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Methyl jasmonate primes defense responses against wounding stress and enhances phenolic accumulation in fresh-cut pitaya fruit

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ABSTRACT

The effect of a pre-cutting methyl jasmonate (MeJA) treatment on wound-induced phenolic accumulation in fresh-cut pitaya fruit and the possible mechanisms were investigated. Results showed that MeJA treatment before cutting significantly enhanced the accumulation of the main individual phenolic compounds and improved the antioxidant activity in fresh-cut pitaya fruit. It is evident that MeJA pretreatment induced a priming mechanism in pitaya fruit, since the MeJA treated intact fruit without subsequent wounding stress did not show any significant physiological responses. However, pitaya fruit pretreated with MeJA and followed by cutting showed an augmented capacity to enhance defense responses against wounding stress. These enhanced responses included reactive oxygen species (ROS) burst, increased gene expression and activity of key enzymes involved in phenylpropanoid pathway and ROS scavenging. These results demonstrated that MeJA treatment can effectively enhance the wound-induced accumulation of phenolic compounds and improve the antioxidant activity in fresh-cut pitaya fruit through priming of defense responses against wounding stress.

1. Introduction

Pitaya fruit (*Hylocereus undatus*) is a popular tropical fruit with great commercial value owing to its special nutritional and functional compositions. The consumption of fresh-cut pitaya fruit has been increasing substantially because of its freshness and convenience. During fresh-cut processing, the tissue is inevitably subjected to wounding stress, which will induce defense responses to produce more secondary metabolites at the injured site or site adjacent to defend and heal the wounding damage (Saltveit, 1997; Cisneros-Zevallos, 2003). It has been confirmed that wounding stress induces phenolic biosynthesis and enhances antioxidant activity in various fresh-cut fruits and vegetables such as mango (Robles-Sánchez et al., 2013), pitaya fruit (Li et al., 2017), lettuce (Zhan et al., 2012), potato (Torres-Contreras et al., 2014) and carrot (Surjadinata and Cisneros-Zevallos, 2012; Han et al., 2017).

The plant defense responses against biotic and/or abiotic stresses have long been recognized to be triggered directly after a stress. However, it is revealed that in addition to direct induction, plant defense responses are able to be induced through the priming mechanism (Conrath et al., 2001). Defense priming is a unique physiological state that can be induced by a series of elicitors such as beneficial microorganisms, chemical agents and biotic/abiotic stresses. Plants do not show any significant physiological responses to these elicitors, however, the primed plants will defend and respond more fasterly and strongerly when subjected to a subsequent biotic or abiotic stress (Conrath et al., 2002, 2006). Recently, many studies have shown that priming is a common immune mechanism of plants to protect them against external environmental stresses including pathogens, insects and abiotic stresses (Conrath, 2009, 2011; Pastor et al., 2013). Several chemicals including 2,6-dichloroisonicotinic acid (INA), β-aminobutyric acid (BABA), salicylic acid (SA) and benzothiadiazole (BTH) have been reported as effective elicitors in inducing tobacco and *Arabidopsis* plants to a primed state against biotic or abiotic stresses (Conrath et al., 2006; Beckers and Conrath, 2007).

As a natural plant regulator, methyl jasmonate (MeJA) has been confirmed to be an important plant signaling molecule involved in plant defense mechanisms (Cheong and Choi, 2003). It has been identified that in addition to directly enhancing defense responses, MeJA can act as an elicitor of priming, leading to more rapid and strong defense responses to subsequent stresses in plants such as *Arabidopsis* (Chen et al., 2011), wheat (Desmond et al., 2006), and harvested fruits such as Chinese bayberry (Wang et al., 2014), sweet cherry (Wang et al., 2015), and grape (Jiang et al., 2015). However, these studies focused on the priming mechanism against pathogen attacks, no information was available about the MeJA-induced priming of defense responses against wounding stress. Heredia and Cisneros-Zevallos (2009a, b)

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Table 1
Primer sequences used for qRT-PCR analysis.

