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Research Paper

Biochemical and cell wall ultrastructural changes in crown tissue of banana (*Musa* AAA 'Berangan') fruit as mediated by UVC irradiation against crown rot fungal infection

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ABSTRACT

The reduction of crown rot disease observed in UVC-irradiated banana fruit, 'Berangan' was associated with activation of the defense response mechanisms, increased peroxidase and polyphenol oxidase activities as well as accumulation of lignin and phenolic compounds in its crown tissue, but not phenylalanine ammonia-lyase activity. Transmission electron microscope micrographs revealed that 0.01 kJ m^{-2} UVC retarded the losses of cell compartments and disintegrations of fibril fraction in cell wall structure of the crown tissue. UVC treatment 24 h after fungal inoculation induced the accumulation of phenol-storing cells that could create an antimicrobial environment in the host tissue for combating the fungal colonization. Moreover, formation of cell wall lignification and heterogenous matrix of cell wall appositions at site of fungal penetration was clearly observed in UVC-irradiated crown tissue, which otherwise absent in the control crowns. These structures might be important physical barriers that were induced by the irradiation to prevent diffusions of toxin and enzyme from the crown rot-infecting fungi.

1. Introduction

The application of UVC irradiation on postharvest commodities is closely related with its germicidal properties and activation of resistance mechanism in plant tissues (Spadoni et al., 2015). Through its germicidal effect, spores and mycelial infections are reportedly destroyed in the outer cell layer of fruit and vegetables, and thus can reduce several diseases (Turtoi, 2013; Escalona et al., 2010; Terry and Joyce, 2004). In plant, UVC induces tissue resistance by increasing phytoalexin compounds (Charles et al., 2008a; D'hallewin et al., 2000), defense enzyme activity (Li et al., 2010), modification of pathogenesis-related proteins (Charles et al., 2009), and formation of structural barriers (Charles et al., 2008b).

Increases of inducible biochemical compounds such as phenolic and lignin have chemically modified the host tissue to become more resistant to cell wall degrading enzymes and diffusion of toxins from pathogens (Mandal, 2010). Both compounds are synthesized in plant host through the phenylpropanoid biosynthetic pathway where phenylalanine ammonia-lyase (PAL) is the key enzyme involved in catalyzing the first step of this pathway (Hahlbrock and Scheel, 1989). The enhancement of plant defense mechanisms against pathogens is also associated with elicitation of oxidative enzyme activities such as polyphenol oxidase (PPO) and peroxidase (POD) (Agrios, 2005). PPO catalyzes the oxidation of phenols into quinones, where this group of compounds possesses antifungal and toxic properties that affects the growth and proliferation of pathogens. POD is primarily involved in the biosynthesis of lignin, which is a component of plant cell wall (Vance et al., 1980), and causes the formation of lignified epidermal cells, and thus is postulated to be an important mechanism in restricting penetration and development of fungus in plant host.

Pombo et al. (2010) have reported that UVC irradiation has the potential to enhance the mechanism of resistance in strawberry fruit against grey mould caused by *Botrytis cinerea* through the induction of PAL, PPO and POD enzyme activities. In another study, UVC irradiation led to the accumulation of phenolic compounds in tomato fruit and strengthened the epicarp and mesocarp tissues through cell wall lignification and suberization (Charles et al., 2008c). However, the potential roles of UVC irradiation on changes in the biochemical and ultrastructure of banana crown tissue in

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relation to susceptibility of the fruit to crown rot disease are unknown. Therefore, the elicitation of defense response in banana fruit of the 'Berangan' cultivar by 0.01 kJ m^{-2} UVC irradiation against crown rot disease caused by *Colletotrichum musae, Fusarium equiseti* and *Lasiodiplodia theobromae* was investigated in this study. 'Berangan' is one of the most popular Malaysian banana dessert cultivars that are highly susceptible to crown rot disease especially during ripening process. The ability of UVC to promote changes in inducible biochemical compounds (total phenolic and lignin), defense enzymes (POD, PPO and PAL) and modification of ultrastructural barriers in crown tissue were analysed during fruit ripening.

2. Materials and methods

2.1. Fruit material

Hand of bananas (*Musa* AAA) 'Berangan' at the mature green stage were used in this study. On average, each hand comprised 10–12 banana fingers. The fruit hands were transported to the laboratory on the same day and then washed with tap water to remove impurities and latex from the crown tissues. The crowns were trimmed carefully to crescent shape by using a sterile curve knife. The fruit was surface sterilized with 0.05% (v/v) sodium hypochlorite solution, followed by three rinses in sterilized distilled water. Fruit were then allowed to dry at ambient temperature (25 ± 2 °C/85% relative humidity).

2.2. Fungal culture and preparation of conidial suspension

L. theobromae, *C. musae* and *F. equiseti* were isolated from naturally fungi infected 'Berangan' banana fruit, collected from a wholesale market at Seri Kembangan, Selangor, Malaysia with high incidence of crown rot. Diseased crown tissue fragments were surface sterilized with 0.05% (v/v) sodium hypochlorite solution for 3 min, rinsed twice with sterile distilled water and then transferred to potato dextrose agar (PDA) (Difco, USA) medium in petri dishes. After 3 d of incubation at 28 ± 2 °C, the actively growing fungus was transferred to fresh PDA plates and re-incubated for 7 d to obtain pure culture. Cultures of the three pathogens were stored and maintained on PDA slants and kept at 4 °C.

