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Crosstalk between calcium and melatonin affects postharvest physiological deterioration and quality loss in cassava

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ABSTRACT

Rapid postharvest physiological deterioration largely reduces the quality and marketability of cassava. The molecular mechanism underlying cassava postharvest physiological deterioration and quality loss is largely unknown. The present study aimed to investigate the role of calcium and its relationship with melatonin in cassava postharvest physiological deterioration. Transcriptomic analyses indicate that most of the calcium ion (Ca^{2+}) sensor genes are upregulated in cassava tuberous roots at different postharvest stages. Exogenous $CaCl_2$ reduces postharvest physiological deterioration, increases the endogenous levels of Ca^{2+} and melatonin, reduces the degradation of ascorbic acid and starch, and induces the expression of genes related to melatonin biosynthesis after harvest. These effects are reversed by the exogenous application of a Ca^{2+} chelator (EGTA). Exogenous melatonin also increases endogenous melatonin levels and reduces ascorbic acid and starch degradation during postharvest physiological deterioration but do not affect endogenous Ca^{2+} content. Together, these findings demonstrate that calcium-induced activation of melatonin biosynthesis plays a role in reducing postharvest physiological deterioration and quality loss in cassava. Additionally, pretreatment with EGTA arrests the melatonin-induced reduction of postharvest physiological deterioration, suggesting the possible crosstalk between melatonin and calcium during postharvest physiological deterioration.

1. Introduction

Cassava is the sixth most important crop in terms of global production following wheat, maize, and rice and is mainly grown for its edible tuberous roots (Zhang et al., 2010; Zidenga et al., 2012). Based on its high starch production, cassava is also considered as a potential biofuel crop (Zidenga et al., 2012). However, the rapid postharvest physiological deterioration of cassava tuberous root that occurs within 72 h postharvest largely reduces its quality and marketability (Zidenga et al., 2012; Vanderschuren et al., 2014). During harvest and storage, physiological deterioration is induced by mechanical injury and progresses from the proximal site of damage to the distal end, resulting in unpalatable roots (Zidenga et al., 2012; Vanderschuren et al., 2014). Extensive studies have been conducted on elucidating the physiological and biochemical mechanisms underlying postharvest physiological deterioration of cassava (Reilly et al., 2001, 2004; Iyer et al., 2010; Zidenga et al., 2012; Xu et al., 2013; Vanderschuren et al., 2014). During postharvest physiological deterioration of cassava, reactive oxygen species (ROS) levels increased, followed by the regulation of genes and activities related to the antioxidant system (Reilly et al., 2004). Moreover, genetic modification of various genes encoding antioxidant enzymes, including AtAOX1A, MeCu/ZnSOD, MeCAT1 and MeGPX, delays postharvest physiological deterioration and extends shelf life of cassava tuberous roots (Zidenga et al., 2012; Xu et al., 2013; Vanderschuren et al., 2014). Collectively, these evidences highlight that ROS-induced oxidation leads to symptoms of postharvest physiological deterioration, and the reduction in ROS accumulation could reduce postharvest physiological deterioration.

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Melatonin (N-acetyl-5-methoxytryptamine) was first isolated from the pineal gland of cow in the late 1950s (Lerner et al., 1958, 1959). In 1995, melatonin was subsequently identified in plants (Dubbels et al., 1995; Hattori et al., 1995), and melatonin has been isolated from various plant species, including *Arabidopsis*, rice, apple, beestrawberry, cucumber, tobacco, and cassava (Ma et al., 2016; Shi et al., 2016). Further studies have shown that melatonin is involved in multiple plant biological processes such as root architecture, photoprotection, flower development, seed germination, leaf senescence, fruit ripening, vegetative growth, and responses to stress (Hu et al., 2016b; Shi et al., 2016). Melatonin also acts as an antioxidant by activating the antioxidant system, directly scavenging ROS, and augmenting the efficiency of other antioxidants (Hu et al., 2016b). A recent study has shown that the exogenous application of melatonin delays cassava postharvest physiological deterioration as well as reduces H_2O_2 accumulation by activating antioxidative enzymes (Ma et al., 2016), which is in agreement with the findings of physiological and transcriptomic analyses (Hu et al., 2016b). Calcium plays an important role in regulating various biological processes. Cellular responses to abiotic stress include a significant increase in Ca^{2+} levels, which in turn activates Ca^{2+} receptors and the Ca^{2+} signaling network (Gilroy et al., 2014). Exogenous application of Ca^{2+} could alleviate stress-induced injury, as it has a protective role in plant responses to abiotic stress. One important event underlying this particular strategy for protection is activation of the antioxidative system and repression of ROS accumulation. Physiological and biochemical studies suggest that the exogenous application of Ca^{2+} increases plants tolerance to cadmium, chilling, acid rain, and hypoxic stresses by activating antioxidant enzymes and inhibiting ROS accumulation (He et al., 2012; Shi and Chan, 2014; Srivastava et al., 2015; Hu et al., 2016a). Further genetic investigations also support the positive role of calcium in inducing the antioxidant system in stress conditions by overexpressing or repressing genes that encode Ca^{2+} receptors (Verslues et al., 2007; Deng et al., 2013a, 2013b; Zou et al., 2015). Calcium is involved in reducing cellular ROS levels and improving plant tolerance to abiotic stress; but, the role and mechanism of calcium in postharvest physiological deterioration of cassava remains unclear. The relationship between calcium and melatonin during cassava postharvest physiological deterioration has yet to be determined.

