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Full Length Research Paper

Tolerance of banana for fusarium wilt is associated with early H_2O_2 accumulation in the roots

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Banana plants derived from a tissue culture process possess a high rate of random variations that were widely used as popular cultivars due to the new desired traits. In this study, two near-isogenic lines, one susceptible (parental Williams-8818) and the other resistant (somaclonal variation progeny Williams-8818-1 from Williams-8818) to *Fusarium oxysporum* f. sp. *Cubense* (Foc4), were inoculated with race 4 of *F. oxysporum* (Fox). Production of $O_2^{\cdot-}$, H_2O_2 and MDA, as well as changes in enzyme activities, and transcript levels of SOD and CAT in root extracts were monitored every 24 h over 4 days. The histochemical location of H_2O_2 was also detected. In the resistant iso-line, the accumulation of $O_2^{\cdot-}$ and H_2O_2, and the activation of SOD occurred in the first 24 h, but activation of CAT reached its maximum only after 48 h. All changes were generally lower in the susceptible iso-line when compared to the resistant iso-line. SOD transcripts were further up-regulated until 72 h in the resistant iso-line, but not in the susceptible iso-line. CAT expression was not affected in any of the two iso-lines. This suggests that expressions of the two key genes in the antioxidant system are less suitable indicators for Foc resistance in banana. In contrast, the first "oxidative burst" is a better indicator for different susceptibility of banana to Foc.

Key words: Banana, *Fusarium oxysporum*, catalase, reactive oxygen species, somaclonal variation, disease resistance.

INTRODUCTION

Banana (*Musa acuminate*) is one of the most important food crops in the world. It provides a staple food and source of income in many households especially in Africa (Jones, 2000). However, in the past decades, banana production world-wide was under the threat of *Fusarium oxysporum*, a plant fungal pathogen causing root rot, or wilt diseases in several plant species, such as tomato (*Solanum lycopersicum*), banana, and asparagus (*Asparagus officinal*is) (Di Pietro, 2003). *F. oxysporum* Schlechtend.:Fr. f. sp. c*ubense* (E.F. Smith) Snyder & Hansen (Foc) is the major banana colonizer. It was first reported in Panama as early as 1890, and is now commonly known as the causal agent of the Panama disease. Among all the races, race 4 of *F. oxysporum* f. sp. *cubense* (Foc4) is the most notorious slaughterer, which destroyed thousands of hectares of bananas in tropical and subtropical countries, such as Indonesia, Malaysia, India, Vietnam and China (Hwang and Ko, 2004; Viljoen, 2002).

Typically, the first symptom of infected plant is yellowing of the lower leaves. This begins along the margin, and sprawls toward the midribs. The leaf subsequently develops brown spots of various shapes and sizes, and the petiole turns brown and buckles. Yellowing and buckling progresses from older to younger leaves resulting to the death of the entire plant (Su et al., 1986). Once it invades its hosts, Foc can persist in affected fields for an extended period of time on plant surfaces as macroconidia, or even survive on soils as dormant chlamydospores in the absence of a suitable host plant, rendering the plots unsuitable for banana planting for several years (Berrocal-Lobo and Molina, 2007). Many efforts have been made to deal with this disease. Unfortunately, neither fungicide application, nor practice system, nor biological control with antagonistic bacteria

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and fungi has received an optimum and grower-acceptable result, though the disease was inhibited to some extent. Therefore, it is now generally believed that the development of new resistant banana cultivars is the best way for the successful control of this disastrous disease.

Theoretically, disease resistant cultivars could be generated by means of conventional breeding, genetic transformation and somatic variation. However, cultivated banana varieties are mostly triploid and can only be propagated asexually, which makes them difficult to be improved genetically via cross-pollination (Pei et al., 2005). Moreover, this process is time-consuming and the final product often suffers from reluctance when recommended to consumers. Similarly, practices of the second method have also been hindered by the lack of resistance mechanisms, resistance markers, genetics of resistance, available resistance genes, and the poor regeneration rate during transformation (Kiggundu, 1999). In contrast, the third method is unexpectedly effective. In the past two decades, it has been widely used in countries like South Africa (De Beer et al., 2001), Malaysia (Ho et al., 2001) and China to obtain fusarium wilt resistant clones from their native banana cultivars, example two clones, GCTCV-215-1 and GCTCV-218 successfully rescued the banana industry in Taiwan from Foc4 damage (Hwang and Ko, 2004). However, this useful method is far from perfection due to its time-consuming and low efficiency in the early selection stage. To get rid of these obstacles requires probing into the basis of disease resistance and characterizing the early responses of plant attacked by *F. oxysporum*.

