Title:

Favorable effects of the weak ethylene receptor mutation *Sletr1-2* on postharvest fruit quality changes in tomatoes

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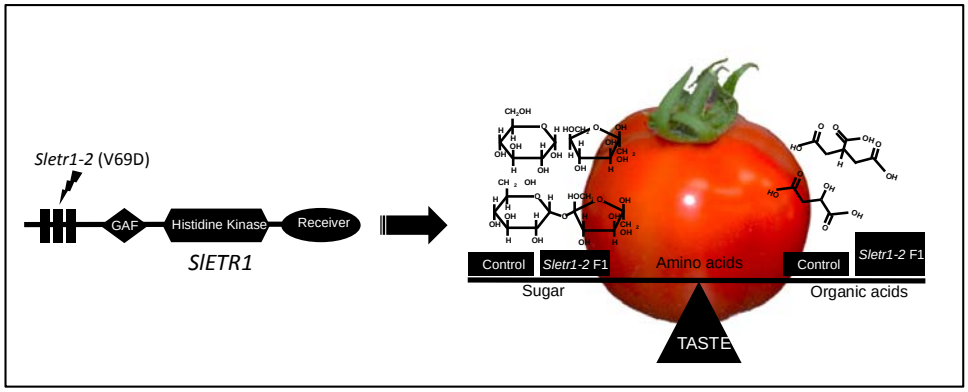
Abstract

Tomatoes with a prolonged fruit shelf life and improved postharvest quality would be an attractive commodity for both breeders and consumers. A weak allele of the tomato ethylene receptor mutant *Sletr1-2* elicits an extended fruit shelf life without prominent undesirable pleiotropic effects. In this study, we elucidate the influences of the *Sletr1-2* mutation on alterations of the postharvest fruit quality of *Sletr1-2* F1 hybrid lines from four different pure-line cultivar parents. The changes in the compositions of metabolic compounds, including sugars, organic acids and amino acids, over 30 days of postharvest storage have been evaluated. The *Sletr1-2* mutation significantly affected the postharvest fruit quality parameters of the *Sletr1-2* F1 hybrid lines in a manner that depended on the pure-line cultivar parental backgrounds. The influence of the *Sletr1-2* mutation was detected only in the reductions and/or increases of individual amino acids and increases in the levels of organic acids, i.e., malate and citrate. In contrast, the sugar level was not changed. Moreover, the *Sletr1-2* mutation significantly reduced the rate of water loss during postharvest storage. These results indicate that the *Sletr1-2* mutation has favorable effects on the postharvest changes of *Sletr1-2* F1 hybrid tomatoes that improve both the shelf life and the taste quality.

Graphical Abstract



Highlights

- The *Sletr1-2* mutation is potentially useful for maintaining the postharvest quality of tomatoes.
- The rate of water loss was slower in the *Sletr1-2* F1 hybrid lines.
- The *Sletr1-2* mutation did not change the sugar content.
- The *Sletr1-2* mutation induced increases in organic acid contents.
- The amino acid contents varied between the *Sletr1-2* F1 hybrid lines.

Key words: amino acid, organic acid, sugar, taste, water loss.

1. Introduction

The tomato (*Solanum lycopersicum* L.) is a major horticultural crop that is used for both fresh and processed product consumption. Tomato fruit have high nutritional value due to the presence of vitamins, carotenoids, and phenolic compounds, which are important for human health (Antunes et al., 2010). One of the major problems related to the postharvest quality of tomatoes is the short shelf life, which influences their transportation and marketability. This short fruit shelf life is associated with the acceleration of the ripening process, which is regulated by ethylene. However, ripening enhances the changes in numerous metabolic pathways that influence the fruit composition, taste, and aroma and affect the consumer's acceptance and the eating quality of the tomato fruit. Because of the high demand for prolonged fruit shelf life and high taste quality of tomato fruit by producers, retailers and consumers, these two characteristics are of major interests in tomato breeding programs (Causse et al., 2003).

Many studies have been undertaken to generate new tomato lines with an extended fruit shelf life based on manipulations of the ripening process. Several ripening mutants that confer extended fruit shelf life, such as *ripening-inhibitor* (*rin*), *non-ripening* (*nor*), and *never ripe* (*Nr*) have been successfully isolated and characterized (Lanahan et al., 1994; Wilkinson et al., 1995). However, many studies have reported that these tomato mutants are generally less tasty than traditional varieties, although they have been utilized in traditional breeding programs (Hobson, 1980; Bartoszewski et al., 2003; Causse et al., 2003; Dorais el al., 2003). In our previous study, we successfully isolated from the Micro-Tom mutant library new mutants, namely *Sletr1-1 and Sletr1-2,* that exhibit altered ripening phenotypes

(Okabe et al., 2011). These two mutants are characterized by a delayed ripening process and extended fruit shelf life; the *Sletr1-1* and *Sletr1-2* mutants exhibit completely and moderately ethylene-insensitive phenotypes, respectively.

Among several known tomato ripening mutants, the *Sletr1-2* mutant line is most suitable as a breeding material to extend the fruit shelf life also considering that it can produce a red ripe fruit and does not show prominent undesirable pleiotropic effects on fruit quality (Okabe el at., 2011). The *Sletr1-2* mutation occurs in the second domain of the transmembrane region of the ethylene receptor gene (*SlETR1*) and exhibits a dominant inheritance pattern (Okabe et al., 2011). Our previous study demonstrated that the *Sletr1-2* mutation exerts a positive effect that extends the fruit shelf life of *Sletr1-2* F1 hybrid lines by 4 to 5 days and results in fruit characteristics that are similar to those of the wild-type F1 hybrid lines without a substantial reduction in red fruit coloration even when the *Sletr1-2* mutant line is crossed with different parental backgrounds (Mubarok et al., 2015). Although the *Sletr1-2* mutant exhibits positive fruit phenotype and shelf life characteristics, the postharvest taste quality has not been clearly evaluated. Therefore, further study is needed to elucidate the alterations in the postharvest fruit taste qualities of *Sletr1-2* F1 hybrid lines that result from the *Sletr1-2* mutation.

Fruit quality is dependent on visual and nonvisual fruit characteristics, and fruit flavor is one of the important nonvisual fruit characteristics because it contributes to fresh fruit and processed product acceptability (Awad and De Jager, 2002; Garg and Cheema, 2011). Fruit flavor is a complex parameter that is determined by the composition of the volatile and non-volatile compounds (Kader, 2008). Taste is affected by the sugar, organic acid, and amino acid contents, which are mainly influenced by environmental factors, cultivation methods, fruit maturity, genetics and

77 postharvest treatments (Paulson and Stevens, 1974; Kader et al., 1977). Sugar
78 contributes to the level of sweetness. Glucose and fructose are the main sugar
79 components of the tomato (Baldwin et al, 1998). Fruit sourness is related to the acid
80 content, particularly the organic acid content, which also has an important function in
81 food nutrition (Tang et al., 2010). Citrate and malate are the major organic acids in the
82 tomato fruit (Baldwin et al, 1998). Amino acids enhance the fruit taste, and glutamic
83 acid, glutamine, aspartic acid and γ-aminobutyric acid are the major amino acids in
84 the tomato fruit (Kader et al., 1977; Nelson et al., 2002; Pratta et al., 2004; Oms-Oliu
85 et al., 2011).

86 The combination of the desired characteristics in terms of postharvest quality,
87 fruit shelf life and taste are needed to produce high-quality tomato fruit with
88 additional value. In this study, we performed metabolic analyses of four tomato F1
89 hybrid lines between the *Sletr1-2* mutant allele and four different pure-line cultivars to
90 elucidate the effects of the *Sletr1-2* mutation on the alteration of the postharvest
91 quality of the fruit. The changes in the metabolic compounds related to the
92 postharvest fruit taste quality, namely sugar, organic acids and amino acids, were
93 evaluated during 30 days of storage. The results suggested that the *Sletr1-2* mutation
94 exhibited favorable effects on the changes in the postharvest fruit quality of the
95 *Sletr1-2* F1 hybrid lines by extending the shelf life without reducing the taste quality.

96 **2. Materials and Methods**

97 **2.1. Fruit preparation**

Fruit from eight F1 hybrid lines of *Sletr1-2* and the wild-type 'Micro-Tom' (WT-MT) crossed with 'Aichi First', 'Ailsa Craig', 'Moneymaker', and 'M82' from the Tomato National Bioresource Project of Japan (Saito et al., 2011) were evaluated. The tomato plants were cultivated under the Nutrient Film Technique (NFT) cultivation system during the winter season from September 2013 to February 2014. Fruit were harvested at similar maturities, i.e., at breaker + 6 days (Br+6) and stored for postharvest analysis at room temperature (20 ± 2 °C) and 80% humidity. The experiment consisted of four biological replicates, and each replicate consisted of three individual plants. Two fruit per individual plant were harvested for each time point of postharvest storage (0, 10, 20, 30, and 40 days postharvest (DPH)).. Because the postharvest quality of the fruit was lost at 40 DPH, the metabolic compounds (i.e., sugar, organic acids, and amino acids) were analyzed up to 30 DPH.

