Expression of α-l-Arabinofuranosidase Genes at Ripening Initiation Potentially Contributes to the Difference in Flesh Juiciness Between Processing and Fresh Tomatoes

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Reconstruction of cell wall polysaccharide is necessary for fruit development and ripening. Arabinose is the one of neutral sugars constituting wall polysaccharides and arabinose-containing polysaccharides play an important role in cellular attachment. In this study, expression patterns of α-l-arabinofuranosidase genes were examined in three tomato cultivars, the processing type ‘OSKAR’, fresh market type ‘Ailsa Craig’, and their hybrid ‘Shonan Pomoron Red’, to elucidate the function of α-l-arabinofuranosidase in fruit traits such as shape, growth, firmness, and juiciness. While there was no significant difference in fruit diameter among the cultivars, longitudinal elongation was observed in ‘OSKAR’ with increasing fruit weight. The hybrid ‘Shonan Pomoron Red’ showed intermediate longitudinal growth. Compared to ‘OSKAR’ at the red ripe stage, fruit flesh softened more in ‘Ailsa Craig’ and much water was released from the pericarp disk. The rate of water release in ‘Shonan Pomoron Red’ was intermediate. The α-l-arabinofuranosidase genes, SlArf/Xyl2, LeXYL1 and LeXYL2, were highly expressed in the young fruits and SlArf/Xyl1 and LeXYL1 were up-regulated when ripening initiated. However, their expression levels did not appear to cause the difference in fruit traits such as growth rate, fruit shape, and firmness among the cultivars. On the other hand, expression levels of SlArf/Xyl1, LeARF1 and LeXYL2 were consistent with the rate of water release from ripe fruit. Therefore, α-l-arabinofuranosidase could influence fruit flesh texture and juiciness in fresh market and processing cultivars.

Key Words: arabinan, arabinose, mealiness, Shonan Pomoron, water release.

Introduction

The tomato is the one of most important, widely grown vegetable fruits with rich nutrients and many options for cooking. Recently, the consumption of both fresh market and processing tomatoes has increased and various cultivars, including F1 cultivars and classic heirloom tomatoes, have been cultivated as cuisine culture has diversified. In Japan, for example, fresh market round tomatoes are preferred, but the processing type has become more popular for cooking. While fresh market tomatoes tend to be soft and watery, processing tomatoes contain a higher percentage of soluble solids without juice, which is suitable for sauces and pastes. Instead of processed canned tomatoes, demand for freshly-harvested processing ones is also increasing as well as fresh-market ones (Kita, 2004). To meet the needs of recent consumption trends, we developed a series of new F1 hybrid cultivars, the Shonan Pomoron series, produced by crosses between fresh market and processing varieties (Hoya et al., 2013).

Tomato fruit enlarges through a process of cell division and cell expansion. Fruit morphogenesis is controlled by a few genes encoding transcription factors, which regulate downstream networks involving diverse
genes (Rodríguez et al., 2011; Azzi et al., 2015). Since plant cells are surrounded by rigid cell walls, reconstruction of the polysaccharide matrix is necessary for cell division and expansion, and subsequent morphogenesis. During fruit enlargement, in which the cell wall is actively synthesized and reconstituted (Terao et al., 2013), multiple enzymes represented by xylan endotransglycosylase/hydrolase (XTH) (Saladié et al., 2006; Miedes and Lorences, 2009) and β-galactosidase (Moctezuma et al., 2003) function to modify the cell wall structure. In ripe fruit, the mechanical strength of the cell wall is reduced because of depolymerization/solubilization of hemicellulose and pectin by a diverse set of proteins including expansin, β-galactosidase and some pectin-metabolizing enzymes (Brummell, 2006). Among them, it was recently revealed that pectate lyase makes a large contribution to tomato fruit softening (Uluisik et al., 2016; Yang et al., 2017). Besides softening, a unique flesh texture is also formed due to a change in the strength of cell-to-cell adhesion during ripening.

