Cross-talk modulation between ABA and ethylene by transcription factor SlZFP2 during fruit development and ripening in tomato

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Abbreviations: SlZFP2, Solanum lycopersicum zinc finger protein 2; HA-SlZFP2, an expression cassette of hemagglutinin-SlZFP2 fusion protein; ACS, 1-aminocyclopropane-1-carboxylic acid synthase; ACO, ACC oxidase; CNR, COLORLESS NON-RIpening; NOT, NOTABILIS; SIT, SITIENS; FLC, FLACCA; SIAO1, Solanum lycopersicum aldehyde oxidase 1; ABA, abscisic acid; dpa, days post anthesis; RNAi, RNA interference; FPKM, Fragments Per Kilobase of transcript per Million mapped reads.

ABA is well known for its roles in seed maturation and germination, in addition to its pivotal roles in stress response. At transcriptional level, ABA biosynthesis is regulated by stresses and also developmental processes, for example, during fruit development. Recently, it has been hypothesized that ABA may be involved in ripening regulation of non-climacteric and climacteric fruits. Understanding the transcriptional regulation of ABA biosynthesis during fruit development is required to dissect the role of ABA in regulation of ripening process. In a recent publication, we characterized the role of the transcription factor SlZFP2, encoding a single C2H2 zinc finger protein, in tomato fruit development. In that study, we found that constitutive expression of HA-SlZFP2 under 35S promoter repressed ABA biosynthesis in leaves and fruits, whereas silencing its expression increased ABA production in young fruits at 5 and 10 dpa. We also revealed that SlZFP2 regulates fruit ripening through transcriptional repression of the ripening regulator CNR. Thus, the SlZFP2 pathway likely modulates crosstalk between ABA biosynthesis and the regulatory network of fruit ripening in tomato.

In tomato, there are two ABA peak levels during fruit development and ripening. ABA level is high in anthesis ovaries, and then declines rapidly after pollination. By monitoring the ABA contents in developing fruits, we also found ABA production decreases to relatively low level around 5 dpa, whereas during the cell expansion phase of fruit development ABA production resumes gradually and reaches its second highest level at mature green stage. Through biochemical and gene expression analysis, we have demonstrated that SlZFP2 suppresses ABA biosynthesis through direct binding to the promoters of the ABA biosynthetic genes NOT, FLC, SIT and SIAO1. Since SlZFP2 is mainly expressed during fruit development, it likely plays an important role in maintenance of the dynamic ABA production post pollination. Indeed, SlZFP2 expression negatively correlates with ABA level during fruit development, for
example, SlZFP2 expression was relatively low in anthesis ovaries and 20 dpa fruits when high ABA production was observed (Fig. 1). Moreover, we found SlZFP2 expression was downregulated in the young fruits of ABA deficient mutants sit and flc, indicating that there is a feedback regulation on SlZFP2 expression by ABA during fruit development. Similarly, its Arabidopsis homolog AtZFP2 can be induced by ABA in seedlings. These results suggest that ABA activates SlZFP2 and the latter in turn represses ABA biosynthesis during fruit development.

Besides its role in fine tuning ABA biosynthesis during fruit development, our study has also revealed that SlZFP2 regulates fruit ripening because overexpressing SlZFP2 or HA-SlZFP2 delayed fruit ripening for 5–7 days, whereas silencing its expression by RNAi accelerated fruit ripening. Fruit ripening in tomato is mainly mediated by ethylene, which its production is transcriptionally regulated by several transcription factors. Among those ripening regulators, CNR inhibits fruit ripening through AP2a mediated negative regulation of ethylene biosynthesis and signaling. In HA-SlZFP2 overexpression and RNAi lines, CNR was respectively repressed and upregulated during ripening process, demonstrating that SlZFP2 regulates ripening process through CNR pathway. Since downregulation of SlZFP2 led to elevated CNR expression in fruits as early as 15 dpa, SlZFP2 likely functions to prevent CNR expression before the onset of ripening process. However, the action of SlZFP2 on fruit ripening is more likely through indirect impact on ethylene production because overexpression of this transcription factor only resulted in increased expression of ethylene biosynthetic genes LeACS6, LeACO1 and LeACO3 in ripe fruits at B10 stage (breaker plus 10 days). Their expression was not impacted at the onset of ripening process by overexpression or RNAi-mediated repression of SlZFP2. Thus, the gene expression analysis suggests that elevated or repressed ABA biosynthesis by manipulating SlZFP2 expression has little impact on ethylene production at the onset of ripening process.

Ethylene is the predominant plant hormone regulating climacteric-fruit ripening. In tomato,
two systems of ethylene biosynthesis have been proposed, which basal ethylene production is maintained in system 1 for normal fruit growth and later its production is increased drastically in system 2 during ripening. In system 1, LeACS1A, LeACS6, and LeACO1, 3 and 4 are responsible for the basal ethylene production. Accordingly, SIZFP2 does not directly regulate the induction of ethylene biosynthesis in system 2. However, we found that SIZFP2 and LeACO4 expression was increased significantly in the 2 dpa fruits of the representative SIZFP2 RNAi line 207 through transcriptome analysis by RNA-seq. The other ethylene biosynthetic genes LeACS1A, LeACS2 and LeACO1, although the LeACS1A and LeACS2 were expressed at low levels, were also expressed at higher levels in the young fruits (Fig. 2A). This suggests that SIZFP2 may regulate ethylene biosynthesis in system 1. Thus, the problematic fruit set observed in these SIZFP2 RNAi lines can be explained by elevated ethylene biosynthesis. Our observation is consistent with early studies that ABA promotes flower and fruit abscission through ethylene biosynthesis. Given the significant increase in ABA content in SIZFP2 RNAi fruits, SIZFP2 likely regulates ethylene biosynthesis during early fruit growth through ABA pathway. To test the possibility, we analyzed the expression of ethylene biosynthetic genes in Arabidopsis. Therefore, if there is an indispensable role for ABA in regulation of fruit ripening, it will possibly lie on its positive effect on basal ethylene production.

Collectively, SIZFP2 plays at least two roles in regulation of fruit development and ripening (Fig. 3). First, SIZFP2, likely induced by high ABA at anthesis, represses ABA biosynthesis after anthesis, and in turn the decrease in ABA level limits ethylene production during fruit set and early fruit growth. Fine-tuning ABA biosynthesis likely helps to maintain ethylene production at its basal level in system 1 for normal fruit growth. Second, SIZFP2 also prevents CNR expression before the onset of ripening process. However, it remains to be determined whether or not the ABA biosynthesis regulated by SIZFP2 is connected with the CNR-mediated ripening regulation.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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Figure 3. A proposed model for SIZFP2 action on fruit development and ripening. During fruit set and development, SIZFP2 acts as a transcriptional repressor to prevent fine tune ABA biosynthesis through direct binding to the promoters of NOT, SIT, FLC, and SIAO1. Decreasing ABA biosynthesis by high SIZFP2 expression leads to relatively lower ethylene production which facilitates fruit set and prevents floral organ senescence. In addition, SIZFP2 also prevents the expression of the ripening regulator CNR before the onset of ripening process, either directly or indirectly.
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