Original Article

Fruit quality of transgenic tomatoes with suppressed expression of LeETR1 and LeETR2 genes*

Bili Bao ME[1], Leqin Ke BE[1,2], Jianmei Jiang BE[1] and Tiejin Ying PhD[1]

[1]*Department of Food Science and Nutrition, Zhejiang University, Hangzhou, China, 310029*
[2]*Department of Chemistry and Life Science, Lishui College, Lishui, China, 323600*

Tomato fruit is renowned for its high concentration of phyto-nutrients such as lycopene and carotenoids, overall contribution to nutrition and human health. The effect of antisense suppression of ethylene receptor genes LeETR1 and LeETR2 over the quality of tomato fruit was investigated in this paper. During the different stages of ripening, the fruit of antisense transgenic tomatoes of *ale1* and *ale2*, compared to their wild type B1, showed higher total soluble solids, acidity and electrolytes accumulations and color development; lower fruit firmness, fruit viscosity and fruit elasticity. However, no significant difference of Vc content, total sugar, fruit pH value and fruit pigments between transgenic lines and B1 were noticed. *ale1* and *ale2* showed shortened shelf life. The data suggest that fruit with suppressed LeETR1 and LeETR2 genes expression have stronger ethylene response, which accelerate fruit ripening and greatly altered tomato variety characteristics.

Key Words: transgenic tomato, LeETR1, LeETR2, fruit quality

Introduction

Tomato is one of the most widely consumed vegetable crops in the world, not only because of its volume, but also because of its overall contribution to nutrition and its important role in human health. The nutritional components of this major crop is of particular concern to researchers and producers through out the world. In recent years, gene modification techniques have been introduced into tomato crop improvement, which greatly altered tomato variety characteristics. There have been some reports on the evaluation of the quality of transgenic tomatoes.[1-4]

The attributes of fruit quality include not only the flavor, color, nutritional content and firmness, but also shelf life, processing qualities and resistance to pre- and postharvest pathogens. Tomato fruit has a rather short post-harvest life. A large annual loss due to spoilage makes the ripening control a great economic importance.[5] Although ripening makes fruit edible and tasty, it also initiates the gradual deterioration of fruit quality, especially in climacteric fruits such as tomato, in which the onset of ripening is considered to be initiated by endogenous ethylene. [6]

The phytohormone ethylene is a key regulator in plant growth and development. In tomato, ethylene is perceived by a family of six membrane-bound receptors: LeETR1-6.[7] Ethylene is considered to bind to the ethylene receptors and initiate the subsequent signal transduction and response. We have obtained two transgenic lines from tomato (*Lycopersicon esculentum* Mill.) cultivar B1 by its transformation with the constructs containing the antisense sequences from tomato ethylene receptor LeETR1 and LeETR2 separately under the control of an enhanced cauliflower mosaic virus 35S promoter. The transgenic lines *ale1* and *ale2* were confirmed by PCR, southern blot for NPTⅡ, GUS activity assay and PCR for the target genes.[8,9] Although morphological and physiological changes of the transgenic lines have been investigated,[10,11] there has no data on fruit quality of the two transgenic tomatoes. The aim of the present study was to investigate the quality of the two transgenic tomato lines.

Materials and methods

Seeds from transgenic lines *ale1* and *ale2* homozygous for the inserted genes were used in this study. *ale1*, *ale2* and their wile type B1 were planted in a greenhouse located in the Institute of Vegetables of Zhejiang University. Fruits at different stages of ripening (mature green (MG), breaker (BK), BK+3, BK+5, BK+7, BK+10, BK+12, BK+15) from *ale1* and *ale2* and their wild type B1 were sampled. Fruit quality parameters include fruit firmness, fruit color, fruit pigments, total soluble solids, titratable acidity, relative electrical conductivity, viscosity, elasticity, Vc content, total sugar content and pH value. Shelf life was also evaluated.

Fruit weight and shape

For measurement of fruit size and shape, a minimum of 150 fruits per genotype were investigated. Fruit shape was recorded as fruit radial diameter /axial height ratio.

