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Tomato Ovary-to-Fruit Transition is Characterized by a Spatial Shift of mRNAs for Cell Wall Invertase and its Inhibitor with the Encoded Proteins Localized to Sieve Elements

William M. Palmer[1,2,4], Lei Ru[1,2,4], Ye Jin[3,4,5], John W. Patrick[1,2] and Yong-Ling Ruan[1,2,*]

[1]School of Environmental and Life Sciences, The University of Newcastle, NSW 2308, Callaghan, Australia

[2]Australia-China Research Centre for Crop Improvement, The University of Newcastle, NSW 2308, Callaghan, Australia

[3]Institute of Plant Physiology and Ecology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, 300 Fenglin Road, Shanghai 200032, China

[4]These authors contributed equally to the article.

[5]Present address: College of Life Sciences, Central China Normal University, Wuhan 430079, China

***Correspondence: Yong-Ling Ruan (yong-ling.ruan@newcastle.edu.au)**

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ABSTRACT

Central to understanding fruit development is to elucidate the processes mediating a successful transition from pre-pollination ovaries to newly set fruit, a key step in establishing fruit yield potential. In tomato, cell wall invertase (CWIN) LIN5 and its inhibitor INH1 are essential for fruit growth. However, the molecular and cellular basis by which they exert their roles in ovary-to-fruit transition remains unknown. To address this issue, we conducted a study focusing on ovaries and fruitlets at 2 days before and 2 days after anthesis, respectively. *In situ* hybridization analyses revealed that *LIN5* and *INH1* exhibited a dispersed expression in ovaries compared with their phloem-specific expression in fruitlets. Remarkably, LIN5 and INH1 proteins were immunologically co-localized to cell walls of sieve elements (SEs) in ovaries immediately prior to anthesis and in young fruitlets, but were undetectable in provascular bundles of younger ovaries. A burst in CWIN activity occurred during ovary-to-fruit transition. Interestingly, the ovaries, but not the fruitlets, exhibited high expression of a defective invertase, SlIdeCWIN1, an ortholog of which is known to enhance inhibition of INH on CWIN activity in tobacco. Imaging of a fluorescent symplasmic tracer indicated an apoplasmic phloem unloading pathway operated in ovaries, contrary to the previously observed symplasmic unloading pathway in fruit pericarp. These new data indicate that (1) a phloem-specific patterning of the *CWIN* and *INH* mRNAs is induced during ovary-to-fruit transition, and (2) LIN5 protein functions specifically in walls of SEs and increases its activity during ovary-to-fruit transition, probably to facilitate phloem unloading and to generate a glucose signal positively regulating cell division, hence fruit set.

Key words: fruit set, invertase, invertase inhibitor, phloem unloading, sugar signaling

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INTRODUCTION

Successful fruit set occurs when an ovary develops to a young fruitlet, accomplished during and soon after anthesis. In most plant species, fruit set co-occurs with seed set marked by the formation of a seed from an ovule following double fertilization. Fruit and seed development follows the sequence of set, growth, and maturation. The set phase is characterized by intensive cell division that determines fruit and seed size and their number, hence yield potential (Ruan et al., 2012). Compared with the latter stages of fruit and seed development, the set phase is highly vulnerable to abiotic

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stresses such as drought, heat, and cold, often leading to fruit and seed abortion, hence irreversible yield loss (Boyer and McLaughlin, 2007; Zinn et al., 2010). Research on fruit and seed development has largely focused on their growth and maturation stages (Klann et al., 1996;Tomlinson et al., 2004; Weber et al., 2005). By contrast, much less is known about the cellular and molecular basis of the transition from ovaries to fruitlets or ovules to seeds (Wang et al., 2009; Wang and Ruan, 2012), a topic of major significance and urgency in achieving global food security under climate change (Barnabás et al., 2008; Eckardt et al., 2009; Ruan, 2014).

Undoubtedly, transition from ovaries to fruitlets and ovules to seeds is under complex regulation exercised by many interacting genes and signals, in particular those involved in rapid cell division and assimilating import and utilization (Wang et al., 2009; Ruan et al., 2012). Strong evidence indicates that invertases, which hydrolyze sucrose (Suc) into glucose (Glc) and fructose (Fru), could play a major role in fruit and seed set. Based on their subcellular localizations they are classified as cell wall, cytoplasmic, and vacuolar invertases (Sturm, 1999) and are abbreviated as CWIN, CIN, and VIN, respectively (Ruan, 2014). Loss of CWIN activity in maize causes stunted grain (fruit) growth (Cheng et al., 1996), or abortion under drought (Boyer and McLaughlin, 2007). Stem infusions of Suc to water-stressed maize plants at anthesis ameliorated grain abortion by restoring CWIN and VIN activities and Glc levels (McLaughlin and Boyer, 2004a, 2004b). This functional reversion, together with the miniature grain phenotype in the mutant lacking ZmCWIN2 (Cheng et al., 1996), demonstrates the critical role played by invertase in maize grain set. Similarly, decreased expression or activity of CWIN and related sugar transporters in reproductive organs correlated with grain abortion in rice under cold (Oliver et al., 2007), wheat under drought (Ji et al., 2010), and floral and fruit abortion in tomato under heat stress (Li et al., 2012). Thus, high CWIN activity in reproductive organs appears to be required for fruit and seed set in both monocot and eudicot species. However, little is known about how CWIN exerts this effect at the cellular and molecular levels. Central to reaching an understanding of how CWIN controls fruit and seed set is to identify the cellular site(s) where CWIN genes and their encoded proteins are expressed during this process.

