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Changes in the physical quality parameters of the lowland transgenic tomato fruit during ripening

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Abstract: Fruit constitutes an important part of the daily diet, thus contributing to its demand in local and worldwide markets. Consumer awareness, relative to food and health, has led to a demand for foods of high nutritional quality. This has encouraged many molecular biologists to study the complexity of fruit ripening so that the plants can be genetically manipulated to enhance the edible quality of the fruits. Tomato fruit from lowland transgenic (RNAi ACO1) line 21 of which have low ethylene production and a long shelf-life of more than 22 days were assessed for nutritional quality, changes in physico-chemical characterization and health-related bioactive compounds. The transgenic fruit were compared with control fruit. The firmness of the transgenic RNAi ACO1 line-21 fruit declined steadily and reached firmness values (5.13 ± 0.12 N) at 22 d compared to the non-transgenic fruit (5.39± 0.22 N) at 10 d. Both the transgenic and non-transgenic tomato fruit developed a similar colour when fully ripe, with average hue angles of approximately 20 degrees. During ripening, the transgenic RNAi ACO1 tomato fruit exhibited 25% and 1% higher levels of lycopene and β-carotene at red ripe stage respectively, compared to the non-transgenic fruit. Furthermore, the transgenic RNAi ACO1-21 fruit only showed delayed increases in ascorbic acid content without altering its level during normal ripening. In conclusion, the results demonstrated that the antioxidant capacity may be partly responsible for prolonging transgenic tomato shelf life as well as its quality characteristics compared to the non-transgenic tomato fruit.
Keywords: Ascorbic acid; β-Carotene; Lycopene; Shelf life; Transgenic tomato fruit.

Introduction

Fruit ripening is a highly regulated and irreversible phenomenon involving a series of physiological, biochemical, and structural changes that lead to an attractive, edible, and ripe fruit[35]. The quality, i.e., the degree of excellence or superiority, of fresh fruits and their products is a combination of characteristics that give each commodity value in terms of food appearance quality, firmness, and shelf-life and are important to wholesale and retail marketers. Tomato fruit ripening is accompanied by changes in colour from green to red, softening, and increased levels of compounds that make up the essential components of a balanced, healthy diet such as ascorbic acid and carotenoids[13, 18]. During ripening, the colour change is due to the unmasking of previously present pigments by the degradation of chlorophyll coupled with the synthesis of different types of anthocyanins and the accumulation of carotenoids such as β-carotene, xanthophyll esters, xanthophylls, and lycopene. Indeed, lycopene is considered a major carotenoid in tomato that provides the red colour[32]. Ascorbate

eliminates reactive oxygen species (ROS) through multiple mechanisms[14]. The capacity of ascorbate to directly eliminate several different ROS including $^1O_2$, $O_2^{\cdot-}$ and $^{\cdot}OH$, make it an important component in protection against oxidative stress[16]. It also maintains the membrane-bound antioxidant α-tocopherol in the reduced state; indirectly eliminates H_2O_2 through the activity of APX[4]; and participates in cell metabolism, growth control[27], cell division[22], and the expansion of cell walls[39].

Fruit texture not only affects consumer acceptance but also influences transportability, disease resistance, and shelf-life. Improving the texture of fruit will encourage a healthier diet, simplify logistics in the food chain, and reduce postharvest waste[7, 40]. Softening is thought to be the result of cell wall disassembly, decreased cell adhesion[23], and the cumulative effects of reducing cellular turgor pressure[34].The changes in cell wall structure are accompanied by the solubilization of pectins and depolymerization of hemicellulosic polysaccharides. In addition to the structural matrices of the cell wall, cellular turgor contributes to texture and fruit firmness. Cellular turgor is governed by the water status within fruit and the relative water distribution within the cell and in the cell wall[33]. A gene expression study has indicated that there is a significant increase in β-galactosidase expression during tomato fruit ripening[37]. The down-regulation of β-galactosidase in tomato results in decreased fruit softening[36]. In addition to these and other enzymes, non-enzymatic factors, such as reactive oxygen species (ROS) may play a role in cell wall degradation[17].

