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Two *GH3* genes from longan are differentially regulated during fruit growth and development

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ABSTRACT

In the present work, two full length cDNAs of *GH3* genes, named *DlGH3.1* and *DlGH3.2* were cloned from pericarp and aril tissues of the longan fruit, respectively. Three conserved motifs, SSGTSAGERK, YASSE and YRVGD, as a characteristic of the acyladenylate/thioester forming enzyme superfamily were observed in *DlGH3.1* and *DlGH3.2* proteins. *DlGH3.1* mainly expressed in pericarp tissues while *DlGH3.2* accumulated in both the pericarp and aril tissues during fruit growth and development. In addition, NAA treatment induced the expression of *DlGH3.1* and *DlGH3.2* in the pericarp tissues at 21 and 77 days after anthesis (DAA), while only *DlGH3.2* in the aril tissues could be induced by NAA at 77 DAA. More importantly, ABA and ethrel treatments suppressed the accumulations of *DlGH3.1* and *DlGH3.2* in the pericarp tissues of longan fruit at 21 DAA (a rapid growth stage of pericarp), but enhanced *DlGH3.2* expression in the aril tissues at 77 DAA (a fruit ripening stage). Furthermore, the expression patterns of *DlGH3.1* and *DlGH3.2* showed different tissue specificity. Thus, our results suggest that *DlGH3.1* gene expression might be associated with pericarp growth, while *DlGH3.2* accumulation is likely to be related to both pericarp growth and fruit ripening, and the responses of *DlGH3s* to plant growth hormones are different and dependent on fruit development stage and fruit tissue.

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1. Introduction

Fruit development is a complex process including organogenesis, expansion, maturation, ripening and senescence stages (Abel and Theologis, 1996; Gillaspy et al., 1993). Phytohormone auxin involves in fruit growth and development through transcriptional regulation of specific genes (Jones et al., 2002; McClure et al., 1989; Wu et al., 2010). The genes that are specifically induced by a short auxin treatment are referred to early or primary auxin-response genes including auxin/indole-3-acetic acid (*Aux/IAA*), small auxin up-regulated (*SAUR*), and Gretchen Hagen3 (*GH3*) (Abel and Theologis, 1996; Hagen and Guilfoyle, 2002; Liu et al., 2011; Wang et al., 2008; 2010). Of these early or primary auxin response genes, *GH3* mRNA discovered firstly in soybean was transcriptionally induced by auxin treatment within 10 min (Hagen et al., 1984; Hagen and Guilfoyle, 2002; Roux and Perrot-Rechenmann, 1997). Further investigations reveal that some *GH3* proteins are crucial in photomorphogenesis, suggesting that *GH3* genes are associated with auxin response and light signal (Nakazawa et al., 2001; Tanaka et al., 2002). It was found that some of *GH3* genes, such as *PBS3* (also referred to as *AtGH3.12*) (Nobuta et al., 2007), *AtGH3.5* (Zhang et al., 2007) and *OsGH3.8* (Ding et al., 2008), play a critical role in regulating salicylic acid (SA) metabolism and inducing defense responses, which proposes that these genes can be regulated by other plant growth substances. For example, *CcGH3* mRNA is enhanced by ethylene but inhibited by 1-methylcyclopropene (an inhibitor of ethylene perception) in non-climacteric fruit of pungent pepper, moreover, tomato fruit overexpressing the pepper *GH3* gene ripened earlier upon an ethylene stimulus (Liu et al., 2005), indicating that *GH3* participates in fruit ripening, which is also confirmed in non-climacteric fruit of grape berry recentely (Böttcher et al., 2010). Also, *GH3.8* transcripts from rice can be rapidly induced by SA and jasmonic acid (JA) treatment (Ding et al., 2008). In addition, the *GH3* genes are also upregulated by abiotic stress. For example, the expression level of *Brassica juncea* L. *GH3* increased in response to Cd (Lang et al., 2005), while *WES1*, a *GH3-5* gene in *Arabidopsis*, is also induced by various stress conditions like cold, heat, high salt, or drought (Park et al., 2007).