2.2. Analysis of postharvest water loss

The postharvest water loss was evaluated over 40 days of postharvest storage and measured gravimetrically in the individual fruit. The fruit were weighed every 10 days up to 40 DPH. The rate of water loss (WRL) was measured as a daily percentage of fruit weight loss with respect to the initial fruit weight as described by Díaz-Pérez et al. (2007).

2.3. Analysis of the sugar contents

The fruit tissue was ground into powder using liquid nitrogen. The sugars were extracted twice from 600 mg of powder with 1 mL of 80% ethanol and te solution was incubated at 85 °C for 10 minutes. Additionally, 1% pentaerythritol was

added to the solution as an internal standard. The extract was evaporated under vacuum at 45 °C. To remove phenolic compounds, 3 mg of polyvinylpolypyrrolidone (PVPP) was added to the aliquots. The extract solution was filtered using a 0.45-µM millipore filter, and 1 mL of extract solution was injected with an autosampler into a high-performance liquid chromatography (HPLC) instrument fitted with a pre-column (Shin-pack SPR-Ca, Shimazdu, Japan) and package column (SC-1011, SHODEX, Showa Denko K.K., Tokyo, Japan) with a reflective index detector (RI-101-SHODEX, Denko K.K., Tokyo, Japan). Ultra-pure water (18 mΩ) was utilized as the mobile phase, and the equipment was set at flow rate of 0.8 mL min^{-1} and a column temperature of 80 °C. The sugars were quantified based on calculations of the peak area relative to the regression curves of the sugar standard.

2.4. Analysis of the organic acid contents

To determine the organic acid contents, 400 mg of frozen fruit powder was mixed with 400 µL of MilliQ water and incubated at 95 °C for 10 minutes. Next, 800 µL of MilliQ water was added and mixed well via vortexing. The solution was centrifuged at 13000 rpm at 4 °C for 30 minutes. Then, 20 µL of supernatant was taken, diluted to 40X and filtered using a GE Healthcare 0.45-µM syringe filter. The extract solution was analyzed using a P/ACE™ MDQ Capillary Electrophoresis System (Beckman Coulter Inc., Brea, CA, USA) equipped with a 75 µ I.D., 60.2-cm-long fused-silica capillary and using an 800-µm aperture in the cartridge. The applied voltage was 30 kV with the cartridge, and the sample temperature was 25 °C.

2.5. Analysis of the amino acid contents

The amino acid extraction was performed according to the following procedure: 50 mg of frozen fruit powder was mixed with 500 µL of 8% TCA solution and centrifuged at 13000 rpm at 4 °C for 20 minutes. Next, 300 µL of supernatant was mixed with 400 µL of diethyl ether and centrifuged at 12000 rpm at 4 °C for 10 minutes. The upper aqueous layer was removed, and this step was repeated twice. The sample was kept at room temperature for 60 minutes with the cap open to completely remove the diethyl ether completely and dried under reduced pressure at 60 °C for 120 minutes. Then, 300 µL of MilliQ water was added to the dried sample, which was then dried again and repeated twice. Next, 300 µL of 0.01 N HCl was added, and the solution was transferred to an Ultrafree® centrifugal filter, i.e., Dupapore®-PVD 0.22 µm and centrifuged at 6x g at 4 °C for 120 minutes. The amino acid analyses were then performed by HPLC as described by Koike et al. (2013).

2.6. Statistical analysis

A completely randomized design was used for this experiment. The normalities of the distributions of the data were analyzed using the Kolmogorov-Smirnov test. Based on the results of this test, we found that the data were normally distributed. The data are represented as the mean values ± SEs of four replicates. For the statistical data analysis, data were subjected to Student's t-tests at $p<0.05$ for comparisons between the investigated data and the control.

3. Results

3.1. The rate of water loss in the *Sletr1-2* F1 hybrid lines was slower than that in the WT-MT F1 hybrid lines

Fruit wrinkling is the first symptom of the loss of postharvest fruit quality. Our previous study demonstrated that the *Sletr1-2* mutation significantly reduced fruit wilting in all of *Sletr1-2* F1 hybrid lines compared with the WT-MT F1 hybrid lines. At 30 days of storage, the controls exhibited wrinkling in the fruit surface, which indicated a loss in fruit quality. However, the *Sletr1-2* F1 fruit were still fresh (Mubarok et al., 2015). In this study, we measured the rate of fruit water loss over 40 days of postharvest storage. The statistical data analyses revealed that the rate of water loss of fruit from all the *Sletr1-2* F1 hybrid lines was significantly slower than that of the WT-MT F1 hybrid lines at 30 and 40 DPH (Fig. 1).

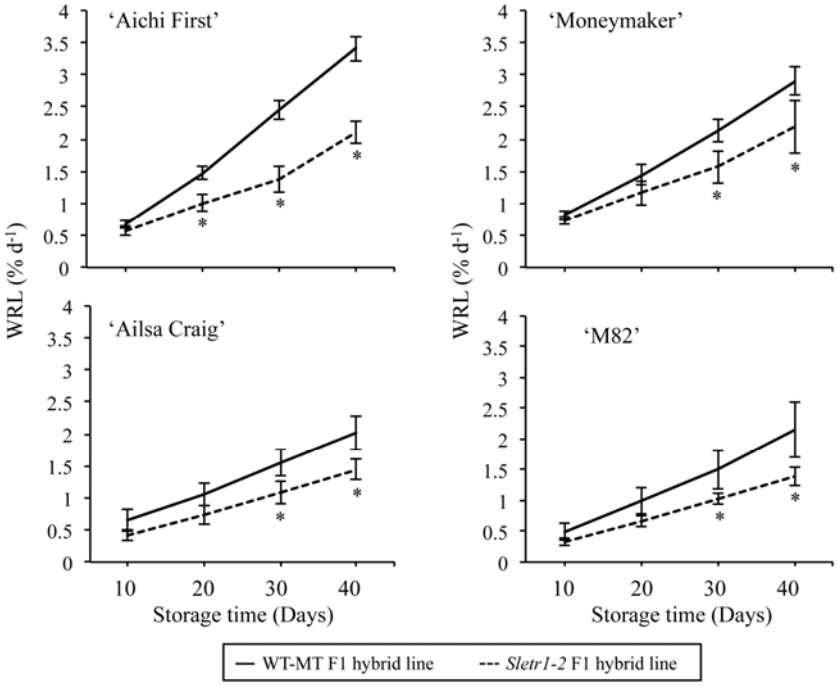


Fig. 1. Fruit water loss (%) in the *Sletr1-2* F1 hybrid lines from four commercial pure-line cultivars ('Aichi First', 'Ailsa Craig', 'Moneymaker', and 'M82') over 40 days of storage. The mean values ± SEs (4 replicates) at the same time points of storage

followed by asterisks are significantly different from the control (WT-MT F1 line) based on Student's t-tests at $p<0.05$.

3.2. The *Sletr1-2* mutation did not affect the postharvest quality changes in sugar contents in *Sletr1-2* F1 hybrid lines

We evaluated the influences of the *Sletr1-2* mutation on the contents of three major sugars (i.e., sucrose, fructose and glucose) in the tomato fruit. The total sugars were analyzed as the total amounts of sucrose, glucose, and fructose. Based on the statistical analysis of the data over 30 days of postharvest storage, no changes in total sugar contents were detected in the *Sletr1-2* F1 hybrid line fruits, with the exception of the *Sletr1-2* F1 hybrid line from 'Aichi First', in which the total sugar was significantly increased to 28.2% greater than that of the WT-MT F1 hybrid line at 30 DPH (8.90 mg g^{-1} DW) (Fig. 2A). During the 30 days of postharvest storage, the trends in the total sugar levels were stable, but they were dependent on the commercial pure-line cultivar parent background. The lowest total sugar content was obtained from *Sletr1-2* crossed with 'M82' (5.59 and 4.72 mg g^{-1} DW at 0 and 30 DPH, respectively), and the highest level was obtained from *Sletr1-2* crossed with 'Ailsa Craig' (10.07 and 10.28 mg g^{-1} DW at 0 and 30 DPH, respectively) (Fig. 2A).

In both of the F1 hybrid lines of *Sletr1-2* and WT-MT, fructose was the sugar present at the highest level, followed by glucose and sucrose. Fructose contributed 53, 52, 53, and 56% to the total sugars, and glucose contributed 41, 43, 41, and 36% of the total sugars in the *Sletr1-2* crossed with 'Aichi First', 'Ailsa Craig', 'Moneymaker', and 'M82', respectively (Fig. 2). Because nearly 83% of the total

sugar was contributed by glucose and fructose, these levels significantly affected the levels of total sugar.

