resistance against *Foc* tropical race 4 in an *in vitro* pathosystem. Consistent with the observation that the SAR provided the highest level of protection when the time interval between primary infection and challenge inoculation was 10 d, the activities of defense-related enzymes such as phenylalanine ammonia lyase (PAL, EC 4.3.1.5), peroxidase (POD, EC 1.11.1.7), polyphenol oxidase (PPO, EC 1.14.18.1), and superoxide dismutase (SOD, EC 1.15.1.1) in systemic tissues also reached the maximum level and were 2.00–2.43 times higher than that of the corresponding controls on the tenth day. The total salicylic acid (SA) content in roots of banana plantlets increased from about 1 to more than 5 $\mu g\,g^{-1}$ FW after the second leaf being inoculated with *Foc* race 1. The systemic up-regulation of *MaNPR1A* and *MaNPR1B* was followed by the second up-regulation of *PR-1* and *PR-3*. Although SA and jasmonic acid (JA)/ethylene (ET) signaling are mostly antagonistic, systemic expression of *PR* genes regulated by different signaling pathways were simultaneously up-regulated after primary infection, indicating that both pathways are involved in the activation of the SAR.

Introduction

Fusarium wilt of banana caused by the soil-borne fungus *Fusarium oxysporum* f. sp. *cubense* (*Foc*) is recognized as one of the most destructive diseases of banana (Ploetz, 2006). Three races (1, 2, and 4) of *Foc* affect edible banana cultivars, while race 3 only affects *Heliconia* (Waite, 1963). Race 1 attacks 'Gros Michel' (*Musa* AAA Gros Michel subgroup) and 'Silk' (*Musa* AAB group). In the 1950s, 'Gros Michel'-based banana export industry was devastated because of the spreading of race 1. Finally, 'Gros Michel' was replaced with cultivars in the Cavendish subgroup, which are still the most common cultivars in commercial production to date. Race 4 was discovered in Taiwan in the 1960s (Hwang and Ko, 2004), it not only attacks Cavendish cultivars but also cultivars susceptible to race 1 and 2. Strains of race 4 are separated into subtropical race 4 and tropical race 4. Although they affect many of the same cultivars, subtropical race 4 only affects plants in the areas with pronounced winters, tropical race 4 affects plants in the absence of predisposing factors (Ploetz, 2006). Since tropical race 4 continues spreading in Southeast Asia, it poses a serious threat to a multibillion dollar industry and the food stability in developing countries. The fact that there are no economically viable biological, chemical or cultural measures of controlling Fusarium wilt in an infected field (Ploetz,

Abbreviations: BTH, benzothiadiazole; ET, ethylene; ETI, effector-triggered immunity; *Foc*, *Fusarium oxysporum* f. sp. *cubense*; FW, fresh weight; HR, hypersensitive response; IAA, indoleacetic acid; INA, 2,6-dichloroisonicotinic acid; ISR, induced systemic resistance; JA, jasmonic acid; MIS, medium for interaction system; MS, Murashige and Skoog medium; NBT, nitroblue tetrazolium; nkat, nanokatals; *NPR1*, nonexpressor of pathogenesis-related genes 1; PAL, phenylalanine ammonia lyase; PAMPs, pathogen associated molecular patterns; PGPR, plant growth-promoting rhizobacteria; pkat, picokatals; POD, peroxidase; PPO, polyphenol oxidase; *PR* gene, pathogenesis-related gene; PRRs, pattern-recognition receptors; PTI, pattern-triggered immunity; SA, salicylic acid; SAR, systemic acquired resistance; SOD, superoxide dismutase.

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2006; Buddenhagen, 2009) leads to search for alternative strategies involving activation of the plant's innate defense system.

Plants have developed a two-layered innate immune system for defense against pathogens. The first line of defense of plants is pattern-triggered immunity (PTI), which is achieved through recognition of pathogen associated molecular patterns (PAMPs) by trans-membrane pattern-recognition receptors (PRRs) (Jones and Dangl, 2006). In response to pathogens that can suppress basal defense, plants have also evolved resistance (*R*) genes that encode cytoplasmic receptors that recognize race-specific pathogen effectors, activating a secondary defense response, effector-triggered immunity (ETI). ETI hence results in gene-for-gene resistance and a hypersensitive response (HR) at the infection site (Jones and Dangl, 2006). Upon pathogen recognition by plants, several signal transduction pathways are activated. The role of the signaling pathway mediated by salicylic acid (SA), jasmonic acid (JA), and ethylene (ET) in the activation of plant defense responses against pathogens is well established (Robert-Seilaniantz et al., 2011). These modulators then control the expression of sets of downstream defense genes encoding antimicrobial proteins or enzymes catalyzing the production of defense metabolites.

According to their lifestyles, plant pathogens are generally divided into biotrophs, necrotrophs, and hemibiotrophs. Biotrophs feed on living host tissue, while necrotrophs kill host tissue and feed on the remains. Hemibiotrophs undergo two distinct phases during invasion, an initial biotrophic phase followed by necrotrophic phase (Perfect and Green, 2001). Although often considered a necrotroph, the means by which *F. oxysporum* infects and promotes diseases suggests that it is better classified as a hemibiotroph (Agrios, 2005). From studies on *Arabidopsis*, McDowell and Dangl (2000) suggested that the lifestyle of the pathogen might be a predictor of plant defense responses, with plant resistance to biotrophic pathogens being mediated through SA signaling, and plant resistance to necrotrophic pathogens being mediated through JA/ET signaling. SA-dependent defense is associated with production of a HR, which is a response generally considered a form of programmed cell death. The HR may restrict the growth of a pathogen that has invaded a living cell but can fail to restrict necrotrophic pathogens (Govrin and Levine, 2000), which derive nutrients from dead or dying cells. Further testing has revealed that while this model is generally true, there are exceptions and additional complexities (Glazebrook, 2005).

