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Characterization of a giant-fruit mutant exhibiting fruit-limited polyploidization in pear (*Pyrus communis* L.)

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ABSTRACT

Fruit size is one of the most important traits in fruit trees. We found a bud sport mutant bearing giant fruits of pear in an orchard in Yamagata prefecture, Japan. The fruit weight of this mutant was more than twice that of an original cultivar 'La France'. The size of the receptacle was already larger in the mutant than in the original cultivar at full bloom, and size differences between the mutant and the original cultivar were observed throughout fruit development. Microscopic observation of fruit cortex cells revealed that the size of cells was larger in the mutant than in the original cultivar, and that the number of cells was comparable between the mutant and the original cultivar. Flow cytometric analysis revealed an increase in the number of cells having doubled amounts of DNA (4C cells) in the fruit cortex, but such an increase was not found in leaves, suggesting chimeric polyploidization or endoreduplication in fruits of the mutant. Mutant fruits exhibited significantly higher titratable acidity and lower firmness than those of the original cultivar. These differences were observed from 110 days after full bloom to 165 days (the commercial harvesting time). However, the concentration of soluble solids was not different between the mutant and the original cultivar. There were no significant differences in polyuronide contents between the mutant and the original cultivar fruits, but hemicellulose content in fruits of the mutant was significantly lower than in those of the original cultivar.

1. Introduction

Fruit size is one of the most commercially important traits in fruit trees, because larger fruits are generally marketed at higher prices. Fruit size is determined by the number and size of fruit cells. In pear (*Pyrus communis* L.) belonging to the subfamily *Maloideae* in the *Rosaceae*, cell division occurs in the period from anthesis, determining the number of cells. Thereafter, cell expansion occurs, and fruit size increases following a sigmoid curve.

In Japanese pear (*Pyrus pyrifolia* Nakai), a closely related to pear, cell number has been reported to be more important than cell size for determining fruit size of cultivars (Zhang et al., 2006). In tomato, which is used as a model plant for the study of fruit development, *FW2.2* has been identified as a gene contributing approximately 30% of phenotypic difference in fruit size between large, domesticated tomatoes and their small-fruited wild relatives. This gene negatively controlling fruit size has been found to be homologous with an oncogene (Frary et al., 2000). Although several reports on the control of cell size have been published, the molecular biological mechanism underlying cell size control has not been elucidated. In a study of endoreduplication of tomato fruits, *Wee1*, a cell cycle-related gene, has been revealed to play an important role in cell expansion by endoreduplication (Gonzalez et al., 2007).

Mutants are important for studying the molecular mechanisms of various traits. In apple (*Malus* × *domestica* Borkh.), a giant-fruit cultivar, 'Grand Gala', has been obtained by spontaneous mutation from cultivar 'Gala'. In 'Grand Gala', higher and lower expression levels of *MdCDKA1* and *MdCYCA2*, respectively, both of which are involved in the cell cycle, than those of 'Gala' have been reported (Malladi and Hirst, 2010). A giant-fruit mutant has been selected in Chinese pear (*Pyrus ussuriensis* Maxim.), and gene expression has been compared between the mutant and the original cultivar (Zhang et al., 2011). In a small-fruit mutant reported in persimmon, a decrease in the number of fruit cells has been observed, suggesting the mutation to be a gene involved in cell division (Yamane et al., 2008). A decrease in malic acid content at the green stage and an increase of sucrose content at ripening stage have been detected in a nonfleshy mutant of grape (Fernandez et al., 2006).

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A bud sport mutant of pear bearing giant fruits (named as 'G. LaF') was found on a tree of 'La France' (shown as 'LaF'), which is considered to be a diploid pear cultivar (Yamamoto et al., 2010), in an orchard in Yamagata prefecture, Japan. Since fruit size is an important trait for fruit markets, this mutant was considered to have a high economic value. Therefore, in the present study, we characterized this mutant morphologically and biochemically.

2. Materials and methods

2.1. Plant materials

Fruits were collected from branches of the bud sport mutant, 'G. LaF' bearing giant fruits, in an orchard in Kaminoyama, Yamagata in 2005 and 2006. Among the three main branches of this tree, one branch was of the mutant, and the other two branches were of the original cultivar 'La France' ('LaF'), which was used for comparison. Only one fruit was set at one flower cluster and fruit thinning was performed to leave one fruit on a spur among four spurs in both 'G. LaF' and 'LaF'.