The *Sletr1-2* mutation did not significantly affect the postharvest quality changes in the metabolic levels of sucrose, fructose and glucose, even when the *Sletr1-2* mutant was crossed with different pure-line cultivar parents (Fig. 2). These levels were comparable to those of the WT-MT F1 hybrid line, with the exception of the *Sletr1-2* F1 hybrid line from the 'Aichi First' at 30 DPH, which exhibited significantly higher levels of glucose and fructose with average values of 3.57 and 4.49 mg g^{-1} DW, respectively (Fig. 2C and 2D). The sucrose levels were comparable with those of the WT-MT F1 hybrid lines in all time points of postharvest storage. The range of the sucrose levels was between 0.54 (*Sletr1-2* crossed with 'M82') and 0.74 mg g^{-1} DW (*Sletr1-2* crossed with 'Ailsa Craig') at 0 DPH and from 0.36 (*Sletr1-2* crossed with 'M82') to 0.48 mg g^{-1} DW (*Sletr1-2* crossed with 'Ailsa Craig) at 30 DPH (Fig. 2B).

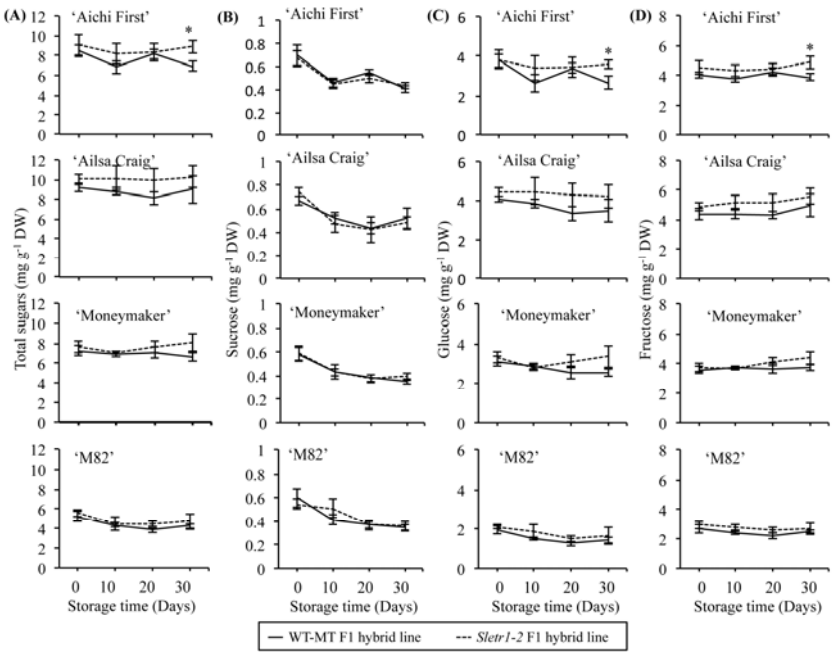


Fig. 2. Total sugar (A), sucrose (B), glucose (C) and fructose (D) content in fruit of

215 the *Sletr1-2* F1 hybrid lines from four commercial pure-line cultivars ('Aichi First',
216 'Ailsa Craig', 'Moneymaker', and 'M82') over 30 days of storage. The mean values ±
217 SEs (4 replicates) at the same time points of storage followed by asterisks are
218 significantly different from the control (WT-MT F1 line) based on Student's t-tests at
219 $p<0.05$.

220 Unlike sucrose, which exhibited a reduction over 30 days of postharvest
221 storage, the levels of glucose and fructose in all of the investigated fruit were stable.
222 The *Sletr1-2* mutation did not affect the changes in the glucose or fructose levels
223 during the postharvest storage; however, data revealed that the pure-line cultivar
224 parent background affected the changes in these levels in both the *Sletr1-2* F1 hybrid
225 lines and the WT-MT F1 hybrid lines. The *Sletr1-2* crossed with 'M82' resulted in the
226 lowest glucose (2.11 and 1.68 mg g^{-1} DW at 0 and 30 DPH, respectively) and fructose
227 (2.94 and 2.68 mg g^{-1} DW at 0 and 30 DPH, respectively) levels. Whereas *Sletr1-2*
228 crossed with 'Ailsa Craig' resulted in the highest levels of glucose and fructose from
229 the initial day of storage (4.44 and 4.89 mg g^{-1} DW for glucose and fructose,
230 respectively) up to 30 DPH (4.22 and 5.57 mg g^{-1} DW for glucose and fructose,
231 respectively) (Fig. 2C and 2D).

232 **3.3. The *Sletr1-2* mutation significantly affected the postharvest fruit quality**
233 **changes in the *Sletr1-2* F1 hybrid lines by increasing the levels of organic acids.**

234 Total organic acid and citrate and malate contents were investigated to
235 evaluate the effects of the *Sletr1-2* mutation on the organic acid levels in the *Sletr1-2*
236 F1 hybrid lines during storage (Fig. 3). Data revealed that the level of organic acid

237 was affected, and we surmised that the change in the organic acid level is an ethylene-
238 dependent process. Compared to control (WT-MT F1 line), higher levels of total
239 organic content as a result of the *Sletr1-2* mutation were detected in three *Sletr1-2* F1
240 hybrid lines, i.e., 'Aichi First', 'Ailsa Craig', and 'Moneymaker'. In the *Sletr1-2* F1
241 hybrid line from 'M82', a significantly increased level of organic acid was detected
242 until 10 DPH only (Fig. 3A). Over 30 days of postharvest storage, the total organic
243 acid contents were 27.6, 29.3, 22.5, and 25.7% higher than those of the WT-MT F1
244 hybrid line for the *Sletr1-2* F1 hybrid lines from 'Aichi First', 'Ailsa Craig',
245 'Moneymaker', and 'M82', respectively (Fig. 3A). The levels of total organic acid
246 varied across the *Sletr1-2* F1 hybrid lines; *Sletr1-2* crossed with 'Ailsa Craig' resulted
247 in the highest level (2.85 and 3.10 μmol g^{-1} DW at 0 and 30 DPH, respectively),
248 whereas the lowest level was detected in the *Sletr1-2* crossed with 'M82' (1.98 and
249 1.15 μmol g^{-1} DW at 0 and 30 DPH, respectively; Fig. 3A). Crossing the *Sletr1-2*
250 mutant with different pure-line cultivar backgrounds resulted in similar tendencies
251 towards higher levels of citrate and malate compared to controls (Fig. 3B and C). This
252 study revealed that malate contributed approximately 9% of the total organic acid.
253 The influence of the *Sletr1-2* mutation in the *Sletr1-2* F1 hybrid lines on the
254 postharvest changes in malate content was dependent on the pure-line commercial
255 parent. In fact, in the *Sletr1-2* F1 hybrid lines 'Moneymaker' and 'M82', changes
256 were detected at 0 DPH, whereas in the *Sletr1-2* F1 hybrid lines from 'Aichi First'
257 and 'Ailsa Craig', 10 and 20 DPH were detected, respectively. After these time points
258 of storage time, no postharvest quality changes related to malate were detected (Fig.
259 3B). At 0 DPH, the malate contents in the *Sletr1-2* F1 hybrid lines from the 'Aichi
260 First', 'Ailsa Craig', 'Moneymaker', and 'M82' lines were 0.19, 0.20, 0.19, and 0.19
261 μmol g^{-1} DW, respectively, which were 99, 37, 42, and 91% greater, respectively, than

the values of the WT-MT F1 hybrid lines. After 30 DPH, the values were 0.22, 0.22, 0.26, and 0.23 μmol g^{-1} DW, respectively (Fig. 3B).