An important horticultural crop is tomato (*Solanum lycopersicum* L.). It is a well-established model system for studying fleshy fruit development and ripening due to its relatively small genome, ease of genetic manipulation, well-characterized developmental mutants, and relatively short life cycle. Tomato belongs to the climacteric class of fruits, which show increased ethylene production at or just before the onset of ripening and require ethylene to complete the process[3]. Therefore, ethylene synthesis is critical for fruit ripening. The pathway of ethylene biosynthesis has been characterized[1], and the key enzyme is 1-aminocyclopropane-1-carboxylic acid (ACC) synthase (ACS), which regulates the production of ACC from S-adenosylmethionine (SAM)[3]. In tomato fruit, the inhibition of ethylene biosynthesis through ACC synthase or ACC oxidase delayed ripening and increased shelf life[6]. The major focus has been on the manipulation of ethylene biosynthesisusing either sense or antisense technology[8].

Until recently, there were few studies on lowland tomato varieties; thus, there is scarce information regarding this variety. The lowland *Solanum lycopersicum* cv. MT1 variety was developed by the Malaysia Agriculture Research and Development Institute (MARDI) by crossing the CL555-10 line with the local white variety. This variety bears smaller-sized fruit with shorter shelf life than the temperate varieties. In this study, we examined the effect of blocking ACC oxidase gene expression (*ACO1*) in lowland tomato during the course of ripening, on physical characteristics (colour and firmness) as well as nutritional composition (ascorbic acid and carotenoid contents)in transgenic tomato fruit. Our findings provided useful insights into the quality of the transgenic tomato fruit compared to non-transgenic tomato.

Materials and Methods

1. Materials

Tomato seeds of transgenic RNAiACO1-21(T_2) and non-transgenic used in this experiment were obtained from UKM Experimental Plots, on May- June 2011. All tomato plants were grown under the same field conditions. Fruits of T2 generation were harvested at different days of ripening. Data for samplings were represented at different days of normal ripening.

2. Physico-chemical characterisation of transgenic RNAi ACO1-21 tomato fruit

2.1. Fruit firmness

Fruit firmness was determined by measuring the amount of force (N) needed to puncture a hole through the fruit. Three measurements were performed along the equatorial region of each fruit using a Texture Analyzer Machine, TAXT Plus (Stable Micro System, England).

2.2. Skin colour

The colour of the fruit was evaluated using a reflectance meter (Minolta Chromameter, Japan) and the colour was recorded at the same location every other day as a*, L*, hue angle, and chroma value. The chroma meter was calibrated with a white standard tile. The colour was recorded as numerical values of a*, indicating a colour range from green to red, whose values are −60 to +60. The fruits were harvested at the mature green stage (MG), at a* value of approximately −17. Breaker (BR) fruit had a* value of approximately −12, and orange (OR) fruit a value of 20. The measurements were continued until the fruit reached the red ripe (RR) stage, with a value of 37[19]. The results are presented as lightness (L*), chroma (C*) and hue angle (H°).

The chroma and hue angle were calculated from the measured a* and b* values using the formulas

$C^* = (a^{*2}+b^{*2})^{1/2}$ and H° = arc tangent (b*/a*) [24].

3. Bioactive compounds of transgenic RNAi ACO1-21 tomato fruit

3.1. Lycopene and β-carotene contents

Lycopene and β-carotene contents were determined according to the method of [26]. Three grams of tomato tissue were homogenized in a cold mortar and pestle with 16 mL of an acetone: hexane mixture (4:6) for 1 min. The absorbance of the filtrate was measured at λ = 453, 505, 645 and 663 nm. The β-Carotene and Lycopene content was calculated according to[25].

