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The cellular physiology of loquat (*Eriobotrya japonica* Lindl.) fruit with a focus on how cell division and cell expansion processes contribute to pome morphogenesis

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ABSTRACT

Develop from flower tube, loquat (*Eriobotrya japonica* Lindl.) fruit differs from apple and other pome on size and shape greatly. To date, cell regulators of loquat fruit morphogenesis are still unknown. Fruit growth, histological observation and regulatory genes expression were investigated for 22 stages in loquat. Growth measurements reveal that loquat contains larger carpel volume and thinner flesh compared to apple. Cellular analyses demonstrate that cell number contributes to size varieties among loquat and other pome. Low cell production rate confers to weak cell proliferation capacity during early development. The correlation of cell number/size changes and several regulatory gene expression implies that: loquat fruit maintained cell division under regulation of *EjFWLs* and other genes from anthesis to 42 days past anthesis; unique temporal expression of *EjWEE1* and *EjKRP3* at 42 and 63 days past anthesis participate in cell cycle exit and polyploidy establishment; complementary expression of *EjCCS52* isoforms promotes cell growth in an endoreduplication dependent pathway like *EjWEE1* and *EjKRP3* may be involved; *EjEXPAs* involved in acid cell growth play crucial role in fruit cell size enlargement. Together, these data indicate that cell division and expansion under complicated regulation, and weak cell proliferation capacity result to less cell layer which confers to thin cortex.

1. Introduction

Multicellular organisms rely on the coordinated progression of cell proliferation and cell differentiation (growth) for organ morphogenesis. Cell proliferation and differentiation are regulated in a coordinated manner through organ development. Generally, cell division activity would gradually decrease as organogenesis proceeds, and most, cells eventually exit division and enter differentiation. The scheduled cessation of cell division and cell growth initiation are critical for the formation of organs with genetically defined sizes and morphologies (Conlon and Raff 1999; Hepworth and Lenhard, 2014).

DREAM complexes (containing the retinoblastoma protein (RB), E2F and its dimerization partner DP, and MYB homologues) have been identified in animals and plants, and they link several distinct transcription factors to coordinate gene expression throughout the cell cycle (Fischer and DeCaprio 2015). (Kobayashi et al., 2015) identified distinct roles for the MYB3R family members during *Arabidopsis* cell cycle. The results revealed that complex bonds to MYB3R4 might activate the expression of G2/M target genes (e.g., *KNOLLE*, *EDE1*, *CYCB1;2*, *CDKB1;2* and *CDKB2;2*), whereas combinations with MYB3R3 would repress cell division. At a given time in development, plant cells at the meristem will exit mitotic cell cycle and start differentiating. To date, the mechanisms that control cell cycle are still not well understood, and the exit from cell cycle also needs more investigation. In *Arabidopsis*, the exit from cell division requires a decrease in the CDK activity (De Veylder et al., 2007). The inhibition of CDK activity by ICK/KRP proteins results in premature cell cycle exit. *WEE1* expression is rapidly induced by DNA stress, resulting in the tyrosine phosphorylation of CDKs and the controls of cell cycle arrest in *Arabidopsis* (De Schutter et al., 2007). CCS52 (cell cycle switch) proteins inhibit the mitotic cycle and drive cells into endocycle for post-mitotic cell growth (Cebolla et al., 1999). Plant cells also arrest cell cycle in response to environmental cues (De Veylder et al., 2007).

In developing from the ovary (ovaries) or other floral organs, fruits protect developing seeds and contribute to seed dispersal for species propagation. In addition, fruits provide humans with nutrition, culinary diversity, and great pleasure (Tanksley 2004). A true fruit develops from the ovary, and the ovary wall becomes the pericarp. For pseudocarpic fruit, organs such as receptacle bracts (or the floral tube and other tissues) participate in fruit formation(Gillaspy et al., 1993).

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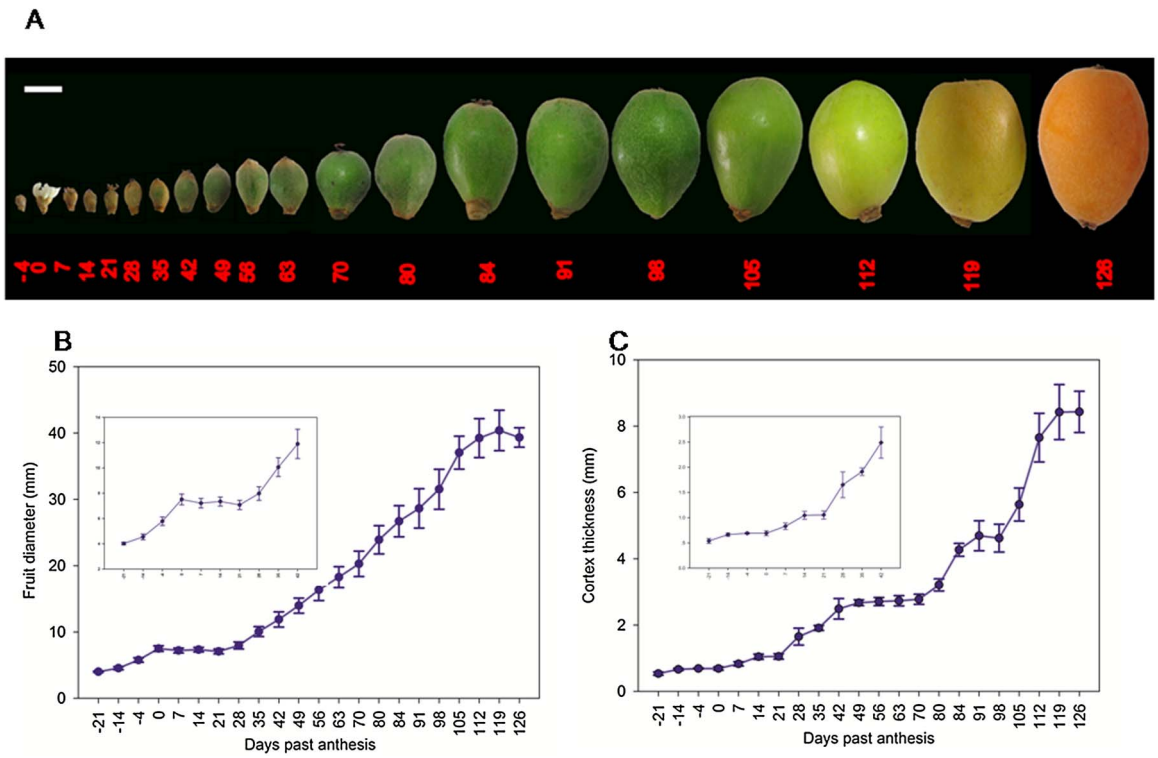