2.2. Morphological observation

Transverse diameters of 30 fruits were recorded at seven stages from full bloom to the commercial harvesting time, 165 days after full bloom, which was determined by starch concentration using I_2-KI solution. Fruit tissues were sliced with a microslicer (ZERO1, Dosaka EM, Kyoto, Japan) to 100-μm thickness, stained with 1% toluidine blue. Five parts in a transverse section of the equatorial planes were observed under a microscope using four fruits harvested at 165 days after full bloom. According to Harada et al. (2005), the number of cells on a line four sides of a 1-mm square was counted. Cell- and space-size index (CSSI) and cell number index (CNI) were calculated according to Harada et al. (2005) as follows:

CSSI (in μm) = the square perimeter/number of cell intersected.
CNI = equatorial diameter/CSSI.

2.3. Flow-cytometric analysis

According to the manufacturer's protocol for the CyStain UV Precise P kit (Partec GmbH, Münster, Germany), nuclei were released by chopping plant tissues in the kit extraction buffer, and passed through a nylon mesh (50-μm pore size). Staining solution was added to the filtrate and left for 5 min at room temperature. Intensity of fluorescence in nuclei and distribution of the intensity were investigated with Ploidy Analyzer (PA, Partec GmbH, Münster, Germany). The position corresponding to 2C was identified by distribution analysis of fluorescence intensity in leaf cells of 'LaF'.

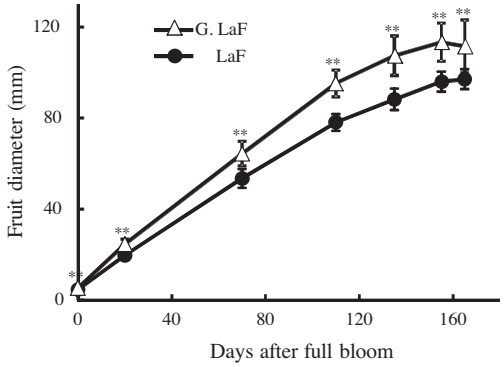

Fig. 1. Fruit diameter of a giant-fruit pear mutant ('G. LaF') and the original cultivar ('LaF') during fruit development in 2006. Bars indicate standard deviation. **: $P < 0.01$ (t-test)

2.4. Fruit quality traits analysis during fruit development

In 2006, the concentration of soluble solids and titratable acidity of three fruits at 110, 140, 155, and 165 days after full bloom were analyzed with a digital sugar content meter (PR101, Atago, Tokyo, Japan) and by titration with 0.1 N NaOH, respectively. The titratable acidity was converted to malic acid content. Firmness and starch concentration were investigated using three fruits harvested 140, 155, and 165 days after full bloom. Firmness was measured with a penetrometer (FT327 5/16 in. tip, Facchini, Italy) after peeling fruit epidermis on the equatorial plane. Starch concentration was quantified according to Murayama et al. (2006). In 2005 and 2006, the soluble solids concentration and titratable acidity, firmness, and seed number were analyzed using 13 fruits harvested 165 days after full bloom.

In 2006, polyuronide (water-soluble, chelator-soluble, and alkaline-soluble) and hemicellulose of cortex in fruits 165 days after full bloom using three fruits for each variety were analyzed in accordance with Murayama et al. (2002).

3. Results

3.1. Morphological investigation of 'G. LaF' and 'LaF'

There was no significant difference in size and thickness of leaves between 'G. LaF' and 'LaF', while the length of vegetative shoots was slightly shorter in 'G. LaF' than that in 'LaF'. Time-course investigation of fruit diameter from the day of full bloom to the commercial harvesting time, 165 days after full bloom, revealed that receptacle sizes of 'G. LaF' were significantly larger than those of 'LaF' at the time of full bloom, and that fruit sizes were significantly larger during the period of fruit development (Figs. 1 and 2). The fruits of 'G. LaF' had slightly depressed shape and more

Fig. 2. Mature fruits of 'G. LaF' and 'LaF'. Bar 10 cm.