Under-expression of the Auxin Response Factor *Sl-ARF4* improves post-harvest behavior of tomato fruits

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Auxin is one of the most prominent phytohormones regulating many aspects of fleshy fruit development including fruit set, fruit size through the control of cell division and cell expansion, and fruit ripening. To shed light on the role of auxin fruit ripening, we have previously shown that *Sl-ARF4* is a major player in mediating the auxin control of sugar metabolism in tomato fruit (*cv MicroTom*). Further extending this study, we show here that down-regulation of *Sl-ARF4* in tomato alters some ripening-related fruit quality traits including enhanced fruit density at mature stage, increased firmness, prolonged shelf-life and reduced water (weight) loss at red ripe stage. These findings suggest that *Sl-ARF4* plays a role in determining fruit cell wall architecture and thus providing a potential genetic marker for improving post-harvest handling and shelf life of tomato fruits.

Fruit ontogeny and ripening are genetically regulated processes involving a complex multi-hormonal control. While the role of ethylene in triggering and regulating the ripening of climacteric fruit have been clearly demonstrated, little is known about the contribution of other hormones.[1] The plant hormone Auxin plays a vital role in all stages of reproductive growth through its important implication in cell division and cell expansion during fruit development.[2-5] This findings were consolidated by the discovery of the involvement of many gene members belonging to two important auxin response gene families; ARFs (auxin response factors) and Aux/IAA in tomato fruit set and growth.[3,6-8] Other studies also showed the involvement of auxin in regulating the fruit ripening process and fruit quality traits in many crop species.[9-11] During fruit ripening, different regulatory factors involved in auxin signaling and response[3,4,12,13] were shown to be also involved in the modification of cell wall structure and composition.[14,15] Qualification and orchestration of these proteins remain an important area of research aimed at uncovering mechanisms of degradation of the cell wall which is responsible of fruit softening.[16] Previous study showed that down-regulation of the *DR12*/*Sl-ARF4* resulted in late-occurring cell divisions in the pericarp tissue and enhanced firmness at the red-ripe stage.[4] Further investigation indicated that the altered firmness does not result from a major impairment of ripening-related pectin metabolism, but rather involves differences in pectin fine structure associated with changes in tissue architecture.[3] We have recently shown that down-regulation of *Sl-ARF4* gene in tomato fruits enhanced photosynthetic activity, chlorophyll and starch accumulation in immature tomato fruits, suggesting that *Sl-ARFs* may play a key role in controlling sugar content, an essential feature of fruit quality.[10] In this work, we show new findings on *Sl-ARF4* down-regulated tomato fruits supporting the role of this gene in the control of fruit softening and shelf life.

Three tomato lines under-expressing *Sl-ARF4* and corresponding to either antisense (ASL1, ASL2) or sense co-suppressed (CSL1) were used in this study.[10] Several parameters such as fruit density, firmness and shelf life were assessed and compared with those of wild type tomatoes. A significant increase in fruit firmness was observed at ten days after breaker in *Sl-ARF4*-AS fruit compared with WT (**Fig. 1A**). On the other hand, transgenic fruit displayed a lower water loss at post-breaker stage (breaker + 10 d) when compared with WT fruit at the same stage. The water loss was assessed by assessing following weight evolution of *Sl-ARF4*-AS and WT harvested fruit and stored at 25 °C for 40 d. The results (**Fig. 1C**) showed a lower water loss between breaker and breaker+10 d stages for the transgenic fruits (0.3 to 0.37 g fresh weight) in comparison to WT fruit at the same stages (0.54 g fresh weight). *Sl-ARF4* down-regulated fruits showed also significantly higher density than that of WT (**Fig. 1B**). The difference in fruit density was also revealed by the fact that when immersed into water, WT fruits