Tomato is ideal for examining cellular localization dynamics of CWINs during ovary-to-fruit transition for several reasons. Of the four CWIN genes in the tomato genome, only one, *LIN5*, is expressed in ovary and fruit and specifically in these organs (Godt and Roitsch, 1997; Fridman and Zamir, 2003). Importantly, silencing *LIN5* led to fruit and seed abortion (Zanor et al., 2009). On the other hand, elevation of CWIN activity by suppressing an invertase inhibitor, INH1, increased seed weight and fruit hexose levels (Jin et al., 2009). These findings collectively demonstrate an essential role for CWIN in tomato fruit and seed set and subsequent growth. However, it remains elusive as to how LIN5 exerts its regulatory control over tomato fruit set, a key stage in fruit development that has not been addressed in previous studies (Ruan and Patrick, 1995; Fridman et al., 2004; Jin et al., 2009; Zanor et al., 2009).

We initiated an investigation on this issue by determining the spatial and temporal patterns of LIN5 expression and its associated regulatory proteins, INH1 (Jin et al., 2009), and a putative defective invertase, SldeCWIN1, during ovary-to-fruit transition by focusing on ovaries and young fruitlets at 2 days before anthesis (DBA) and 2 days after anthesis (DAA). The expression analysis was complemented by estimating changes in the catalytic activity of LIN5 and of the cellular pathway of phloem unloading across this developmental window of fruit set. Our analyses led to a number of novel findings. First, a phloem-specific expression of *LIN5* and *INH1* mRNAs was established during ovary-to-fruit transition. Both genes exhibited a dispersed spatial expression in ovaries that became phloem specific in fruitlets. Second, LIN5 and INH1 proteins were found to be co-localized to sieve elements (SEs) of ovaries prior to anthesis, with the pattern persisting in young fruitlets. Third, CWIN exhibited a burst in activity during ovary-to-fruit transition, probably through post-translational regulation imposed by an interplay with INH1 and a newly identified defective invertase, SldeCWIN1. These data, coupled with the finding of an apoplasmic phloem unloading pathway operating in ovaries, advance our understanding of how CWIN regulates the transition from ovary to fruit.

RESULTS

LIN5 and *INH1* Expression Shifts from a Dispersed to a Phloem-Specific Pattern during Ovary-to-Fruit Transition

To determine the spatial and temporal expression pattern of the CWIN gene *LIN5* during ovary-to-fruit transition, histological sections prepared from ovaries and fruitlets at 2 DBA and 2 DAA, respectively, were screened for *LIN5* transcripts by *in situ* hybridization. Figure 1A shows that the *CWIN* mRNA in 2 DBA ovaries exhibited a dispersed pattern. Compared with the sense control, bound antisense probe was detected in ovules, adjacent placenta, and columellar tissue connecting the receptacle (Figure 1A versus 1B). This cellular distribution of *LIN5* expression contrasted with that at 2 DAA where the *CWIN* mRNA was restricted to the fruit vasculature (Figure 1C versus 1D), consistent with a previous study showing that *CWIN* mRNA was localized to fruit phloem parenchyma cells (Jin et al., 2009). Noticeably, *LIN5* transcript levels were higher in phloem located in the columella and placenta connecting the young seed, and much less in pericarp phloem (Figure 1C). Transcript was not detected in any other regions of the developing fruit (Figure 1C).

Expression of the CWIN inhibitor gene *INH1* displayed a dispersed pattern similar to that of *LIN5*. Transcripts were detected in the ovule, adjacent placenta, and columella (Figure 2A versus 2B). Interestingly, *INH1* mRNA levels were most abundant at the ovary base adjoining the receptacle (Figure 2A). By 2 DAA, *INH1* transcript was localized to phloem of developing seeds, placenta, and columella. Detectable levels of *INH1* transcript were much lower (Figure 2C) than those of *LIN5* (Figure 1C) in young fruitlets as previously reported (Jin et al., 2009). These observations clearly show that the phloem-specific expression of *LIN5* and *INH1* mRNAs observed in fruitlets was absent in ovaries prior to anthesis.