Arabinose is a neutral sugar that forms major cell wall polysaccharides such as arabinan and arabinofuranosyl xylan. Several studies have indicated that a matrix network consisting of arabinose-containing polysaccharides plays an important role in cellular attachment (Iwai et al., 2001; Orfila et al., 2002) or wall flexibility (Jones et al., 2003; Moore et al., 2008). Modification of arabinose-containing polysaccharides is assumed to be involved in the resulting fruit texture of tomato fruit (Orfila et al., 2001, 2002), as well as in apple (Nara et al., 2001; Peña and Carpita, 2004; Nobile et al., 2011) and peach (Yoshioka et al., 2011). α-L-Arabinofuranosidase is an enzyme that releases arabinofuranosyl residues from polysaccharides. In tomato, there are at least six putative α-L-arabinofuranosidase genes and each gene shows a differential expression pattern in fruit development and ripening (Itai et al., 2003; Tateishi et al., 2014). Consistent with this, α-L-arabinofuranosidase activity is observed throughout the fruit developmental stages (Sozzi et al., 2002), suggesting α-L-arabinofuranosidase functions in fruit morphogenesis through modification of arabinose-containing polysaccharides.

As described above, differences in fruit morphogenesis, firmness and flesh texture are found between fresh market tomatoes and processing tomatoes. In this study, we characterized the fruit traits of three cultivars: ‘OSKAR’ as a representative processing tomato (San Marzano), ‘Ailsa Craig’ as a fresh market tomato, and the newly developed F1 hybrid ‘Shonan Pomoron Red’, an intermediate phenotype of the other two types. Here, we consider the transition in α-L-arabinofuranosidase gene expression level throughout fruit development and ripening in three cultivars and discuss the involvement of α-L-arabinofuranosidase in fruit traits.

Materials and Methods

Plant materials

The tomato (Solanum lycopersicum) cultivars ‘Ailsa Craig’, ‘Shonan Pomoron Red’ and ‘OSKAR’ were grown in a greenhouse at Kanagawa Agricultural Technology Center under conventional conditions. Fruits were harvested at distinct development and ripening stages (mature green, turning and red ripe). Ripening stages were determined according to the definition of Grierson and Kader (1986). After measuring fruit diameters (longitudinal and side) and fresh weight, fruit firmness was measured by a handy hardness meter (KM-1 type; FUJIWARA SCIENTIFIC CO., Ltd., Tokyo, Japan). Equatorial positions were examined at least three times per fruit. To measure the water release rate, six disks (10 mm diameter) were prepared from pericarp tissue of one fruit using a cork borer and the exocarp and endocarp were removed with a scalpel. The disk was placed on three-layered filter paper and crushed with a plunger (40 mm diameter) equipped with a creep meter (RHEONER II; YAMADEN Co., Ltd., Tokyo, Japan) at a 90% deformation. Released juice from the disk was absorbed by a filter paper and the weight was measured after removal of the residues. The ratio of excluded juice amount to entire amount was determined.

Phylogenetic tree

A phylogenetic tree of tomato α-L-arabinofuranosidases and related proteins with higher plant α-L-arabinofuranosidases, whose substrates were elucidated, was constructed with Clustal W (Larkin et al., 2007). The tree was drawn by Interactive Tree Of Life, an online tool (Letunic and Bork, 2019).

RNA extraction and reverse transcription

Tissue samples were ground using a mortar and a pestle in the presence of liquid nitrogen. Fruit total RNA was isolated with Plant RNA Isolation Reagent (Life Technologies Japan Ltd., Tokyo, Japan) according to the manufacturer’s instructions. Before reverse transcription, total RNA was treated with recombinant DNase I (Takara Bio Inc., Shiga, Japan). cDNA was synthesized using PrimeScript reverse transcriptase (Takara Bio) with Not I oligo d(T)18 primer (5’-ACCTG GAAGAATTCGCGGCCGCAGGAA(T)-3’).

Quantitative RT-PCR

Transcript levels of genes were determined by quantitative reverse transcribed polymerase chain reaction (qRT-PCR) using the StepOnePlus Real-Time PCR system (Applied Biosystems, CA, USA). The reactions were performed in a final volume of 20 μL consisting of 1 μL of diluted cDNA, 0.4 μM of specific primers and 10 μL of SYBR Premix Ex Taq II (Takara Bio). PCR conditions were 95°C for 30 s, and 40 cycles of 95°C
for 5 s and 60°C for 30 s. The expression levels were normalized using the clathrin adaptor complexes medium subunit/endocytic pathway (CAC) (Expósito-Rodríguez et al., 2008). The expression level was normalized using the Clathrin adaptor complexes medium subunit/endocytic pathway (CAC) (Expósito-Rodríguez et al., 2008) expression level as an internal control.