Primer name	Forward primer sequence (5′ to 3′)	Reverse primer sequence (5′ to 3′)
HuUBQ	TGAATCATCCGACACCAT	TCCTCTTCTTAGCACCACC
HuPAL	AAGGAACTTCGGCTATCCCG	ACTCCTCCCCAGGAGACTTC
HuC4H	AAGTTGAAGCTCCCACCAGG	CTGGCCCATCCTAAGCAACA
Hu4CL	TCTTCAAATCACGCCTCCCC	GTTGGAGATGAGACAGGGGC
HuSOD	ACGCTCCGAGAATCTATCCA	CTTTGCACGTACAGTAGGGGA
HuCAT	TGCATCCAATACAGGGGAACT	GTTTGGCTTGAATGCGTGGA
HuAPX	AAGGAACGCAACCCTTCCAA	AACCTCTGCCATGGGAAACC

Table 2
Effect of MeJA pretreatment and wounding stress on changes of main individual phenolic compounds and antioxidant activity in pitaya fruit during 48 h of storage at 15 °C[a].

Storage time (hour)	Treatment	Phenolic compounds content (g kg^{-1})					Antioxidant activity (%)
		Gallic acid	Protocatechuic	*p*-Hydroxybenzoic	Caffeic acid	*p*-Coumaric	
0	Control	0.041 ± 0.003	0.045 ± 0.008	0.097 ± 0.004	0.021 ± 0.001	0.169 ± 0.002	57.330 ± 1.390
12	Control	0.042 ± 0.005aA	0.040 ± 0.003cA	0.099 ± 0.006bB	0.021 ± 0.001aA	0.228 ± 0.004bC	56.675 ± 0.695bA
	MeJA	0.044 ± 0.004aA	0.053 ± 0.005bA	0.101 ± 0.010bB	0.023 ± 0.003aA	0.249 ± 0.004bA	60.975 ± 2.027bA
	Wounding	0.044 ± 0.003aB	0.062 ± 0.002abA	0.125 ± 0.007aC	0.023 ± 0.002aA	0.315 ± 0.023aB	67.649 ± 1.506aA
	MeJA + Wounding	0.045 ± 0.010aB	0.073 ± 0.006aA	0.141 ± 0.008aC	0.024 ± 0.003aA	0.346 ± 0.003aC	70.557 ± 0.405aB
24	Control	0.041 ± 0.012bA	0.044 ± 0.005bA	0.115 ± 0.007cAB	0.020 ± 0.003aA	0.233 ± 0.004dC	55.569 ± 0.637cA
	MeJA	0.039 ± 0.009bA	0.047 ± 0.002bA	0.121 ± 0.005cA	0.023 ± 0.004aA	0.257 ± 0.002cA	58.927 ± 1.216cA
	Wounding	0.060 ± 0.008aA	0.058 ± 0.008bA	0.146 ± 0.004bB	0.025 ± 0.004aA	0.390 ± 0.001bA	68.059 ± 3.359bA
	MeJA + Wounding	0.067 ± 0.001aA	0.075 ± 0.002aA	0.196 ± 0.007aB	0.027 ± 0.006aA	0.417 ± 0.003aA	75.839 ± 0.695aAB
36	Control	0.045 ± 0.004cA	0.042 ± 0.003cA	0.120 ± 0.005cA	0.021 ± 0.006aA	0.282 ± 0.005cA	59.992 ± 1.679cA
	MeJA	0.042 ± 0.003cA	0.049 ± 0.002cA	0.123 ± 0.005cA	0.019 ± 0.005aA	0.253 ± 0.015dA	57.576 ± 0.695cA
	Wounding	0.066 ± 0.007bA	0.069 ± 0.007bA	0.190 ± 0.004bA	0.020 ± 0.006aA	0.341 ± 0.010bB	72.563 ± 0.927bA
	MeJA + Wounding	0.077 ± 0.003aA	0.083 ± 0.009aA	0.229 ± 0.010aA	0.019 ± 0.003aA	0.395 ± 0.006aB	78.501 ± 0.985aA
48	Control	0.043 ± 0.002bA	0.049 ± 0.002cA	0.115 ± 0.009cAB	0.019 ± 0.004aA	0.253 ± 0.014bB	55.610 ± 1.274bA
	MeJA	0.041 ± 0.004bA	0.059 ± 0.005bA	0.129 ± 0.004cA	0.024 ± 0.003aA	0.263 ± 0.015bA	59.459 ± 3.359bA
	Wounding	0.063 ± 0.003aA	0.059 ± 0.002bA	0.156 ± 0.003bB	0.023 ± 0.005aA	0.344 ± 0.008aB	70.311 ± 0.637aA
	MeJA + Wounding	0.066 ± 0.004aA	0.077 ± 0.004aA	0.209 ± 0.008aB	0.018 ± 0.003aA	0.346 ± 0.006aC	75.184 ± 0.811aAB

