Phytochemistry 142 (2017) 11–20

Contents lists available at ScienceDirect

Phytochemistry

journal homepage: www.elsevier.com/locate/phytochem

Postharvest changes in LIN5-down-regulated plants suggest a role for sugar deficiency in cuticle metabolism during ripening

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ARTICLE INFO

Article history:
Received 26 February 2017
Received in revised form
6 June 2017
Accepted 14 June 2017

Keywords:
Solanum lycopersicum
Solanaceae
Invertase
Fruit
Cuticle
LIN5 gene
Postharvest

ABSTRACT

The cell wall invertase gene (*LIN5*) was reported to be a key enzyme influencing sugar uptake of tomato (*Solanum lycopersicum*) fruit. It was additionally revealed to be a key regulator of total soluble solids content in fruit as well as for reproductive development, being mainly involved in flower development, early fruit and seed development but also in ripening. Here, we demonstrate that silencing of the *LIN5* gene promotes changes affecting fruit cuticle development which has a direct effect on postharvest properties. Transformants were characterized by reduced transpirational water loss in mature fruits accompanied by several other changes in the cuticle. Quantitative chemical composition, coupled with microscopy of isolated cuticle fruits revealed that the cuticle of the transformants were characterized by an increase of the thickness as well as significant increase in the content of cuticle components (cutin, phenolic compounds, and waxes). Furthermore, detailed analysis of the waxes revealed that the transformants displayed changes in waxes composition, showing higher levels of *n*-alkanes and triterpenoids which can shift the proportion of crystalline and amorphous waxes and change the water flux through the cuticle. Expression of the genes involved in cuticle biosynthesis indicated that *LIN5* influences the biosynthesis of components of the cuticle, indicating that this process is coupled to sugar uploading via a mechanism which links carbon supply with the capacity for fruit expansion.

1. Introduction

Fruit growth, ripening and postharvest are complex developmental processes involving many events that contribute to their final composition, texture and storage potential. Tomato has become a model of fleshy fruit development and ripening because of its economic importance and impact on the human diet (Seymour et al., 2013). Cell wall disassembly is one of the main processes occurring at the end of the ripening period and its rate and extent are crucial for the maintenance of fruit quality and integrity (Matas et al., 2009). The cuticle, the lipophilic membrane layer that covers the outer epidermal cell wall of the aerial parts of higher plants (Jeffree, 2006; Nawrath, 2006), has been largely disregarded with respect to its putative influence in modulating fruit development, ripening and postharvest performance. Recent research has highlighted the cuticle as an important element in the plant's detection and transmission of osmotic stress signals (Wang et al., 2011). The cuticle is synthesized by epidermal cell layer of the fruit pericarp. It is composed of two main components: cutin, an insoluble, amorphous, and high-Mr matrix of esterified hydroxyl fatty acids or diacids, and cuticular waxes (Jeffree, 2006; Nawrath, 2006). Phenolic compounds, mostly cinnamic acid derivatives and flavonoids, are also present (Hunt and Baker, 1980). In addition, a significant amount of polysaccharides can be found, which represent the portion of epidermal cell wall to which the cuticle is attached (Jeffree, 2006). Tomato fruit cutin is mainly composed of C16 hydroxy fatty acid monomers, while cuticular wax, predominantly comprises fatty acids, alcohols, alkanes, and esters derived from aliphatic very long-chain fatty acids with chain lengths

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http://dx.doi.org/10.1016/j.phytochem.2017.06.007
0031-9422/

ranging from C20 to C34 and a variety of other lipophilic metabolites (Jenks and Ashworth, 1999; Vogg et al., 2004; Samuels et al., 2008). During fruit development, the cuticle undergoes several biochemical changes and enlarges considerably, surrounding the epidermal cells. The degree of cutin that is deposited in the cell wall and the relative contribution of each cuticle component affect its biomechanical behavior as well as the physiological properties of the whole fruit pericarp (Matas et al., 2004; López-Casado et al., 2007; Dominguez et al., 2011). Furthermore, the chemical properties of the cuticle are dynamic with both cuticle deposition and wax composition altering in response to water stress and abscisic acid (Kosma et al., 2009; Wang et al., 2011).

Genomic and proteomic-based approaches have identified genes and proteins preferentially expressed in fruit peel and perturbation of several genes through mutagenesis has revealed altered fruit cuticle phenotypes (Vogg et al., 2004; Leide et al., 2007; Mintz-Oron et al., 2008; Isaacson et al., 2009; Yeats et al., 2010; Nadakuduti et al., 2012; Giménez et al., 2015). Among them, the *Cutin Deficient* (*CD2*) gene encodes a member of the class IV homeodomain-Leu zipper family that regulates cutin biosynthesis (Isaacson et al., 2009; Nadakuduti et al., 2012). *SlCER6*, *β-ketoacyl-Coenzyme A synthase* gene which is involved in cuticular wax composition during fruit development (Vogg et al., 2004; Leide et al., 2007). Cuticle deposition must be coordinated with organ growth and epidermal cell differentiation to ensure continuous surface coverage and maintenance of structural integrity. Therefore, some regulatory genes that have been described to be involved in a broader regulation of organ development and cell identity, have also been identified as influencing cutin and wax formation. Such is the case for the tomato transcription factor genes *SlSHN1, SlSHN2*, and *SLSHN3*, described as transcriptional regulators of cutin and wax biosynthetic pathways (Mintz-Oron et al., 2008; Shi et al., 2013) and the orthologs of the Arabidopsis AP2 transcription factor, which have been described as regulators of floral cutin and epidermal cell morphology (Shi et al., 2011). Recently, the transcription factor TOMATO AGAMOUS-LIKE 1 (*TAGL1*) a key regulator of tomato fruit development, has been demonstrated affect cuticle production, including cutin, waxes, and polysaccharides (Giménez et al., 2015). Similarly, some key components of the flavonoids pathway are involved in tomato cuticle development. That is the case of the transcription factor encoded by *SlMYB12* (Adato et al., 2009; Ballester et al., 2010); this regulates flavonoid biosynthesis by controlling *chalcone synthase 1* and *2* (*CHS1* and *CHS2)* the first step in the flavonoid pathway (España et al., 2014). Similarly, transcription factors that participate in fruit development and ripening regulation such as AP2/ethylene-responsive element binding protein, MADS box, MYB, and homeodomain-Leu zipper families, have recently been involved in different processes of tomato cuticle formation (Hen-Avivi et al., 2014). In addition, a recent screening of a tomato ethyl methanesulfonate has facilitated the isolation of novel genes related to