As it is well know, plants ward off pathogen infections by eliciting a wide array of defense strategies, which include reinforcement of the cell wall (Bradley et al., 1992), synthesis of phytoalexins (Hahlbrock and Scheel, 1989), and expression of defense-related genes (Bowles, 1990; Dixon and Harrison, 1990). These responses are usually associated with a rapid and transient production of reactive oxygen species (ROS), such as the superoxide anion radical ($O_2^{\bullet-}$), hydroxyl radical ($^{\bullet}OH$) and hydrogen peroxide (H_2O_2). H_2O_2 is known to be an early messenger molecule in signaling cascades activated by several external and developmental stimuli (Lamb and Dixon, 1997; Bolwell, 1999). In the case of plant-pathogen interactions, it not only qualifies as the oxidative agent in killing, or inhibiting the growth of pathogens, but also it is centrally involve in the induction of pathogen defense genes, such as genes encoding pathogenesis-related (PR) proteins, genes regulating accumulation of phenylpropanoid compounds, and genes encoding ROS detoxifying enzymes (Levine et al., 1994; Schenk et al., 2000). For example, elevated levels of H_2O_2 mediated resistance to soft rot caused by *Erwinia carotovora* sp. *carotovara* in transgenic potato plants (Wu et al., 1995); early H_2O_2 accumulation in mesophyll cells led to hypersensitive cell death in resistance barley lines attacked by *Blumeria graminis* (Vanacker et al., 2000); early H_2O_2 accumulation was common in regulating gene expression and cell death induced by *Pseudomonas syringae* pv *syringae* in birch (Pellinen et al., 2002).

To date, plant-Foc interaction has been studied profusely in tomato. El-Khallal (2007) reported that production of ROS, mainly H_2O_2 and $O_2^{\bullet-}$, and activity of antioxidant enzymes (SOD, APX and CAT), increased in tomato leaves in the late stage of infection; Farag Hanaa et al. (2011) also found the increasing level of lipid peroxidation and marked increase in the activities of antioxidant defensive enzyme, that is, POX, CAT, and SOD in seedling plants. However, the early events of plant infection by Foc seem quite different. For example, ROS was quickly produced following Fox infection in *Arabidopsis*, which mediated cell death and might contribute to disease development (Berrocal-Lobo and Molina, 2007); whereas a rapid activation of anti-oxidative system was associated with restriction of *Fusarium oxysporum* f. sp. *asparagi* growth (He et al., 2001). These contradictory results indicate that the oxygen radical production and activation of the anti-oxidative system might have different effects depending on the interaction.

In order to establish biochemical markers for the identification of Foc resistance banana clones, Kavino et al. (2007) documented the roles of series of oxidative enzymes like peroxidase (PO), polyphenol oxidase (PPO), superoxide dismutase (SOD) and catalase at the late stage of infection, finding that their activity were relatively higher in resistant hybrids than susceptible hybrids. Obviously, the events that occurred in the very early stage of infection might be the more key factors to determine a clone as the resistance one or not. As known, these early responses have not been reported in banana.

Williams-8818 is a popular banana cultivar wildly planted in China, but it is susceptible to Foc. In order to breed resistant line, we carried out a somaclonal variation project during which ethyl methane sulfonate (EMS) was used to mutate the Williams-8818 explants in tissue culture.

The survival plantlets from EMS treated experiments were planted in soil with controlled inoculum density in glasshouse for the selection of disease resistant clones. In turn, the second-, and third-generation (suckers) from the resistant clones were planted again in infected field for further test of the stability of resistance and other selected traits. Finally, a line named as Williams-8818-1 was obtained for its effectiveness in disease resistance in comparison with the GCTCV-218, and good agricultural traits comparable with its parental Williams-8818. Here, we reported that these two banana lines exhibited quite different responses against Foc4 during the early infection stage.

MATERIALS AND METHODS

Race 4 of *F. oxysporum* f. sp. *Cubense* was collected from infested field in Xuwen city, Guangdong province, China. Two banana iso-

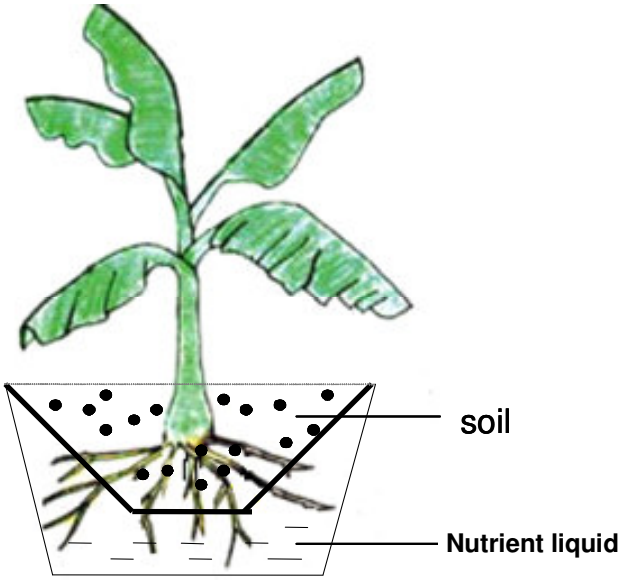

Figure 1. A schematic view for double-cup culture system.

lines were used for this experiment: a susceptible iso-line (the parental Williams-8818), and a resistant iso-line (Williams-8818-1), which is a somaclonal variation progeny from Williams-8818.

Treatment

Adventitious shoots of both susceptible and resistant iso-lines were propagated by tissue culture. Rooting seedlings were first planted in small plastic cups (250 mL) in glasshouse, T = 24 to 36℃. After three months, morphological identical plantlets, which produced about seven leaves, were transplanted to bigger plastic cups (2000 mL) for further growth for about two weeks. All the plantlets were later on finally cultivated using the "double-cup cultural system"——a device consisting of two plastic cups, of which a shallow inner cup (2000 mL) is suspending on a deep outer cup (3000 mL). The inner cup contains a plantlet, and the outer one holds about 800 mL MS (Murashige and Skoog, 1962) medium at its bottom (Figure 1). This device enables the plantlet roots to go through the holes of inner cup, and grow well within the nutrient liquid of the outer cup.