Table 1. Primer sequences used in this study. Accession numbers from GenBank database or Sol Genomics Network (SGN). SlArf/Xyl1-3 primers were followed the method of Tateishi et al. (2014). LeXYL1-2 and LeARF1 primers were designed by PRIMER 3 software (Rozen and Skaletsky, 2000) with some modified optional conditions. The expression level was normalized using the clathrin adaptor complexes medium subunit/endocytic pathway (CAC) (Expósito-Rodríguez et al., 2008) expression level as an internal control.

| Gene Name   | Accession Number | Primer Name         | Sequence          |
|-------------|------------------|---------------------|-------------------|
| SlArf/Xyl1  | AB612972         | Tomato_GHF3_ARA1_F  | 5′-GCTTTATCAGCCGGAGTCAC-3′ |
|             |                  | Tomato_GHF3_ARA1_R  | 5′-CCTTTCCCTGTAAGCTCAATCG-3′ |
|             |                  | TomatoRT_GHF3_ARA2_F| 5′-TGGCGTCTAAATGGGAGCTG-3′ |
|             |                  | TomatoRT_GHF3_ARA2_R| 5′-GAGGCGGTCCATGATTCTTA-3′ |
|             |                  | TomatoRT_GHF3_ARA3_F| 5′-CAGGGAGAAAGGCTGGTAA-3′ |
|             |                  | TomatoRT_GHF3_ARA3_R| 5′-GCCCTCAATGAGGCTCACA-3′ |
| LeXYL1      | AB041811         | TomatoRT_GHF3_XYL1_F| 5′-GGTCTTAATGCAATCTTCTCG-3′ |
|             |                  | TomatoRT_GHF3_XYL1_R| 5′-CCTTTGAGGAAAGGACATGCT-3′ |
|             |                  | TomatoRT_GHF3_XYL2_F| 5′-TGTTAAGTGAGGTTGTCAAA-3′ |
|             |                  | TomatoRT_GHF3_XYL2_R| 5′-TCCCAACTCAATGCTTCTCG-3′ |
| LeXYL2      | AB041812         | TomatoRT_GHF51_ARF1_F| 5′-GCCCTGAATGCATATCTTCTCG-3′ |
|             |                  | TomatoRT_GHF51_ARF1_R| 5′-GCCCTGAATGCATATCTTCTCG-3′ |
|             |                  | TomatoRT_CAC_F      | 5′-GCCCTGAATGCATATCTTCTCG-3′ |
|             |                  | TomatoRT_CAC_R      | 5′-GCCCTGAATGCATATCTTCTCG-3′ |

Results

The fruit of all three cultivars rapidly enlarged from 15 days after flowering to the mature green stage (Fig. 1). At the mature green stage, there was no significant difference in fruit side diameter among the cultivars (Fig. 1B). However, ‘OSKAR’ showed sharp increases in longitudinal diameter and fresh weight during the developmental stage compared with ‘Ailsa Craig’ and ‘Shonan Pomoron Red’ (Fig. 1A, C). Thus, the ratio of longitudinal/side diameter was higher in ‘OSKAR’ (2.24 ± 0.15) and lower in ‘Ailsa Craig’ (0.86 ± 0.05). ‘Shonan Pomoron Red’ had a spindle-shaped fruit with an intermediate ratio (1.69 ± 0.06) (Fig. 2).

Fruit firmness of all the cultivars was 7.41–7.97 N at the mature green stage and then slightly decreased at the turning stage (Fig. 3). During this period, there was no significant difference in fruit firmness among the cultivars. However, considerable fruit softening during ripening clearly progressed in ‘Ailsa Craig’. It showed a significant decrease in firmness at the ripening stage (3.32 ± 1.09 N), whereas the fruit firmness of ‘OSKAR’ was relatively maintained even as ripening progressed (6.26 ± 0.43 N). ‘Shonan Pomoron Red’ had an intermediate firmness (5.36 ± 0.68 N) with no significant difference in ‘OSKAR’ (Fig. 3).