Fruit firmness, viscosity and elasticity

The method given by Fan *et al*[12] was used to determine fruit firmness with a Texture Analyzer model TA-XT2i (Stable

Corresponding Author: Professor Tiejin Ying, Department of Food Science and Nutrition, Zhejiang University, 268 Kaixuan Road,Hangzhou, Zhejiang, China 310029
Tel: 86 571 8697 1162; Fax: 86 571 8603 2848
Email: tjying@hzcnc.com

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Table 1. Fruit weight, shape and shelf life of the transgenic lines *ale1*, *ale2* and their wild type B1

	Fruit weight (g) n=150	Fruit shape index n=150	Shelf life (day) n=48
ale1	228 ± 41.4[a]	1.19 ± 0.07[a]	13.5 ± 2.6[b]
ale2	190 ± 34.7[a]	1.14 ± 0.09[a]	13.9 ± 2.9[b]
B1	82.1 ± 16.2[b]	1.04 ± 0.12[b]	18.4 ± 3.2[a]

Data of fruit weight and shape are means ± S.E. of a minimum of 150 fruits per genotype. Data of shelf life are means ± S.E. of six replicates each of eight fruits per genotype.

Micro System, UK) fitted with a diameter 5 mm plunger. The force required for the plunger to press into the fruit to a depth of 4 mm was recorded, expressed in N/mm. Thirty fruits per genotype were sampled and four readings were taken from each fruit and their average value was taken as the firmness.

Fruit color

Changes in fruit color were monitored using a colorimeter of model Minolta TC-P11G. The color index was based on the Hunter's color system in which *a* is the reading on the green to red scale (pure green as −80, and pure red as 100), *b* is the blue to yellow scale (pure blue as −80; and pure yellow as 70), and *L* indicates the brightness. Thirty fruits per genotype at each ripening stage were assessed. Three readings were taken from each fruit equator area and their average value was taken as the color value.

Fruit pigments

The fruit pigments lycopene (i), carotenoids (ii) and total chlorophyll (iii) contents were analyzed. The assessment was done with a spectrophotometer according to the methods described by Tomes[13] (for lycopene), Kirk[14] (for chlorophyll) and Davis[15] (for carotenoids), with some modifications in extraction. Fruit pericarp tissue (1 g) was ground in 14 mL extraction solvent of n-hexane and acetone (3:2, V/V), and then centrifuged at 10 000 × *g* for 10 min in a BR 4i centrifuge (JOUAN, France). The supernatant was collected and the precipitate was extracted repeatedly until it became totally white. The absorbances of supernatants were determined at 502, 450, 645, and 663 nm. The concentration of each pigment was calculated from the following empirical equations, and then converted into μg/g fresh weight (FW) of fruit pericarp, (i) Lycopene concentration (μg/mL) = 3.12 × OD_{502}; (ii) Carotenoids concentration (μg/mL) = 4 × OD_{450}; (iii) Chlorophyll (a + b) concentration (μg/mL) = (20.2 × OD_{645}) + (8.2 × OD_{663}). Ten fruits per genotype at each ripening stage were assessed.

Total soluble solids

Total soluble solids were checked on the homogenized fruit juice using a RFM81 digital refractometer. Ten fruits per genotype at each ripening stage were tested.

Titratable acidity and pH

Titratable acidity was measured by titrating the tomato slurry with 0.1 N NaOH to pH 8.1. pH value was measured with a pH meter. Ten fruits per genotype at each ripening stage were assessed.

Relative electrical conductivity

The relative electrical conductivity was determined according to the method of Campos *et al*[16], with modifications. Ten gram of fresh pericarp tissues was taken from each fruit, sliced into 5 mm square strips and put into a 250 mL flask before washed by deionized water for three times. Then 100 mL deionized water was added. After gently shaken for 3 h, the electrical conductivity was measured as "*a*". The sample was then placed in an oven (90℃) for 2 h, the electrical conductivity was measured as "*b*". Relative electrical conductivity (%) =*a* ×100% / *b*. Ten fruits per genotype at each ripening stage were tested.

