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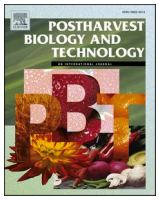

Phenotyping of a diverse tomato collection for postharvest shelf-life

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ABSTRACT

In all fruit and vegetable crops, reduction in quality during postharvest storage leads to substantial losses of primary production with enormous economic consequences. Also in tomato, fruit shelf-life is an important quality trait. In this study a collection of tomato accessions, consisting of 92 *S. lycopersicum* landraces and old cultivars and several *S. pimpinellifolium* accessions, was phenotyped for several shelf-life parameters and biochemical characterization was performed during the postharvest shelf-life of fruit from selected accessions. This collection was selected based on available genotypic data and represents the genetic diversity present in the EU–SOL tomato core collection (Roohanitaziani, 2020). The core collection was grown in a greenhouse, and fruit were harvested at the breaker-turning stage and stored in a controlled climate chamber for 42 d at 18 ºC. The shelf-life attributes firmness loss, weight loss, as well as color pigments, were measured once a week and evaluated over time. All three shelf-life-related parameters varied markedly among accessions, resulting in fruit with different shelf-life. The most promising accessions of the first screen were re-grown and analyzed to validate the initial results and six accessions with contrasting shelf-life were selected for metabolite analysis. Fruit were harvested at the breaker stage and stored for 35 d at 18 ºC. Samples were taken at weekly intervals and analyzed for volatile compounds, primary metabolites and cell wall polysaccharide monomers. During storage long and short shelf-life accessions showed considerable differences in their content of sugars, such as galactose and polyamines, such as putrescine in their pericarp. The content of three cell wall sugars, galactose, arabinose and galacturonic acid, underwent considerable changes during postharvest storage. The short shelf-life accessions contained a higher amount of arabinose and galactose in their cell wall than other accessions which is indicative of highly branched pectin. This knowledge provides a better understanding of the difference in pectin structure between short and long shelf-life fruit during the ripening process.

1. Introduction

Managing postharvest losses of fruit in modern agriculture is of great importance due to globalization of the markets and often very long distances between producers and consumers. In tomato the early solution to manage postharvest losses has been picking green or unripe fruit instead of picking ripe fruit, which are more prone to damage during the delivery period. Although picking unripe fruit extends its shelf life, it will degrade its quality on the other hand (Beckles, 2012). Today, various physical and chemical postharvest treatments enhance the fruit shelf-life by slowing down physiological processes of ripening and senescence. However, each of these treatments has its limitations (Mahajan et al., 2014; Sandarani et al., 2018). The first breeding strategy to improve the tomato fruit shelf-life was the use of ripening mutants and deploying their alleles in the development of hybrids with extended shelf-life. F1 hybrids of ripening inhibitor (*rin*/+), non-ripening (*nor*/+) and alcobaca (*alc*/+) mutants have been widely commercialized and they have played an important role in increasing the availability of "vine-ripened" tomatoes in the market (Kopeliovitch et al., 1979; McGlasson et al., 1983; Nguyen et al., 1991; Markovic et al., 2012). The second breeding strategy, which recently has gained more attention, is looking for varieties with extended shelf-life, which are not ripening mutants but remain firm for an extended time after ripening and allow harvesting at a more advanced color and thus riper stage. Tomato

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germplasm collections, fortunately, offer a valuable gene pool for both fruit and crop-related traits. There are many tomato accessions currently held in tomato gene banks that have greatly different fruit shelf-life characteristics. These natural variations can be exploited for improved fruit shelf-life through breeding programs (Rick and Chetelat, 1995; Blanca et al., 2015).

Fruit shelf-life is determined by series of physiological, biochemical and organoleptic changes during ripening to make fruit edible and desirable for consumers. These changes generally include softening of the flesh by modification of the cell wall structure and loss of turgor, alterations in pigment biosynthesis, increase in the levels of aromatic volatiles and nutrients, alteration in cuticle architecture and composition and increase in susceptibility to postharvest pathogens (Giovannoni, 2001). Decrease in fruit firmness is the result of the dissolution of the middle lamella and structural changes in cell wall polysaccharides (mainly in pectin but also to a minor extent in hemicellulose and cellulose), in an ordered series of modifications by cell wall degrading enzymes (Brummell, 2005, 2006; Brummell and Harpster, 2001; Lahaye et al., 2012; Posé et al., 2018; Wang et al., 2018). Although many details about cell wall modification and changes in the structure of polysaccharides during ripening of tomato fruit are still unclear, several earlier studies have shown that loss of galactorunic acid (Gal A), galactose (Gal) and arabinose (Ara) residues from the cell wall and concomitant increase of free galactose and galactorunic acid in the pericarp occur (Gross and Wallner, 1979; Labavitch, 1981; Gross, 1983; Gross and Sams, 1984; Prasanna et al., 2007). Galactorunic acid, galactose and arabinose are major components of pectic polysaccharides and changes in their content during ripening have shown to be different from fruit to fruit and especially between wild-type tomatoes and ripening mutants (Gross and Wallner, 1979; Gross, 1983; Gross and Sams, 1984; Lunn et al., 2013). Pectin in the fruit cell wall consists of acidic polysaccharides made of variable proportions of three structural domains: homogalacturonan (HG), rhamnogalacturonan I (RG-I) and rhamnogalacturonan II (RG-II). HG consists of linearly linked 1, 4-α-D-galacturonic acids. RG-I is the major component of the primary cell wall and middle lamella in dicot plants and it is responsible for the structural diversity of pectin. The backbone of RG-I consists of repeating disaccharides of rhamnose and galactorunic acid. Almost 50% of rhamnose residues in the backbone of RG-I are branched with side chains consisting of D-galactose and L-arabinose residues. RG-II is a HG decorated with complex side chains, some of which containing rhamnose. It is invariably present as a minor component of the cell wall and their relevance to ripening related cell wall metabolism is unknown (Houben et al., 2011; Posé et al., 2018; Prasanna et al., 2007; Wang et al., 2018).

The aim of the present study was to evaluate a core collection of 92 tomato accessions (Roohanitaziani et al., 2020) for their postharvest shelf-life, in order to identify novel sources of improved shelf-life for future breeding programs. The shelf-life attributes firmness loss, weight loss, and fruit decay, as well as color pigments, were monitored during a 42 d storage period. The other objective of this study was to characterize and compare changes in cell wall sugar composition, primary metabolites and volatile compounds of fruit with long and short postharvest shelf-life.

2. Materials & methods

2.1. Plant materials and growth conditions

The collection for this project consisted of 92 *S. lycopersium* landraces and old cultivars and several *S. pimpinellifolium* accessions, and was described extensively in Roohanitaziani et al., 2020. For the first shelf-life trial seeds were sown in spring 2014, mid-November, after a temperature treatment of 48 hs at 75 °C to kill seed-borne viruses. Five weeks later, the plants were transplanted to the greenhouse. Three plants per accession for round and cherry tomatoes and 6 plants per accession for beef tomatoes were grown.

For the repetition of the experiment of 2014 with promising accessions, seeds of 17 accessions were sown in mid-January 2015 and fruit were harvested from mid-May onwards. All accessions were grown in three blocks, using a complete randomized block design. Each plot contained 2–6 plants of one accession grown alongside each other. In total, 15 plants per accession were grown for beef tomatoes (five plants per plot in each block), nine plants per accession for round (three plants per plot in each block) and six plants per accession for cherry tomatoes (two plants per plot in each block). Five fruit per accession were harvested from each block at breaker-turning stage (in total 3 ×5 =15 fruit per accession) for the shelf-life analysis.

For biochemical characterization of the six selected accessions, 30 plants per accession were grown in three blocks of 10 plants in the greenhouse as described for the trial in 2015. In total 45 fruit per accession were harvested, six fruit in green stage and 39 fruit in breaker stage. The latter were stored in a climate chamber (18 °C, 85% Humidity). From this batch of stored fruit, 15 fruit per accession were subjected to a shelf-life trial for 35 days and firmness, weight loss and pigment measurements were conducted once a week (results not shown). The other 30 fruit (6 fruit harvested at green stage and 24 fruits harvested at B stage) were used for metabolite analysis: at different time points 6 fruits were pooled to make a representative sample for Green stage (GS), Breaker+ 1 (one day after storage), Breaker+ 14, Breaker+ 21 and Breaker + 35.

2.2. Postharvest shelf-life trial

All fruit analyzed in this study were harvested by breaking the joint and therefore had a pedicel. Harvested fruit were stored in a controlled climate chamber (18 °C, 85% Humidity) for 42 d. Firmness, fruit weight and chlorophyll and lycopene were measured once a week and evaluated over time.

Firmness was expressed as the average of four measurements per fruit around the equator of the fruit using a handheld Fruit Hardness Tester (53215, Turoni Italy).

For weight loss evaluation, each fruit was weighed with a precision balance.

A hand-held photodiode array spectrophotometer (Pigment Analyzer PA1101, CP, Germany) was applied to measure the chlorophyll and lycopene levels non-destructively. Remittance was assessed between 350 and 1100 nm to calculate the normalized difference vegetation index (NDVI) and the normalized anthocyanin index (NAI) which are indicators for chlorophyll and lycopene respectively and produces a normalized value between − 1 and + 1 (Schouten et al., 2014). Measurements were carried out on two positions on the equator of each tomato and reported as an average NDVI and NAI value per tomato. Measurements were performed at the day of harvest and subsequently once a week until the end of the storage period.

During the storage period, the appearance of wrinkles or loss of shape and infections with pathogens were monitored every week. A picture of each analyzed fruit was taken before each measurement.

2.3. Measurements of volatile compounds

Six fruit from each accession at each postharvest time point were cut in small pieces, pooled together and immediately frozen in liquid nitrogen. Frozen fruit pieces were subsequently ground in an analytical electric mill (IKA A11 basic, Germany) and stored at − 80 °C before analysis. Volatile compounds were quantified and identified using Gas Chromatography/Mass Spectrometry (GC/MS) as described in (Tikunov et al., 2005).