Fig 1. Fruit growth of 'Zaozhong-6' loquat.

(A) A time series of loquat receptacle size. Bar = 2 cm. (B) Changes in fruit diameter. The inset presents a magnified view of fruit diameter during early pome development. (C) Changes in the cortex. The inset presents a magnified view of the cortex thickness during early pome development. For each time point fruit diameter and cortex thickness were measured with 5 biological replicates.

Different types of fruit display various developmental programmes. For example, the avocado pericarp continues its cell division until shortly before ripening (Schroeder, 1953) while most fruits such as peaches (Ognjanov et al., 1995), tomatoes (Joubes et al., 1999) and apples (Bain and Robertson 1951; Malladi and Hirst 2010) continue to engage in cell proliferation during early fruit development stage, and later, the cells enlarge for a long period.

GS3, *GS5*, *GW8* and *GLW7* have been demonstrated to play important roles in the grain width and length regulation of rice and other grass (Mao et al., 2010; Li et al., 2011; Wang et al., 2012; Si et al., 2016), and many genes have been functionally identified to act as pivotal regulators during fruit size and shape formation in dicots. *FW2.2* was first cloned in tomato as a negative regulator of ovary wall cell division during tomato fruit weight evolution (Frary et al., 2000). Another QTL (quantitative trait loci) is *FW3.2*, which promotes tomato pericarp cell division (Chakrabarti et al., 2013). Additionally, natural variations in *POS1* and *AtARF18* were successively shown to have diverse capacities for cell size regulation during tomatillo and silique development, respectively (Wang et al., 2014; Liu et al., 2015). Moreover, two cloned loci called *Fascinated* (*FAS*) and *Locule number* (*LC*) were discovered to have functions in tomato shape domestication by controlling the locule number in the ovary (Cong et al., 2008; Munos et al., 2011). The natural variations in the above genes all contribute to fruit size or shape evolution. Unfortunately, few genes have been determined to specifically regulate woody perennial fruit development. The expression patterns of two *MdANT*s during early fruit growth were found to coincide with cell production period, and they were positively correlated with that of the cell cycle regulatory genes. The data implied that *MdANT*s may participate in cell production in apples (Dash and Malladi 2012). *PpKNOPE1* represses *GA3ox1* expression during peach mesocarp cell differentiation and ectopic *KNOPE1*expression in *Arabidopsis*, resulting in decreased cell size (Testone et al., 2015). A bHLH (basic helix loop helix) transcription factor called VvCEB1 has been determined to be grape berry-specific, and it strongly stimulates cell expansion in *VvCEB1*-overexpressing embryos (Nicolas et al., 2013). In pears (Hiwasa et al., 2003), grapes (Ishimaru et al., 2007; Suzuki et al., 2015) and peaches (Cao et al., 2016), the expression of *EXPA*s has been analysed during fruit late development; some of these genes are associated with cell division or expansion. In addition, a SNP marker for peach fruit size control was identified in the promoter region of one of the *Expansin* genes by (Cao et al., 2016) recently. However, the molecular mechanisms of cell division and cell expansion regulation in woody fruit trees still requre further study.

The molecular mechanisms involved in regulating fruit morphogenesis and final size in loquats are not yet understood. The growth pattern of the loquat has been studied by (Blumenfeld 1980), (Ding and Zhang 1988) and (Cuevas et al., 2003), and it was divided into 3 stages by (Ding and Zhang 1988). To date, no information has been reported about quantitative changes in gene expression with cell division or cell enlargement during loquat fruit morphogenesis. The primary goal of this work is to establish exhaustive cytological kinematics for loquat fruit, and to determine the key cellular program shift points to illustrate how cell division and cell expansion coordinate and relate to gene expression profiles during pome formation. To understand how cell division and cell growth coordinate with one another throughout fruit development, the receptacles or cortexes from 22 stages were collected and sectioned, and numerous genes associated with cell division, cell cycle exit and cell expansion were selected for quantitative analysis.

2. Materials and methods

2.1. Plant materials

In this study, five mature 'Zaozhong-6' trees under normal management in the loquat germplasm resource preservation garden (South China Agricultural University, Guangzhou, China) were used. Fully open flowers at full-bloom stage were tagged. Measurements and samples were collected before or after tagging. Samples were collected