In addition to fruit firmness, fresh market tomatoes tend to be accepted as ‘watery’, and processed tomatoes are not juicy. We addressed the actual amount of released water from fruit pericarp tissue by mechanical pressure. At the turning stage, there was no significant difference in released water among the cultivars (37.5–43.8%). However, at the red ripe stage, less water was released in ‘Shonan Pomoron Red’ (19.7 ± 4.12%) and ‘OSKAR’ (10.9 ± 3.74%) compared to ‘Ailsa Craig’ (32.9 ± 3.25%). ‘OSKAR’ was the least juicy fruit among the cultivars (Fig. 4). Water content in the fruits was not measured but the rate of released water could directly influence the mouth-feel of fruit texture.

Since we observed differences in some traits including fruit size, shape, firmness, and water release among the cultivars (Figs. 1, 2, 3, and 4), we analyzed the expression levels of α-L-arabinofuranosidase genes to elucidate α-L-arabinofuranosidase function in fruit morphogenesis. The tomato genome contains several α-L-arabinofuranosidases and related genes, which are classified into glycoside hydrolase (GH) family 3 (SlArf/Xyl1-3, LeXYL1, 2) and GH family 51 (LeARF1) based on their primary structure and three-dimensional folds, rather than substrate specificity (Fig. 5) (Davies et al., 2005; Cantarel et al., 2009). The enzymes hydrolyze glycosidic bonds with net retention of an anomeric configuration (retaining type) (Hrmova et al., 1996; Pitson et al., 1996). Among them, SlArf/Xyl1 and SlArf/Xyl2 have been indicated to have α-L-arabinofuranosidase activity, while others are assumed to have similar functions based on sequence similarity.
which had higher sequence homology to SlArf/Xyl3, was excluded from expression analysis in this study since it predominantly showed β-xylosidase activity (Tateishi et al., 2014).

SlArf/Xyl1 expression was maintained at a low level until the mature green stage, but sharply increased after ripening with the maximum level at the turning stage (Fig. 6A). Interestingly, the expression level in ‘OSKAR’ at the turning stage was 10 times higher than in ‘Ailsa Craig’.

‘Shonan Pomoron Red’ had an intermediate expression level (Fig. 6A). In contrast, SlArf/Xyl2 was highly expressed only in immature fruit (Fig. 6B). SlArf/Xyl3 was constantly expressed throughout all development stages (Fig. 7A). The expression levels of SlArf/Xyl2 and SlArf/Xyl3 differed slightly in the cultivars, but no clear tendency was found among them. The LeXYL1 expression tended to be higher at the immature stage (5 DAF) and red ripe stage (Fig. 7B). At the ripening stage, the LeXYL1 expression level differed among the cultivars with the lowest in ‘Shonan Pomoron Red’, while the
level was high in ‘Ailsa Craig’ at the immature stage (Fig. 7B). Similar to the SlArf/Xyl2 expression pattern, higher expression of LeXYL2 was observed in immature fruit and the expression gradually decreased with fruit development and ripening (Fig. 7C). The expression level was higher in ‘OSKAR’ at both the immature stage (5 DAF) and turning stage (Fig. 7C). The LeARF1 expression was completely stable throughout all stages, but was higher at the turning stage in ‘Shonan Pomoron Red’ and ‘OSKAR’ (Fig. 7D).

Discussion

Desired traits differ between fresh market tomatoes and processing tomatoes. While fruit shape is an important visual trait for attractiveness to consumers, fruit firmness and water release from the flesh tissue determine mouth feeling and flavor sensation. Clear differences were found between ‘Ailsa Craig’ and ‘OSKAR’ in terms of fruit shape, firmness, and the rate of water release (Figs. 1, 2, 3, and 4). Since arabinose-containing polysaccharides are assumed to be involved in cellular attachment and cell wall flexibility in plant tissues (Iwai and..., 2000).

Fig. 4. Rates of water release from tomato pericarp disks. Each disk was crushed with a plunger and the amount of released juice was measured. Each experiment was done using six disks from one fruit. The values are the means of five individual experiments. Vertical bars indicate the standard error (n = 5). Different letters indicate significant differences by Tukey’s HSD test (P < 0.05).