Vc content and total sugar content

The method given by Han YS[17] was used to determine Vc content and total sugar content. Ten fruits per genotype at each ripeness stage were tested.

Shelf life

Fruits of transgenic lines *ale1* and *ale2* and their wild type B1 were harvested at breaker ripening stage, then surface washed in large volumes of water containing 2.5 mL commercial bleach per litre, and finally, rinsed with tap water. Fruit were blotted then surface-dried and sorted. Fruits with any abnormalities or damage were discarded. Fruits were packed in random blocks in flat cardboard shipping boxes containing plastic liners holding 25 (5 × 5) fruit. The boxes were stored at 13℃ in refrigerator. Six replicates consisting of eight fruits each were used per genotype. Fruit were assessed for deterioration on a five-point scale twice per week. At packing, all fruit had a score of 0. A fruit deterioration index of 1.0 was taken as the limit of marketable quality.[1]

Statistical analyses

Data were subjected to ANOVA with the software package SAS 6.12 (GLM) PC, and significance of differences between means at the level of $p = 0.05$ was determined by the Tukey's test.

Results

Fruit weight and shape

The individual fruit of the transgenic lines *ale1* and *ale2* showed a significantly higher weight than wild type B1 ($p<0.05$), while the shape of the fruits was much flatter than B1 ($p<0.05$) (Table 1).

Fruit firmness, viscosity and elasticity

As shown in Fig 1A, the tomato firmness at green and breaker stages were much higher than the following stages, and showed a downtrend during ripening. The

Fig 1A

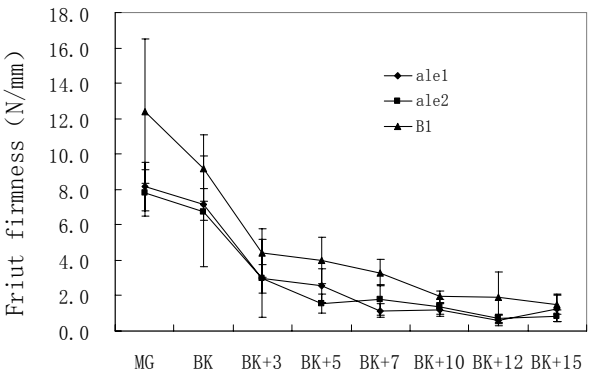

Fig 1B

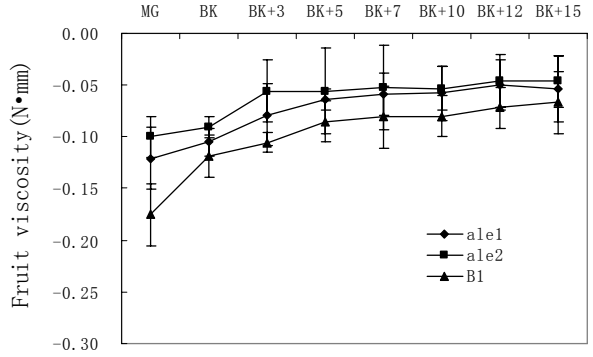

Fig 1C

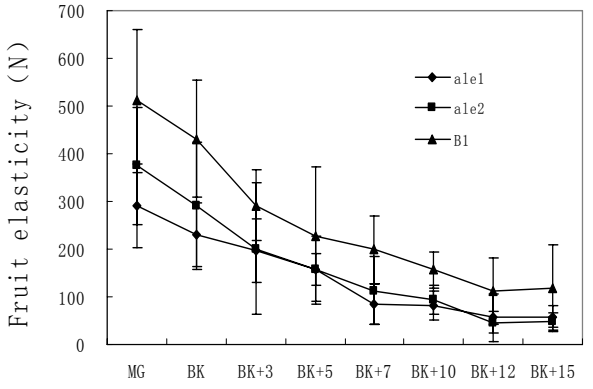

Figure 1. The firmness (1A), viscosity (1B) and elasticity (1C) of fruit during ripening. Data are means ± S.E. of four replicates each of 30 fruits per genotype.

fruit firmness of the transgenic lines was lower than B1 (p<0.05), while there was no significant difference between *ale1* and *ale2*. The fruit viscosity of the transgenic lines showed a similar trend with firmness (Fig 1B), and was significantly lower than that of B1 (p<0.05). The elasticity of transgenic lines showed a downtrend during fruit ripening (Fig 1C) and was significantly lower than B1 (p<0.05).