Three weeks later, plantlets with well-developed roots were deemed suitable for inoculation. After gently crushing for wounds, the plantlet root system was inoculated by totally immerging into a spore suspension (5×10^6 spores mL^{-1}) for 5 min. Sterile distilled water was replaced in the control plantlets. Inoculated plantlets were replaced into the double-cup system containing 800 mL MS medium and kept in a separate greenhouse (T = 24 to 36℃) for root sampling (0, 24, 48, 72, and 96 h). Each sample consisted of six plantlets. The whole experiment was repeated three times

Assay of $O_2^{\bullet-}$ production rate in roots

The method described by Elstner and Heupe (1976) was used to determine superoxide free radical ($O_2^{\bullet-}$) generation rate by monitoring the nitrite formation from hydroxylamine in the presence of $O_2^{\bullet-}$. The absorbance in the aqueous solution was read at 530 nm. A standard curve with NO_2^- was used to calculate the production rate of $O_2^{\bullet-}$ from the chemical reaction between $O_2^{\bullet-}$ and hydroxylamine.

Assays of hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) contents in roots

Extraction of H_2O_2 from roots was done as described by Rao et al. (2000) with some modifications. Quantification of H_2O_2 was based on the method of Tana et al. (2009), and using an Amplex Red Hydrogen Peroxide Assay kit (Molecular Probes, Invitrogen Detection Technologies, Leiden, Netherlands). Briefly, 50 µL of extract was mixed with 50 µL of the solution containing 1 $U\cdot mL^{-1}$ horseradish peroxidase in 50 $mmoL\cdot L^{-1}$ sodium phosphate buffer, pH 7.4. This was incubated for 15 min at 25℃. Fluorescence was measured with a fluorescence microplate reader (Fluostar Optima, BMG Labtechnologies, Germany) equipped for excitation at 520 nm and emission at 590 nm. Blank readings were taken before the reaction was initialized (T = 0 min). The concentration of H_2O_2 was calculated using a standard curve obtained with known concentrations of pure H_2O_2 (Molecular Probes) diluted in 0.2 $moL\cdot L^{-1}$ $HClO_4$.

A modified thiobarbituric acid (TCA) reactive substance assay was used as an alternative assessment of lipid oxidation according to the method of Gutteridge and Wilkins (1982). Root (1.0 g) was ground in 80:20 ethanol:methanol (V/V) containing 0.01% (W/V) butylated hydrosytoluen (BHT). After centrifugation at 13000 × *g* for 15 min at 4℃, the supernatant was recovered (200 mL) and added to 1 mL of a solution of 20% (W/V) TCA and 0.01% (W/V) BHT containing 0.65% (W/V) thiobarbituric acid for 25 min at 95℃. After centrifugation, sample absorbance was measured at 532 nm. Blank measurements were performed using reagent solution without thiobarbituric acid. Nonspecific turbidity was subtracted from the 532 nm signal by using the measurements at 600 and 440 nm. The results were expressed as MDA equivalent.

Histochemical detection of H_2O_2 in root tips

Hydrogen peroxide production in roots was monitored using the H_2O_2-sensitive fluorescent probe H_2DCFDA (Sigma, St Louis, MO, USA) as described by Desikan et al. (2002, 2004b). Root sample was floated in MES-KCl buffer (10 mmoL/L MES, 5 $mmoL\cdot L^{-1}$ KCl, 50 $mmoL\cdot L^{-1}$ $CaCl_2$, pH 6.15) under light for 2 h, and then floated in the MES-KCl buffer containing 5 mm DMTU for scavenging of H_2O_2 under light for 25 min, followed by loading with 50 $mmoL\cdot L^{-1}$ H_2DCFDA for 15 min. After washing with MES-KCl buffer for 20 min, the epidermal fragments were visualized for imaging using Fluorescence microscope (OLYMPUS BX51, Tokyo, Japan).

Assays of superoxide dismutase (SOD) and catalase (CAT) activities in roots

Extraction of SOD and CAT in roots was based on the description of Mo et al. (2008). Roots (1.0 g) was ground in a mortar using a pestle in 5 mL of 50 $mmoL\cdot L^{-1}$ cool phosphate buffer (pH 7.8). The homogenate was centrifuged at 13 000 × *g* for 15 min at 4℃ and the supernatant was used for assays of antioxidant enzyme activities of SOD and CAT.

SOD activity was determined according to the method of Giannoplitis and Ries (1977) in a 3 mL reaction volume solution containing 13 $\mu moL\cdot L^{-1}$ methionine, 63 $\mu moL\cdot L^{-1}$ nitroblue tetrazoliumchloride (NBT), 1.3 $\mu moL\cdot L^{-1}$ riboflavin, 50 mmoL/L phosphate buffer (pH 7.8) and 50 µL enzymes extract. The reaction solution was incubated for 10 min under fluorescent light with 80 $\mu moL\cdot m^{-2}\cdot s^{-1}$. Absorbance at 560 nm was determined by spectrophotometer (Beckman DU-7, Beckman Co. Ltd., California, USA). Blank readings were taken before the reaction was initialized (T = 0 min). One unit of SOD activity was defined as the amount of enzyme required for inhibiting photochemical reduction of NBT by 50%.