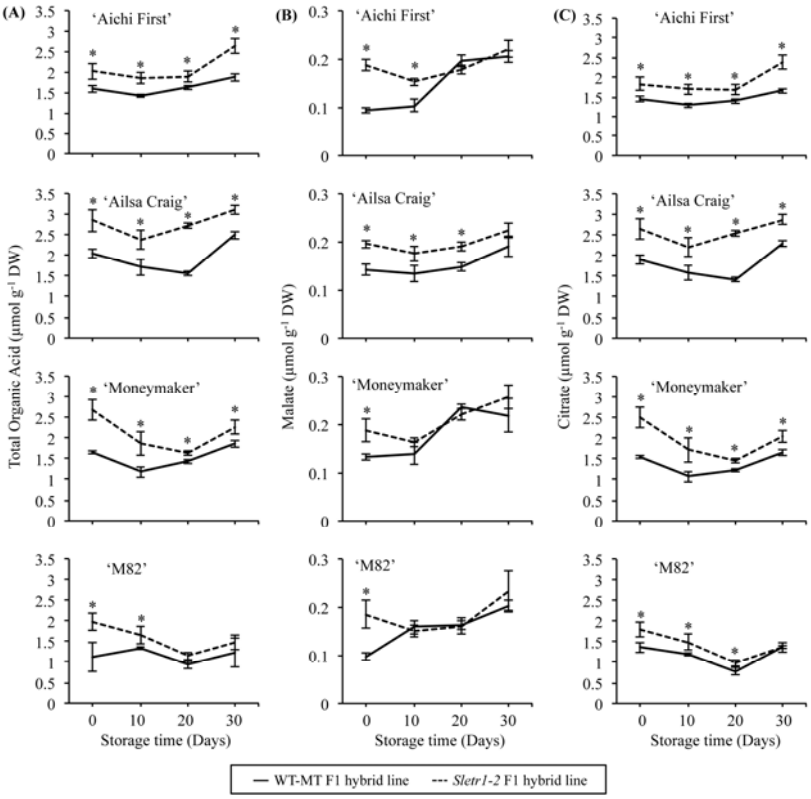


Fig. 3. Total organic acid (A) malate (B) and citrate (C) contents in fruit of the *Sletr1-2* F1 hybrid lines from four commercial pure-line cultivars ('Aichi First', 'Ailsa Craig', 'Moneymaker', and 'M82') over 30 days of storage. The mean values ± SEs (4 replicates) at the same time points of storage followed by asterisks are significantly different from the control (WT-MT F1 line) based on Student's t-tests at $p<0.05$.

Citrate has the highest contribution to the investigated fruit and constitutes approximately 92% of the total organic acids (Fig. 3C). In three *Sletr1-2* F1 hybrid lines from 'Aichi First', 'Ailsa Craig', and 'Moneymaker', significant increases in the citrate levels were detected for up to 30 days of postharvest storage, with average values of 2.50, 2.65, and 1.83 μmol g^{-1} DW at 0 DPH and 2.05, 2.88, and 2.38 μmol g^{-1} DW at 30 DPH, respectively (Fig. 3C). In the *Sletr1-2* F1 line from 'M82', the

275 increase in citrate content was detected until 10 DPH with an average value of 1.79
276 μmol g^{-1} DW, whereas after 20 DPH, the citrate level was comparable with that of the
277 WT-MT F1 line at an average value of 1.18 μmol g^{-1} DW (Fig. 3C).

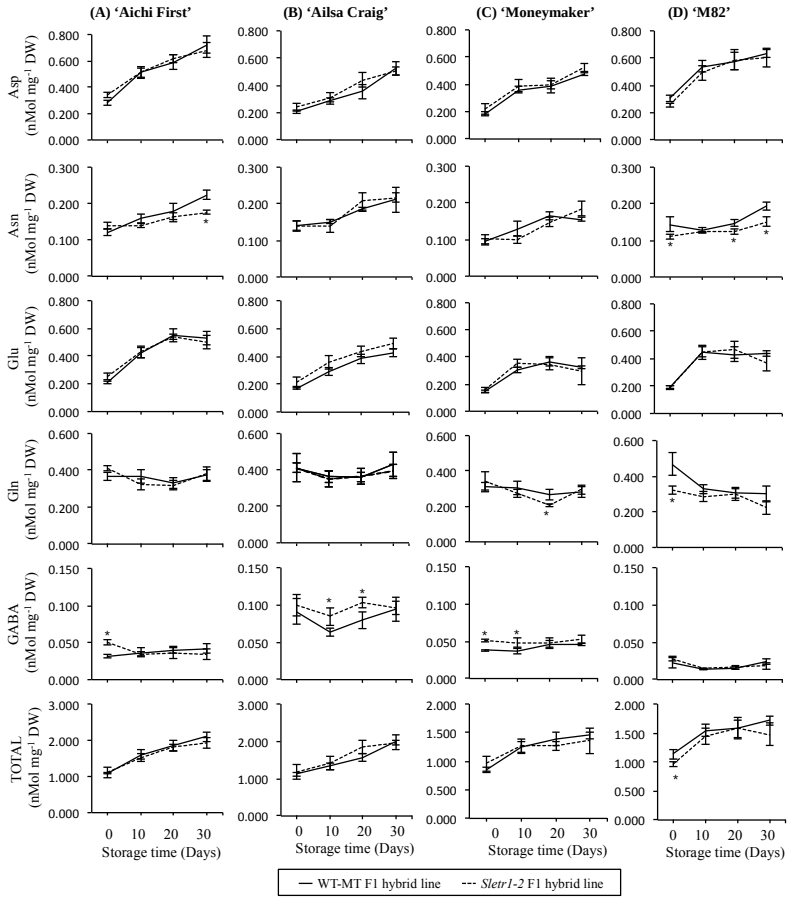
278 **3.4. The influence of the *Sletr1-2* mutation on the alteration of the amino acid**
279 **contents varied between the *Sletr1-2* F1 hybrid lines.**

280 Amino acids are another group of metabolic compounds in tomato fruit that
281 may affect the fruit taste. The total amino acids were measured via totaling the
282 amounts of 19 individual amino acids. Over 30 days of storage, the levels of total
283 amino acids in all of the investigated fruit showed a general increasing trend.
284 However, the values varied between the F1 hybrid lines: *Sletr1-2* crossed with 'Aichi
285 First' resulted in the highest level (1.59 nmol mg^{-1} DW), whereas the lowest level was
286 detected in the *Sletr1-2* F1 hybrid line from 'Moneymaker' (1.22 nmol mg^{-1} DW)
287 (Fig. 4A and C). No influence of the *Sletr1-2* mutation on the change in total amino
288 acid during 30 days of postharvest storage was detected in the *Sletr1-2* F1 hybrid
289 lines, except in the *Sletr1-2* F1 line from 'M82' at 0 DPH, which exhibited a reduction
290 in total amino acids of 14.78% lower than that of WT-MT F1 (1.13 nmol mg^{-1} DW),
291 and after 10 DPH, no difference from control was detected (Fig. 4D).

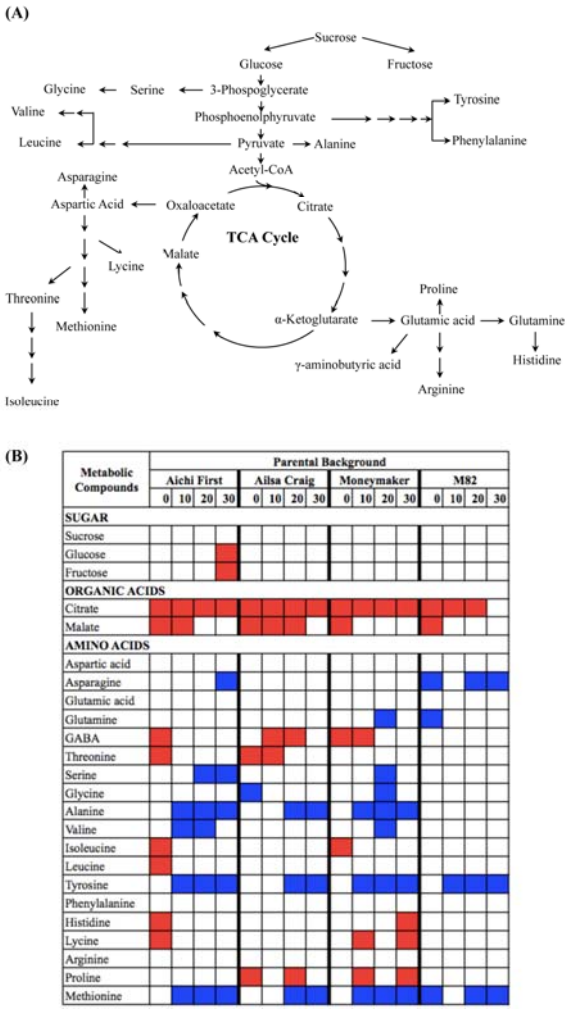
292 Although the *Sletr1-2* mutation did not directly affect the total amino acids, it
293 did significantly induced some changes in the individual amino acid levels, and the
294 influence varied depending on the commercial pure-line cultivar background and the
295 storage time (Fig. 4A-D and Supplementary Fig. S1-S4). The model of the alteration
296 of the metabolic compounds (Fig. 5) revealed that during the 30 days of postharvest
297 storage, the changes in the 19 examined amino acids were different according to the
298 parental background. In total, 12, 7, 12, and 4 individual amino acids were increased

and/or decreased by the *Sletr1-2* mutation in the *Sletr1-2* F1 hybrid lines from 'Aichi First', 'Ailsa Craig', 'Moneymaker', and 'M82', respectively. Aspartic acid, asparagine, glutamine, glutamic acid and γ-aminobutyric acid (GABA), were detected in fruit of all the investigated *Sletr1-2* F1 hybrid lines. The total contribution of these five individual amino acids over 30 DPH were 95.14, 89.96, 93.33, and 93.65% for the *Sletr1-2* F1 hybrid lines from the 'Aichi First', 'Ailsa Craig', 'Moneymaker', and 'M82', respectively. In the *Sletr1-2* F1 hybrid lines from 'Aichi First', 'Moneymaker', and 'M82', aspartic acid was the greatest component and contributed for more than 30% of the total amino acid content, followed by glutamic acid, glutamine, asparagine and GABA (Fig. 4).