2.4. Primary metabolite analysis

The detection of amino acids, sugars and organic acids was performed by GC-TOF-MS. For extraction of samples 700 μL methanol

containing 0.5 g/L ribitol (as an internal standard) was added to 300 mg frozen fruit powder in a 2 mL Eppendorf vial. Samples were vortexed for 20 min and centrifuged at max speed for 8 min 500 μL of the methanol extract was transferred to a new vial to which 450 μL water and 250 μL of chloroform were added. The samples were shortly vortexed and centrifuged at max speed for 8 min. Supernatant was diluted 8 times with pure methanol. 40 μL of supernatant was transferred in to a crimp cap with insert and dried overnight in a speed vac and capped under argon. Samples were analyzed by GC-TOF-MS as described in (Carreno-Quintero et al. 2012) using a detector voltage of 1700 V. Leco Chroma TOF software 2.0 was used for pre-processing of the raw data.

2.5. Preparation of cell wall (alcohol insoluble residue; AIR)

The preparation of cell wall material (alcohol insoluble residue; AIR) was based on the procedure described in (Uluisik et al., 2016). Briefly, fresh tomato pericarp (100 g) was peeled, seeds and jelly materials were discarded, and the pericarp was diced and heated in 95% ethanol (100 mL) at 80 °C for 30 min. The samples were cooled to room temperature, homogenized using a coffee grinder, then filtered through Miracloth and washed successively with hot 85% ethanol (200 mL), chloroform/methanol (1:1 v/v) (200 mL) and 100% acetone. The samples were then air dried overnight.

2.6. Preparation of monosaccharide fraction from cell walls

Acid hydrolysis of the cell wall was carried out as described in (Van Wychen and Laurens, 2016; Rashidi and Trindade, 2018) with minor modifications. 25–30 mg of each cell wall sample was weighed in a 15 mL pressure glass tube. Each sample was weighed in triplicate and the weight of each sample was recorded. The glass tubes were labeled with permanent marker. 1 mL of 72% sulfuric acid was added to each tube and vortexed until the sample was thoroughly mixed. The tubes were placed in a thermomixer set at 30 °C for 1 h. After removing the tubes from the thermomixer, acid was diluted to 6% final concentration with MQ water. The samples were mixed by inverting the tubes several times, and then heated for 1 h at 121 °C, and subsequently allowed to cool to room temperature. 1 mL of each sample was filtered through a 0.2 μm filter, using a syringe and transferred to vials with inserts for monosaccharide analysis. A set of sugar recovery standards (including glucose, galactose, arabinose, mannose, xylose, rhamnose, glucuronic acid and galacturonic acid) were prepared and analyzed along with the samples.

2.7. Assays of monosaccharide composition of cell wall fractions

Monosaccharide composition was determined by high-performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) on a CarboPac PA1, 4- × 250-mm column preceded by a guard column (CarboPac PA 1, 4 × 50 mm) mounted in a DX-500 system, Dionex®. Saccharides were separated as described by (van Arkel et al., 2012). The elution times and eluent concentrations were modified. The monosaccharides from the cell wall were eluted using a linear increase in concentration of NaOH (5–20 mM) during the first 25 min of the program, then the concentration of NaOH is kept constant at 20 mM, but now supplemented with an linear increasing concentration of sodium acetate from 0 to 100 mM, during an additional 25 min. The flowrate is kept constant during chromatography at one mL per min. Peaks were identified by co-elution of standards.

2.8. Statistical and data analysis

Averages, standard deviations and least significant differences (LSD), were calculated with the IBM-SPSS statistic software. Descriptive statistics and data visualization was performed in R (version 4.0.2).

3. Results and discussion

3.1. Shelf-life trial

Fruit of a diverse tomato collection, consisting of 92 S. lycopersicum landraces and old cultivars and several *S. pimpinellifolium* accessions, were evaluated for the shelf-life attributes firmness loss, weight loss and decay. This collection had been selected from a phylogenetic tree, based on genotypic data of a set of 343 genetically diverse tomato accessions from the EU–SOL tomato core collection (Roohanitaziani, 2020). Fruit were harvested at breaker-turning stage and stored in a climate controlled chamber (18 °C, 85% Humidity) for 42 d. Measurements were performed once a week and fruit showing visual signs of pathogen infection or over-ripening were discarded. In 2014, the entire collection was evaluated and in 2015 a more extensive analysis was carried out on 17 accessions selected from the 2014 screen.

3.1.1. Firmness measurements

In the 2014 trial, the firmness scores (in Newton), measured by a fruit hardness tester, ranged from 36 (cv. Rote Beere, RF_016) to 85 (cv. The Dutchman, RF_028; cv. Flora Dade, RF_ 140013; cv. S. esc. A0011–6–3, RF_140014, cv. Mao Tao Shi Zi, RF_218) at the day of harvest and from 15 (cv. Kecskemeti Koria Bibor, RF_235) to 81 (cv. EZ 033-rin, RF_231) after 21 d (Table S1). Scores were calculated based on the average of five fruit per accession, although the shelf-life of some fruit did not extend to 21 d and for those accessions the average is representative of less than five fruit. The firmness loss (FL) of each accession was evaluated during storage and is expressed as the average of the % of FL and was calculated as $FL = 100-(F_{(21)} * 100/F_{(0)})$. Where $F_{(21)}$ is the firmness at 21 d of storage and $F_{(0)}$ the firmness at the harvesting time. Based on their FL value, accessions were scored as 1) high FL (FL > 50%), 2) medium FL (FL from 30% to 50%), 3) low FL (FL from 10% to 30%), 4) very low FL (FL < 10%). The lowest FL after 3 weeks storage was found for cv. EZ 033-rin, RF-231 (2%) and the highest FL was found for. *S. pimpinellifolium*, RF_046 and cv. Mao Tao Shi Zi, RF_218 (72%) (Table 1, Fig. S1).

We observed that there are significant differences among accessions in firmness at the day of harvest and that firmness at harvest does not guarantee the maintenance of fruit firmness during storage. In some accessions the firmness at harvest was not high, but loss of firmness was low and after 21 d they still exhibited an acceptable firmness, which was empirically set at > 40 N. On the other hand there were accessions that showed very high firmness at the day of harvest but lost considerable firmness during storage. Neither firmness at harvest, nor firmness after 21 d was related to the size of the fruit (fruit weight) or fruit type, since we observed a diverse range of firmness scores in both small and big fruit.

3.1.2. Weight loss

Weight loss (WL) was expressed as the % of weight loss and calculated by subtracting the fruit weight after 21 d from the initial fruit weight, divided by the initial fruit weight. The performance of accessions for weight loss is shown in Table S1 and Fig. S2. The best performing accession was cv. Italian cherry, RF_215 (3% weight loss) which seems to be a ripening mutant, since its fruit did not get completely ripe until the end of storage (data not shown). Accession *S. pimpinellifolium* (RF_046) lost most weight during three weeks storage (27%). There was

Table 1
Sugar ratios based on sugar composition data.

	Sugar ratio	property
1	$\frac{\text{Gal A}}{Rha + Ara + Gal}$	Linearity of pectin
2	$\frac{\text{Rha}}{Gal\ A}$	Contribution of RG to pectin population
3	$\frac{Ara + Gal}{Rha}$	Branching of RG-I

no strong correlation between weight loss and firmness loss (Fig. S3). Neither did we find a significant correlation between the initial fruit weight and percentage weight loss (Fig. S4): in both big and small fruit varying weight losses were observed. Weight (water) loss can also be expressed according to surface area. We also calculated water loss per surface area, based on weight calculations, and observed a fair correlation between % weight loss and water loss per surface area and therefore only used % weight loss in this study.

3.1.3. Fruit decay (FD)

Before each of the shelf-life measurements, fruit showing signs of mold infection, wounds, shrivelling of the skin or extreme softening were discarded, as they no longer had an acceptable consumption quality. Fruit decay (FD) was calculated each week as the % of fruit with an unacceptable consumption quality in relation to the number of fruit at harvesting time and the results are presented in Table S1. The accessions were classified based on their shelf-life behavior as follows; 1) Very high decay: accessions for which > 50% of the fruit were discarded after 2 weeks storage, 2) High decay: accessions for which > 50% of fruit were discarded after 3 weeks storage, 3) Medium decay: accessions for which > 50% of fruit were discarded after 4 weeks storage, 4) Low decay: accessions for which > 50% were discarded after 5 weeks storage 5) very low decay: accessions for which 50% or more of the fruit remained of acceptable consumer quality without apparent signs of decay until the end of the experiment. For accessions cv. Italian cherry tomato (RF_215), cv. DL/67/248 (RF_226), cv. EZ 033-rin, (RF_231) and cv. Floradade (RF_140013) at least 50% of the fruit remained of acceptable consumption quality during the 6 weeks storage period.

3.1.4. Chlorophyll and lycopene contents

The levels of chlorophyll and lycopene, based on Normalized Difference Vegetation Index (NDVI) and Normalized Anthocyanin Index (NAI) obtained from remittance VIS spectroscopy, are given in Table S2. Previously it has been reported that NDVI and NAI obtained from remittance VIS spectroscopy is closely related to the lycopene and chlorophyll content in pericarp tissue as measured by HPLC and other biochemical methods (Kuckenberg et al., 2008; Farneti et al., 2012). The level of lycopene reached its peak 14–21 d after harvest and after that remained constant. The higher lycopene content was found for cv. Black cherry (RF_029), cv. Purple Russian (RF_202) and cv. Indian Striped (RF_205). Chlorophyll had disappeared after 7–14 d of storage (reached its lowest level) for most of the accessions. In some accessions, such as cv. Black cherry (RF_029), cv. Indian Striped (RF_205), cv. Purple Russian (RF_202), *L. esculentum* (RF_037) and cv. DL/67/24 (RF_226), the decrease in chlorophyll content was very low and they did not lose all of the chlorophyll until the end of the storage period, One of these accessions (RF_029, cv. Black cherry) is a known color mutant and holds two mutations; the *old-gold-crimson* mutation affecting *LYCOPENE β-CYCLASE* leading to an increased lycopene content and the *green flesh* allele gf^4 leading to retention of chlorophyll during ripening (Aflitos et al., 2014; Roohanitaziani et al., 2020), but the other two sequenced accessions in this class (RF_037 and RF_226) are not among known color and/or ripening mutants, while for RF_202 and RF_205 this information could not be retrieved due to lack of sequence information. In yellow and orange tomatoes the level of lycopene was much lower than in red tomatoes, but chlorophyll break down was the same as in normal red tomatoes. No correlation between lycopene content and firmness loss was found (Fig. S5). The variation in patterns of lycopene accumulation and chlorophyll breakdown is illustrated for 5 representative accessions in Fig. 1 for a ripening mutant (RF_231), a color mutant (RF_029), an orange tomato (RF_008) and one of our long shelf-life accessions (RF_226) in comparison to cv.Moneymaker (RF_001).