For isolates of *C. musae* and *F. equiseti*, the conidia were harvested from 7-d-old cultures, while the conidia of *L. theobromae* were harvested from 4-wk-old cultures. To harvest the conidia, the surface of media was flooded with sterilized distilled water and then gently agitated using a bent glass rod to dislodge the spores. The resulting suspension was filtered through two layers of sterile muslin cloth and the concentration of conidia was adjusted to 5×10^5 conidia mL^{-1} by using a haemacytometer (Neubauer, Germany) (Mercier et al., 2001).

2.3. UVC treatment and fungal inoculation

A dose of 0.01 kJ m^{-2} UVC was used to treat fruit on the day after harvest. This optimum dose was selected based on previous findings on 'Berangan' banana (Ding et al., 2015). A stainless steel chamber equipped with UVC irradiation facility provided by Vision Scientific Co. Ltd. (Malaysia) was used in this study. The chamber contained a low pressure mercury vapor discharge lamp (90 cm long, 220 V and 36 W) and emitted 254 nm of UVC light (VER Bright, Vision Scientific, Korea). The interior of chamber was lined with reflective mirrors which designed to minimize any shadowing effect on irregularly shaped samples. The lamp was turned on for 30 min prior to the treatment application in order to stabilize the radiation. UVC dose rate was measured by using a digital radiometer (UVC-254, Lutron Electronic Enterprise, Taiwan) which calibrated to read specifically at 254 nm. The dose of 0.01 kJ m^{-2} was determined based on a specific irradiation time i.e. 30 s under the obtained fixed dose rate of 0.336 W m^{-2} as according to Stevens et al. (1998). The calculation used was:

$$\text{UVC dose (kJ m}^{-2}\text{)} = \text{Dose rate (W m}^{-2}\text{)} \times \text{Irradiation time (s)} \times 10^{-3}.$$

A total of five treatments were investigated, which included: i) UVC treatment at 24 h before fungal inoculation (indicated by UVC 24 h before inoculation); ii) UVC treatment at 24 h after fungal inoculation (indicated by UVC 24 h after inoculation); iii) inoculation first with fungus and followed by the application of 1.0 g L^{-1} Octave® fungicide solution (active ingredient: 50% w/w prochloraz) (indicated by positive control); iv) inoculation with fungus without irradiation with UVC (indicated by negative control) and v) non-irradiation with UVC and without fungal inoculation (indicated by without treatment).

Inoculation with a specific fungus conidial suspension was performed at the crown region of the fruit hand by puncturing three fresh wounds to a depth of 5 mm using a sterile dissecting needle. Each banana hand was inoculated separately with 40 μL conidial suspension of each *L. theobromae*, *C. musae* and *F. equiseti* at concentration of 5×10^5 conidia mL^{-1}. Thereafter, the inoculation point at crown tissue was covered with sterilised wet cotton and then sealed with parafilm to promote germination of the conidia.

2.4. Ripening process

Banana hands that had been treated as described above were treated with 10 mL L^{-1} of ethylene in airtight polyethylene bag (10 L) and left for 24 h at room temperature (25 ± 2 °C) (Tee et al., 2012). After 24 h, the ethylene gas was released and the fruit were allowed to ripen at the same condition with 85% relative humidity. Analysis was performed on d 0 (before ripening initiation) and d 1, 3 and 5 after ethylene treatment. Every ripening day, twelve banana hands represented four replications (three hands per replicate) for each treatment were used for biochemical analysis.

2.5. Preparation of banana crown tissue

Crown tissue from each treatment replicate was separated from the banana hands, cut into smaller pieces, and the bulked samples (from the three crowns) were frozen in liquid nitrogen. The frozen tissues were then ground using a dry kitchen blender (Tefal La Moulinette DPA171, France) for 10 s and kept at −20° C until analysis. Samples were taken during 0, 1, 3 and 5 days of banana ripening at 25 ± 2 °C.

2.6. Determination of total phenolic content

Total phenolic concentrations were measured according to Singleton and Rossi (1965). Five grams of frozen powder was dissolved in 70% (v/v) acetone. The homogenate was extracted in darkness by swirling on orbital shaker at 180 rpm for 1 h and then centrifuged at 12,000 × *g* for 10 min before decanting the supernatants for the phenolic assays. A 300 μL aliquot of supernatant extract and 1.5 mL of 10% (v/v) Folin-Ciocalteu reagent were mixed in a test tube and incubated for 5 min in dark, followed by addition with 1.2 mL of 6% (w/v) of sodium carbonate (Na_2CO_3). The mixture was again incubated in dark for 1 h at room temperature before measuring the absorbance at 765 nm by using a spectrophotometer (S1200, Spectrowave spectrophotometer, Cambridge, England). The total phenolic content was expressed as gallic acid equivalents (GAE) per mass of crown tissue on a fresh weight basis (g kg^{-1}).

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