In all parental backgrounds during the 30 days of postharvest storage, the *Sletr1-2* mutation did not change the levels of aspartic acid and glutamic acid. However, this mutation changed the levels of asparagine, glutamine and GABA. The statistical data analyses revealed significant changes in terms of reductions in the amounts of asparagine in the *Sletr1-2* F1 hybrid lines from 'Aichi First' (0.176 nmol mg^{-1} DW at 30 DPH) and 'M82' (0.11, 0.12, and 0.15 nmol mg^{-1} DW at 0, 20, and 30 DPH, respectively), whereas changes in glutamine were detected in the *Sletr1-2* F1 hybrid lines from 'Moneymaker' (0.21 nmol mg^{-1} DW at 20 DPH) and ''M82' (0.32 nmol mg^{-1} DW at 0 DPH). In contrast, the *Sletr1-2* mutation significantly increased the amounts of GABA in the *Sletr1-2* F1 hybrid lines from 'Aichi First' (0.05 nmol mg^{-1} DW at 0 DPH), 'Ailsa Craig' (0.08 and 0.104 nmol mg^{-1} DW at 10 and 20 DPH, respectively), 'Moneymaker' (0.05 and 0.05 nmol mg^{-1} DW at 0 and 10 DPH, respectively) (Fig. 4A-D).



322 **Fig. 4.** Levels of five individual amino acids (Asn, Asp, Glu, Gln, and GABA) and
323 total amino acids in fruit of the *Sletr1-2* F1 hybrid lines from four pure-line cultivars,
324 i.e., (A) 'Aichi First', (B) 'Ailsa Craig', (C) 'Moneymaker', and (D) 'M82', over 30
325 days of storage. The mean values ± SEs (4 replicates) at the same time points of
326 storage followed by asterisks are significantly different from the control (WT-MT F1
327 line) based on Student's t-tests at $p<0.05$.



328 **Fig. 5.** A model of the metabolic pathways of sugars, organic acids and amino acids
329 (A). The model of the alterations of the metabolic compounds in fruit of the *Sletr1-2*
330 F1 hybrid lines in four pure-line commercial cultivar backgrounds (B). The changes
331 in these metabolic compounds are illustrated in the *Sletr1-2* F1 hybrid lines from four
332 commercial pure-line cultivar parents, i.e., 'Aichi First', 'Ailsa Craig',
333 'Moneymaker', and 'M82', at 0, 10, 20, and 30 DPH. The red boxes indicate
334 significantly increased metabolic levels, and the blue boxes indicate significantly
335 decreased metabolic levels compared with the control (WT-MT F1 hybrid line).

336 Other minor individual amino acids were also altered by the *Sletr1-2* mutation.
337 The statistical data analysis revealed that significant reductions of the levels of

methionine and tyrosine were detected in all of the *Sletr1-2* F1 hybrid lines from the four parental backgrounds; however, the changes in the levels of the other amino acids varied according to the parental background and storage time point. The *Sletr1-2* mutation decreased the levels of serine, glycine, alanine, and valine, whereas this mutation increased the levels of threonine, isoleucine, leucine, histidine, lycine and proline (Supplementary Fig. S1-S4).

4. Discussion

Several physiological and metabolic processes, such as respiration and the synthesis of pigments, flavors and phenolic compounds, change continuously after the harvesting of fruits. During postharvest storage, fruits have no capability to compensate for water loss due to transpiration, which accelerates the wilting and softening processes. Therefore, water loss during storage determines the reduction of the fruit shelf life of the tomato. In other fruits, such as citrus fruits, bell peppers and eggplants, it has been reported that fruit softening and reduced shelf life result from excessive fruit water loss (Ben-Yehoshua et al., 1983; Lownds et al., 1994). Nakano et al. (2003) reported that water loss is involved in the initiation of ethylene biosynthesis in detached young persimmon. Mutations in the ethylene receptor gene *Sletr1-2* significantly prevented water loss in the *Sletr1-2* F1 hybrid line, and the effect was similar among different parental backgrounds (Fig. 1). Low rates of water loss in the *Sletr1-2* F1 hybrid lines indicated that the fruit could maintain the water content and reduce excessive transpiration, which led to slow wilting and softening. Based on this study, we believe that the prolonged fruit shelf life in the *Sletr1-2* F1 hybrid lines is correlated with the low rate of water loss during storage.

Ethylene plays an important role in modulating many processes during plant growth and development, including the ripening process of climacteric fruit and organ senescence. One of the roles of ethylene in the ripening process is the regulation of the changes in metabolites in the fruit that contribute to the alteration of the postharvest fruit quality, such as flavor. Flores et al. (2001) stated that some of the changes in metabolites are ethylene-dependent processes, whereas others are ethylene-independent processes. Fruit flavor is a complex parameter that represents the combination of taste (i.e., the balance between sweetness and sourness or acidity, and low or no astringency) and aroma sensations (i.e., the concentrations of odor-active volatile compounds) (Yilmaz, 2001; Kader, 2008). Both non-volatile and volatile compounds are important for determining overall fruit flavor quality. This study focused on the contribution of the *Sletr1-2* mutation to the non-volatile compounds of the tomato that are related to organoleptic quality. However, because volatile compounds are also important to tomato fruit flavor, we need to measure volatile compounds in future studies.

376 Taste is one of the most important non-visual tomato qualities, and sugars,
377 organic acids, and amino acids are the non-volatile constituents of tomato flavor with
378 the greatest contributions to the postharvest quality of the tomato fruit (Grierson and
379 Fray, 1994; Yilmaz, 2001). This study revealed that the *Sletr1-2* mutation had
380 favorable influences on the postharvest quality of *Sletr1-2* F1 hybrid fruit. Over 30
381 days of postharvest storage, the *Sletr1-2* mutation significantly affected the level of
382 organic acid, although it did not affect the levels of total sugar or the levels of
383 individual sugars. Based on these results, we surmise that fruit with high organic acid
384 contents and similar sugar contents result in good postharvest fruit quality with
385 favorable mouth feel and taste.

386 Kader (2008) stated that fruits containing high levels of sugar and acid are
387 preferred by consumers. Therefore, increased organic acid levels favorably influence
388 consumer preference. In contrast, reduced sugar content negatively influences
389 consumer preference due to reduced postharvest fruit quality and results in reduced
390 consumer preference due to a reduction in the sweetness of the fruit. In this study,
391 over 30 days of fruit storage, the *Sletr1-2* mutation did not alter the total sugar
392 concentration or the concentrations of the individual sugars sucrose, glucose, or
393 fructose in the *Sletr1-2* F1 hybrid lines that were crossed with different commercial
394 pure-line cultivar parents. These sugar contents were similar to those of the WT-MT
395 F1 hybrid lines (Fig. 2). These results contrast with reports that have indicated that
396 other ethylene-ripening mutants exhibit unfavorable results due to reductions in sugar
397 content. Hobson (1980) demonstrated that the *Nr* mutants with a mutation in the
398 *SlETR3* gene result in lower sugar content. Another study by Osorio et al. (2011)
399 demonstrated that reductions in the levels of sucrose, glucose and fructose are also
400 present in *Nr, nor,* and *rin* mutants. The *Sletr1-2* mutation did not result in prominent

401 reductions in the sugar contents of its hybrid lines during postharvest storage even
402 when crossed with different commercial pure-line cultivar backgrounds. These results
403 contrast with those for the *Nr, nor,* and *rin* mutants, which exhibit undesirable effects
404 due to reduced sugar levels (Osorio et al., 2011). Therefore, based on these studies,
405 we concluded that the *Sletr1-2* mutation is favorable for maintaining the postharvest
406 fruit quality of tomato fruits without reducing the sugar content that affects fruit
407 sweetness.

