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RESEARCH ARTICLE

Cloning, localization and expression analysis of two *fw2.2-like* genes in small- and large-fruited pear species

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Abstract

Fruit size is one of the most important agronomic characters, which is mainly determined by cell number and cell size. However, our current knowledge about pear is largely unknown. Through counting of pear mesocarp cells at different stages, we found that the cell number, rather than the cell size, is responsible for the differences between small- and large-fruited cultivars. *Fruit weight-2.2* (*fw2.2*) is an important quantitative trait locus (QTL) affecting fruit weight in tomato and functions as a negative regulator in carpel cell division. To get more insights into this QTL in pear fruit development, we isolated two putative homologous *fw2.2* genes, which were designated as *fw2.2-like* (*PbFWL*) genes. PbFWLs encode Cys-rich proteins with the CCXXXXCPC motif and belong to the PLAC8 superfamily. In addition, results from the subcellular localization indicated that PbFWLs were localized in the plasma membrane. The expression profile of the *PbFWL* genes by qRT-PCR showed they expressed higher in small-sized fruit cultivar than that in large-sized fruit cultivar during the cell division period. In summary, our data suggest that these two *PbFWLs* might be negatively related to the cell division in pear fruit.

Keywords: pear, fruit size, *fw2.2*, subcellular localization, gene expression, cell division

1. Introduction

Fruit size is an important agronomic character for plant breeders. However, little is known about the physiological characteristics and molecular mechanisms of the variability of fruit size which is a very complicated trait determined by a number of key genes and quantitative trait loci (QTLs) (Liebhard *et al*. 2003; Kenis *et al*. 2008).

A previous study in *Arabidopsis thaliana* showed that overexpression of the *AINTEGUMENTA* (*ANT*) gene and *AUXIN REGULATED GENE INVOLVED IN ORGAN SIZE* (*ARGOS*) gene could increase fruit size by extending the cell proliferation period (Krizek 1999; Mizukami and Fischer 2000; Hu *et al*. 2003). In avocado, it was reported that two genes encoding mitotic A- and B-type cyclins, together with a gene encoding a putative proliferating cell nuclear antigen protein, might provoke early cessation of fruit cell division (Dahan *et al*. 2010). In rice, several QTL genes related to yield had been cloned, such as *GW2*, *GS3* and *GW5*, all

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of which negatively regulated grain size (Fan *et al*. 2006; Song *et al*. 2007; Shomura *et al*. 2008). A study in tomato found 28 QTLs affecting the weight and size of tomato fruit among large-sized cultivars and related small-sized wild species (Alpert *et al*. 1995; Grandillo *et al*. 1999). One of the QTLs, the *fruit weight 2.2* (*fw2.2*) gene which was significantly affective, has been cloned and characterized: results showed that the *fw2.2* was proven to control 30% of the variance in fruit weight by negatively regulating cell division (Frary *et al*. 2000). Alleles in wild relatives were found to cause a reduction in fruit size, whereas alleles in domesticated tomato increased fruit size (Liu *et al*. 2003; Tanksley 2004), and the *fw2.2* gene was reported to be involved in modulating the size of pre-anthesis ovaries (Kortstee *et al*. 2007). The genome sequences of 14 plant species have subsequently been mined based on similarity to the tomato *fw2.2* gene, with homologs containing a PLAC8 domain (Libault *et al*. 2010). PLAC8 motif-containing proteins form a large family, members of this protein family in plants typically range from 100 to 150 amino acids, though some are >400 amino acids in length (Libault and Stacey 2010). Although the overall protein sequence identity/similarity among family members is relatively low, there is strong conservation of key domains, suggesting a conservation of the core biochemical function of these proteins (Libault *et al*. 2010). Indeed, in tomato, the difference between 'small' and 'large' alleles of *fw2.2* lied in the promoter sequence, and the *fw2.2* mRNA levels were higher in small-fruit tissues than that in large-fruit tissues; moreover, the high level of *fw2.2* mRNA in small-fruit tissues was negatively correlated with mitotic activity (Frary *et al*. 2000; Cong and Tanksley 2006). Similar conclusions were drawn in avocado (Dahan *et al*. 2010). In maize, the homologous genes of *fw2.2* are named *Cell Number Regulators* (*CNRs*), and transgenic experiments showed that *CNR1* actually functions by affecting cell number (Guo *et al*. 2010). In sweet and sour cherry, *PavCNR12* and *PavCNR20* are potential candidates genes for the control of fruit size (De Franceschi *et al*. 2013). In addition to the determination of fruit size, in *Arabidopsis thaliana*, some members of this family are called plant cadmium resistance (PCR), which played an important role in transport of heavy metals, such as cadmium or zinc, through the plasma membrane (Song *et al*. 2011).