[a] Data are expressed as the mean ± SE (n = 3). Values with different letters indicate statistically significant differences at $p < 0.05$. Lowercase letters represented significant difference among treatment factors, capital letters represented significant difference among storage time factors.

demonstrated that a post-cutting MeJA treatment induced phenolic accumulation in several wounded fruits and vegetables, but whether MeJA can prime defense responses against wounding stress was not determined, since they did not include a pre-cutting MeJA treatment plus wounding in their studies. Just recently, we investigated the effect of pre-cutting MeJA treatment on phenolic accumulation and the possible action mechanisms in fresh-cut pitaya fruit. We found that MeJA was effective in enhancing wound-induced accumulation of total phenolics by regulating sugar content and energy status (Li et al., 2018). In this paper, we provided data to determine if the MeJA-induced phenolic accumulation is associated with priming of defense responses against wounding stress in pitaya fruit.

2. Materials and methods

2.1. Chemical reagents

L-phenylalanine, nitrotetrazolium blue chloride (NBT) and ascorbic acid were obtained from Shanghai Ryon Biological Technology Co., Ltd. (Shanghai, China). Hydroxylamine hydrochloride, *p*-aminobenzene sulfonic acid, α-naphthylamine, 2,2-diphenyl-1-picrylhydrazyl (DPPH) were obtained from Beijing Solarbio Science & Technology Co., Ltd. (Beijing, China). PrimeScript™ RT Master Mix kit and SYBR Premix Ex Taq™ kit were purchased from Takara Biomedical Technology Co., Ltd. (Beijing, China). MeJA, β-mercaptoethanol, *p*-coumaric acid, and other chemicals were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Acetonitrile and methanol were reagents of HPLC-grade; the other chemical reagents were of reagent grade.

2.2. Fruit materials and MeJA treatment

Fruit materials, treatment and sampling procedure were the same as described in our previous paper (Li et al., 2018). The experiment included four treatments: (1) control, intact pitaya fruit without MeJA or cutting treatment; (2) MeJA, intact pitaya fruit treated with 100 μM of MeJA vapor at 20 °C for 12 h; (3) wounding, pitaya fruit cut into quarter-slices of 1 cm thickness; (4) MeJA + wounding, pitaya fruit pretreated with 100 μM of MeJA vapor at 20 °C for 12 h and then cut into quarter-slices. After treatment, all groups of fruit were stored at 15 °C for 48 h. Fruit samples were taken in control fruit before storage (time 0 in all figures and tables) and at 12 h interval in all four groups for further for further analysis. Each treatment was replicated three times, the experiment was conducted twice.

2.3. Phenolic compounds assay

2.3.1. Extraction

Frozen samples (5 g) were extracted with methanol (15 mL) in the dark at 4 °C for 12 h, centrifuged at 12,000 × *g* for 20 min afterwards. The obtained supernatant was gathered in a 50 mL of flask and concentrated by rotary evaporation to about 5 mL in volume. The concentrated supernatant was added into an activated C_{18} sep-pak cartridge, with the solvent flowed out and the sample stayed in the solid phase. The cartridge was washed with 3 mL of methanoic acid solution (3%, v/v), which was discarded after outflowing from the cartridge. Afterwards, the phenolic compounds staying in the cartridge was eluted with 3 mL of methanol solution which contained 3% methanoic acid. The purified extraction of phenolic compounds was passed through a nylon syringe filter (0.22 μm) and prepared for chromatographic