3.1.5. Repetition of the shelf-life experiment with selected promising accessions

Based on the results of the shelf-life experiment in the first season, 13 promising accessions (these accessions have been highlighted in Table S1) were selected and grown together with two reference accessions with “normal” ripening, cv. Moneymaker (RF_001) and cv. Gardeners delight (RF_003), and two negative control accessions cv. Mao Tao Shi Zi (RF_218) and cv. Madara (RF_220) which showed a short shelf-life performance in the first season. The result of firmness measurements for these 17 accessions is shown in Fig. 2. In Fig. 2A the black bars show firmness at the day of harvest and the gray bars show firmness after 21 d of storage. As is clear from this figure all of the 13 selected promising accessions had acceptable firmness after 21 d (we empirically determined with a commercial cultivar that the lower threshold for an acceptable firmness was 40 N). The two negative controls and the two reference accessions had the lowest firmness after 21 d. These four accessions also showed higher firmness loss after 21 d (Fig. 2B). Of all accessions cv. Floradade (RF_140013) showed the lowest firmness loss. Performance of accessions for weight loss after 21 d of storage is shown in Fig. 2C. The

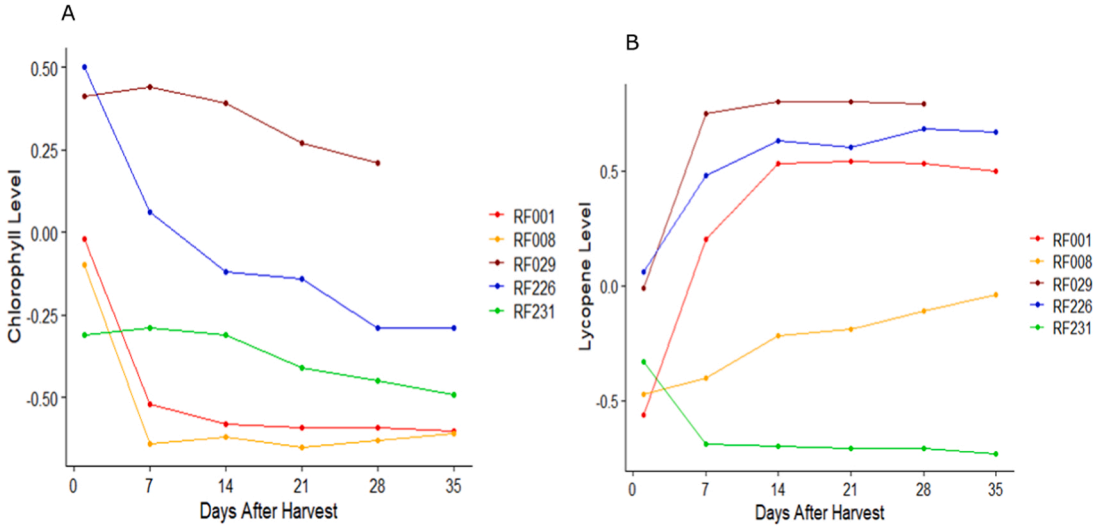

Fig. 1. Illustration of the different lycopene accumulation and chlorophyll breackdown patterns observed in this study. Lycopene accumulation and chlorophyll breakdown in selected accessions in comparison to the performance of cv. Moneymaker (RF001). A) Chlorophyll breakdown, B) Lycopene accumulation. Accessions are presented by their RF number. RF_231: a ripening mutant; RF_029: a color mutant,; RF_008: an orange tomato, RF_226: one of the long shelf-life accessions in our experiment. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

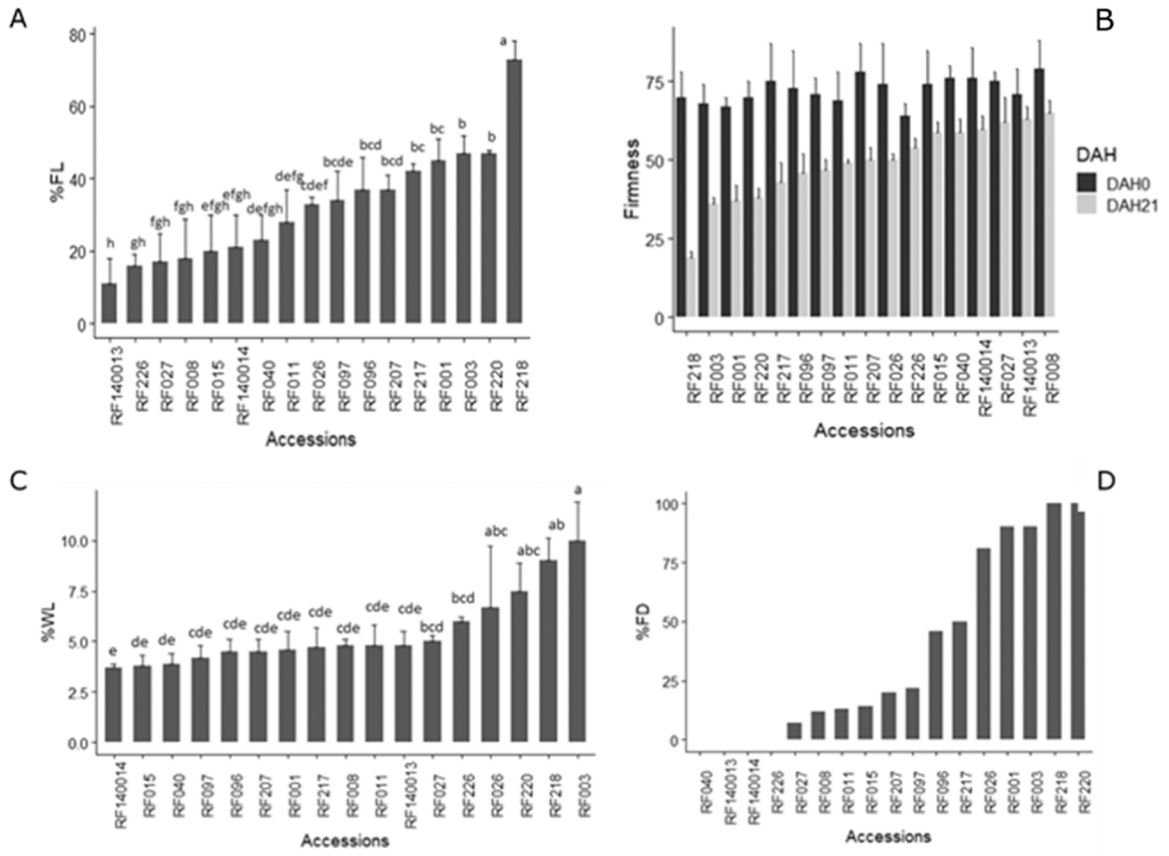

Fig. 2. Performance of 17 accessions for weight loss, firmness and fruit decay during 42 d of storage. A: Firmness at the day of harvest and after 21 d of storage. B: percentage of firmness loss after 21 d. C: Percentage of weight loss after 21 d. D: Percentage of fruit decay after 42 d. FL: firmness loss; WL: weight loss; FD: fruit decay; DAH: days after harvest. Data represent the average + - SD of three biological replicates. Each biological replicate is based on the average of 5 fruits. Significant differences in %FL and %WL are indicated with different letters, based on 2-way ANOVA and LSD test.

highest weight loss was found for RF_003 and the lowest weight loss for RF_140014. For four accessions (RF_140013, 140014, 226, 040) fruit decay after 42 d was zero and all of the fruit harvested kept an acceptable consumer quality until the end of the experiment (see Fig. 2D). Fruit decay for the two negative control accessions RF_218 and 220 was 100%. Finally, considering all shelf-life parameters, we selected four accessions (RF_226, RF_140013, RF_027, RF_040) as long shelf-life (LSL), RF_218 as a short shelf-life (SSL) and RF_001 (cv. Moneymaker) as a reference genotype to study the mechanisms underlying shelf-life in more detail through biochemical characterization. Except for RF226, a cherry tomato, all other accessions were round type tomatoes with 3–4 locules.

3.2. Cell wall analysis

3.2.1. Changes in cell wall sugar composition

The sugar composition of the six accessions at the five postharvest time points, expressed in mg/kg AIR is shown in Table S3. Components of the pectic polysaccharides (arabinose, galactose, rhamnose, galacturonic acid) comprised approximately 60% of the cell wall of mature green tomatoes.