Pear (*Pyrus spp*), which belongs to the subfamily Pomoideae in the family Rosaceae, is the third most important temperate fruit species, after grape and apple, and has been cultivated for over 3000 years. Pear fruit is believed to benefit human health because of the rich in vitamin C, dietary fiber, sugar, protein, and many other nutrients. In the Xinjiang Uygur Autonomous Region of China, Korla fragrant pear is a well-regarded cultivar grown in the Bayingol Mongolian Autonomous Prefecture and Aksu region. The fruit of Korla fragrant pear is crisp and succulent, with a mild fragrant taste. Pear fruit display a variety of sizes (Kajiura and Sato 1990; Gan *et al*. 2006): whereas some wild pears weigh only a few grams, cultivated pears range from dozens of grams to hundreds of grams and even up to one kilogram. A study showed that sugar accumulation in larger-sized fruit was significantly higher than that in small-sized fruit (Wu *et al*. 2011). In Korla fragrant pear, the research showed that xenia could affect fruit size (Xie *et al*. 2013). Nonetheless, the molecular mechanism determining fruit size in pear fruit remains poorly understood. Before the release of draft genome of pears (*Pyrus bretschneideri* Rehd.) (Wu *et al*. 2013), to decipher the function of *fw2.2* genes, two genes were isolated in pear fruit and named as *fw2.2-like* (*PbFWL*) genes using a homology-based cloning method. The phylogenetic analysis and expression profiling of these *PbFWL* genes were also characterized. These results shed light on the mode by which *PbFWL*s could regulate fruit size in pears.

2. Results

2.1. Fruit development and examination of cell size and number

There are obvious differences in fruit size between Duli pear and another two pear cultivars (Fig. 1), while Duli pear is the smallest, and Yali pear is the largest. The growth curves of fruit volume of Yali pear and Korla fragrant pear displayed as an "S" shape, exhibiting the pattern "slow-quick-slow", whereas the growth curve of Duli pear did not show an obvious "S" shape (Fig. 2-A). The growth curve of fruit weight was similar to that of fruit volume (Fig. 2-B). Thus, cell size and cell number were tested during the entire fruit development stage to ascertain which determines pear size. Through observations and measurements based on paraffin sections of mesocarp cells at different stages (Fig. 3-A and B), the cell volume and cell number were calculated (Fig. 4-A and B). The results showed that from full bloom to 45 days after full bloom (DAFB), the mesocarp cells in the three cultivars were tightly arranged, without intercellular spaces in cell division. During this period, the cell volume was small, and the increase was slow; the increase in cell number mainly resulted in fruit swelling. At 45 DAFB, the pear mesocarp cells stopped dividing, and the fruit grew slowly. At 75 DAFB, the volume of the mesocarp cells began to swell rapidly, and the growth of the fruit in this period mainly depended on cell volume. All these results show that the mesocarp cell volumes of the three cultivars in every stage were similar; thus, the difference of fruit sizes depended on the cell number.

2.2. Cloning of full-length *PbFWLs*

As there has been no information about the pear *fw*2.2