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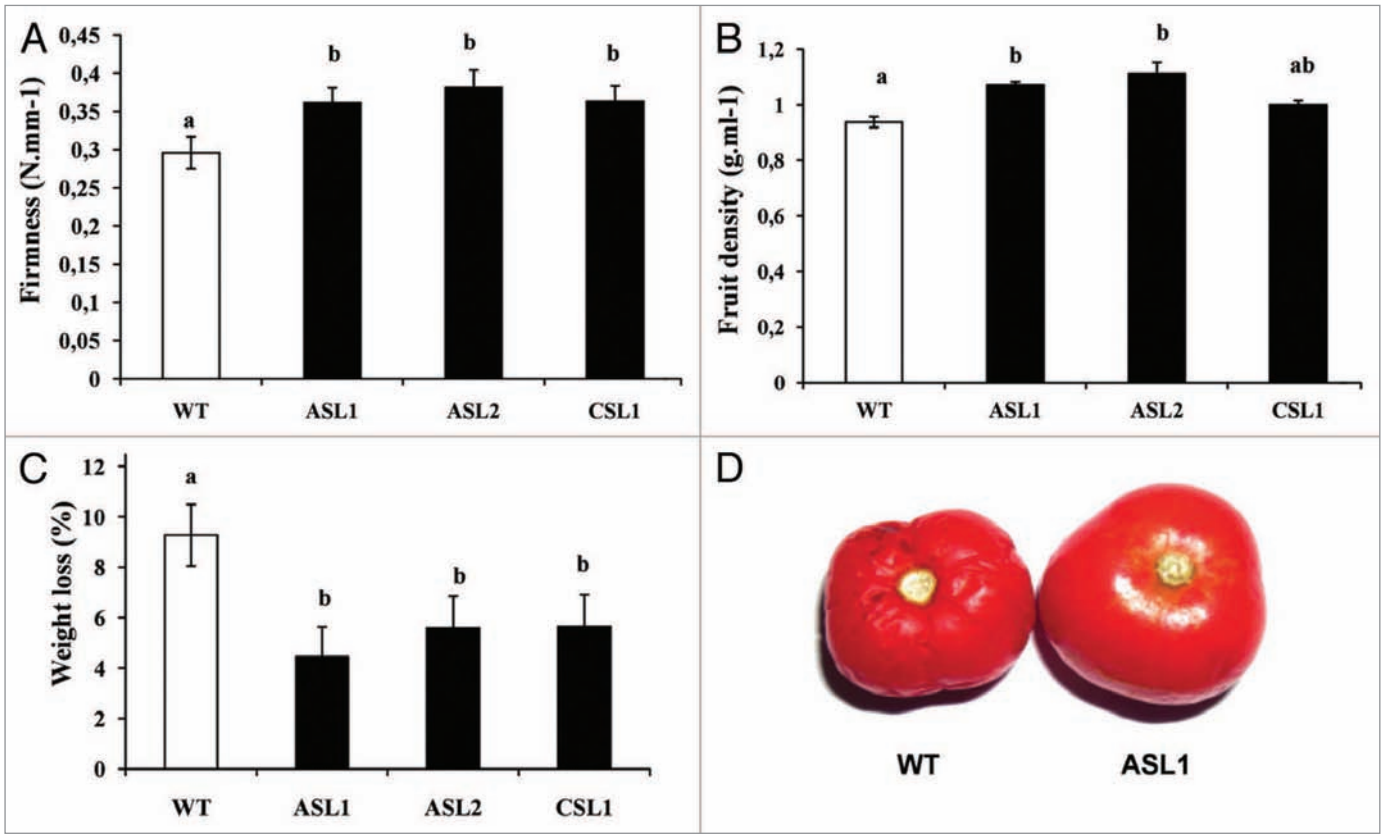

Figure 1. Density, firmness and weight loss measured on WT and *Sl-ARF4* down-regulated fruits. (**A**) Firmness of WT and *Sl-ARF4* down-regulated fruits measured at br + 10 d (**B**) Density of WT and *Sl-ARF4* down-regulated fruits measured at br + 10 d (**C**) Weight loss measured on WT and *Sl-ARF4* down-regulated fruits between the two stages breaker and breaker + 10 d. Small letters show significant differences between the WT and every downregulated line using LSD at $P < 0.05$. Values represent means ± SE (n = 30). (**D**) Prolonged conservation of *Sl-ARF4* down-regulated fruit. Fruits were harvested at br stage and conserved at 25 °C for 45 d. (br = breaker stage, br + 10= ten days after breaker stage)

float while transgenic ones sink (data not shown). These observations are consistent with the elevated total soluble solids concentration in the *Sl-ARF4*-AS fruits shown previously.[10]

The enhanced fruit firmness and lower water loss of *Sl-ARF4*-AS fruits prompted us to assess the post-harvest behavior of *Sl-ARF4* downregulated fruit to determine shelf-life potential, WT and *Sl-ARF4-AS* fruits were harvested at the breaker stage (45 d after anthesis) and stored at 25 °C until they reached complete deterioration. As shown in **Figure 1D**, when stored at 25 °C, WT fruits first displayed severe shrinking and then undergo effusion of juice contents associated with loss of texture and integrity at 20 d after storage. By contrast, in the same conditions, the *Sl-ARF4* down-regulated fruits did not display such signs of deterioration even after 45 d of storage (**Fig. 1D**).

Altogether, our study show that fruits under-expressing *Sl-ARF4* significantly exhibit enhanced density, firmness, prolonged shelf life and lower water (weight) loss at red ripe fruit stage.

