Plant Science 274 (2018) 109–120

Contents lists available at ScienceDirect

Plant Science

journal homepage: www.elsevier.com/locate/plantsci

Functional characterization of persimmon β-galactosidase gene *DkGAL1* in tomato reveals cell wall modification related to fruit ripening and radicle elongation

Qiuyan Ban[1], Ye Han[1], Yiheng He, Mijing Jin, Shoukun Han, Jiangtao Suo, JingPing Rao*

College of Horticulture, Northwest A&F University, Yangling, Shaanxi 712100, PR China

ARTICLE INFO

Keywords:
Persimmon
β-Galactosidase
Ripening
Germination
Radicle elongation
Cell wall

ABSTRACT

Cell wall metabolism during fruit ripening is a highly organized process that involves complex interplay among various cell wall hydrolases. Among these cell wall hydrolases, β-galactosidase has been identified to participate in cell wall metabolism via its ability to catalyze galactosyl metabolism from the large and complex side chains of cell walls. In this study, the galactose content in the pericarp increased during persimmon fruit ripening, but cell wall galactosyl residues decreased, indicating a relationship between galactose metabolism and persimmon fruit ripening. Expression of a previously isolated β-galactosidase gene, *DkGAL1*, increased 25.01-fold during fruit ripening. Heterologous expression of *DkGAL1* under the CaMV 35S promoter in tomato accelerated on-plant and postharvest fruits ripening. The fruit firmness of one of transgenic line, OE-18, was 23.83% lower than that of WT at the breaker stage. The transgenic fruits produced more ethylene by promoting the expression of ethylene synthesis-related genes and cell wall degradation-related genes. Overexpression of *DkGAL1* in tomato also reduced cell-to-cell adhesion and promoted both wider intercellular spaces and less cell compaction in transgenic fruit structures. Moreover, *DkGAL1* was involved in seed germination and radicle elongation in transgenic tomato seeds. These results confirm the role of *DkGAL1* in fruit ripening and suggest that this gene alters galactose metabolism in the fruit, which can promote ripening and reduce cellular adhesion. In addition, the role of *DkGAL1* is not limited to fruit softening; *DkGAL1* was also involved in seed germination and radicle elongation in transgenic tomato seeds.

1. Introduction

The plant cell wall matrix is complex and comprises highly organized structures that are composed mostly of polysaccharides such as cellulose, hemicellulose, and pectin; more than a hundred genes are involved in cell wall synthesis, modification, and breakdown [1]. Among these genes, the glycosyl hydrolases, β-galactosidase (EC3.2.1.23) has been extensively studied and is believed to participate in cell wall modification [2]. In higher plant, β-galactosidase is a member of the class of glycosyl hydrolases belonging to the GH35 family, and is identified by its ability to hydrolyze terminal, non-reducing β-D-galactosyl residues from numerous β-D-galactoside substrates [2]. β-Galactosidase is involved in various biological processes, including plant growth [3], seed germination [4,5], flower senescence [6], fibers development [7,8] and fruit ripening [9,10]. However, enzymes exhibiting β-galactosidase activity belong to a multigene family with at least 17 genes isolated from tomato and *Arabidopsis thaliana*, respectively [2]. Transcriptions of these genes exhibited diverse patterns during various physiological processes [11,12]. It is imperative to identify the role of specific genes in plant growth. In persimmon, four full-length cDNAs encoding β-galactosidase genes *DkGAL1* to *DkGAL4* was isolated from fruit, all of them could be detected in both fruits and other vegetative tissues [13]. Only *DkGAL1* showed an expression pattern that could be related to the fruit ripening process, and *DkGAL2* expressed mainly in development fruit, besides, transcripts accumulation of *DkGAL3* and *DkGAL4* were low during persimmon fruit ripening [13].