408 Organic acids are additional metabolic compounds that affect postharvest
409 tomato fruit quality. Several factors affect the levels of organic acids in tomato fruit,
410 and ethylene is one of the influencing factors. Oms-Oliu et al. (2011) stated that the
411 metabolisms of citrate and malate are subjected to ethylene regulation. The mutation
412 in the ethylene receptor gene (*Sletr1-2*) significantly improved fruit acidity by
413 inhibiting the degradations of the organic acids malate and citrate in the TCA cycle.
414 Our study corroborates another finding from a study of transgenic antisense *LeACS2*
415 with suppressed ethylene biosynthesis; the transgenic fruit exhibited higher levels of
416 citrate and malate, the contents of which are returned to normal levels following
417 treatment with ethylene. These findings demonstrate that the levels of citrate and
418 malate are subject to ethylene regulation (Gao et al., 2007). Increased organic acid
419 levels have also been found in a study of the *Nr* mutant, but the opposite result has
420 been found in the *nor* mutant (Osorio et al., 2011).

421 Citrate and malate are the major organic acids in ripe tomato fruits, and the
422 concentration of citrates are higher than those of malate (Yilmaz, 2001; Kader, 2008;
423 Oms-Oliu et al., 2011). Oms-Oliu et al. (2011) stated that increases in the catabolic
424 activity of the malic enzyme, which catalyzes the decarboxylation of L-malic acid to
425 pyruvate, and the continued activities of malate dehydrogenase and citrate synthase

426 result in a decline in malate and the accumulation of citrate. In the present study,
427 malate was present in smaller quantities than citrate (Fig. 3), therefore the sour taste
428 of the tomato fruit might be mainly related to the citrate content. Organic acids
429 interact with sugar to determine the quality of fruit taste. Many studies have reported
430 that high sugar and moderate to high organic acid contents are characteristics of better
431 tomato tastes and are preferred by consumers (Grierson and Fray, 1994; Kader, 2008).
432 Based on consumers' preferences, the *Sletr1-2* mutation had favorable influences on
433 the postharvest quality changes that should result in better taste quality compared with
434 the WT-MT F1 fruit because the *Sletr1-2* F1 fruit exhibited similar sugar content and
435 higher levels of organic acid compared with the WT-MT F1 hybrid lines (Figs. 2 and
436 3). All of the *Sletr1-2* F1 hybrid lines with improved fruit shelf life exhibited high
437 levels of organic acid; however, the changes varied between the *Sletr1-2* F1 hybrid
438 lines and according to postharvest storage time. Moreover, at 30 DPH the fruit of the
439 *Sletr1-2* F1 hybrid line were still fresh, whereas the control fruit exhibited skin
440 wrinkling, which indicated losses in fruit quality (Mubarok et al., 2015). A study by
441 Humayun (2014) reported that citric acid concentration is directly related to the shelf
442 life of oranges. Moreover, organic acids function as food additives and preservatives
443 that prevent food deterioration and extend the shelf life of perishable food ingredients
444 by controlling contamination from bacteria and the dissemination of foodborne
445 pathogens during the preharvest, postharvest, and food processing periods (Ricke,
446 2003). Based on these studies, we assume that the increased organic acid levels during
447 storage in the *Sletr1-2* F1 hybrid line resulted in extended fruit shelf life.
448 Another class of metabolic compounds affecting fruit taste quality is
449 represented by the amino acids. The results of our study demonstrated that the
450 influence of the *Sletr1-2* mutation on the postharvest quality changes in the levels of

451 total amino acids and individual amino acids varied between the pure-line commercial
452 cultivar backgrounds and with storage time (Fig. 4). Different behaviors in the
453 accumulation of individual amino acids were also observed in the *Nr*, *rin* and *nor*
454 mutants (Osorio et al., 2011; Osorio and Fernie, 2013). Therefore, we concluded that
455 the changes in amino acids were dependent on the genetic background. Several
456 studies have demonstrated that four predominant amino acids are present in ripened
457 tomato fruits; i.e., glutamic acid, glutamine, aspartic acid, and GABA (Kader et al.,
458 1977; Pratta et al., 2004; Oms-Oliu et al., 2011). These results corroborate those of
459 our present study, which demonstrated that these amino acids were predominant in all
460 of the investigated fruit (Figs. 4 and 5). Regarding fruit taste quality, glutamic acid
461 has a substantial contribution; it enhances taste perception and fruitiness intensity and
462 is correlated with fruit shelf life (Yilmaz, 2001; Oms-Oliu et al., 2011). Fruits with
463 lower relative glutamic acid contents exhibit long shelf life (Pratta et al., 2004).
464 Associations between long fruit shelf life and lower levels of glutamic acid has also
465 been demonstrated in the *Nr*, *rin* and *nor* mutants (Pratta et al., 2004; Osorio et al.,
466 2011). These findings contrast with those of our study, which demonstrated that
467 although the shelf life of the *Sletr1-2* F1 hybrid fruit was longer than that of the WT-
468 MT F1 fruit, there was no change in the level of glutamic acid, and the levels were
469 comparable with those of the WT-MT F1 hybrid line fruit (Figs. 4 and 5). The report
470 from Oms-Oliu et al. (2011) stated that during fruit development, the concentration of
471 glutamic acid is very low in small green, immature, and mature green fruit, but this
472 level increases during the ripening process. Based on this study, we conclude that at
473 harvest, the *Sletr1-2* F1 fruit was in a similar fruit development stage as that of the
474 WT-MT F1 fruit with some red coloration but exhibited a delayed ripening process.
475 Moreover, *Sletr1-2* can produce red fruit; therefore, the level of glutamic acid was not

476 changed and did not influence the postharvest fruit quality. Amino acids are
477 correlated with fruit taste and play roles as taste-enhancers (Nelson et al., 2002).
478 Glutamic acid and aspartic acid are known to enhance sourness, whereas alanine,
479 asparagine and glutamine contribute to a sweet taste (Kader et al., 1978). In addition
480 to sweetness and sourness levels, fruitiness is another quality that is affected by high
481 levels of sugar and low levels of glutamic acid (Bucheli et al., 1999). Because the
482 *Sletr1-2* F1 and WT-MT hybrid lines exhibited similar levels of sugar and glutamic
483 acid (Figs. 2 and 4), we hypothesize that these fruit had similar fruitiness levels.

484 **5. Conclusions**

485 The influences of the *Sletr1-2* mutation on postharvest taste quality changes in *Sletr1-*
486 *2* F1 hybrid fruit related to non-volatile compounds were nearly undetected in terms
487 of the total and individual contents of sucrose, glucose and fructose and the total
488 amino acid content. However, changes were detected in the levels of some individual
489 amino acids, and the levels of total organic acids and the individual organic acids
490 citrate and malate were increased. Moreover, the *Sletr1-2* mutation elicited a positive
491 influence on reducing fruit water loss during postharvest storage that improved the
492 fruit shelf life. We conclude that the *Sletr1-2* mutation results in a favorable influence
493 on the non-volatile compounds that effect the tomato flavor and contributes to the
494 improvement of the postharvest fruit quality of the *Sletr1-2* F1 hybrid lines.

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614 **Figure Captions**

615 **Fig. 1.** Fruit water loss (%)in the *Sletr1-2* F1 hybrid lines from four commercial pure-
616 line cultivars (‘Aichi First’, ‘Ailsa Craig’, ‘Moneymaker’, and ‘M82’) over 40 days of
617 storage. The mean values ± SEs (4 replicates) at the same time points of storage
618 followed by asterisks are significantly different from the control (WT-MT F1 line)
619 based on Student’s t-tests at $p<0.05$.

620 **Fig. 2.** Total sugars (A), sucrose (B), glucose (C) and fructose (D) content in fruit of
621 the *Sletr1-2* F1 hybrid lines from four commercial pure-line cultivars (‘Aichi First’,
622 ‘Ailsa Craig’, ‘Moneymaker’, and ‘M82’) over 30 days of storage. The mean values ±

623 SE (4 replicates) at the same time point of storage followed by asterisks are
624 significantly different from the control (WT-MT F1 line) based on Student's t-tests at
625 $p<0.05$.

626 **Fig. 3.** Total organic acids (A), malate (B) and citrate (C) contents in fruit of the
627 *Sletr1-2* F1 hybrid lines from four commercial pure-line cultivars ('Aichi First',
628 'Ailsa Craig', 'Moneymaker', and 'M82') over 30 days of storage. The mean values ±
629 SE (4 replicates) at the same time point of storage followed by asterisks are
630 significantly different from the control (WT-MT F1 line) based on Student's t-tests at
631 $p<0.05$.

632 **Fig. 4.** Levels of five individual amino acids (Asn, Asp, Glu, Gln, and GABA) and
633 total amino acids in fruit of the *Sletr1-2* F1 hybrid lines from four pure-line cultivars,
634 i.e., (A) 'Aichi First', (B) 'Ailsa Craig', (C) 'Moneymaker', and (D) 'M82', over 30
635 days of postharvest storage. The mean values ± SEs (4 replicates) at the same time
636 points of storage followed by asterisks are significantly different from the control
637 (WT-MT F1 line) based on Student's t-tests at $p<0.05$.