The present study aimed to elucidate the role of calcium in regulating postharvest physiological deterioration and tuberous root quality of cassava, as well as establish the relationship between calcium and melatonin during cassava postharvest physiological deterioration using transcriptome and physiological analyses.

2. Material and methods

2.1. Plant materials and treatments

Ten-month-old cassava tuberous roots (*Manihot esculenta* Crantz cv. SC8) were harvested to cut into 0.005-m-thick slices that were subsequently transferred into Petri dishes lined with wet filter paper (Vanderschuren et al., 2014). To investigate the role of calcium in postharvest physiological deterioration, root slices were incubated in water (control), 0.01 M $CaCl_2$, or 0.01 M ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA). All the slices of each treatment were placed in a 2 L volume of the solution for 2 h. Then, the root slices were removed from the solution and placed in an incubator in the dark (28 °C and 60% relative humidity). After incubation for 0 h, 2 h, 6 h, 12 h, 24 h, 48 h, and 72 h, the slices were frozen in liquid nitrogen until total RNA extraction or physiological analysis. Each sample (each treatment at each time point) had three replicates (each replicate contained one slice from one tuberous root), which constituted one biological experiment. Totally, three biological experiments were performed for each sample. To study the role of melatonin in postharvest physiological deterioration, root slices were incubated in water (control) or different concentrations (1×10^{-4} M, 3×10^{-4} M, and 5×10^{-4} M) of melatonin for 2 h. To test the combined effect of melatonin and EGTA on postharvest physiological deterioration, root slices were pretreated with 0.01 M EGTA for 2 h, then exposed to 1×10^{-4} M melatonin for 2 h. The material composition and the subsequent incubation were the same as earlier described. To test the effect of calcium on whole tuberous roots, roots were incubated in water (control), 0.01 M $CaCl_2$, or 0.01 M EGTA for 2 h. Then, the roots were placed in an incubator in the dark (28 °C and 60% relative humidity). After incubation for 0 d, 14 d, and 21 d, the roots were cut into 0.005-m-thick slices and then imaged.

2.2. Transcriptomic analysis

Total RNA was isolated using a plant RNA extraction kit (TIANGEN, China) and used for cDNA library construction. Sequencing was performed with an Illumina GAII system following the manufacturer's instructions. Adapter sequences were removed using a FASTX-toolkit. Clean reads were generated by removing low-quality sequences using FastQC. Tophat v.2.0.10 was used to map the clean reads to the cassava genome (Trapnell et al., 2009). Transcriptome data was assembled using cufflinks (Trapnell et al., 2012). Fragments Per Kilobase of transcript per Million mapped reads (FPKM) was employed to calculate gene expression levels. Differentially expressed genes were identified with DEGseq (Wang et al., 2010). Each sample had three biological replicates (each replicate contained one slice from one tuberous root).

2.3. Quantitative real-time PCR analysis (qRT-PCR)

Changes in the expression of *MeTDC1* (Manes.12G079500), *MeTDC2* (Manes.12G038600), *MeSNAT* (Manes.08G168900), *MeT5H* (Manes.06G111700), *MeASMT1* (Manes.13G140900), *MeASMT2* (Manes.17G050500), *MeASMT3* (Manes.13G140500) were measured by qRT-PCR on a Stratagene Mx3000P real-time PCR system using SYBR® Premix Ex Taq™ (TaKaRa, Japan). The primer pairs were examined based on the melting curve, agarose gel electrophoresis, and sequencing PCR products (Table S1). The qRT-PCR was conducted as follows: 95 °C for 10 min; followed by 40 cycles at 95 °C for 10 s, 55 °C for 15 s, and 72 °C for 20 s. The amplification efficiency was within the range of 0.92–1.05. The expression of the target genes was normalized with the *TUB* and *EF1* genes. The $2^{-\Delta\Delta Ct}$ method was employed to calculate the relative expression of the target genes. Each sample had three biological replicates (each replicate contained one slice from one tuberous root).

2.4. Evaluation of postharvest physiological deterioration of cassava tuberous roots

Postharvest physiological deterioration evaluation was determined according to the method of Zidenga et al. (2012). Tuberous roots were carefully harvested, and then cut into slices approximately 0.005-m-thick, followed by various treatments. After treatment, the slices were stored at a growth chamber with 28 °C and 60% relative humidity in the dark. After incubation up to 72 h, the slices were collected to evaluate the deterioration rate using ImageJ image processing and analysis software (http://rsb.info.nih.gov/ij/, NIH, MD, USA). The root slices were photographed under standard illumination settings. Color images were converted into gray images. Gray values that represent the area of postharvest physiological deterioration in each root slice were calculated. The entire area of the root slices was also calculated. The ratio between gray values and entire area in each root slice was used to evaluate deterioration rate, which ranged from 0 to 1.

2.5. Quantification of endogenous melatonin, Ca^{2+}, ascorbic acid, and starch

Samples from treated or control root slices were frozen in liquid

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