Fruit cell walls are usually highly enriched in pectin, which is the most abundant component in middle lamella [14]. Pectin comprises three main domain: homogalacturonan (HG), type I rhamnogalacturonan (RGI) and type II rhamnogalacturonan (RGII) [15]. HG is known as the “smooth region” of pectin and consists of α-(1,4)-linked

Abbreviations: RG, rhamnogalacturonan; OE, overexpression; ORF, opening reading frame; CWM, cell wall material; 1-MCP, 1-methylcyclopropene

* Corresponding author.
E-mail address: raojingpingxn@163.com (J. Rao).

[1] These two authors contributed equally to this work.

https://doi.org/10.1016/j.plantsci.2018.05.014
Received 19 January 2018; Received in revised form 14 April 2018; Accepted 17 May 2018
Available online 21 May 2018
0168-9452/

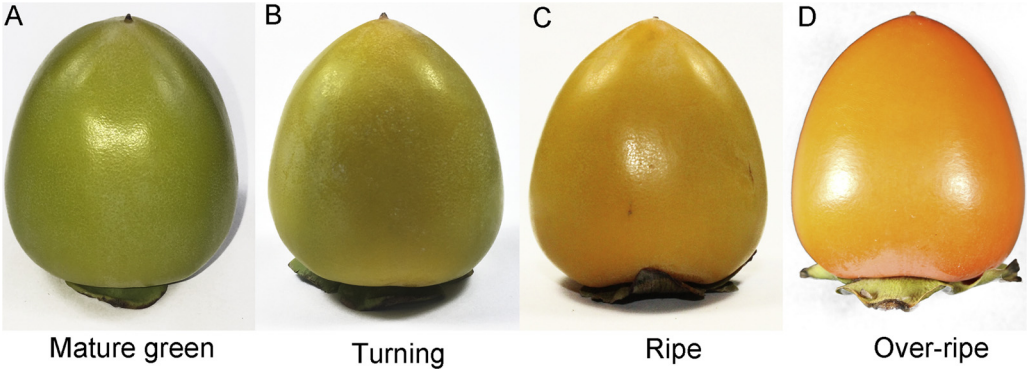

Fig. 1. Photograph of persimmon fruit in different stages. (A) mature green stage; (B) turning stage; (C) ripe stage; (D) over-ripe stage. Mature green, corresponding to a fruit firmness of ~160 N (fruit was fully development and before the onset of ripening, ~120 days after anthesis (DAA)); Turning, corresponding to a fruit firmness of ~120 N (commercial harvest stage, ~150 DAA); Ripe, corresponding to a fruit firmness of ~60 N (respiration burst stage, ~160 DAA) and over-ripe, corresponding to a fruit firmness of ~20 N (the end of shelf life, ~170 DAA) (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.).

galacturonic acid [15]. RGI and RGII constitute what is also known as the "hair region" because of the rhamnose residues of rhamnogalacturonans (RGs) that are decorated by attached neutral sugar side chains, among which arabinan and galactan are the most abundant [15]. A pectin-rich middle lamella constitutes the key factor in maintaining intercellular adhesion and preventing cell separation, and depolymerization of pectin is the main cause of fruit softening [15]. The most evident alteration of persimmon fruit cell wall ultrastructure during softening is swelling of the middle lamella; this swelling is accompanied by slight degradation of the primary wall [16,17]. Redgwell et al. [18] reported that the loss of neutral sugar side chains could increase wall porosity; this increased porosity could allow better movement of other hydrolase enzymes to access their substrates, resulting in cell wall degradation [18]. In addition to the depolymerization of pectin galacturonans, the loss of galactan, arabinan or arabinogalactan side chains from RGI and RGII may contribute to fruit texture because of the ability of these side chains to anchor pectins to both xyloglucan and cellulose to form a tight cell wall structure [19]. Suppressing the tomato β-galactosidase gene *TBG4* reduces fruit softening [9]. Moreover, silencing of α-mannosidase (*α-Man*) or β-D-*N*-acetylhexosaminidase (*β-Hex*) results in firmer fruits [20,21]. Together, these results demonstrate that pectin side chains are involved in fruit softening.