RF_218, the SSL accession, remained firm enough until 21 d after storage but after that time point the quality of the fruit was not good enough anymore for cell wall extraction. Therefore the amount of sugars for this accession has only been reported until B+ 21. Arabinose (Ara), galactose (Gal) and galacturonic acid (Gal A) showed the most changes during the postharvest storage. Arabinose levels decreased 2–3-fold in RF_ 140013, 040, 027, 226 and 001 from the first day of storage until 14 d and after that remained constant. Interestingly, for RF_218, the SSL accession, no change in cell wall arabinose was observed during storage (Table S2 and Fig. 3A), and its level after 21 d storage is up to 2-fold higher than in the LSL accessions. Galactose showed the most drastic decline (3–5-fold) during storage compared to the other sugars although this decline was different among the different accessions. The decline occurred from B+ 1 until B+ 14 and after that there was no further change (Table S3 and Fig. 3B). The cell wall galactose content in RF_218, the SSL accession, was significantly higher than in the other accessions. RF_226 had the lowest galactose content in green stage and B+ 1. The amount of galacturonic acid increased for all the accessions, except RF_001, until 14 d storage and after that it decreased (Table S3 and Fig. 3C). For RF_001 the amount of galacturonic acid did not change until B+ 14 and after that it decreased. After 35 d of storage the galacturonic acid level in RF_001 was lower than those found in the four LSL accessions. Unfortunately, it was not possible to harvest RF_218 at B+ 35 stage, since all fruit were already spoiled. Therefore we cannot make any comparison regarding the loss of galacturonic acid for this accession at B+ 35 d. Among the accessions RF_130014 was the only accession in which the amount of galacturonic acid remained constant during 35 d storage.

We were not able to separate the mannose and xylose by Dionex HPLC and the amount of these two sugars combined is reported. The content of xylose+mannose (Xyl+Man) remained the same or showed an apparent increase during storage. No remarkable difference between LSL and SSL accessions was observed for the rhamnose, xylose+mannose, and glucose content (Table S2 and Fig. 3D, E, F). The reported amount of glucose is for total glucose and we did not separate the cellulosic and non-cellulosic glucose.

The four LSL accessions behaved quite similar to each other during storage with respect to changes in their cell wall sugar composition,

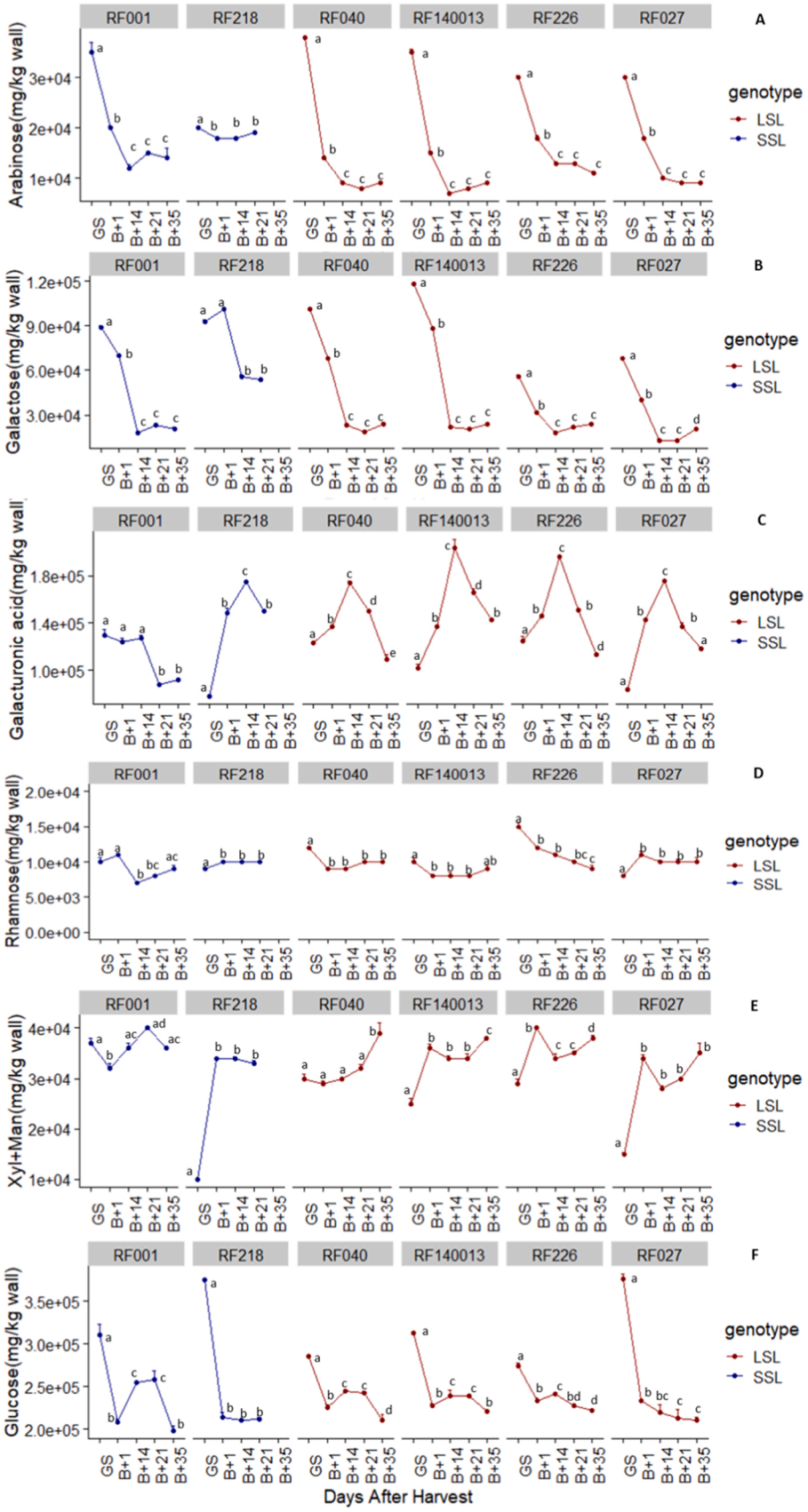

Fig. 3. Changes in cell wall arabinose (A), galactose (B), galacturonic acid (C), rhamnose (D), xylose+mannose (E), and glucose (F) content during 35 d of postharvest storage in six selected tomato accessions. Data shown are means of three replicates. Points (on a curve) which are labeled with the same letter are not significantly different from each other at the $P < .05$ level (LSD). GS: green stage; B+ 1: breaker+ 1; B+ 14: breaker+ 14; B+ 21: breaker+ 21; B+ 35: breaker+ 35. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

although their sugar content was slightly different. The SSL accession differed from LSL accessions with respect to changes and amount of the pectin sugars arabinose and galactose: the SSL accession contains significantly higher levels of these two sugars in its ripe fruit (B+14 and B+21 time points), suggesting a higher branching of pectin in SSL compared to LSL accessions.

3.2.2. Sugar ratios

To asses the polymerization level during postharvest storage, we defined three 'sugar ratios' based on (Houben et.al., 2011) that are used as an expression for the occurrence and properties of certain pectin structures from sugar composition data (Table 1). The first sugar ratio is embodied by the ratio of the pectic backbone sugar galacturonic acid (Gal A) to the neutral pectic sugars involved in side chains, thus being a measure for the linearity of pectin. The second ratio is the proportion of rhamnose (Rha) relative to galacturonic acid, indicative for the contribution of RG (rhamnogalacturonan) to the entire pectin population. Ratio 3 compares the amount of RG-I side-chain sugars to rhamnose, as a measure for the extent of branching of RG-I.

The sugar ratios for the different accessions, calculated based on the sugar content are displayed in Table S4. Summarizing the sugar composition data as sugar ratios reveals that in all six accessions, the linearity of pectin (sugar ratio 1) increases as fruit ripen and after fruit get fully ripe (at B+14 time point) the amount of linear pectin decreases. The SSL accession, RF_218, and RF_001 (MM) exhibit the lowest linearity of pectin in their ripe fruit (from B+14 onwards)(Table S4 and Fig. 4A). The contribution of RG to the pectin population (sugar ratio 2) goes down slightly during ripening until fruit get fully ripe and then goes up again. However, no difference in RG contribution was observed between LSL and SSL accessions (Table S4 and Fig. 4B). In all six accessions RG-I branching (sugar ratio 3) was highest in unripe fruit and decreased as fruit ripened. As soon as the fruit was fully ripe, the branching of RG-I remained constant, but the extent of RG-I branching was considerably higher in ripe fruit of the SSL accession and RF_001 (MM) compared to the LSL accessions (Fig. 4C). According to these sugar ratios, green and unripe fruit have more pectin branches than ripe fruit and we can conclude that early fruit softening is primarily caused by a decrease in pectin branches and pectin complexity. B.

The second stage of fruit softening starts when fruit are completely ripe and is characterized by a decrease in the proportion of linear pectin content, which may be due to breakdown or a reduction in de novo synthesis of this polysaccharide, in line with earlier reports (Goulao and Oliveira, 2008; Posé et al., 2015, Paniagua et al., 2017). In conclusion, our results revealed that the LSL accessions had more linear pectin compared to the two accessions with shorter shelf-life and a lower extent of RG-I branching, particularly at later stages of fruit ripening (B+21) and suggest that the structure of pectin is an important determinant of postharvest firmness loss: more branched and less linear pectin may result in fruit losing their firmness faster. In addition, loss of neutral sugar side chains from RG-I has been considered to be an important component of the changes that alter fruit firmness and textural properties during ripening of several plants, including tomato (McCartney et al., 2000; Smith et al., 2002; Ulvskov et al., 2005; Brummell, 2006; Paniagua et al., 2016). However, it is still unclear how this modification in RG-I structure leads to loss of textural firmness and fruit softening (Wang et al., 2018). The recently revised cell wall model suggests that RG-I is located in the primary cell wall, likely coating cellulose microfibril surfaces to interlink the pectin and cellulose network (Park and Cosgrove, 2012; Posé et al., 2018; Wang et al., 2018; Méndez-Yañez et al., 2020). However, the precise structure of the pectin domains and their exact role in fruit textural alteration is not completely understood yet (Round et al., 2010; Paniagua et.al., 2017; Posé et al., 2018; Wang et al., 2018). Therefore, based on the current knowledge about the fruit cell wall and its role in fruit softening it is difficult to predict the effect of differences in cell wall monomer composition observed between soft and firm fruit on fruit softening. In this respect, several studies revealed that