Fig. 5. Phylogenetic tree of higher plant α-L-arabinofuranosidases. Based on their primary structure the enzymes are divided into two groups: glycoside hydrolase families 3 and 51. SlArf/Xyl1-4, LeXYL1-2, and LeARF1 are derived from Solanum lycopersicum with accession numbers, AB612972-612975, AB041811-041812, and AB073310. Several α-L-arabinofuranosidases in GH family 3 possess both α-L-arabinofuranosidase and β-xylosidase activities. Among them, SlArf/Xyl1 and SlArf/Xyl2 possess both activities against artificial substrates; SlArf/Xyl4 is an β-xylosidase with a similar sequence. The other enzymes were not evaluated for α-L-arabinofuranosidase activity. AtBXL1 (Arabidopsis thaliana, BAB09906), AtBXL3 (A. thaliana, BAB11424), AtAsd1 (A. thaliana, AAF19575), ARA-I (Hordeum vulgare, AAK38481), AXAH-I (H. vulgare, AAK21879), and AXAH-II (H. vulgare, AAK21880), MsXyl1 (Medicago sativa, ABQ45227), PpARF2 (Pyrus pyrifolia, BAD98523) and RsAraf1 (Raphanus sativus, BAE44362) also showed α-L-arabinofuranosidase enzymatic activity when assayed with 4-nitrophenyl-α-L-arabinofuranoside as a substrate.

Fig. 6. Expression of α-L-arabinofuranosidase genes during the fruit growth stage (5, 15, and 30 DAF) and ripening stage. Abbreviations (MG, Tr, and Red) are the same as in Figure 1. A; SlArf/Xyl1, B; SlArf/Xyl2. The values are the means of three individual experiments. Vertical bars indicate the standard error (n = 3). Different letters in each stage indicate significant differences by Tukey’s HSD test (P < 0.05). NS: not significant.
et al., 2001; Jones et al., 2003; Moore et al., 2008), it was of interest to elucidate how these polysaccharides are involved in the important tomato fruit traits. To address this issue, we chose the two cultivars, ‘Ailsa Craig’ and ‘OSKAR’, which represent a fresh market type and processing type, respectively. ‘Shonan Pomoron Red’ was also used because it is a hybrid of the two types and showed intermediate traits between ‘Ailsa Craig’ and ‘OSKAR’ (Figs. 1, 2, 3, and 4).

We monitored the expression levels of α-1-arabinofuranosidase-related genes in the three cultivars (Figs. 6 and 7). SlArf/Xyl2, LeXYL1, and LeXYL2 were highly expressed in the young fruits (Figs. 6B and 7B, C), in which cell wall reconstitution actively occurs (Terao et al., 2013). Consistent with this, α-1-arabinofuranosidase genes have been found to be highly expressed in young proliferating tissues such as the root meristem in carrots (Tanaka et al., 2001), barley seedlings (Lee et al., 2003), elongating stems in Arabidopsis (Minic et al., 2004), developing seeds of radish and Arabidopsis (Kotake et al., 2006; Minic et al., 2006) and expanding leaves in avocado (Tateishi et al., 2015). Given that SlArf/Xyl2 and LeXYL1, 2 are highly expressed in young fruit, the encoded α-1-arabinofuranosidases presumably contribute to cell wall degradation to allow the temporal loosening of the rigid structure that is required for fruit enlargement. Meanwhile, synthesis and reconstruction of cell wall polysaccharides with the loosening also occur during fruit development (Terao et al., 2013). Family 3- and 51-glycoside hydrolases are of the retaining type (Hrmova et al., 1996; Pitson et al., 1996). Such retaining type-enzymes also possess transglycosylation activity under certain conditions. Thus, the high expressions of SlArf/Xyl2 and LeXYL1, 2 in young fruit may be related to polysaccharide polymerization rather than degradation.

However, their expression patterns did not account for the unique fruit shape formation because no clear difference was found among the three cultivars. It has been reported that fruit shape is defined by several genes. Ovate is the most critical gene to produce an obovoid fruit shape (Rodríguez et al., 2011). ‘Ailsa Craig’ has a functional Ovate whereas ‘OSKAR’ possesses a mutation in that gene (Rodríguez et al., 2011), most likely explaining the shape difference. α-1-Arabinofuranosidase presumably has a role to facilitate cell division and enlargement, irrespective of fruit shape. SlArf/Xyl3 showed constant expression throughout fruit development (Fig. 7A). We previously detected a considerable amount of SlArf/Xyl3 transcript in flowers (Tateishi et al., 2014), suggesting its function is related to morphogenesis of floral organs rather than fruit.

