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Multiple pre-harvest treatments with acibenzolar-S-methyl reduce latent infection and induce resistance in muskmelon fruit

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ABSTRACT

Muskmelons (cv. Yindi) were sprayed with 100 mg a.i. L^{-1} acibenzolar-S-methyl (ASM) four times at four different stages: flowering, the young fruit, the fruit enlarging period and the netting periods. Analyses were performed 1 week after each ASM treatment. Results showed that the incidence of total latent infection (caused by all observable fungi) and the incidence of relative latent infection (caused by *Alternaria alternata* or *Fusarium* spp.) were significantly lower in sprayed muskmelons than in the control fruit. Moreover, the reduction in the incidence of latent infection was greater with increased ASM treatments. The control of latent infection by ASM resulted in reduced incidence of postharvest decay and improved fruit appearance and firmness after 10 d of storage. In addition, the activities of peroxidase, phenylalanine ammonia lyase, β-1,3-glucanase and chitinase increased significantly in treated muskmelons. ASM treatments also contributed to the accumulation of phenolic compounds, lignin and flavonoids. Increases in defense-related enzyme activities and in particular metabolite levels were observed in plants with more ASM treatments. These results suggest that multiple ASM treatments could induce disease resistance in muskmelons and could be an ideal strategy for preventing latent infection in fruits.

1. Introduction

Muskmelon (*Cucumis melo* L.) is an economically important crop in Northwest China (Bi et al., 2007a). The fruit is susceptible to postharvest decay caused by several pathogenic fungi. Alternaria rot and Fusarium rot, caused by *Alternaria alternata* (*A. alternata*), and *Fusarium* spp., respectively, are two of the principal postharvest diseases of muskmelons (Ge et al., 2005). Both pathogens can infect the flowers and young fruit and remain quiescent until storage, during which time lesions progressively appear (Ge et al., 2005). The rot can be effectively controlled with iprodione and azoxystrobin spraying before harvest (Ma et al., 2004), or by postharvest dipping in hot iodine and guazatine (Bokshi et al., 2007). However, due to problems related to fungicide toxicity, development of fungicide resistance by pathogens and potential harmful effects on the environment and human health, new strategies for controlling postharvest diseases have been proposed (Spadaro et al., 2004; Elmer, 2006).

One promising approach for disease management is the use of an elicitor to induce pathogen resistance in plant tissues (Terry and Joyce, 2004; Bi et al., 2007b). Acibenzolar-S-methyl (ASM), a plant hormone-like compound and an analogue of salicylic acid that acts as a molecular signal to trigger systemic acquired resistance (SAR) against a wide range of pathogens, from viruses to parasitic weeds (Iriti et al., 2007; Véronési et al., 2009). ASM has been extensively studied and is commercially available under the trade names Bion or Actigard (Hammerschmidt et al., 2001). Unlike most chemical elicitors, ASM is not phytotoxic at low dose and it has been proven to be one of the most effective resistance elicitors in many harvested crops, such as cucumber (Cools and Ishii, 2002; Bokshi et al., 2006), potato (Bokshi et al., 2003), apple (Spadaro et al., 2004; Quaglia et al., 2011), peach (Liu et al., 2005), pear (Cao and Jiang, 2006), tomato (Małolepsza, 2006; Iriti et al., 2007) and muskmelon (Huang et al., 2000; Bi et al., 2006; Bokshi et al., 2006, 2007; Ge et al., 2008; Wang et al., 2008).

Terry and Joyce (2000, 2004) demonstrated that either multiple or single preharvest ASM treatments (0.25–2 mg a.i. mL^{-1}) applied during the winter growing conditions delayed the development of gray mold disease by 2 d in harvested strawberry fruit, but were inefficient when applied in the summer. Huang et al. (2000) reported that a single ASM foliar spray (25 or 50 mg a.i. L^{-1}) prior to flowering reduced *Alternaria* spp. and *Fusarium* spp. postharvest incidence and severity in 'Eldorado' and 'South Cross' rock melons stored for 3 weeks after harvest at 2–8 °C in Australia. However, no significant effect was found in postharvest disease incidence and severity caused by *A. alternata* and *Fusarium* spp. in 'Yindi' muskmelons when a single ASM foliar spray (50 or 100 mg a.i. L^{-1}) was applied prior to flowering in China (Bi et al., unpublished data).

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Therefore, growing conditions and plant variety may be important factors affecting the efficacy of ASM.

The objective of this study was to determine whether ASM suppresses latent infection in muskmelon and whether it improves the postharvest qualities when applied repeatedly during fruit development. Another objective was to analyze whether defensive responses were activated in treated fruit by assaying the activities of the defense-related enzymes peroxidase (POD), phenylalanine ammonia lyase (PAL), β-1,3-glucanase (GLU) and chitinase (CHT), as well as levels of certain metabolites (total phenolic compounds, lignin and flavonoids). Changes in microstructure due to ASM treatments were studied to determine whether induced resistance is the main mechanism behind the observed protection.

2. Materials and methods

2.1. Plant material

The melon seeds (cv. Yindi) were provided by the Hexi Institute of Watermelons and Melons in Gansu. They were seeded in the seedling trays on March 5, 2005, transplanted in the open field of Quanshan Town, Minqin County in Gansu Province of China on April 20, 2005, and then cultivated until June 15, 2005. The area typically has dry weather and high infection pressure from fungi.

2.2. Preparation of ASM and pre-harvest treatments

Acibenzolar-S-methyl (ASM) was applied in the form of Bion 50 WG (Novartis Crop Protection Australia), and 100 mg a.i. L^{-1} suspension in distilled water was added with 0.01% (v/v) Tween 20. Whole muskmelon plants were sprayed with droplets of ASM (approximately 20 mL $plant^{-1}$) during the flowering (May 18, 2005), young fruit (May 25, 2005), enlarging (June 1, 2005) and netting periods (June 8, 2005). Plants sprayed with water served as controls. Three replicates of approximately 60 plants were sprayed for each treatment at every period.