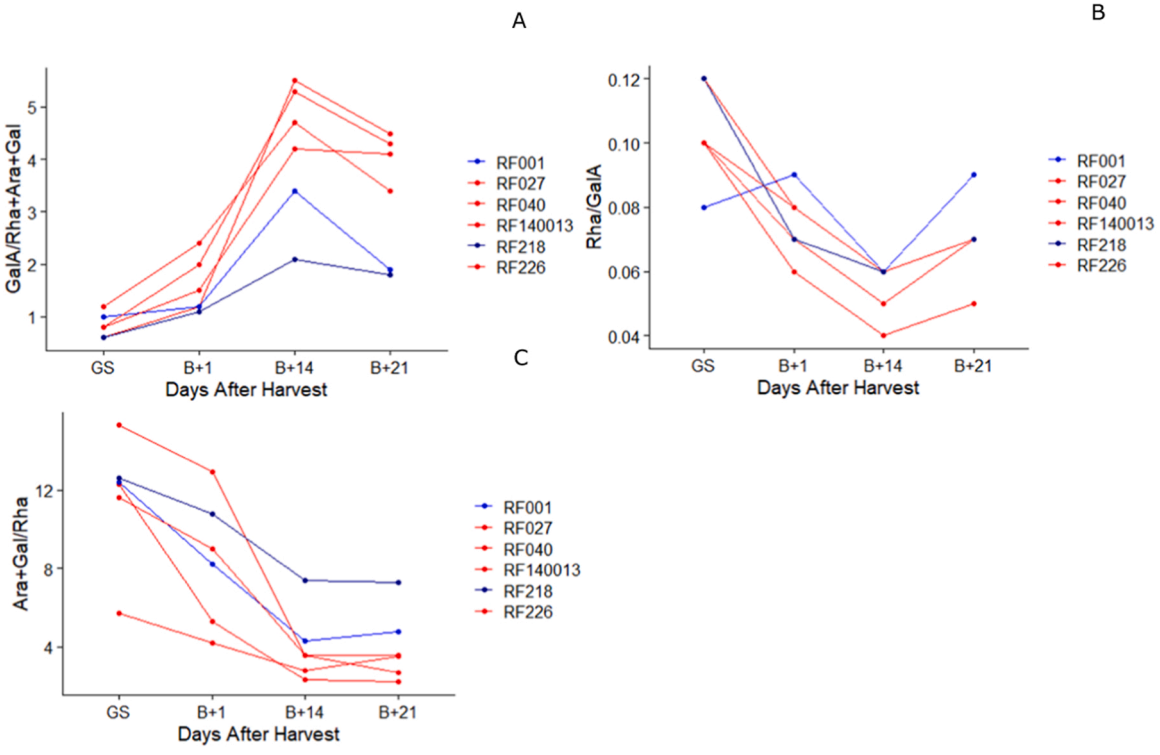

Fig. 4. Change in three pectin properties; linearity, RG contribution, and branching of RG1 which were derived from sugar ratios. Fig. 5 A shows the change in linearity of pectin during ripening and Fig. 5B and 5 C shows the change in contribution of RG and branching of RG-I, respectively. GS: green stage; B+ 1: breaker+ 1; B+ 14: breaker+ 14; B+ 21: breaker+ 21; B+ 35: breaker+ 35. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

pectin depolymerization and solubilization play an important role in the softening of various fruits, including tomato (Brummel et al., 2006). A detailed investigation of the relation between cell wall monomer composition, pectin (ultra) structure and pectin depolymerization/solubilization in a larger set of short and long shelflife genotypes may lead to a better understanding on how differences in cell wall monomer composition reflect and may predict differences in the physiology of fruit softening.

In the past decade, several enzymes acting on the RG-I backbone and side chains, such as beta galactosidases, arabinofuranosidases and rhamnogalacturonan lyase, have been characterized (Trainotti et al., 2001; Smith et al., 2002; Rosli et al., 2009; Tateishi et al., 2014; Paniagua et al., 2016; Ochoa-Jiménez et al., 2018; Méndez-Yañez et al., 2020). Silencing of the genes encoding these enzymes did not show a major effect on the prevention of fruit softening. It is hypothesized that the activity of the enzymes acting on RG-I side chains may enhance the access of other pectin modifying enzymes such as PL (Pectate lyase; RG-lyases), PME (Pectin methylesterase), and PG (Polygalacturonase) to the backbones of the RG-I domain itself or other pectin domains, which results in weakening of the pectin network and disassembly of the cell wall (Liu et al., 2018; Posé et al., 2018; Wang et al., 2018; Méndez-Yañez et al., 2020). In transgenic tomato fruit suppressed in endo-PG enzyme accumulation, only small reductions in fruit softening were detected (Kramer et al., 1992; Langley et al., 1994), the only major effects on fruit texture so far reported for tomato are by changes in PL (Uluisik et al., 2016, Yang et al.,2017) and to a lesser extent expansin (Brummell et al. 1999). These relatively small improvements in fruit softening by silencing of single genes reflects the complexity of the fruit softening process. It is expected that silencing of different combinations of cell wall modification genes may lead to more promising shelf-life improvements, but this remains to be demonstrated.

3.2.2.1. Primary metabolites. GC-MS analysis of primary metabolites led to the identification of 37 metabolites (Table S5 and S 6), including several sugars, organic acids and amino acids. Based on principal components analysis (Fig. 5) 2 groups of metabolites could be recognized. The first group of metabolites (PC1) showed variation as a function of ripening stage, the major source of variation in the data set (32%). The second group (PC2) showed variation between LSL and SSL accessions (9.5%). Ripe tomato samples (B+14, B+21 and B+35 postharvest time points) are located on the positive side of the X axis (PC1), which is characterized by high levels of amongst others, lysine, asparagine, alanine, galacturonate, aspartate, glutamate, glutamine, galactose and methyl glucose, whereas mature green and breaker fruit samples are located towards the negative side of the X axis which is characterized by high levels of, amongst others, malate, citrate, sucrose, mannose, glucose, and threonine. The further a variable is located from the axis origin the more influential the variable is on the principal component. Progression of ripening from the green stage to the postharvest ripe and over-ripe stages seems to be characterized by a decrease in organic acids (citrate and malate), several sugars (sucrose, glucose, mannose) and an increase in amino acids and sugars such as galactose and methyl glucose. The key metabolites to distinguish LSL and SSL accessions (PC2) are putrescine, galactose, myo-inositol, fructose, glucose, mannose and methyl glucose. LSL fruit are located on the negative side of the Y axis, while SSL fruit are located towards the positive side. In three of our LSL accessions (RF_130014, 040, 226) the putrescine level went up during ripening and ripe fruit of these accessions contained 2–3 times higher putrescine levels compared to the green and breaker stages (Fig. 5, Fig. 6A, Table S6). In contrast, the remaining one of the LSL accessions (RF-027) did not accumulate these high levels of putrescine in its ripe fruit. The amount of putrescine in RF-001 did not change during the storage period but in RF_218 putrescine levels showed a steady decrease and green fruit of this accession contained more putrescine than ripe fruit.

Enhanced putrescine levels have previously been reported in fruit of the tomato landrace *alcobaca (alc)* which ripens slowly and has prolonged storage qualities (Dibble et al., 1988). It has been suggested that the enhanced putrescine levels in *alc* may be responsible for the ripening and storage features. *Alc* fruit produces less ethylene compared to

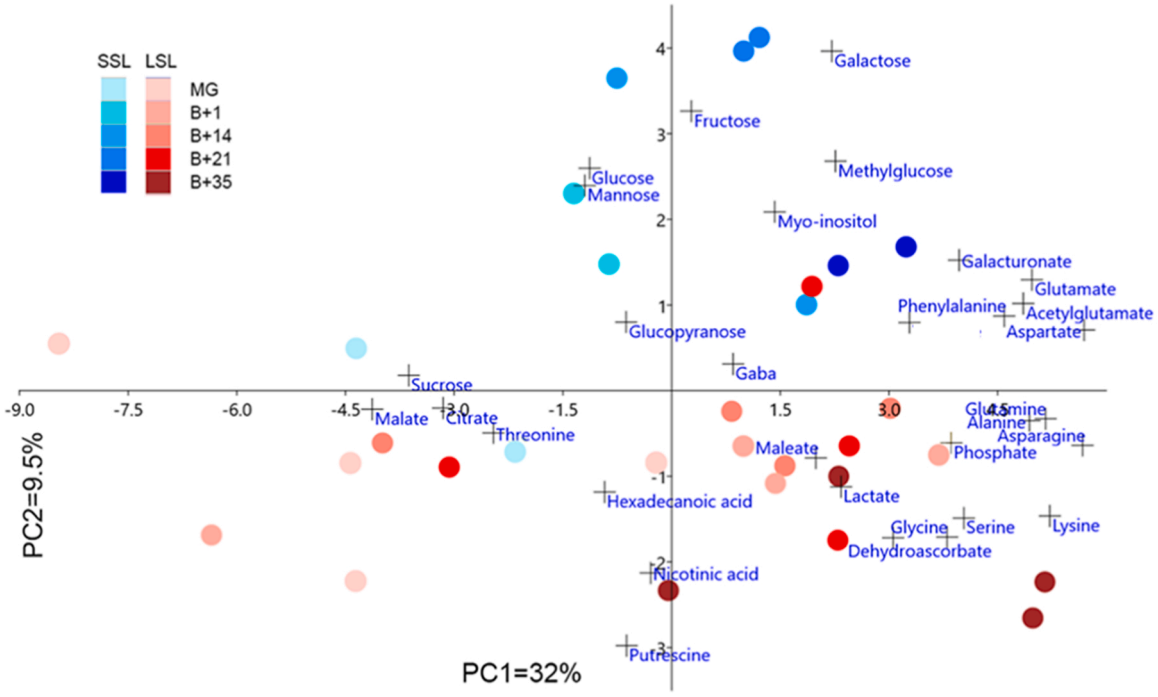

Fig. 5. Principal components analysis of the variation of primary metabolite accumulation between SSL and LSL fruit and between postharvest time points. LSL accessions (cv. Moneymaker and RF_218) are colored from light to dark blue depending on their ripening stages. LSL accessions are depicted in pink to dark red. MG: mature green stage; B+ 1: breaker+ 1; B+ 14: breaker+ 14; B+ 21: breaker+ 21; B+ 35: breaker+ 35. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