Fruit color

The *a* value was a good parameter for red color development and the degree of ripening in tomato. A significant

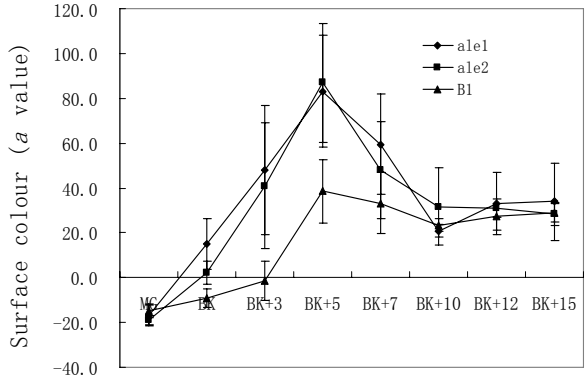

Figure 2. The color changes of fruit during ripening. Data are means ± S.E. of three replicates each of 30 fruits per genotype.

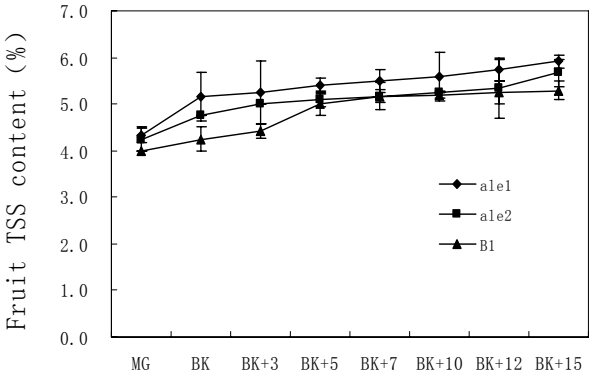

Figure 3. The total soluble solids of fruit during ripening. Data are means ± S.E. of ten fruits at each ripening stage per genotype.

increase of the *a* value was observed in the transgenic fruits, and *a* value came to its maximum at the BK+ 5 stage. The transgenic lines had a deeper red color compared with B1 (p<0.05), but after BK+10, the color of the three lines came to identical. No significant difference between *ale1* and *ale2* was observed (Fig 2).

Fruit pigments

Synthesis of lycopene and carotenoids and chlorophyll decomposition are the main reasons for color change of tomato fruit from green to red. The transgenic lines *ale1*, *ale2* and wild type B1 showed an identical change trend in fruit pigments. The content of chlorophyll, lycopene and carotenoids in transgenic lines showed no significant difference to B1 (data not shown).

***Total soluble solids** (TSS)*

The content of TSS has a very important influence in tomato flavour. The content of the total soluble solids of *ale1* was significantly higher than *ale2* and B1 (p<0.05) while there was no significant difference between *ale2* and B1 (Fig 3).

Titratable acidity

Acidity is a main factor attributes to fruit quality. The titratable acidity of all plants increased during fruit ripening, and came to maximum at the BK stage. The content of titratable acidity of transgenic lines were much higher than B1 (p<0.05) (Fig 4).