3.2. Ascorbic acid content

Extraction of ascorbic acid from tomato fruit is based on a previously described method[10].Five grams of the tomato sample was homogenized with 25 mL of water (HPLC grade).Then, the sample was centrifuged at 20,500 xg for 15 minat 4°C. The resulting supernatant was filtered through Whatman paper No. l. Next, 10 mL of the sample was again filtered with a filter needle, size 0.2 µm nylon membrane with a radius of 25 mm (Sartorius). Samples resulting from the second filtration were ready for HPLC analysis. Analytical HPLC (high performance liquid chromatography) was performed for ascorbic acid using a Shimadzu HPLC system (Tokyo, Japan) with radiation detector diodes (diode detector arrays; DAD).The HPLC separation method is based on a previously described method[42].The 20 µL samples were separated at 40ºC on a Waters Symmetry C18 column (3.9 mm × 150 mm id; 5µm particle size, Milford, MA, USA) using a mobile phase of 5% acetic acid at a flow rate of 1 mL/min. The amount of ascorbic acid was calculated from the absorbance at 254 nm using ascorbic acid (20, 40, 60, 80 and 100 mg/L) as a standard. The results were expressed as milligrams of ascorbic acid per gram of fresh weight.

4. Statistical analyses

All of the experiments were conducted with six replicates in a completely randomized design. Analysis of variance (ANOVA) was performed using IBM SPSS statistics 20. Bars indicate significant difference in the means from post-hoc Tukey tests at a significance level of 0.05.

Results and Discussion

1. Physico-chemical characterization of transgenic RNAi ACO1-21 tomato fruit.

1.1. Fruit firmness

The firmness of most fruits varies according to the type of fruit and its stage of maturity, and fruit ripening is an irreversible process once initiated. Figure 1 shows the changes in firmness of transgenic RNAi ACO1-21 and non-transgenic tomato fruit, where the firmness decreased with an increase in ripening period at a slower rate in the RNAiACO1-21 tomato fruit. At the beginning of ripening (first day of harvest), all fruit were very firm (firmness from 35 N to 45 N). However, the non-transgenic fruit underwent a large reduction in firmness early and reached 5N on day 8, whereas the RNAiACO1-21 tomato fruit lost their firmness slowly, reaching 5.25 N at the end of the ripening period on day 22 (Fig. 1).There was a significant ($P \leq 0.05$)

difference in the firmness between the non-transgenic and transgenic RNAiACO1-21 tomato fruit during ripening.

In our results, the non-transgenic tomato fruit lost their textural integrity faster than the transgenic RNAiACO1-21 fruit. The maintenance of firmness in transgenic fruit may be due to the reduction in respiration and other ripening processes during ripening[8].During the ripening of most fruits, the enzymatic degradation of the cell walls results in a significant decrease in firmness. A previous study reported a significant decrease in tomato firmness likely caused by a loss of tissue integrity in the cell wall polysaccharides pectin and hemicellulose related to the role of β-galactosidase in softening[38].

The correlation between firmness and ethylene was established in a previous study [21] where apple and kiwi exhibited a similarly slower initial softening at early maturity stages. In soursop, ethylene enhanced the changes in firmness during ripening, where a significant increase was observed only at more advanced stages of ripening[29]. Investigation of the relationship between ethylene and the firmness of apples revealed that fruit obtained from plants silenced for either ACC synthase or ACC oxidase had reduced fruit softening[15].