In many plants seeds, cell wall polysaccharides are the principal storage compounds [22,23]. Galactose exists as galactans or as branching residues of many polysaccharides, such as glucomannans, galactomannans and xyloglucans, indicating multiple potential functions of galactose metabolism during seed germination [23]. Seed germination is accompanied by irreversible embryo cell wall loosening, leading to embryo elongation and eventually radicle emergence [22]. Turgor pressure created by water imbibition contributes to cell-wall loosening, but this loosening is restricted by cell wall extensibility [24]. Therefore, modification of the complex polysaccharide wall is necessary for both relaxation of wall stress and cell expansion. Owing to their ability to degrade tissue surrounding the embryo, several hydrolytic enzymes are involved in both seed germination and radicle elongation [22]. Enzymess that may be involved in cell wall loosening including expansin [25], xyloglucan endotransglycolases/hydrolases [26,27], and α-xylosidase [28]. Nevertheless, β-galactosidase can also release stored energy for rapid growth and associated cell wall modifications during plant cell elongation and differentiation [29,30]. Besides, β-galactosidase is involved in seed germination in *Arabidopsis* [31], rice [5], *Lupinus angustifolius* [32], and radish [33]. Together, β-galactosidase functions in various physiological processes and cell wall-associated processes in different tissues.

Persimmon (*Diospyros kaki* L.) not only is a high-economic-value crop but also displays evident texture changes during fruit ripening, making this species ideal for studying fruit softening. In our previous study, four β-galactosidase gene were amplified, and *DkGAL1* was identified as the major β-galactosidase gene in involved in persimmon fruit softening [13]. However, there is still a lack of direct genetic evidence of a role of *DkGAL1* in fruit softening. In this study, by overexpressing *DkGAL1* in tomato, we functionally characterized *DkGAL1* (GenBank accession No. KJ764874) by overexpressing it in tomato. To assess changes in their cell walls, transgenic fruits were phenotyped for a range of biophysical features and microstructures. Furthermore, we found that seed germination and radicle elongation were promoted in transgenic tomato seeds, possibly due to the higher activity of β-galactosidase in the meristem.

2. Materials and methods

2.1. Plant materials

Astringent persimmon (*Diospyros kaki* L. cv. Fupingjianshi) were collected from eight persimmon trees from a commercial orchard in Fuping (109°17′, 34°76′), Shaanxi, China. Fruit of four ripening stage were collected in 2014 based on fruit skin color (Fig. 1), namely mature green, corresponding to a fruit firmness of ~160 N (after the fully development and before the onset of ripening, ~120 days after anthesis (DAA)); turning, corresponding to a fruit firmness of ~120 N (commercial harvest stage, ~150 DAA); ripe, corresponding to a fruit firmness of ~60 N (respiration burst stage, ~160 DAA) and over-ripe, corresponding to a fruit firmness of ~20 N (the end of shelf life, ~170 DAA). Fruits on east sides of tree canopy were harvested between 10–12 a.m. on a cloudless day and transported within hours to the postharvest laboratory at Northwest A&F University, Yangling, Shaanxi, China.

Tomato plants (*Solanum lycopersicum* cv. 'Micro-Tom') were used for *DkGAL1* transformation. Tomato plants growth and postharvest fruit storage conditions setting were according to Hou et al. [34]. The wild type (WT) and T_3 generation overexpression (OE) lines were cultivated in a growth chamber with conditions setting as follows: 14:10 h light/dark with a light intensity of 280 μmol m^{-2} s^{-1}, 25 ± 1: 20 ± 1 °C and 70–80% relative humidity. In order to improve the percentage of fertile fruit and achieve stable high yield, tomato flowers were pollinated by hand shaking and tagged at anthesis. Breaker date and red-ripe date of fruits were recorded for ripening time measurement. Fruits for postharvest storage were harvested at the breaker stage when fruits display a definite "break" in color from green to yellow but on no more than 10% of the skin. And the harvested fruits were stored at 25 ± 1 °C for 21 days, 85–95% relative humidity. Fruits were sample at seven days intervals during storage. Three biological replicates were used for each determination and each biological replication contained at least five fruits.