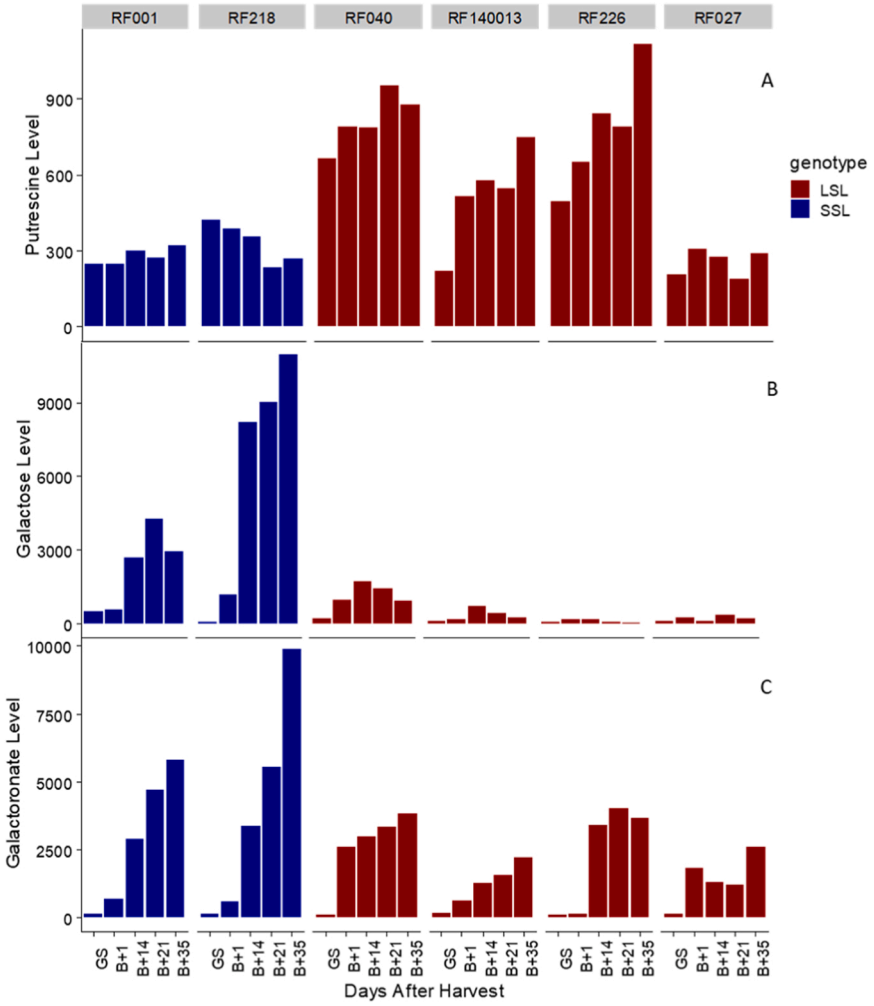

Fig. 6. the levels of putrescine (A), galactose (B) and galacturonic acid (C) during storage. The two SSL accessions are depicted with blue color and the four LSL accessions with red. The metabolite levels are expressed as MS detector response. GS: green stage; B+ 1: breaker+ 1; B+ 14: breaker+ 14; B+ 21: breaker+ 21; B+ 35: breaker+ 35. Each data point represents the level observed in a representative sample obtained from 6 pooled fruits per genotype. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

normal ripening tomatoes (Dibble et al., 1988; Kumar et al., 2018; Wang et al., 2019) and the long keeping quality of *alc* may be related to its lack of ethylene-induced over-ripening. Other studies have shown that for senescence the functions of ethylene and polyamines are antagonistic, not only in tomato fruit of the *alc* landrace, but also in apple fruit and tobacco leaves (Apelbaum et al., 1982; Smith, 1985). The two biosynthesis pathways share the common intermediate, S-adenosyl-methionine. The elevated putrescine levels in *alc* fruit have been reported not to be due to changes in putrescine conjugation or metabolism, but rather to an increase and rise in free putrescine levels (Rastogi and Davies, 1990, 1991). Elevated levels of free polyamines have similarly been observed in the pericarp of tomato cv Liberty, which ripens slowly and has a prolonged keeping quality. Pericarp of Liberty also shows decreased climacteric ethylene production in ripening fruit as compared to pericarp of normal ripening tomatoes (Saftner and Baldi, 1990). It is not clear yet whether the elevated level of free polyamines is responsible for the reduction in ethylene production in cv. Liberty and *alc* fruit or vice versa. In our study, fruit of the three accessions exhibiting high putrescine levels in their pericarp were fully ripe and mature and they showed normal ripening processes. However, we did not measure ethylene in these accessions. Polyamines may also increase fruit shelf-life and attenuation of over-ripening through other mechanisms, as they are involved in many aspects of plant development and considered as important molecules associated with both abiotic and biotic stress tolerance. The exogenous application of polyamines including putrescin either pre-harvest (during fruit growth and ripening on the vine) or after harvest to delay the postharvest ripening process is a common method of postharvest handling in several fruit such as pomegranate, strawberry, plum, apricot, mango, tomato and cucumber (Barman, et al., 2011; Koushesh saba et al., 2012; Jia et al., 2018; Wannabussapawich and Seraypheap, 2018). The exogenous application of polyamines has been shown to increase the levels of antioxidant compounds and the activity of antioxidant enzymes, which results in a decrease in accumulation of ROS (reactive oxygen species) and in this way delays senescence processes and postharvest over-ripening in fruit (Serrano et al., 2003; Sharma et al., 2017; Serrano and Valero, 2018).

Galactose was another metabolite which showed a large quantitative difference between LSL and SSL accessions. Galactose levels were similar in all accessions at green and breaker stage, but in SSL genotype RF-218 and the standard tomato cv. Moneymaker, free galactose levels increased dramatically upon postharvest storage, while this increase was only minor in LSL accessions (Table S6, Fig. 5, Fig. 6B). Although the exact source(s) of free galactose observed in the SSL accessions remains as yet unknown, the cell wall is by far the major galactose-containing organelle, suggesting that free galactose in the fruit pericarp, as observed for SSL accession RF-218, is most likely due to galactosyl solubilization from the cell wall (Kim et al., 1991; Prasanna et al., 2007). However, our results failed to show a correlation between the decrease in cell wall-bound galactose and the increase in free galactose, neither with respect to timing during postharvest storage nor with respect to the levels found in the SSL and LSL genotypes (Figs. 3 and 6; Table S6). Similar results were obtained in previous studies in which the cell wall sugars of *rin* and *nor* mutants had been analyzed in comparison to normal ripening tomato cv. Heinz and cv. Rutgers: the decrease in cell wall galactose content was

noticed for both ripening mutants and normal ripening tomatoes but the increase in free galactose was only noticed in normal ripening tomatoes (Gross and Wallner, 1979; Gross, 1983; Gross and Sams, 1984; Gross, 1985; Seymour et al., 1990; Kim et al., 1991). In line with our results, this suggests that there is no direct relation between the cell wall-bound galactose content and the presence of free galactose in the pericarp. Therefore we hypothesize that the cell wall-bound sugar levels represent the levels present in intact cell walls, while the free sugar levels reflect decomposed, degraded cell walls. A similar lack of correlation between cell wall-bound and free levels of galacturonic acid (Figs. 3 and 6; Table S6) confirm this hypothesis: despite a similar decrease in cell wall galacturonic acid levels in the SSL genotype RF-218 and the three LSL genotypes at later stages of ripening, RF-218 accumulates by far the highest levels of free galacturonic acid in its pericarp.

3.3. Volatile compounds

In total 174 volatile compounds were identified and semi-quantified in fruit of six accessions at five postharvest time points. Six major biochemical classes of volatiles were detected: 1) lipid-derived, 2) phenolic volatiles derived from phenylalanine, 3) phenylpropanoid volatiles 4) terpenoids, 5) volatiles derived from the amino acids Leu and Ile, 6) open chain carotenoid derived volatiles. The PCA shows no variation for volatile compounds between SSL and LSL fruit and fruit of both types of accessions were distributed as a function of ripening stages (Fig. S6).

4. Conclusions

In this study we evaluated a collection of tomato accessions consisting of 92 landraces and heirlooms of *S. lycopersicum* and *S. lycopersicum* var. *cerasiforme,* and several *S. pimpinellifolium* for their fruit postharvest shelf-life. The collection presented a wide range of diversity for shelf-life attributes and color pigments. We have provided a valuable source for improvement of tomato fruit shelf-life through breeding programs. Several of these accessions have been already resequenced which make these materials an interesting source to study the genetics and physiology of fruit shelf-life. Biochemical characterization of selected lines with contrasting shelf-life showed considerable differences in their cell wall sugars and pericarp primary metabolites such as galactose and polyamines. These results provide novel insight in the metabolic changes occurring in cell walls and fruit pericarp during postharvest storage of short and long shelflife genotypes.

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CRediT authorship contribution statement

Raana Roohanitaziani: Conceptualization, Methodology, Formal analysis, Writing – original draft. **Michiel Lammers**: Methodology. **Jos Molthoff**: Methodology. **Yuri Tikunov:** Methodology. **Fien Meijer-Dekens**: Methodology. **Richard G.F. Visser**: Conceptualization, Funding acquisition, Writing – review & editing. **Jeroen van Arkel**: Methodology. **Richard Finkers**: Conceptualization, Funding acquisition, Project administration. **Ruud A. de Maagd**: Conceptualization, Funding acquisition, Supervision, Writing – review & editing. **Arnaud G. Bovy**: Conceptualization, Funding acquisition, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.postharvbio.2022.111908.

References

Aflitos, S., Schijlen, E., de Jong, H., de Ridder, D., Smit, S., Finkers, R., Peters, S., 2014. Exploring genetic variation in the tomato (Solanum section Lycopersicon) clade by whole-genome sequencing. Plant J. 80 (1), 136–148. https://doi.org/10.1093/jxb/erm037.