Material and Methods

Tomato plants (cv *MicroTom*) were grown and transformed as described previously.[10] Density was determined by measuring fruit weight in air and in water (volume), this parameter was calculated following the equation (density = mass/volume). Firmness was determined by measuring the diameter and deformability of red ripe fruits (breaker + 10 d) using a compression clamp, which determines the difference between fruit diameter without pressure and the diameter after clamp pressure. Firmness value was calculated as described in Ecarnot et al. 2013.[17] For shelf life, fruits at the breaker stage were detached and kept at room temperature (25 °C and 55~60% relative humidity) for approximately 45 d. Six replicates were taken for each individual plant. Average fresh weight loss was determined between the breaker and breaker + 10 d stages.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References

1. Bouzayen M, Latché A, Nath P, Pech JC. Mechanism of Fruit Ripening. In: Pua EC, Davey MR, eds. Plant Developmental Biology - Biotechnological Perspectives: Springer Berlin Heidelberg, 2010:319-39.
2. Abel S, Theologis A. Early genes and auxin action. Plant Physiol 1996; 111:9-17; PMID:8685277; http://dx.doi.org/10.1104/pp.111.1.9
3. Guillon F, Philippe S, Bouchet B, Devaux M-F, Frasse P, Jones B, et al. Down-regulation of an Auxin Response Factor in the tomato induces modification of fine pectin structure and tissue architecture. J Exp Bot 2008; 59:273-88; PMID:18267945; http://dx.doi.org/10.1093/jxb/erm323
4. Jones B, Frasse P, Olmos E, Zegzouti H, Li ZG, Latché A, et al. Down-regulation of DR12, an auxin-response-factor homolog, in the tomato results in a pleiotropic phenotype including dark green and blotchy ripening fruit. Plant J 2002; 32:603-13; PMID:12445130; http://dx.doi.org/10.1046/j.1365-313X.2002.01450.x
5. Ruan YL, Patrick JW, Bouzayen M, Osorio S, Fernie AR. Molecular regulation of seed and fruit set. Trends Plant Sci 2012; 17:656-65; PMID:22776090; http://dx.doi.org/10.1016/j.tplants.2012.06.005
6. de Jong M, Wolters-Arts M, Feron R, Mariani C, Vriezen WH. The Solanum lycopersicum auxin response factor 7 (SlARF7) regulates auxin signaling during tomato fruit set and development. Plant J 2009; 57:160-70; PMID:18778404; http://dx.doi.org/10.1111/j.1365-313X.2008.03671.x
7. Vriezen WH, Feron R, Maretto F, Keijman J, Mariani C. Changes in tomato ovary transcriptome demonstrate complex hormonal regulation of fruit set. New Phytol 2008; 177:60-76; PMID:18028300
8. Wang H, Schauer N, Usadel B, Frasse P, Zouine M, Hernould M, et al. Regulatory features underlying pollination-dependent and -independent tomato fruit set revealed by transcript and primary metabolite profiling. Plant Cell 2009; 21:1428-52; PMID:19435935; http://dx.doi.org/10.1105/tpc.108.060830
9. Cohen JD. In vitro tomato fruit cultures demonstrate a role for indole-3-acetic acid in regulating fruit ripening. J Am Soc Hortic Sci 1996; 121:520-4
10. Sagar M, Chervin C, Mila I, Hao Y, Roustan JP, Benichou M, et al. SlARF4, an auxin response factor involved in the control of sugar metabolism during tomato fruit development. Plant Physiol 2013; 161:1362-74; PMID:23341361; http://dx.doi.org/10.1104/pp.113.213843
11. Vendrell M. Dual effect of 2, 4-D on ethylene production and ripening of tomato fruit tissue. Physiol Plant 1985; 64:559-63; http://dx.doi.org/10.1111/j.1399-3054.1985.tb08539.x
12. Wang H, Jones B, Li Z, Frasse P, Delalande C, Regad F, et al. The tomato Aux/IAA transcription factor IAA9 is involved in fruit development and leaf morphogenesis. Plant Cell 2005; 17:2676-92; PMID:16126837; http://dx.doi.org/10.1105/tpc.105.033415
13. Zegzouti H, Jones B, Frasse P, Marty C, Maitre B, Latch A, et al. Ethylene-regulated gene expression in tomato fruit: characterization of novel ethylene-responsive and ripening-related genes isolated by differential display. Plant J 1999; 18:589-600; PMID:10417710; http://dx.doi.org/10.1046/j.1365-313x.1999.00483.x
14. Brownleader MD, Jackson P, Mobasheri A, Pantelides AT, Sumar S, Trevan M, et al. Molecular aspects of cell wall modifications during fruit ripening. Crit Rev Food Sci Nutr 1999; 39:149-64; PMID:10198752; http://dx.doi.org/10.1080/10408399908500494
15. Brummell DA, Harpster MH. Cell wall metabolism in fruit softening and quality and its manipulation in transgenic plants. Plant Mol Biol 2001; 47:311-40; PMID:11554479; http://dx.doi.org/10.1023/A:1010656104304
16. Seymour GB, Manning K, Eriksson EM, Popovich AH, King GJ. Genetic identification and genomic organization of factors affecting fruit texture. J Exp Bot 2002; 53:2065-71; PMID:12324530; http://dx.doi.org/10.1093/jxb/erf087
17. Ecarnot M, Baczyk P, Tessarotto L, Chervin C. Rapid phenotyping of the tomato fruit model, Micro-Tom, with a portable VIS-NIR spectrometer. Plant Physiol Biochem 2013; 70:159-63; PMID: 23774377; http://dx.doi.org/10.1016/j.plaphy.2013.05.019.