638 **Fig. 5.** A model of the metabolic pathways of sugars, organic acids and amino acids
639 (A). The model of the alterations of the metabolic compounds in fruit of the *Sletr1-2*
640 F1 hybrid lines in four pure line commercial cultivar backgrounds (B). The changes in
641 these metabolic compounds are illustrated in the *Sletr1-2* F1 hybrid lines from four
642 commercial pure-line cultivar parents, i.e., 'Aichi First', 'Ailsa Craig',
643 'Moneymaker', and 'M82' at 0, 10, 20, and 30 DPH. The red boxes indicate
644 significantly increased metabolic levels, and the blue boxes indicate significantly

645 decreased metabolic levels compared with the control (WT-MT F1 hybrid line).

Supplementary data:

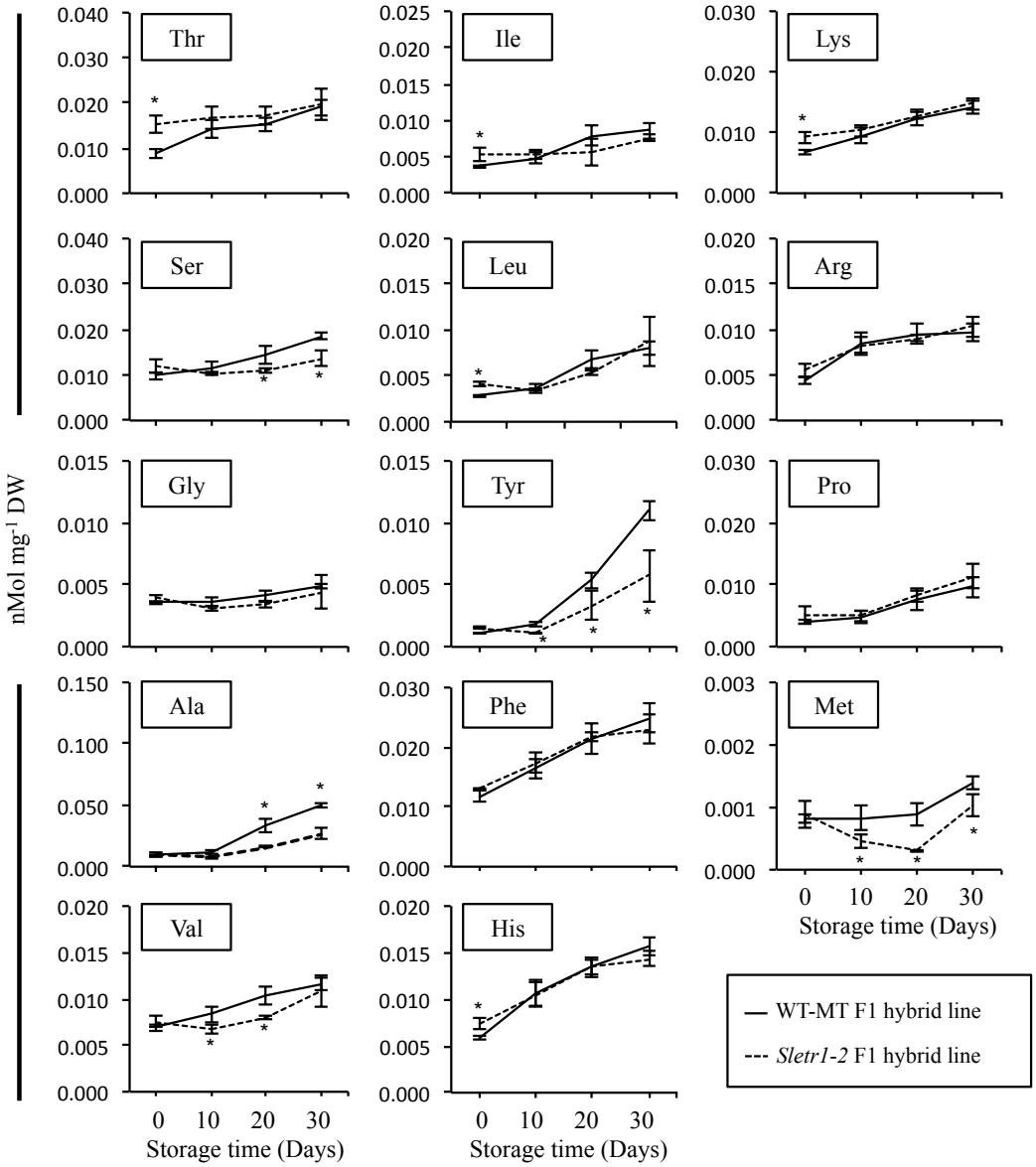

Fig. S1. Levels of other amino acids in fruit of the *Sletr1-2* F1 hybrid line from the pure-line cultivar ('Aichi First') over 30 days of storage. The mean values ± SEs (4 replicates) at the same time points of storage followed by asterisks are significantly different from the control (WT-MT F1 line) based on Student's t-tests at $p<0.05$.

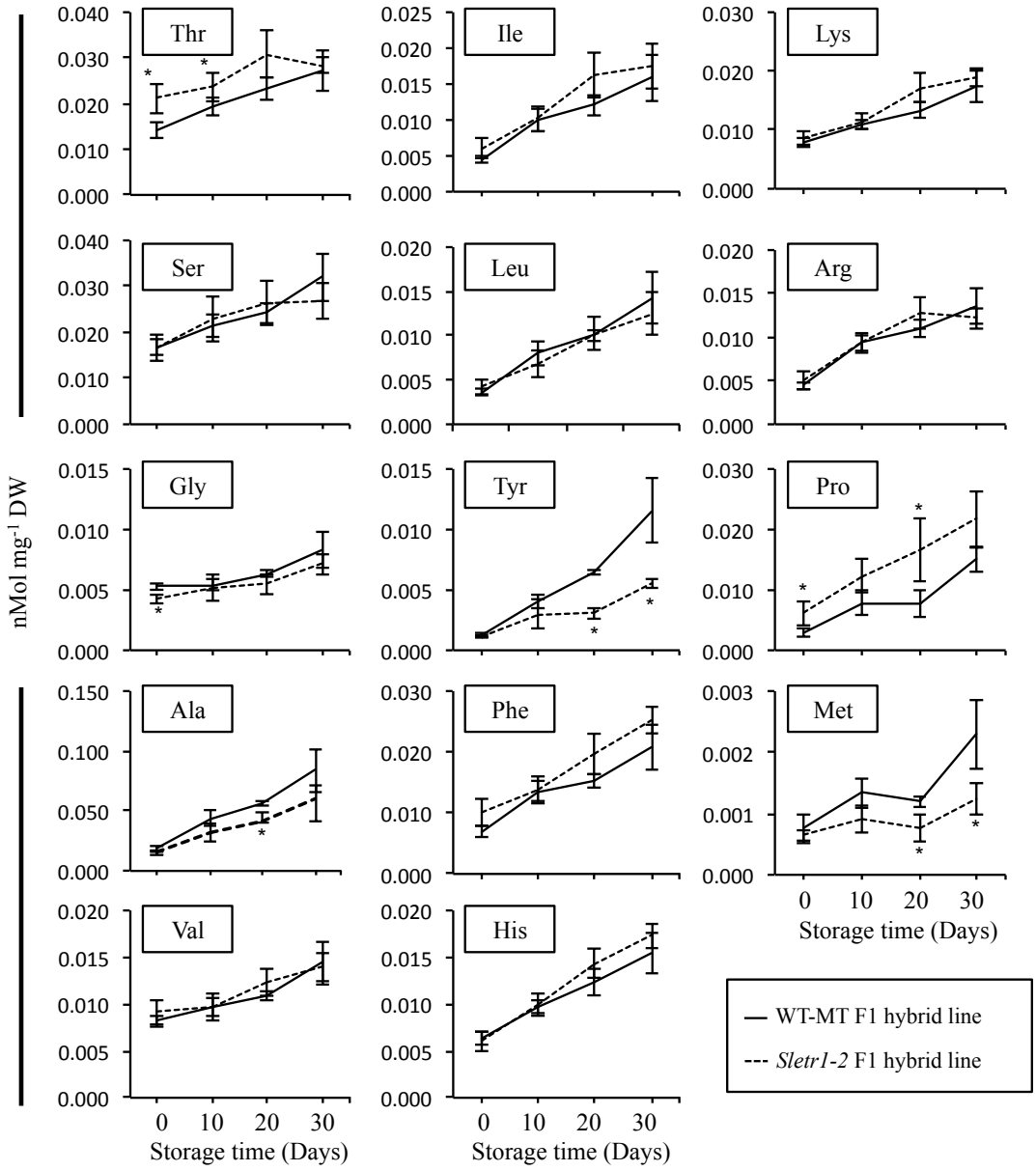

Fig. S2. Levels of other amino acids in fruit of the *Sletr1-2* F1 hybrid line from the pure-line cultivar ('Ailsa Craig') over 30 days of storage. The mean values ± SEs (4 replicates) at the same time points of storage followed by asterisks are significantly different from the control (WT-MT F1 line) based on Student's t-tests at $p<0.05$.