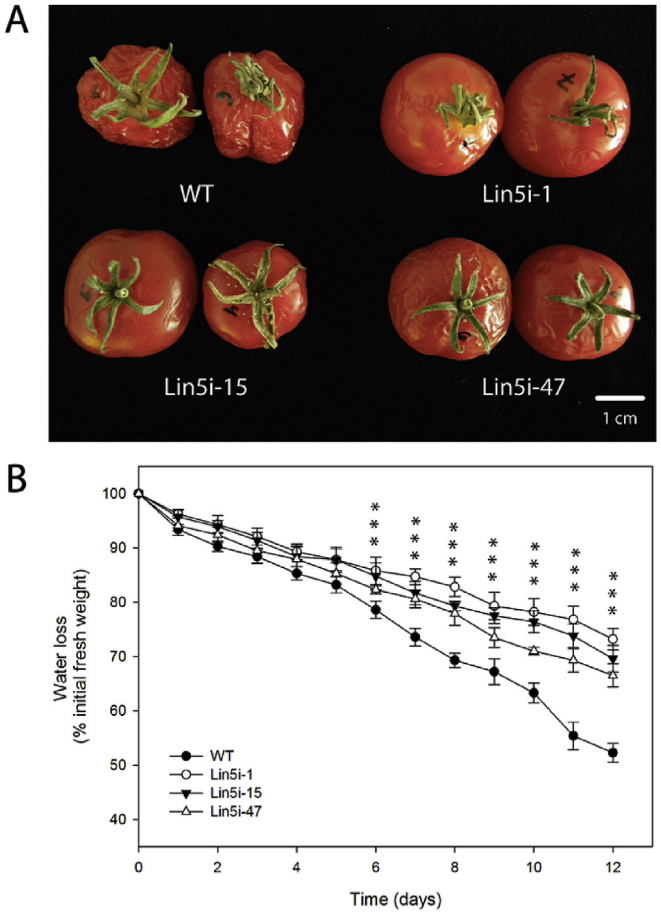

Fig. 1. Comparison of the Lin5i-RNA transgenic fruit phenotypes. (A) Fruits after 12 days of storage at room temperature. Scale bar = 1 cm. (B) Percentage of weight loss from detached fruits. Asterisks indicate statistically significant differences from WT as determined by *t*-test $P < 0.05$ (mean ± SD, n = 18).

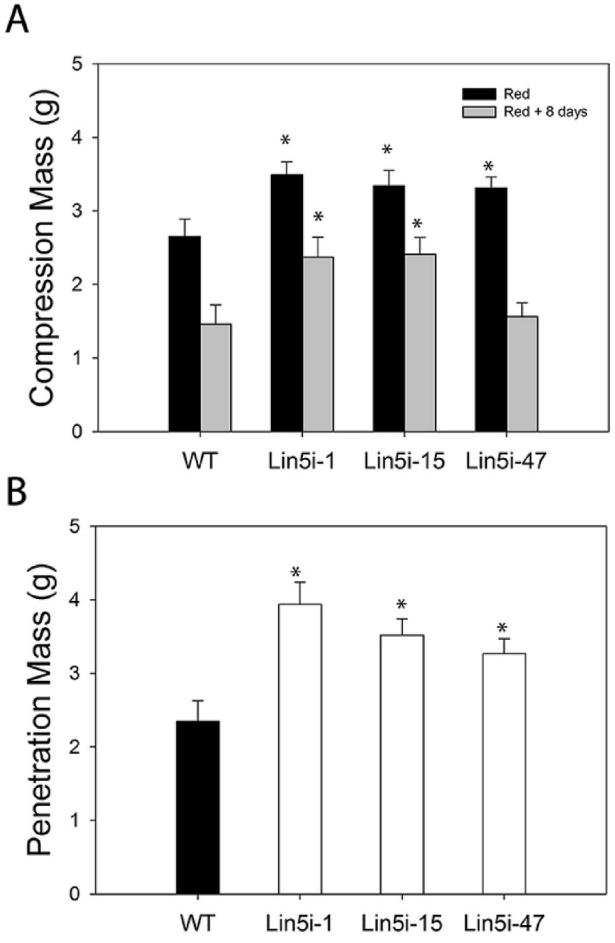

Fig. 2. Textural analysis of tomato fruit. (A) Compression test of intact tomato fruit at red and red + 8 days. (B) Penetration analysis of combined pericarp and cuticle at red stage. Asterisks indicate statistically significance differences from WT as determined by *t*-test (mean ± SD, n = 25 $P < 0.05$).