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Research article

Cell wall composition of tomato fruit changes during development and inhibition of vesicle trafficking is associated with reduced pectin levels and reduced softening

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ABSTRACT

Fruit development entails a multitude of biochemical changes leading up to the mature green stage. During this period the cell wall will undergo complex compositional and structural changes. Inhibition of genes encoding elements of the machinery involved in trafficking to the cell wall presents us with a useful tool to study these changes and their associated phenotypes. An antisense *SlRab11a* transgene has previously been shown to reduce ripening-associated fruit softening. *SlRab11a* is highly expressed during fruit development which is associated with a period of pectin influx into the wall. We have analysed the cell wall polysaccharides at different stages of growth and ripening of wild type and antisense *SlRab11a* transgenic tomato (*Solanum lycopersicum* cv, Ailsa Craig) fruit. Our results demonstrated intriguing changes in cell wall composition during the development and ripening of wild type Alisa Craig tomato fruit. Analysis of *SlRab11a* expression by TaqMan PCR showed it to be expressed most strongly during growth of the fruit, suggesting a possible role in cell wall deposition. The *SlRab11a* antisense fruit had a decreased proportion of pectin in the cell wall compared with the wild type. We suggest a new approach for modification of fruit shelf-life by changing cell wall deposition rather than cell wall hydrolytic enzymes.

1. Introduction

Fruit ripening is of interest to research groups and industry alike. The obvious phenotypic changes that occur during this process are brought about by a multitude of factors. These complex and highly regulated processes have been studied extensively in tomato [1]. Cell wall modifying enzymes have been considered to be the main contributors to the softening of the fruit and consequently, many groups have tried to alter this aspect of ripening by genetic constructs designed to inhibit the synthesis of these specific enzymes. However, despite the success of these techniques at the genetic level, phenotypically these have generally resulted in minor alterations to the softening process. Gene silencing to reduce polygalacturonase activity to as low as 1% of normal levels had relatively little effect on fruit softening [2–6]. Similar studies have also been conducted to elucidate the role of PE [7,8], endo-glucanase [9,10] and xyloglucan endotransglycosylase [11] in fruit softening. However, all of these studies resulted in only minor or no reduction in fruit softening, with one particular study by Phan et al. [12] showing an increase in softening. However, more significant effects have been achieved through inhibition of galactanase gene expression [13] and inhibition of the ripening specific expansin [14]. Fruits in which polygalacturonase and expansin had been inhibited simultaneously showed a greater effect on fruit softening and tomato paste viscosity than fruits with either alone inhibited [15,16]. These studies show the complexity of ripening and give evidence for multiple enzymes working in concert. Therefore, the alteration of softening may depend on reduction of multiple enzymes simultaneously and this is supported by the observation that inhibition of ethene biosynthesis [17] to simultaneously inhibit many aspects of ripening has a more dramatic effect upon softening. For these reasons it has been suggested that blocking the trafficking route to the cell wall might bring about just such a simultaneous inhibition of the effect of many enzymes and thus a more significant effect on softening [18].

Trafficking of cell wall precursors and cell wall modifying enzymes requires control to maintain the balance of developmental cues, particularly for metabolically active tissue such as tomato

Abbreviations: AIS, acetone insoluble solids; CDTA, 1,2-cyclohexylenedi nitrilotetraacetic acid; DTT, dithiothreitol; EtOH, ethanol; FAM, 6-carboxyfluorescein; HPAEC-PAD, high-performance anion exchange chromatography with pulsed amperometric detection; IPA, isopropanol; PE, pectinesterase; TAMRA, 6-carboxytetramethyl-rhodamine; TGN, trans-Golgi network.

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fruit. Rab GTPase enzymes give rise to this control by regulating vesicle docking through action as a molecular switch [19]. It has now been shown that there are many related clades of Rabs across many species throughout nature and these regulate docking with different parts of the endomembrane system [20–23]. In particular, the plant RabA GTPase class is deserving of investigation. Plant species possess an expanded RabA class compared with their mammalian Rab11 counterparts. In *Arabidopsis* there are 57 individual *RAB* genes of which 26 belong to the *RABA* class [22]. *SlRab11a* (formerly *LeRab11a*), which is orthologous to *RABA1a* in *Arabidopsis* is associated with the TGN and dominant negative versions of the *SlRab11a* gene showed inhibited exocytosis of secreted GFP [24]. In addition, an antisense *SlRab11a* construct reduced tomato fruit softening, producing firmer fruit in comparison to the Ailsa Craig wild type (AC^{++}) and reduced levels of several cell wall modifying enzymes in the fruit [25]. However, as well as enzymes, many other compounds are transported through the TGN to the plasma membrane and the apoplast. Therefore, we decided to analyse other possible components of the cell wall that might have been affected by inhibition of Rab11 activity.

2. Results

2.1. Confirmation of G4 antisense homozygous lines

Wild type and G4 antisense lines [25] were grown from seed and PCR was undertaken to confirm the presence of the antisense *Rab11a* construct in the fruit. The results are shown in Supplementary Fig. S1. As a confirmation of the expression of the antisense phenotype, fruit was harvested at fixed times during ripening and firmness was assessed using texture analysis (Supplementary Fig. S2). This confirmed the slow softening phenotype of the G4 line. Photographs of fruit at the 40 day post breaker stage further confirm this phenotype (Supplementary Fig. S3). At this point the AC^{++} tomatoes had lost their smooth structure and had cracked exocarps which accounts for the infection shown.