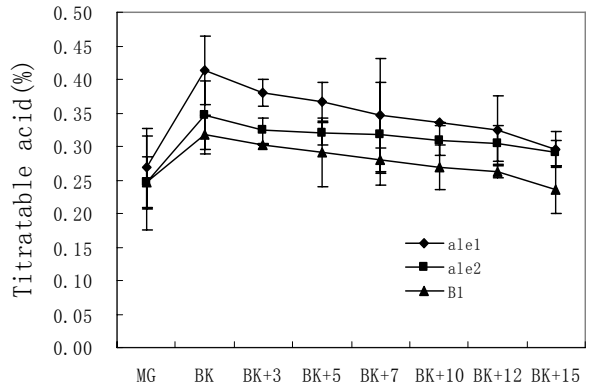

Figure 4. The titratable acid content of fruit during ripening. Data are means ± S.E. of ten fruits at each ripening stage per genotype.

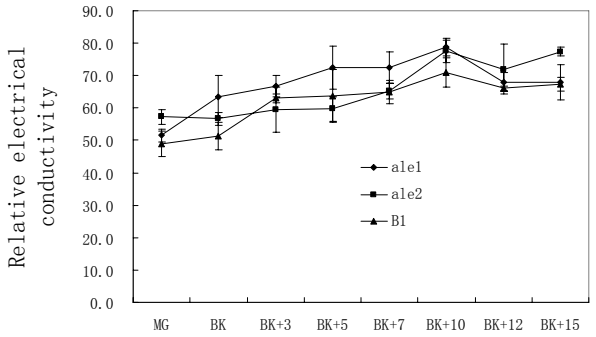

Figure 5. The relative electrical conductivity of fruit during ripening. Data are means ± S.E. of ten fruits at each ripening stage per genotype.

Tissue electrical conductivity

The relative electrical conductivity is an important index for integrity of the fruit structure. Relative electrical conductivity of transgenic lines and wild type B1 showed an uptrend during fruit ripening. Relative electrical conductivity of *ale1* was significantly higher than *ale2* and B1 (p<0.05) (Fig 5).

Vc and total sugar contents

No significant differences in contents of Vc and total sugars were observed among the fruits of different lines (data not shown).

Shelf life

As shown in Table 1, the shelf lives of the transgenic lines were shorter than that of B1 (p<0.05).

Discussion

In this study, the individual fruit weight of the transgenic lines were significantly heavier than wild type B1, while the shape of the fruits was much flatter than B1, which indicated that suppression of the LeETR1 and LeETR2 gene expression had a significant effect on fruit size. In contrast, the suppression of the expansin gene LeExp1[1] and pectin methylesterase (PME) activity[18] of transgenic lines showed no significant effect on fruit size.

The fruit firmness, elasticity and viscosity of the transgenic lines *ale1* and *ale2* were lower than their wild type B1 (p<0.05) at different stages of ripening. It is known that excessive softening is the main factor responsible for the deterioration that limits shipping, storage and marketability. Fruits with suppressed polygalacturonase (PG) accumulation were slightly firmer than controls during ripening.[19] As PG expression in fruit is positively modulated by ethylene, it is possible that the suppression of ethylene receptors resulted in increase of PG activity.

For fresh tomatoes, texture and skin color are the two quality attributes that are most important to buyers and consumers.[20] Tieman *et al*[21] reported that lines with reduced LeETR4 expression initiated earlier and faster ripening and more synthesis of lycopene. In contrast, though fruits of antisense LeETR1 and LeETR2 transgenic lines ripened more quickly, no significant increase in lycopene accumulation was observed.

It was reported that suppression of PG activity caused only a very small reduction in fruit softening in ripening, but resulted in extended fruit shelf life and increased viscosity of juice and paste prepared from these fruit.[2,3,19] Similarly, transgenic suppression of PME activity had little effect on fruit softening during ripening, but resulted in higher soluble solids content and increased viscosity in processed juice and paste.[4,22] The present study indicated that the antisense suppression of ethylene receptor LeETR1 and LeETR2 also resulted accelerated fruit ripening, shortened storage life, and significantly higher total soluble solids(except *ale2*), acidity and electrolytes.

According to the negative regulation model of ethylene receptor, the loss of function of ethylene receptor may result in stronger ethylene response, which may accelerate fruit ripening. Our results were in consistency with the negative regulation model. The nutritional implication of these changes remains to be further investigated.

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