Fig. 7. Expression of putative α-1-arabinofuranosidase genes during the fruit growth stage (5, 15, and 30 DAF) and ripening stage. Abbreviations (MG, Tr and Red) are the same as in Figure 1. A; SlArf/Xyl3, B; LeXYL1, C; LeXYL2, D; LeARF1. The values are the means of three individual experiments. Vertical bars indicate the standard error (n = 3). Different letters in each stage indicate significant differences by Tukey’s HSD test (P < 0.05). NS: not significant.
development. Considering the dynamic transition in α-L-arabinofuranosidase gene expressions, α-L-arabinofuranosidase appears to function during fruit enlargement in tomato, together with other cell wall-modifying enzymes. However, the extent of α-L-arabinofuranosidase’s contribution to each important fruit trait has been unclear. In this study, there was no clear effect of α-L-arabinofuranosidase on fruit shape in the tested cultivars.

Fruit firmness decreased as ripening progressed (Fig. 3). In the case of tomato fruits, the α-L-arabinofuranosidase genes, SLArf/Xyl1 and LeXYL1, were up-regulated when ripening initiated (Figs. 6A and 7B). α-L-Arabinofuranosidase is therefore involved in cell wall degradation during ripening. However, their expression levels did not appear to cause the difference in fruit firmness among the cultivars since the softest cultivar, ‘Ailsa Craig’, had the lowest SLArf/Xyl1 level (Fig. 6A) and the LeXYL1 level of ‘Ailsa Craig’ was almost the same as the firmest cultivar ‘OSKAR’ (Fig. 7B). Rather than fruit softening, SLArf/Xyl1 could potentially influence the juiciness of fruits. Consistent with the SLArf/Xyl1 levels, the rate of water release from ripe fruit tissue was the highest in ‘Ailsa Craig’ and the lowest in ‘OSKAR’ (Figs. 4 and 6A). LeARF1 also displayed differential expression levels, as well as those of SLArf/Xyl1, in the cultivars at the turning stage (Fig. 7D) and LeXYL2 showed the highest expression level in ‘OSKAR’ at the turning stage (Fig. 7C). These results lead us to propose a similar role for these genes according to their expression patterns. A modification of arabinosyl side chains has been observed in tomato fruit with a mealy texture (Orfila et al., 2001, 2002). In mealy fruit tissues in which middle lamella is not strong enough to provide adherence between neighboring cells, cell separation, rather than cell rupturing, easily occurs during chewing, resulting in a lower release of liquid content (Areﬁ et al., 2016). Loss of arabinofuranosyl residues from side chains of polysaccharides has been observed in mealy texture apple fruit (Nara et al., 2001) and a mutated tobacco cell line (nolac-14) with weaker cell adhesion (Iwai et al., 2001). The release of arabinofuranosyl residues catalyzed by SLArf/Xyl1, LeARF1, and LeXYL2 may lead to the development of a non-juicy texture. The modification probably occurs with the synergetic action of multiple cell wall modifying enzymes since SLArf/Xyl1 and LeXYL2, which do not have a carbohydrate binding module, presumably have higher activities against soluble arabinose-containing polysaccharides (Karita, 2016). Also, α-L-arabinofuranosidase in GH family 51 derived from Arabidopsis has been shown to have higher arabinose-releasing activity from oligosaccharides than polysaccharides (Ichinose et al., 2010). LeARF1, a member of GH family 51, is predicted to have a similar property. On the other hand, in the Cnr mutant tomato with a mealy texture, arabinosyl residues are deposited in cellulose and alkaline-soluble hemicellulose (Orfila et al., 2002). In this case, potential transglycosylation activity of the retained glycoside hydrolase could be involved in arabinose deposition, which strengthens cell wall rigidity and leads to a lack of juiciness. In either case, based on the expression patterns, we speculate that differential expression patterns of SLArf/Xyl1, LeARF1, and LeXYL2 in tomato cultivars cause arabinose-related modifications in cell wall polysaccharides, creating unique flesh textures, juiciness and mealiness in each cultivar.

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