2.2. mRNA expression profiling of SlRab11a during fruit development and ripening

The expression of *SlRab11a* has only previously been determined during ripening and only by northern blot. We determined the expression profile for *SlRab11a* in the wild type during both fruit development and ripening using quantitative real-time PCR and specific primers. Fig. 1 shows that the mRNA level was at its maximum at 15 days-post-anthesis (15 dpa) which was the earliest time point taken during the analysis. The level reached its lowest at 45 dpa and remained at a relatively low level throughout the fruit ripening period.

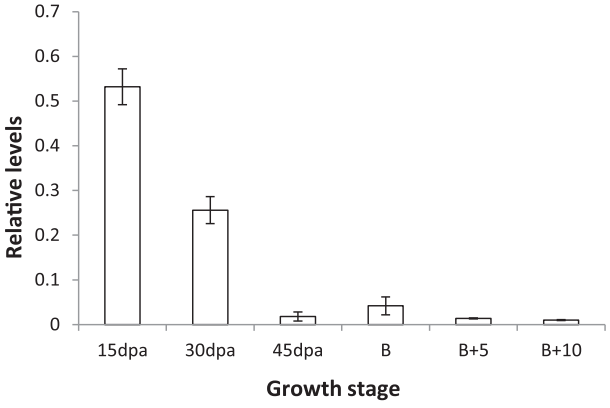

Fig. 1. Expression profile of *SlRab11a* during tomato fruit development and ripening. *SlRab11a* RNA levels are relative to 18S RNA. *SlRab11a* mRNA levels measured by real-time PCR at 15 dpa, 35 dpa, 45 dpa, breaker, breaker +15 days and breaker +35 days.

2.3. Cell wall analysis of developing tomato fruit

The composition of the cell wall during the development of the fruit was assessed. To do this acetone insoluble solid (AIS) was prepared from tomato fruit pericarp and fractionated to give a profile of the cell wall composition. The composition (mg/g) of the pectin, hemicellulose and cellulose rich fractions at 15 dpa, 35 dpa and breaker stages of development can be seen in Fig. 2. After correction for the starch component of the cellulose rich fraction, the total mass balance recovered during the fractionation was between 830 and 890 mg/g of the total AIS, the breakdown of which can be seen in Table 1. For wild type (AC^{++}) fruit at 15 dpa the hemicellulose rich fraction represented the largest component of the cell wall, accounting for around 45% of the recovered cell wall mass. This compared to 21% pectin and 33% cellulose rich fraction.

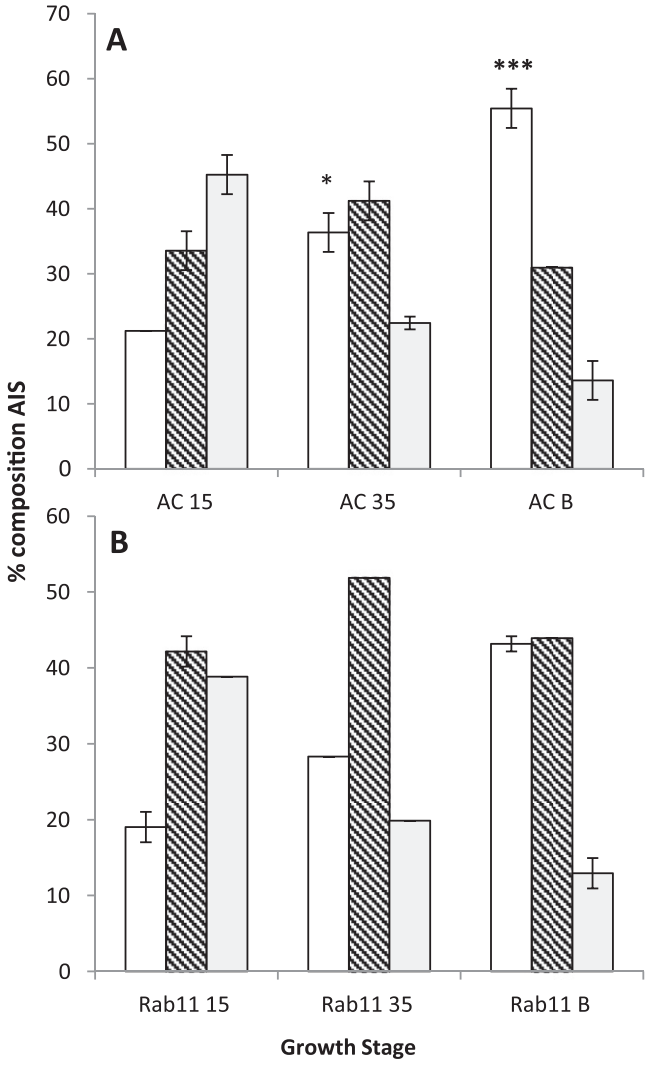

Fig. 2. Cell wall composition of AC^{++} and G4 fruit. Fruit was harvested at 15 dpa, 35 dpa and breaker stage. Pericarp cell wall material was prepared as an acetone insoluble solid and fractionated into pectin, hemicellulose and cellulose enriched fractions. AC^{++} (A) and G4 antisense (B). Statistical significance level at 35 dpa $p < 0.05$ and breaker $p < 0.001$. Cell wall components are as follows: pectin (□), cellulose (▨), hemicellulose (▩).