CAT activity was determined using a spectrophotometer according to the method of Chance and Maehly (1955) in a 3 mL reaction volume solution containing 15 mmoL·L^{-1} H_2O_2, 50 mmoL·L^{-1} phosphate buffer (pH 7.0) and 100 µL of enzyme extract. The reaction was initiated by adding enzyme extract at 25℃ and determined with absorbance at 240 nm by Beckman DU-7. Readings were taken at 0 and 1 min. One unit of catalase activity is defined as that amount of enzyme which breaks down 1 µmoL H_2O_2·min^{-1} under the assay conditions described.

Quantitative RT-PCR check of SOD and CAT transcripts in roots

Quantitative real-time PCR was performed in triplicates with SYBR Green PCR Master Mix (TAKARA, Tokyo, Japan) using an iQ5 multicolor Real-Time PCR Detection System (BioRad, California, USA). Efficiency of each pair of primers was determined based on its standard curve obtained from a series of 4-fold diluted template cDNAs. The difference between the cycle threshold (Ct) of target gene and the Ct of *β-Actin* ($\triangle Ct = Ct_{\text{target gene}} - Ct_{\beta\text{-Actin}}$) was used to obtain the normalized expression of target gene, which corresponds to $2^{-\triangle Ct}$. This value was finally expressed as fold-change relative to a 'calibrator', which is the expression level for the particular transcript in control Williams-8818 roots at 0 h post innoculation (hpi). Primer sequences used for *β-Actin*, *SOD* and *CAT* were as follows: SODs (5'-CCGAGGTTCTCTTCACTTCATCC-3') and SODan (5'-GAGTTGCAGCCGTTGGTGGTATC-3') for *SOD* (Genbank accession number DQ866813); CATs (5'-ATGCAGAGA GATTCCCCATTCC-3') and CATans (5'-CTTGCCTATCAGGTGCC CAGGT-3') for *CAT* (Genbank accession number EU139298); ACTINs (5'-GCCATACAGTGCCAATCTACGAGG-3') and ACTINan (5'-ATGTCACGAACAATTTCCCGCTCA-3') for *β-Actin* (Genbank accession number AB022041).

Statistical analysis

Statistical analysis was conducted using SPSS V11.5 (SPSS Inc., Chicago, USA). After an analysis of variance (ANOVA), significant difference ($P < 0.05$) among means was determined by Duncan's multiple range tests. All values in the figures were shown as mean±standard deviation.

RESULTS

Early responses of ROS in root attacked by Foc4

In order to get insight into the early responses of ROS in banana inoculated with Foc4, two key members of radical chain reactions family ($O_2^{\bullet-}$ and H_2O_2) were tracked in the root systems of Williams-8818 and williams-8818-1. The results show that $O_2^{\bullet-}$ production rate increased sharply in Williams-8818-1, which got the highest level of 5.60 nmol g^{-1} FW min^{-1} at 24 hpi, then promptly dropped to its primal level at 48 hpi. By contrast, in Williams-8818, $O_2^{\bullet-}$ increased slowly within the first 24 h and then creped gently to the peak of 5.12 nmol g^{-1} FW min^{-1} at 72 hpi, the remaining unchanged at 96 hpi (Figure 2). Similarly, H_2O_2 content in williams-8818-1 rose swiftly from 0 to 24 hpi, by 0.14 to 0.32 nmol g^{-1} FW, then decreased to basal levels at 72 hpi. In the case of Williams-8818, there was a slower increase in H_2O_2 content until 72 hpi (Figure 3). Therefore, it was a resistant variety William-8818-1 rather than susceptible parental Williams-8818 that exhibited an early and quick "first oxidative burst", likely inducing disease resistance mechanisms to set in. To further confirm this event, we examined the visual imagine of H_2O_2 accumulation in root tip. Interestingly, fluorescence in Williams-8818-1 was little brighter than that in Williams-8818 at 0 hpi, but it was much stronger at 24 hpi (Figure 4). The changes correlated with that of $O_2^{\bullet-}$ production rate and H_2O_2 accumulation.

Early response of anti-oxidant activity in root systems

It is well know that SOD and CAT serve as key catalysts for H_2O_2 production and elimination in the plant antioxidant system. We therefore monitored their activities in root system in the early interaction stage. Both SOD and CAT activities first increased before declining in each iso-line. However, the increments were always higher in the resistant iso-line than in the susceptible iso-line during the whole phase tested. In detail, SOD activity in Williams-8818-1 ascended rapidly from 31.6 U g^{-1} FW h^{-1} at the start of the experiment to a peak value of 76.0 U g^{-1} FW h^{-1} at 24 hpi and then descended slightly at 48, 72 and 96 hpi, but without attaining basal levels. In variety Williams-8818 however, an increase in SOD activity began only after 24 hpi, slowing attaining a peak value of 54.7 U g^{-1} FW h^{-1} at 72 hpi (Figure 5). CAT activity in Williams-8818-1 increased within the first 48 h from 10.6 to 26.3 U g^{-1} FW min^{-1} then experienced a downward trend afterwards; whereby it peaked mush slowly first and then dropped to a value below the basal level in Williams-8818, that is, the values increased from 10.4 U g^{-1} FW min^{-1} at 0 hpi to 15.2 U g^{-1} FW min^{-1} at 48 hpi and then dropped to 6.4 U g^{-1} FW min^{-1} at 96 hpi (Figure 6).