2.3. Sample collection

Fruit from treated plants were collected at various stages: collected at the young fruit period (May 25, 2005) after being ASM-sprayed once at the flowering period, collected at the enlarging period (June 1, 2005) after being ASM-sprayed twice at the flowering and young fruit period, collected at the netting period (June 8, 2005) after being ASM-sprayed three times at the flowering, young fruit and enlarging period, collected at the mature period (June 15, 2005) after being ASM-sprayed four times at the flowering, young fruit, enlarging and netting period. After sorting for size uniformity and the absence of obvious injuries, the fruits were packed individually, stored in standard melon shipping boxes, and transported to the laboratory (stored at 25 ± 2 °C, 55–60% RH) within 24 h.

2.4. Assessment of latent infection

Latent infection of muskmelons at all growth stages was assessed according to the method of Prusky et al. (1981), with some modifications. Fruits were washed thoroughly and dried, and then 2-mm thick discs of tissue with peel were cut from around the top, middle, and bottom zones of each fruit using a 0.5 cm stainless steel cork borer. The discs were immediately sterilized by soaking in 75% ethanol for 30 s followed by 2 min in mercuric chloride. After washing with sterile distilled water 3 times, 10 discs from the same zone of every fruit were placed peel side down in a Petri dish containing PDA as a culture medium, and they were incubated at 25 °C for 6 d. The percentage of latent infection was assessed with the following formula. The incidence of total latent infection (%) = (Number of discs with isolates/Number of all discs) × 100%. The incidence of relative latent infection (%) = (Number of discs infected by *A. alternata* or *Fusarium* spp./Number of all discs) × 100%. Three replicates of 3 fruits were used for each treatment at every period.

2.5. Evaluation of postharvest decay incidence and fruit qualities

Postharvest decay incidence and the qualities of fruits collected at the mature period were evaluated on the 10th day of storage. Fruit were considered to be decayed if there was fungal growth on the surface of the stem end and the rind. The percentage of fruit with decay was determined. General appearance was evaluated visually as described by Aharoni et al. (1997), with freshness of the fruit, decay and skin blemishes scored on a scale of 1–5, where 1 = poor, 3 = good, and 5 = excellent quality. Fruit with a rating of less than 2.5 were considered unfit for human consumption. Total soluble solids (TSS) were determined with the analysis of juice from the central section of the flesh using Abbe refractometer (10481 S/N, Division of Warner-Lambert Technologies, Inc., USA). Three TSS readings were obtained from each melon. Firmness was measured at the equator of the fruit where a section of rind (4 cm × 4 cm and 1 cm deep) had been removed, using a hand-held penetrometer (GY-1, Fruit Firmness Tester, Zhejiang, China). Three firmness readings were obtained for each melon. The fruit without pre-harvest ASM sprays were regarded as the control. Three replicates of 10 fruits were used for each treatment on the 10th day after harvest at mature period.

2.6. Enzyme assay

For muskmelon fruits collected at each period, 3 g of tissue were taken from 5 to 10 mm below the skin around the equator of each fruit, using a stainless steel cork borer. Samples were packed in aluminum foil and immediately frozen in liquid nitrogen, and then kept at −80 °C before use.

Frozen tissues were homogenized with a mortar and pestle in various buffers mixed with 10% (w/v) of polyvinyl polypyrrolidone (PVPP). These buffers were 3 mL of sodium phosphate buffer (0.05 M, pH 5.9) for POD, 3 mL of 0.2 M sodium borate buffer (pH 8.8, containing 5 mM β-mercaptoethanol and 1 mM EDTA) for PAL, 3 mL of sodium acetate buffer (0.1 M, pH 5.0) for GLU and 3 mL of sodium acetate (0.1 M, pH 5.0) for CHT. The homogenates were centrifuged at 15,000 × *g* at 4 °C for 20 min, and the supernatants were assayed for enzyme activities.

POD activity was determined according to the method of Hammerschmidt and Kuć (1982), with some modifications. The reaction mixture included 200 μL of crude extract, 1.8 mL of guaiacol substrate (0.05 M) mixed with sodium phosphate (0.05 M, pH 5.9) and 100 μL of H_2O_2 (2%). The POD activity was determined by measuring the increase in absorbance at 470 nm. The enzyme activity was expressed as U_{470}, where $U_{470} = 0.01\ \Delta A_{470}$ mg $protein^{-1}$ min^{-1}.

PAL activity was assayed using the method of Assis et al. (2001), with some modifications. Enzyme extract (200 μL) was incubated with 2 mL of borate buffer (50 mM, pH 8.8) containing 20 mM L-phenylalanine for 10 min at 37 °C. The reaction was stopped with 1 mL HCl (6 M). The reaction in the control mixture was stopped by adding 1 mL HCl immediately after mixing the crude enzyme preparation with L-phenylalanine. The PAL activity was measured at 290 nm absorbance and activity units were expressed as U_{290}, where $U_{290} = 0.01\ \Delta A_{290}$ mg^{-1} protein h^{-1}.

GLU activity was assayed by measuring the amount of reducing sugar released from the substrate using the method of Ippolito et al. (2000), with some modifications. Enzyme preparation (500 μL) that had been dialyzed for 12 h was incubated with 500 μL of laminarin (0.5%, w/v) for 60 min at 37 °C. Afterward, 50 μL of mixture was removed and diluted 1:4 with sterile distilled water. The reaction