Apelbaum, A., Icekson, I., Burgoon, A.C., Lieberman, M., 1982. Inhibition by polyamines of macromolecular synthesis and its implication for ethylene production and senescence processes. Plant Physiol. 70 (4), 1221–1223. https://doi.org/10.1104/pp.70.4.1221.

Barman, K., Asrey, R., Pal, R.K., 2011. Putrescine and carnauba wax pretreatments alleviate chilling injury, enhance shelf life and preserve pomegranate fruit quality during cold storage. Sci. Hortic. 130, 795–800. https://doi.org/10.1016/j.scienta.2011.09.005.

Beckles, D.M., 2012. Factors affecting the postharvest soluble solids and sugar content of tomato (Solanum lycopersicum L.) fruit. Postharvest Biol. Technol. 63, 129–140. https://doi.org/10.1093/jxb/erm037.

Blanca, J., Montero-Pau, J., Sauvage, C., Bauchet, G., Illa, E., Díez, M.J., Cañizares, J., 2015. Genomic variation in tomato, from wild ancestors to contemporary breeding accessions. BMC Genom. 16 (1), 257. https://doi.org/10.1186/s12864-015-1444-1.

Brummell, D.A., Harpster, M.H., Civello, P.M., Palys, J.M., Bennett, A.B., Dunsmuir, P., 1999. Modification of expansin protein abundance in tomato fruit alters softening and cell wall polymer metabolism during ripening. Plant Cell 11, 2203–2216.

Brummell, D.A., Harpster, M.H., 2001. Cell wall metabolism in fruit softening and quality and its manipulation in transgenic plants. Plant Mol Biol 47 (1–2), 311–340.

Brummell, D.A., 2005. Regulation and genetic manipulation of ripening in climacteric fruit. Stewart Postharvest Rev. 1 (3), 1.

Brummell, D.A., 2006. Cell wall disassembly in ripening fruit. Funct. Plant Biol. 33 (2), 103–119. https://doi.org/10.1071/fp05234.

Carreno-Quintero, N., Acharjee, A., Maliepaard, C., Bachem, C., Mumm, R., Bouwmeester, H., Keurentjes, J., 2012. Untargeted metabolic quantitative trait loci (mQTL) analyses reveal a relationship between primary metabolism and potato tuber quality. Plant Physiol. 158 (3), 1306–1318. https://doi.org/10.1016/j.postharvbio.2012.05.008.

Dibble, A.R.G., Davies, P.J., Mutschler, M.A., 1988. Polyamine content of long-keeping alcobaca tomato fruit. Plant Physiol. 86 (2), 338–340. https://doi.org/10.1016/j.postharvbio.2012.05.008.

Farneti, B., Schouten, R.E., Woltering, E.J., 2012. Low temperature-induced lycopene degradation in red ripe tomato evaluated by remittance spectroscopy. Postharvest Biol. Technol. 73, 22–27. https://doi.org/10.1016/j.postharvbio.2012.05.008.

Giovannoni, J., 2001. Molecular biology of fruit maturation and ripening. Annu. Rev. Plant Biol. 52, 725–749. https://doi.org/10.1146/annurev.arplant.52.1.725.

Goulao, L.F., Oliveira, C.M., 2008. Cell wall modifications during fruit ripening: when a fruit is not the fruit. Trends Food Sci. Technol. 19 (1), 4–25. https://doi.org/10.1016/j.tifs.2007.07.002.

Gross, K.C., 1983. Changes in free galactose, myo-inositol and other monosaccharides in normal and non-ripening mutant tomatoes. Phytochemistry 22 (5), 1137–1139. https://doi.org/10.1016/0031-9422(83)80207-6.

Gross, K.C., 1985. Promotion of ethylene evolution and ripening of tomato fruit by galactose. Plant Physiol. 79 (1), 306–307. https://doi.org/10.1104/pp.79.1.306.

Gross, K.C., Sams, C.E., 1984. Changes in cell wall neutral sugar composition during fruit ripening: a species survey. Phytochemistry 23 (11), 2457–2461. https://doi.org/10.1111/j.1399-3054.1984.tb05918.x.

Gross, K.C., Wallner, S.J., 1979. Degradation of cell wall polysaccharides during tomato fruit ripening. Plant Physiol. 63 (1), 117–120. https://doi.org/10.1104/pp.63.1.117.

Jia, B., Zheng, Q., Zuo, J., Gao, L., Wang, Q., Guan, W., Shi, J., 2018. Application of postharvest putrescine treatment to maintain the quality and increase the activity of antioxidative enzyme of cucumber. Sci. Hortic. 239, 210–215. https://doi.org/10.1016/j.scienta.2018.05.043.

Houben, K., Jolie, R.P., Fraeye, I., Van Loey, A.M., Hendrickx, M.E., 2011. Comparative study of the cell wall composition of broccoli, carrot, and tomato: structural characterization of the extractable pectins and hemicelluloses. Carbohydr Res. 346 (9), 1105–1111. https://doi.org/10.1016/j.carres.2011.04.014.

Kim, J., Gross, K.C., Solomos, T., 1991. Galactose metabolism and ethylene production during development and ripening of tomato fruit. Postharvest Biol. Technol. 1 (1), 67–80. https://doi.org/10.1016/0925-5214(91)90020-C.

Kopeliovitch, E., Rabinowitch, H.D., Mizrahi, Y., Kedar, N., 1979. The potential of ripening mutants for extending the storage life of the tomato fruit. Euphytica 28 (1), 99–104. https://doi.org/10.1007/BF00029179.

Koushesh saba, M., Arzani, K., Barzegar, M., 2012. Postharvest polyamine application alleviates chilling injury and affects apricot storage ability. J. Agric. Food Chem. 60 (36), 8947–8953. https://doi.org/10.1021/jf302088e.

Kuckenberg, J., Tartachnyk, I., Noga, G., 2008. Evaluation of fluorescence and remission techniques for monitoring changes in peel chlorophyll and internal fruit characteristics in sunlit and shaded sides of apple fruit during shelf-life. Postharvest Biol. Technol. 48 (2), 231–241. https://doi.org/10.1016/j.postharvbio.2007.10.013.

Kumar, R., Tamboli, V., Rameshwar Sharma, R., Sreelakshmi, Y., 2018. NAC-NOR mutations in tomato Penjar accessions attenuate multiple metabolic processes and prolong the fruit shelf life. Food Chem. 259, 234–244. https://doi.org/10.1007/s00438-011-0671-7.

Labavitch, J.M., 1981. Cell wall turnover in plant development. Ann. Rev. Plant Physiol. 31, 385–406. https://doi.org/10.1146/annurev.pp.32.060181.002125.

Lahaye, M., Quemener, B., Causse, M., Seymour, G.B., 2012. Hemicellulose fine structure is affected differently during ripening of tomato lines with contrasted texture. Int. J. Biol. Macromol. 51 (4), 462–470. https://doi.org/10.1016/j.postharvbio.2012.09.009.

Liu, H., Qian, M., Song, C., Li, J., Zhao, C., Li, G., Wang, A., Han, M., 2018. **Down-regulation of *PpBGAL10* and *PpBGAL16* delays fruit softening in peach by reducing polygalacturonase and pectin methylesterase activity**. Front. Plant Sci. 9, 1015. https://doi.org/10.1016/j.plantsci.2014.12.006.

Lunn, D., Phan, T.D., Tucker, G.A., Lycett, G.W., 2013. Cell wall composition of tomato fruit changes during development and inhibition of vesicle trafficking is associated with reduced pectin levels and reduced softening. Plant Physiol. Biochem. 66, 91–97. https://doi.org/10.1016/j.plaphy.2013.02.005.

Mahajan, P.V., Caleb, O.J., Singh, Z., Watkins, C.B., Geyer, M., 2014. Postharvest treatments of fresh produce. Philos. Trans. A Math. Phys. Eng. Sci. 372 (2017), 20130309 https://doi.org/10.1098/rsta.2013.0309.

Markovic, Z., Zdravkovic, J., Cvikic, D., Pavlovic, N., Zdravkovic, M., 2012. Breeding new F1 tomato hybrids of rin genotype. Acta Hortic. 960, 165–168. https://doi.org/10.17660/ActaHortic.2012.960.23.

McCartney, L., Ormerod, A.P., Gidley, M.J., Knox, J.P., 2000. Temporal and spatial regulation of pectic (1–>4)-beta-D-galactan in cell walls of developing pea cotyledons: implications for mechanical properties. Plant J. 22 (2), 105–113. https://doi.org/10.1046/j.1365-313x.2000.00719.x.

McGlasson, W., Sumeghy, J., Morris, L., McBride, R., Best, D., Tigchelaar, E., 1983. Yield and evaluation of F1 tomato hybrids incorporating the non-ripening nor gene. Aust. J. Exp. Agric. 23 (120), 106–112. https://doi.org/10.1071/EA9830106.

Méndez-Yañez, A., González, M., Carrasco-Orellana, C., Herrera, R., Moya-León, M.A., 2020. Isolation of a rhamnogalacturonan lyase expressed during ripening of the Chilean strawberry fruit and its biochemical characterization. Plant Physiol. Biochem. 146, 411–419. https://doi.org/10.1016/j.plaphy.2019.11.041.

Nguyen, V.Q., Ashcroft, W.J., Jones, K.H., McGlasson, W.B., 1991.). **Evaluation of F1 hybrids incorporating the rin (ripening inhibitor) gene to improve the storage life and fruit quality of fresh market tomatoes (Lycopersicon esculentum Mill.)**. Aust. J. Exp. Agric. 31, 407–413. https://doi.org/10.1071/ea9910407.

Ochoa-Jiménez, V., Berumen-Varela, G., Burgara-Estrella, A., Orozco-Avitia, J., Ojeda-Contreras, A., Trillo-Hernández, E., Tiznado-Hernández, M., 2018. Functional analysis of tomato rhamnogalacturonan lyase gene Solyc11g011300 during fruit development and ripening. Plant Physiol. 231, 31–40. https://doi.org/10.1016/j.jplph.2018.09.001.