Inoculation induced MDA production

Radical chain reaction results in lipid oxidation which in turn produces terminal products of MDA. Thus, accumulation of MDA is an indicator of membrane injury. Our results indicate that MDA content was increased continuously in Williams-8818-1 and Williams-8818 after inoculation, but the potential was quite different (Figure 7). At the beginning of the experiment, both Williams-8818 and Williams-8818-1 had basal content of 0.8 and 0.9 nmol g^{-1} FW respectively of MDA which increased to 2.2 and 1.3 nmol g^{-1} FW respectively at the end the fourth day, suggesting that Williams-8818-1 was better at maintaining membrane integrity.

SOD and CAT expressions on pathogen attack

In order to verify whether the expressions of H_2O_2-related

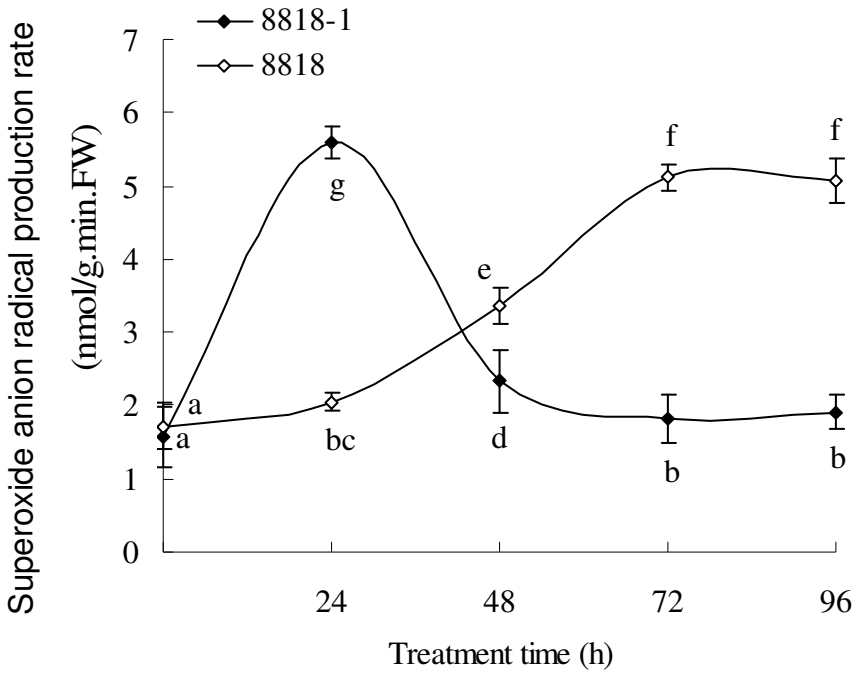

Figure 2. Effects of inoculation with Foc4 on the production rate of superoxide anion radical.

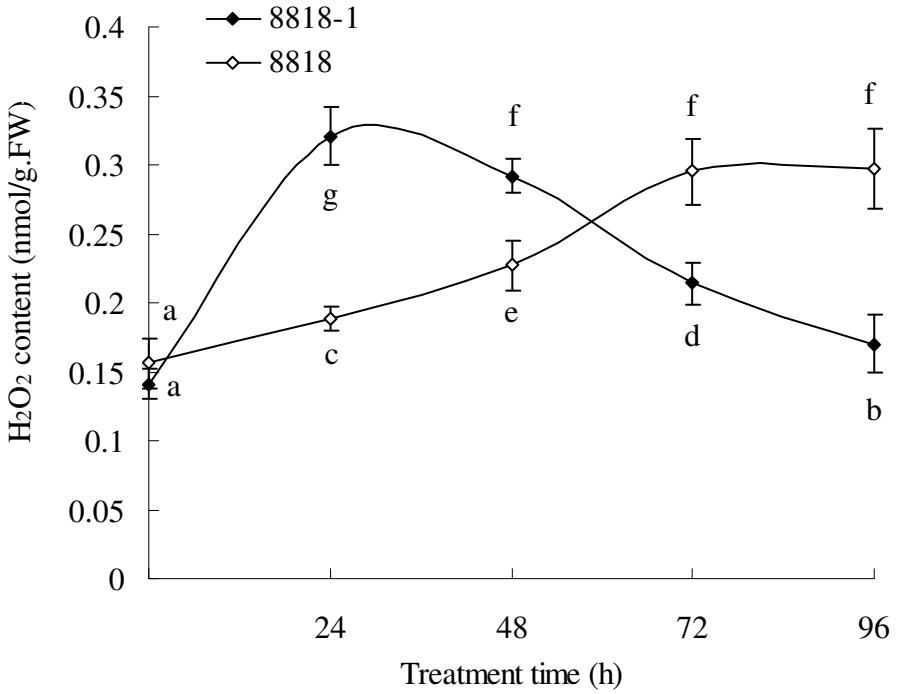

Figure 3. Effects of inoculation with Foc4 on the H_2O_2 content.