Abbreviations: ABA, abscisic acid; Aux/IAA, auxin/indole-3-acetic acid; DAA, days after anthesis; EGase, endo-1,4-ß-glucanase; EXP, expansins; *GH3*, gretchen hagen3; IAA, indole-3-acetic acid; JA, jasmonic acid; NAA, α-naphthalene acetic acid; SA, salicylic acid; SAUR, small auxin-up RNA; TDZ, thidiazuron; XET, xyloglucan endotransglucosylase.

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However, the expression of *GH3* genes expression regulated by other plant growth substances such as abscisic acid (ABA) requires further investigation.

Longan as a non-climacteric fruit is gaining in popularity for its nutritional and commercial virtue (Jiang and Li, 2001; Huang, 1995). In previous studies, we reported that xyloglucan endotransglucosylase (*XET*), expansins (*EXP*) and endo-1,4-b-glucanase (*EGase*) from longan fruit were involved in the fruit growth and can be regulated by plant growth substances, including α-naphthalene acetic acid (NAA) and thidiazuron (TDZ) (Chen et al., 2009; Feng et al., 2008; Xie et al., 2009). Goetz et al. (2007) suggested that auxin could play an important role in fruit growth and development. Thus, it is proposed that auxin-responsive *GH3* may involve in fruit growth and development. Unfortunately, there is little information on the *GH3* expression regulated by exogenous growth substances in relation to fruit growth and development of longan.

The aim of the present study was to isolate *GH3* cDNAs from longan fruit and then analyze their mRNA accumulation profiles in pericarp and aril tissues of the fruit during growth, development and ripening. The *GH3* expression of longan fruit regulated by plant growth substances, including NAA, ABA and ethephon (the ethylene releasing compound), was also investigated. This study can improve the understanding of the role of *GH3* in fruit growth, development and ripening of non-climacteric longan fruit.

2. Materials and methods

2.1. Plant materials

Ten longan trees (*Dimocarpus longan* Lour. cv. Shixia) of 5-year-old from an orchard in South China Agricultural University, Guangzhou, China were chosen for this experiment. Developing fruit located in different positions in each tree was collected and sampled once a week for a period of 9 weeks, beginning at 14 days after anthesis (DAA) and ending at 77 DAA. Roots, stems and leaves were also collected from the same longan tree to compare the expression difference of *GH3* in different organs. The whole fruit before 42 DAA (14, 21, 28 and 35 DAA), and the separated pericarp and aril tissues at 42 DAA, and thereafter were then frozen in liquid nitrogen and stored at −80 °C until use.

2.2. NAA treatment

Longan fruit was treated with NAA at two different developmental stages. In the first time, about 400 fruits at 21 DAA located in different directions of each tree were tagged and dipped for 1 min in solution containing 0 (control), or 100 mg/l NAA. After 0, 2, 6, 12, 24 and 48 h of each treatment, 60 randomly-selected fruits were collected, then frozen in liquid nitrogen and finally stored at −80 °C until use. The second treatment was conducted at 77 DAA by the above-mentioned method.

2.3. ABA and ethephon treatment

Treatment with ABA or ethephon (the ethylene releasing compound) was conducted at two different developmental stages, 21 and 77 DAA, respectively. About 400 fruits at 21 and 77 DAA located in different directions of each tree were tagged and dipped for 1 min in a solution containing 0 (control), 200 mg/l ABA or 500 mg/l ethephon. After 0, 2, 6, 12, 24 and 48 h of each treatment, 60 randomly-selected fruits were detached and excised, then frozen in liquid nitrogen and finally stored at −80 °C prior to analysis.

2.4. RNA extraction

Frozen tissues (10 g) were ground to a fine powder in a mortar using a pestle in the presence of liquid nitrogen. Total RNA was extracted using the hot borate method of Wan and Wilkins (1994).