Paniagua, C., Blanco-Portales, R., Barcelo-Munoz, M., Garcia-Gago, J.A., Waldron, K.W., Quesada, M.A., 2016. Antisense down-regulation of the strawberry beta-galactosidase gene FabetaGal4 increases cell wall galactose levels and reduces fruit softening. J. Exp. Bot. 67, 619–631. https://doi.org/10.1093/jxb/erv462.

Paniagua, C., Kirby, A.R., Patrick Gunning, A., Morris, V.J., Matas, A.J., Quesada, M.A., Mercado, J.A., 2017. Unravelling the nanostructure of strawberry fruit pectins by endo-polygalacturonase digestion and atomic force microscopy. Food Chem. 224, 270–279. https://doi.org/10.1016/j.foodchem.2016.12.049.

Park, Y.B., Cosgrove, D.J., 2012. A revised architecture of primary cell walls based on biomechanical changes induced by substrate-specific endoglucanases. Plant Physiol. 158 (4), 1933–1943. https://doi.org/10.1104/pp.111.192880.

Posé, S., Kirby, A.R., Paniagua, C., Waldron, Kw, Morris, V.J., Quesada, M.A., Mercado, J.A., 2015. The nanostructural characterization of strawberry pectins in pectate lyase or polygalacturonase silenced fruit elucidates their role in softening. Carbohydr. Polym. 132, 134–145. https://doi.org/10.1016/j.carbpol.2015.06.018.

Posé, S., Paniagua, C., Matas, A., Gunning, A.P., Morris, V.J., Quesada, M.A., Mercado, J. A., 2018. A nanostructural view of the cell wall disassembly process during fruit ripening and postharvest storage by atomic force microscopy. Trends Food Sci. Technol. 87, 47–58. https://doi.org/10.1016/j.tifs.2018.02.011.

Prasanna, V., Prabha, T.N., Tharanathan, R.N., 2007. Fruit ripening phenomena-an overview. Crit. Rev. Food Sci. Nutr. 47 (1), 1–19. https://doi.org/10.1080/10408390600976841.

Rashidi, B., Trindade, L.M., 2018. Detailed biochemical and morphologic characteristics of the green microalga Neochloris oleoabundans cell wall. Algal. Res. 35, 152–159. https://doi.org/10.1016/j.algal.2018.08.033.

Rastogi, R., Davies, P.J., 1990. Polyamine metabolism in ripening tomato fruit: I. Identification of metabolites of putrescine and spermidine. Plant Physiol. 94 (3), 1449–1455. https://doi.org/10.1104/pp.94.3.1449.

Rastogi, R., Davies, P.J., 1991. Polyamine metabolism in ripening tomato fruit: II. Polyamine metabolism and synthesis in relation to enhanced putrescine content and storage life of tomato fruit. Plant Physiol. 95 (1), 41–45. https://doi.org/10.1104/pp.95.1.41.

Rick, C.M., Chetelat, R.T., 1995. utilization of related wild species for tomato improvement. Acta Hortic. 412 https://doi.org/10.17660/ActaHortic.1995.412.1.

Roohanitaziani, R., de Maagd, R.A., Lammers, M., Molthoff, J., Meijer-Dekens, F., van Kaauwen, M.P.W., Finkers, R., Tikunov, Y., Visser, R.G.F., Bovy, A.G., 2020. Exploration of a resequenced tomato core collection for phenotypic and genotypic variation in plant growth and fruit quality traits. Genes 11, 1278. https://doi.org/10.3390/genes11111278.

Rosli, H.G., Civello, P.M., Martínez, G.A., 2009. alpha-l-Arabinofuranosidase from strawberry fruit: cloning of three cDNAs, characterization of their expression and analysis of enzymatic activity in cultivars with contrasting firmness. Plant Physiol. Biochem. 47, 272–281. https://doi.org/10.1016/j.plaphy.2008.12.009.

Round, A.N., Rigby, N.M., MacDougall, A.J., Morris, V.J., 2010. A new view of pectin structure revealed by acid hydrolysis and atomic force microscopy. Carbohydr. Res. 345 (4), 487–497. https://doi.org/10.1016/j.carres.2009.12.019.

Saftner, R., Baldi, B.G., 1990. Polyamine levels and tomato fruit development: possible interaction with ethylene. Plant Physiol. 92 (2), 547–550. https://doi.org/10.1104/pp.92.2.547.

Sandarani, M., Dasanayaka, D., Jayasinghe, C., 2018. Strategies used to prolong the shelf Life of fresh commodities. J. Agr. Food Res. 9 (1), 1–6.

Schouten, R.E., Farneti, B., Tijskens, L.M.M., Alarcón, A.A., Woltering, E.J., 2014. Quantifying lycopene synthesis and chlorophyll breakdown in tomato fruit using remittance VIS spectroscopy. Postharvest Biol. Technol. 96, 53–63. https://doi.org/10.1016/j.postharvbio.2014.05.007.

Serrano, M., Martinez-Romero, D., Guillen, F., Valero, D., 2003. Effects of exogenous putrescine on improving shelf life of four plum cultivars. Postharvest Biol. Technol. 30, 259–271. https://doi.org/10.1016/S0925-5214(03)00113-3.

Serrano, M., Valero, D., 2018. Application of polyamines to maintain functional properties in stored fruit. Methods Mol. Biol. 1694, 449–458. https://doi.org/10.1007/978-1-4939-7398-9_37.

Seymour, G.B., Colquhoun, I.J., Dupont, M.S., Parsley, K.R., Selvendran, R., 1990. Composition and structural features of cell wall polysaccharides from tomato fruit. Phytochemistry 29 (3), 725–731. https://doi.org/10.1016/0031-9422(90)80008-5.

Sharma, S., Pareek, S., Sagar, N.A., Valero, D., Serrano, M., 2017. Modulatory effects of exogenously applied polyamines on postharvest physiology, antioxidant system and shelf life of fruit: a review. Int. J. Mol. Sci. 18 (8) https://doi.org/10.3390/ijms18081789.

Smith, T.A., 1985. Polyamines. Annu. Rev. Plant Biol. 36 (1), 117–143. https://doi.org/10.1146/annurev.pp.36.060185.001001.

Smith, D.L., Abbott, J.A., Gross, K.C., 2002. Down-regulation of tomato beta -galactosidase 4 results in decreased fruit softening. Plant Physiol. 129 (4), 1755–1762. https://doi.org/10.1104/pp.011025.

Tateishi, A., Kamiyoshihara, Y., Matsuno, J., Miyohashi, F., Shiba, H., Kanayama, Y., Watanabe, K., Nomura, K., Inoue, H., 2014. Heterologous expression of tomato glycoside hydrolase family 3 α-L-arabinofuranosidase/β-xylosidases in tobacco suspension cultured cells and synergic action of a family 51 isozyme under antisense suppression of the enzyme. Physiol. Plant 150 (2), 238–251. https://doi.org/10.1111/ppl.12079.

Tikunov, Y., Lommen, A., de Vos, C.H.R., Verhoeven, H.A., Bino, R.J., Hall, R.D., Bovy, A.G., 2005. **A novel approach for nontargeted data analysis for metabolomics.** large-scale profiling of tomato fruit volatiles. Plant. Physiol. 139 (3), 1125–1137. https://doi.org/10.1104/pp.105.068130.

Trainotti, L., Spinello, R., Piovan, A., Spolaore, S., Casadoro, G., 2001. Beta Galactosidases with a lectin-like domain are expressed in strawberry. J. Exp. Bot. 52, 1635–1645.

Ulvskov, P., Wium, H., Bruce, D., Jorgensen, B., Qvist, K.B., Skjot, M., Sorensen, S.O., 2005. Biophysical consequences of remodeling the neutral side chains of rhamnogalacturonan I in tubers of transgenic potatoes. Planta 220 (4), 609–620. https://doi.org/10.1007/s00425-004-1373-8.

Uluisik, S., Chapman, N.H., Smith, R., Poole, M., Adams, G., Gillis, R.B., Seymour, G.B., 2016. Genetic improvement of tomato by targeted control of fruit softening. Nat. Biotechnol. 34 (9), 950–952. https://doi.org/10.1038/nbt.3602.

van Arkel, J., Vergauwen, R., Sévenier, R., Hakkert, J.C., van Laere, A., Bouwmeester, H. J., Koops, A.J., van der Meer, I.M., 2012. Sink filling, inulin metabolizing enzymes and carbohydrate status in field grown chicory (Cichorium intybus L.). Plant Physiol. 169, 1520–1529. https://doi.org/10.1016/j.jplph.2012.06.005.

Van Wychen, S., Laurens, Lieve M.L., 2016. Determination of Total Carbohydrates in Algal Biomass. Laboratory Analytical Procedure (LAP) N. P., United States https://doi.org/10.2172/1118073.

Wang, D., Trevor, H., Yeats, T.H., Uluisik, S., Jocelyn, K.C., Rose, J., Seymour, G.B., 2018. Fruit Softening: Revisiting the Role of Pectin. Trends Plant Sci. 23 (4), 302–310. https://doi.org/10.1016/j.tplants.2018.01.006.

Wang, R., Tavano, E.Cd.R., Lammers, M., Martinelli, A.P., Angenent, G.C., de Maagd, R. A., 2019. Re-evaluation of transcription factor function in tomato fruit development and ripening with CRISPR/Cas9-mutagenesis. Sci Rep 9 (1696). https://doi.org/10.1038/s41598-018-38170-6.

Wannabussapawich, B., Seraypheap, K., 2018. Effects of putrescine treatment on the quality attributes and antioxidant activities of 'Nam Dok Mai No.4' mango fruit during storage. Sci. Hortic. 233, 22–28. https://doi.org/10.1016/j.scienta.2018.01.050.

Yang, L., Huang, W., Xiong, F., Xian, Z., Su, D., Ren, M., Li, Z., 2017. **Silencing of *SlPL*, which encodes a pectate lyase in tomato, confers enhanced fruit firmness, prolonged shelf-life and reduced susceptibility to grey mould**. Plant Biotechnol. 15, 1544–1555. https://doi.org/10.1111/pbi.12737.