genes were quickly reprogrammed after inoculation, we further determined the *SOD* and *CAT* transcript levels. The results show that *SOD* transcripts in Williams-8818-1 were restricted at basal level in the first half period tested, followed by an abrupt increase in the second half phase, that is, it was 75 folds higher than that of Williams-8818 at the very beginning. By contrast, it kept a much more gentle wave in Williams-8818 during the whole determining

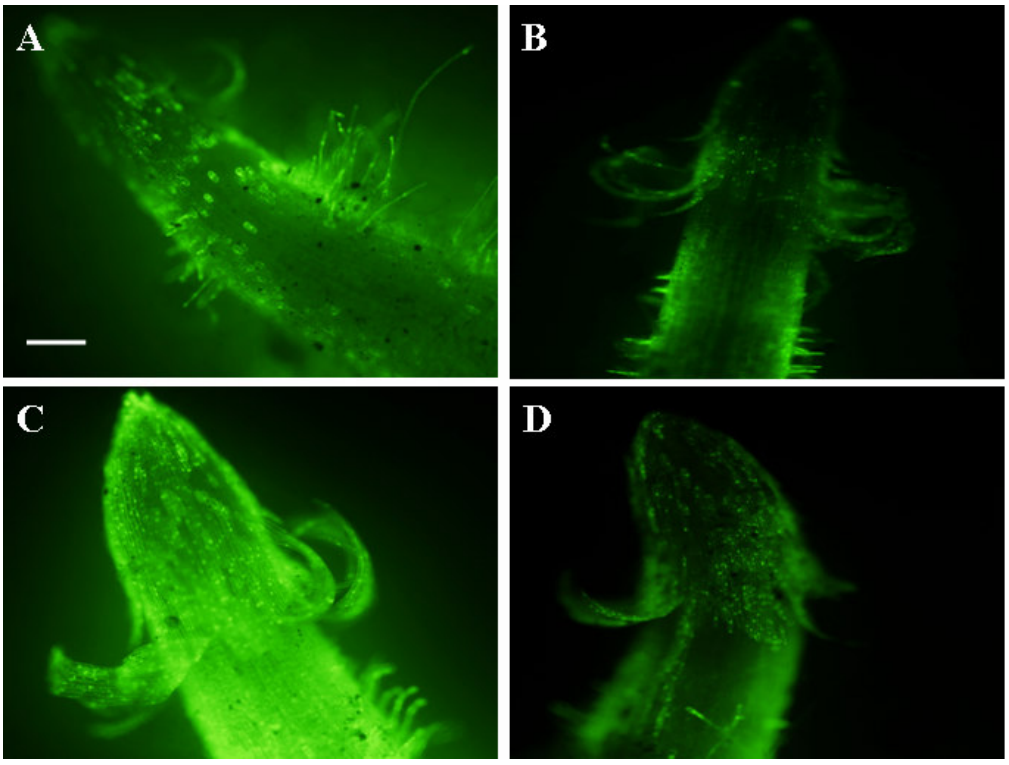

Figure 4. H_2O_2 accumulation in root tips after inoculation; (A) 8818-1, 0 h; (B) 8818, 0 h; (C) 8818-1, 24 h; (D) 8818, 24 h. The bar is 100 μm in length.

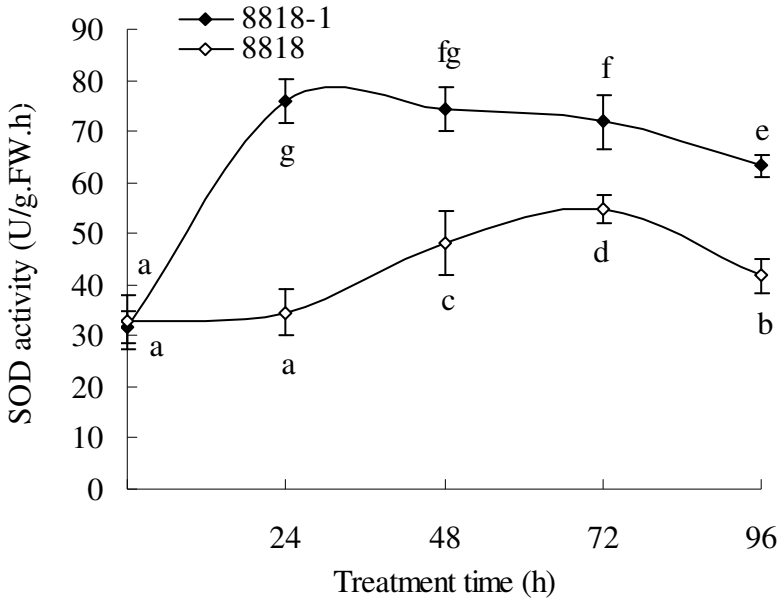

Figure 5. Effects of inoculation with Foc4 on changes of SOD activity of banana.

times. Unexpectedly, the *CAT* transcripts were likely kept at its initial level in each line and only a little statistical significant difference was observed between the two lines during the whole process (Figure 8).