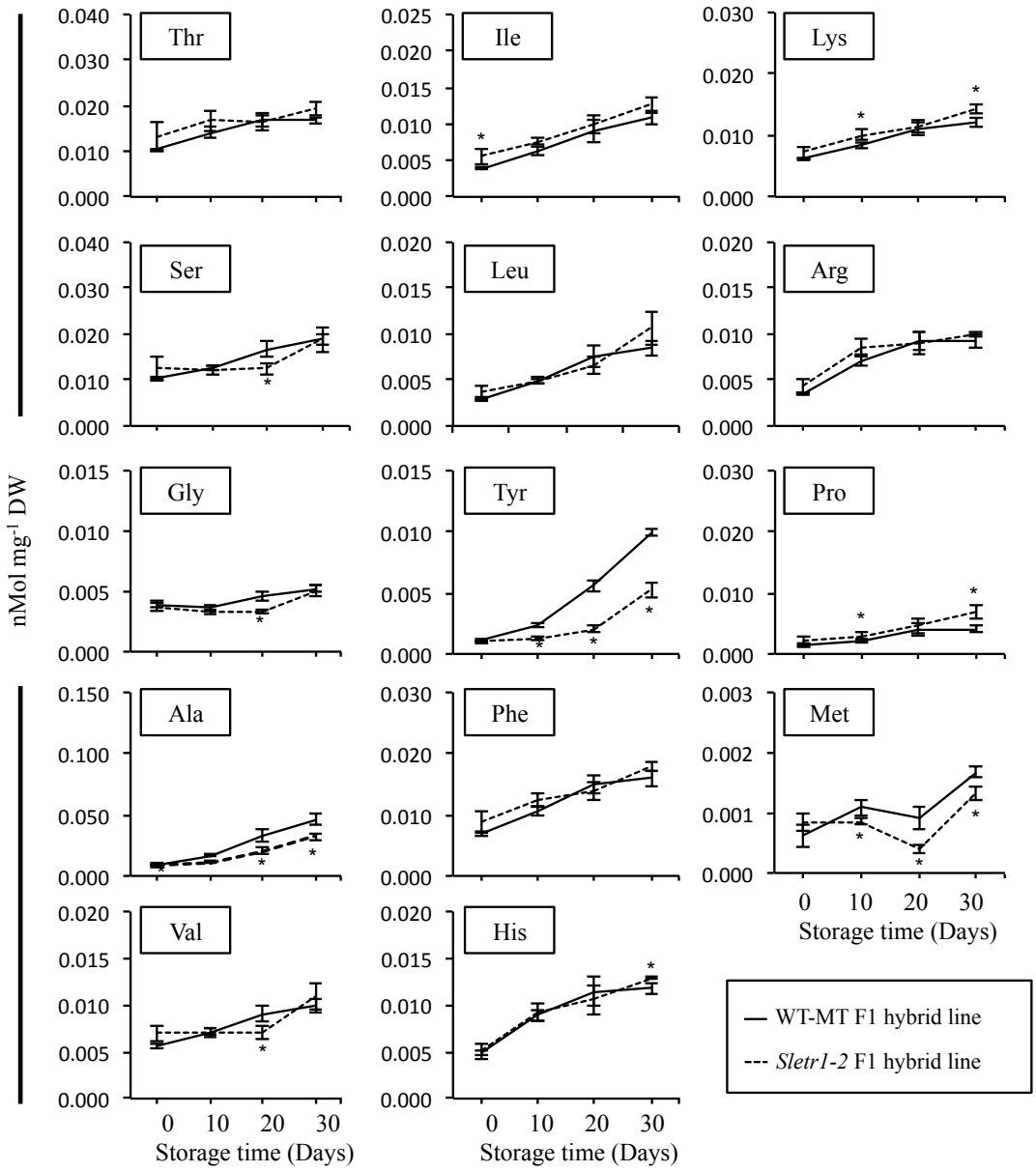

Fig. S3. Levels of other amino acids in fruit of the *Sletr1-2* F1 hybrid line from the pure-line cultivar ('Moneymaker') over 30 days of storage. The mean values ± SEs (4 replicates) at the same time points of storage followed by asterisks are significantly different from the control (WT-MT F1 line) based on Student's t-tests at $p<0.05$.

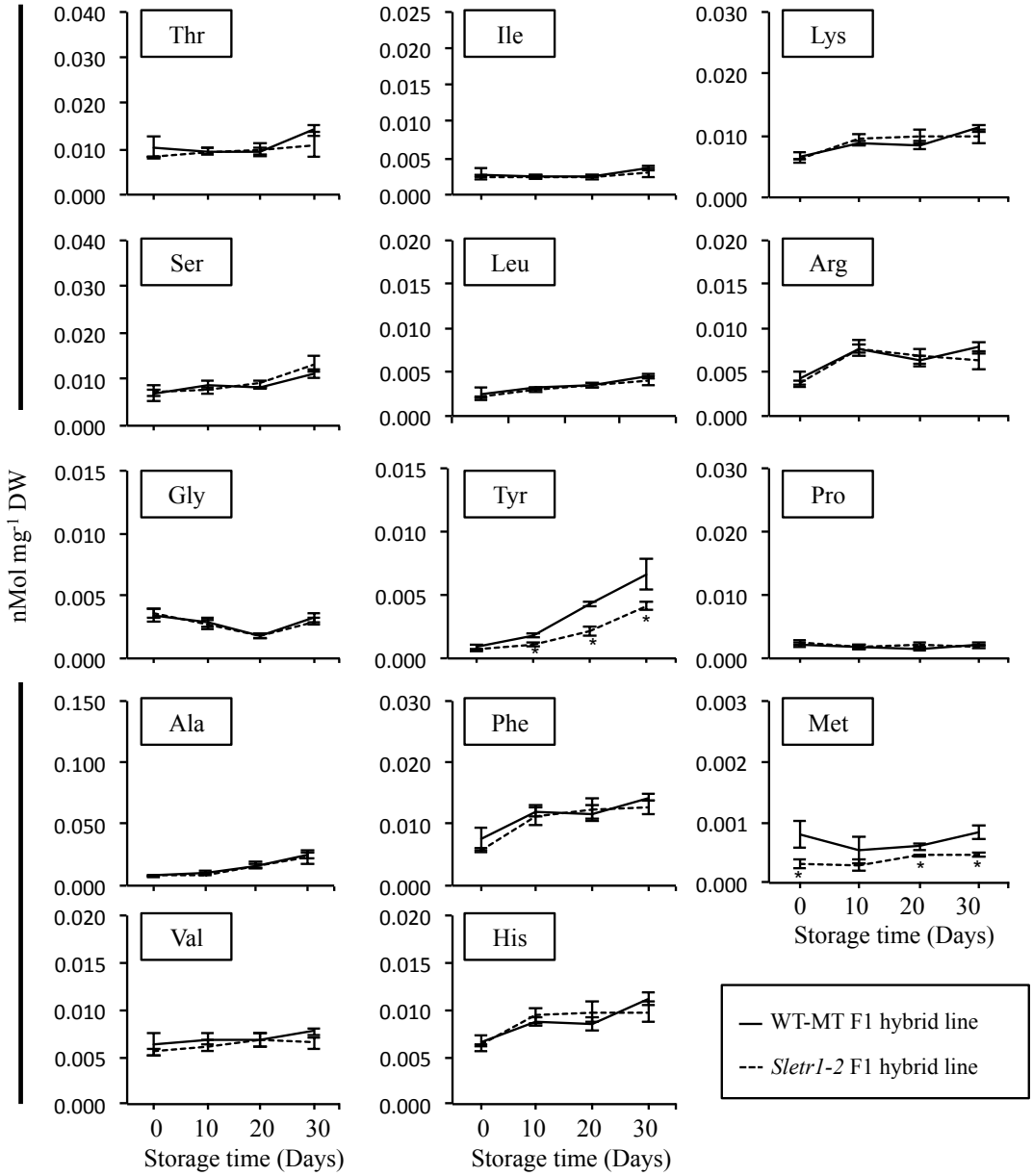

Fig. S4. Levels of other amino acids in fruit of the *Sletr1-2* F1 hybrid line from the pure-line cultivar ('M82') over 30 days of storage. The mean values ± SEs (4 replicates) at the same time points of storage followed by asterisks are significantly different from the control (WT-MT F1 line) based on Student's t-tests at $p<0.05$.