Besides basal resistance responses that act at the infection site, plants are capable of developing a systemic resistance that is effective against further pathogen infections. Systemic acquired resistance (SAR) is an inducible defense mechanism in plants, resulting from an incompatible reaction with a pathogen or non-pathogen, which confers immunity to a broad spectrum of pathogens (Durrant and Dong, 2004). Biologically induced SAR has been reported in several plant species (Cameron et al., 1994; Mauch-Mani and Slusarenko, 1994; Summermatter et al., 1995; Dann and Deverall, 1995, 2000; Díaz et al., 2005), most of them are dicots. There are very few reports on biologically induced SAR in monocots. Being one of monocots, banana and plantain are famous tropical and subtropical fruit around the world, and also staple food in developing countries. It is reported that exogenous application of plant growth regulators such as indoleacetic acid (IAA) (Fernández-Falcón et al., 2003) and menadione sodium bisulphite (Vitamin K_3) (Borges et al., 2004) induce resistance to Fusarium wilt of banana, while biologically induced SAR to the disease has not been described.

SAR requires the signal molecule SA and is manifested by the induction of a subgroup of the pathogenesis-related (*PR*) genes in systemic tissues. It is demonstrated that elevated levels of endogenous SA in the uninfected portions of the plant is essential for the activation of SAR, but SA itself is not the mobile signal (Park et al., 2007). Consistent with the key role of SA, SAR can also be induced by exogenous SA and its functional analogues, such as benzothiadiazole (BTH), and 2,6-dichloroisonicotinic acid (INA). The chemicals are translocated throughout the plant (Oostendorp et al., 2001), and as such chemical induction of SAR does not completely reflect the situation during biological induction of the response. The SA-dependent expression of *PR* genes also relies on the nonexpressor of pathogenesis-related genes1 (*NPR1*), which has been identified as a positive key regulator of SAR. *Arabidopsis* plants with a defective *NPR1* gene are unable to induce the expression of *PR* genes, and are compromised in disease resistance in response to SA (Durrant and Dong, 2004). Over-expression of *Arabidopsis NPR1* in rice (Chern et al., 2001) and wheat (Makandar et al., 2006) confers enhanced resistance to fungal, viral, and bacterial pathogens. In recent years, two *PR* genes (Van den Berg et al., 2007) and two *NPR1*-like genes (Endah et al., 2008) have been isolated from Cavendish banana and no data exist about the expression profile of these four genes at the onset of SAR.

The mechanisms underlying SAR are much less understood in monocots than in dicots. In rice, the non-host pathogen *Pseudomonas syringae* pv. *syringae*, that activates resistance to rice blast, induces a gene expression pattern that clearly differs from those induced by BTH and INA (Schweizer et al., 1999). Likewise, gene expression profiles induced by incompatible *Erysiphe graminis* f. sp. *hordei* in barley and wheat overlap only slightly with those induced by BTH and INA (Schaffrath et al., 1997; Beßer et al., 2000; Jansen et al., 2005). Since systemic protection of plants by attenuated or avirulent pathogens is a typical SAR response, the establishment of a biologically induced SAR model in banana is helpful to investigate the mechanism of SAR to Fusarium wilt. The objective of this study was to establish one such model using incompatible *Foc* race 1 to induce resistance against further infection by *Foc* tropical race 4 in an *in vitro* pathosystem recently reported by Wu et al. (2010), and to study the mechanism of biologically induced SAR at biochemical and molecular level.

Materials and methods

Plant material and inoculum preparation

Suckers of 'Brazil Xiangjiao' (*Musa* AAA Cavendish subgroup), which is not affected by *Foc* race 1 but highly susceptible to *Foc* tropical race 4, were micropropagated using shoot-tip meristem culture (Arinaitwe et al., 1999). Rooted plantlets were then transferred to 150 mL Erlenmeyer flasks containing modified medium for interaction system (MIS), one plantlet in each flask. MIS consisted of half-strength MS (Murashige and Skoog, 1962) salts and 6 g L^{-1} agar. After growing on MIS for 1–2 weeks, plantlets for primary infection were selected according to the morphological standards proposed by Wu et al. (2010).

One isolate of *Foc* race 1 (VCG 0124/5, ACC 31277) and one isolate of *Foc* tropical race 4 (VCG 01213, ACC 31282) were procured from the Agricultural Culture Collection of China, which were collected from diseased Fenjiao (*Musa* ABB group Pisang Awak) and Xiangyajiao (*Musa* AAA Cavendish subgroup), respectively, in Hainan Province in 2005. For primary infection and challenge inoculation, spore suspensions of *Foc* race 1 and tropical race 4 at a concentration of 10^6 conidia mL^{-1} were prepared, respectively, as previously described (Wu et al., 2010).

Primary infection and challenge inoculation

Before primary infection, 5 mm diameter filter paper discs were soaked in a conidial suspension of *Foc* race 1. Then the second fully expanded leaf from the top of plantlet was stabbed with a syringe