DISCUSSION

ROS production could occur extracellularly through the activities of plasma membrane-resident Rboh proteins,

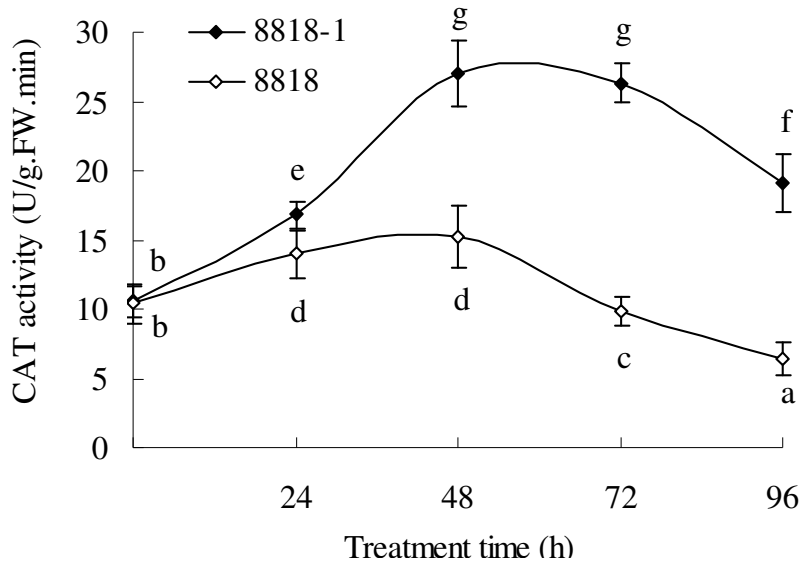

Figure 6. Effects of inoculation with Foc4 on changes of CAT activity.

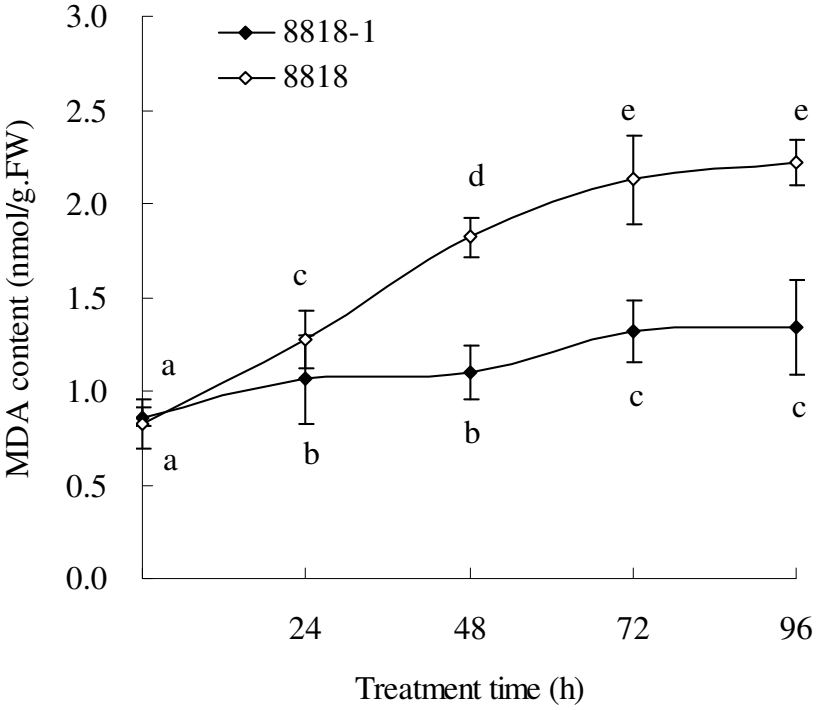

Figure 7. Effects of inoculation with Foc4 on MDA content.

which are homologs of mammalian NADPH oxidase 2, known to be the predominant mediators of apoplastic ROS production (Torres et al., 1998; Galletti et al., 2008). The first ROS production, termed as “oxidative burst”, is usually rapid and transient in resistant plants. ROS can oxidize lipid that finally result in MDA production through a series of redox reactions, in which SOD converts $O_2^{\bullet-}$ into H_2O_2, which in turn, was removed usually by CAT. Therefore, the accumulations of $O_2^{\bullet-}$ and H_2O_2 have been considered as the early events in plant-pathogen recognition. More importantly, their profiles in temporal and spatial environments are the key factors that decide the plant resisted to a certain pathogen or not.

Our results show that $O_2^{\bullet-}$ and H_2O_2 contents in resistant iso-line Williams-8818-1 were changed radically following inoculation. They speedily elevated to a