2.5. GH3 cDNAs isolation

Total RNA extracted from pericarp and aril tissues was reverse transcribed to cDNA using the PrimeScript™ 1st Strand cDNA Synthesis Kit (TaKaRa, Shiga, Japan) by the manufacturer's instructions, respectively. The product (the first-strand cDNA) was subjected to PCR amplification. Degenerate primers were designed with reference to the following conserved amino acid sequences of *GH3* genes: forward: 5′-CTA CGA CAC CTA CCA RCA GWT STA YWS NCA-3′ and reverse: 5′-CAG CTC CCA GAA GAT CAY GTR RTS NCC NGG-3′ (R = A/G; Y = C/T; S = C/G; W = A/T; N = A/C/G/T). Reactions for the RT-PCR were subjected to one cycle of 94 °C for 3 min, 35 cycles each at 94 °C for 1 min, 55 °C for 1 min and 72 °C for 2 min, and then one cycle of 72 °C for 10 min. PCR products of the predicted size (about 750 bp in length) were purified and then cloned into pMD-20T vector (TaKaRa, Shiga, Japan) by TA cloning. The nucleotide sequences of the cDNA inserts were determined using the thermo sequenase dye terminator cycle sequencing kit and a 3730 DNA sequencer (Perkin-Elmer Applied Biosystems).

2.6. Amplification of full length cDNA by RACE

RACE-PCR was performed using 3′-Full RACE Core Set Ver.2.0 Kit and 5′-Full RACE Kit (TaKaRa, Shiga, Japan) according to the manufacturer's protocol. To amplify 3′-end fragments, 3′-RACE nested primers were designed according to the sequences of cDNA fragments of *DlGH3.1* and *DlGH3.2*, respectively, while 5′-end fragments were amplified using 5′-RACE nested primers derived from cDNA sequences of *DlGH3.1* and *DlGH3.2*.

2.7. RNA gel blot analysis

Total RNA (10 µg per lane) from each sample was separated by electrophoresis on 1.0% agarose-formadehyde gel in 1 × MOPS (morpholinopropanesulfonic acid) buffer and capillary blotted onto positively-charged nylon membrane (Biodyne® B, 0.45 µm, PALL Co. Sarasota, FL). The RNA was fixed to the membrane by baking for 2 h at 80 °C and then cross-linked to the membranes using an ultraviolet cross linker (Amersham Biosciences, Piscataway, NJ). The membranes were prehybridized for more than 3 h in SDS buffer [50% deionized formamide (v/v), 5 × SSC, 7% SDS, 2% blocking reagent (Roche Diagnostics, Mannheim, Germany), 50 mM sodium-phosphate (pH 7.0) and 0.1% N-lauroylsarcosine (w/v)] and hybridization was then performed overnight in the same buffer containing the gene-specific DIG-labeled probes at 45 °C. Probes were prepared with a DIG probe synthesis kit (Roche Diagnostics) by following the manufacturer's instructions. Both probes containing 3′ un-translated regions of the genes were synthesized. Following hybridization, membranes were washed twice for 10 min with 2 × SSC containing 0.1% SDS at 37 °C, followed by washing twice in 0.1 × SSC containing 0.1% SDS for 30 min at 62 °C. The signals were detected with chemiluminescence using CDP-Star™ (Roche Diagnostics, Gmbh, Mannheim, Germany) as described by the manufacturer. For each treatment (membrane), three repeats were performed. The specific primers used for synthesis of DIG-labeled probes are as follows: *DlGH3.1*, forward (5′-3′) GCAATCCCTCTCCAACGACT; reverse (5′-3′) GGCTGTAACCAACTGGACCA; *DlGH3.2*, forward (5′-3′) ACACCAGTTACGCTGACACC; reverse (5′-3′) TTCCTCCGTTGAATCCCCTC.

3. Results and discussions

3.1. Isolation and analysis of GH3 cDNAs

GH3 genes are multigene families consisting of 20 members in *Arabidopsis* (Hagen and Guilfoyle, 2002) and 13 members in rice (Terol et al., 2006). In this study, two full-length cDNAs of *GH3* genes

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