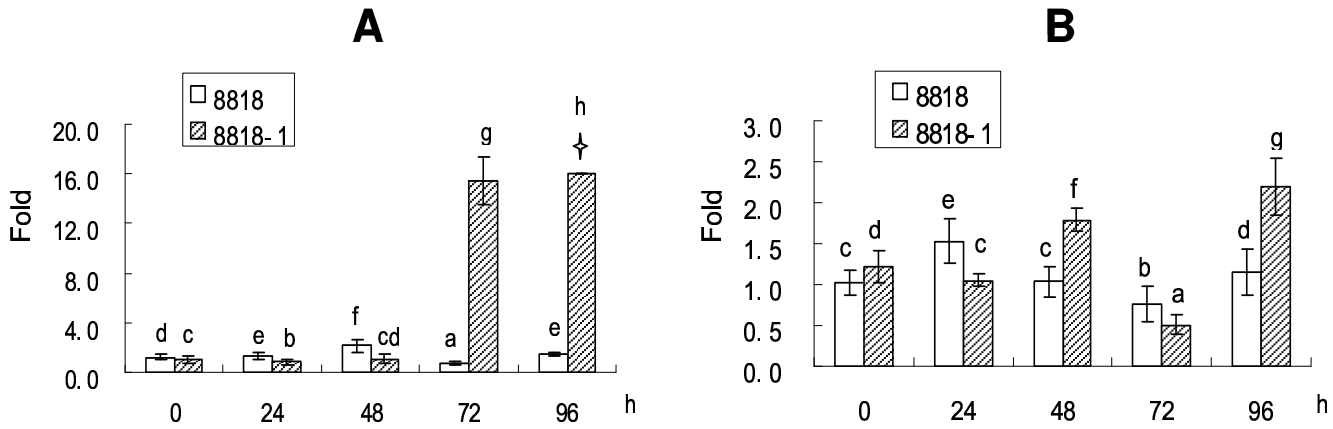

Figure 8. Effects of inoculation on *SOD* (A) and *CAT* transcripts levels (B). The values of fold-change were expressed relative to a 'calibrator', the expression level for the particular transcript in control Williams-8818 roots at 0 hpi. Asterisk in (A) indicate a 75-fold increase.

remarkable high level within one day, then crumpled back in the next day, which was accorded with an "oxidative burst". Following ROS accumulation, SOD activity also peaked at 24 h, but it receded gradually and maintained steadily at a high level; CAT activity, however, took one day more in attaining a peak value. Correspondingly, MDA content was restricted with only a little expansion by the end of the fourth day. Oppositely, susceptible iso-line Williams-8818 lacked the first "oxidative burst". Its ROS accumulation was slowly strengthened as the time passed by; the SOD and CAT activities showed a slow slight increase before decreasing, resulting in a high level of MDA accumulation.

H_2O_2 has been postulated to play a very important role in plant defense (Baker and Orlandi, 1995). For example, the antimicrobial activity of H_2O_2 has been shown *in vitro* against plant pathogenic bacteria and fungi (Peng and Kuc, 1992; Kiraly et al., 1993). H_2O_2 accumulation has also been detected following pathogen treatment in soybean (Levine et al., 1994), barley (Thordal-Christensen et al., 1997), birch (Pellinen et al., 2002), and in transgenic CAT-antisense tobaccos (Chamnongpol et al., 1998). In line with these reports, resistant iso-line Williams-8818-1 exhibited a typical response of first "oxidative burst", in which H_2O_2 accumulation was greatly increased. This early event might suggest that H_2O_2 was one of the key factors in banana resistance to Foc4 invasion; while on the other hand, ROS itself is a toxin. For this reason, it carries equal weight for plant survival to activate the antioxidative system following ROS accumulation. Here, resistant iso-line exhibited a highly efficient antioxidant capacity as soon as ROS accumulated as a result, MDA accumulation was well controlled. This reaction implied that Williams-8818-1 could repair the destruction of cell membranes by reducing lipid oxidation in time. These results are in line with that of previous study (Mo et al., 2008).

It is common that transcript levels of genes encoding antioxidant enzymes are modified when plants are challenged by adverse condition, because of their involvement in regulating ROS accumulation (Schenk et al., 2000). Of those genes, copper/zinc superoxide dismutase (*Cu/Zn-SOD*) and catalase (*CAT*) could delimit excess ROS accumulation in plants under various biotics stress (Schenk et al., 2000). Unfortunately, up till now, limited information is available in banana about genes that control the defense responses, let alone the timing of expression of these genes. Our results suggest that *Cu/Zn-SOD* was not up-regulated until the second day in each iso-line, but it was strikingly highly expressed in the resistant iso-line from then one (Figure 8). In the case of *CAT*, transcripts waved slightly around the basal level all through the days tested. Paparu et al. (2007) also showed that *CAT* transcripts were not up-regulated in the tolerant banana cultivar Kayinja until 33 days after endophyte colonized the roots. Possibly, *SOD* expression lag was good for H_2O_2 accumulation at the early stage after inoculation, by which defense arrangements were onset in our resistance iso-line; and a background level of *CAT* transcripts was enough for detoxifying ROS generated in the first "oxidative burst".

In conclusion, we show here that the characteristics of $O_2^{\bullet -}$ and H_2O_2 elevations, SOD and CAT activations, low MDA accumulation, and *SOD* transcripts enhancement were distinguished from the resistant iso-line to the susceptible iso-line in temporal process in banana roots at the early stage of Foc4 infection. These indicators now enable us to select more directly the new germplasm with resistance to Foc4 in mutation breeding. Now that the first "oxidative burst" has represented the occurrence of up-stream defense cascade, the following questions emerged: Did the oxidative burst eventually elicit downstream defense responses in banana especially pathogenesis-related genes resulting to less sensitivity to the pathogen? Also, how did the anti-oxidative system

and oxygen radical change during the whole infection period? These are interesting questions to be further studied.

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