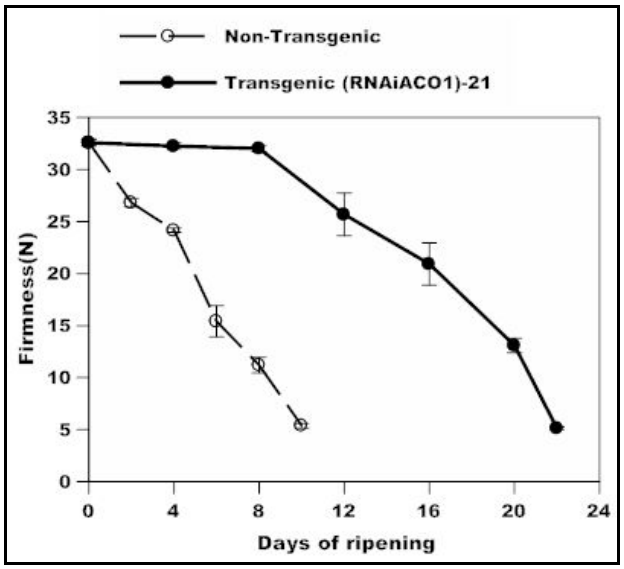

Fig.1. Changes in firmness of transgenic RNAiACO1-21 and non-transgenic tomato fruit during days of naturally ripening .Data are the mean ± S.E (n=6) Data were analysed by 2 way ANOVA.

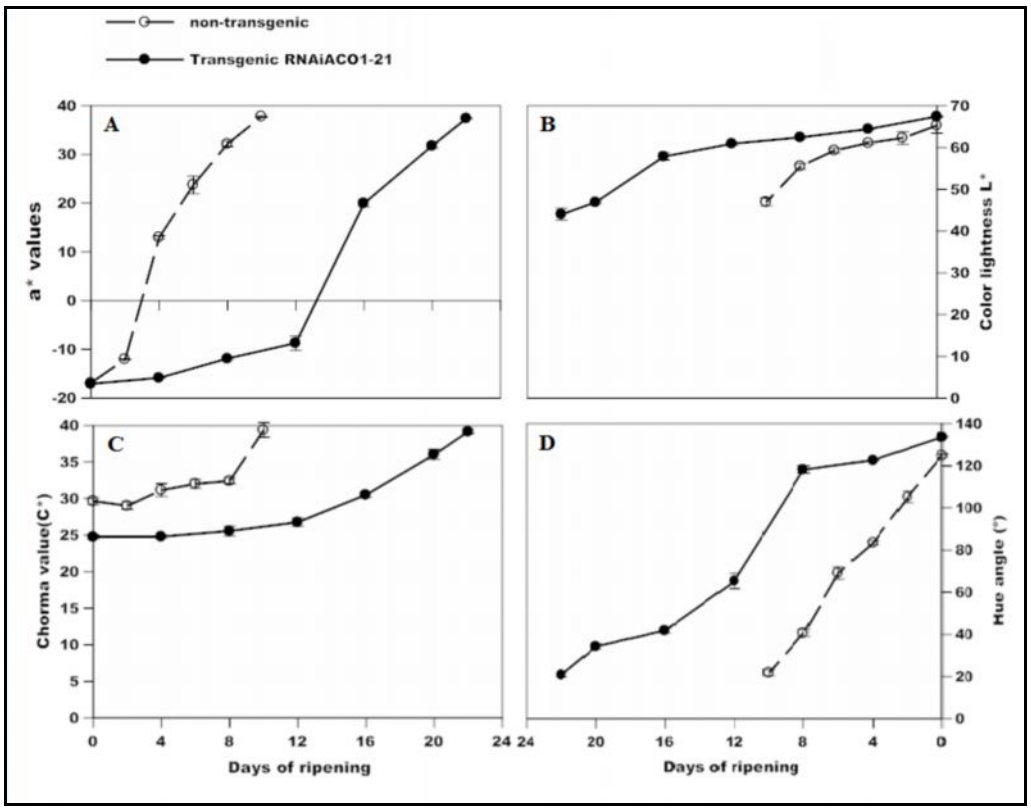

Fig.2. Changes in skin color of the transgenic RNAi ACO1-21 and non-transgenic tomato fruit during naturally ripening .a* index (A), colour lightness L*(B), chroma C* value (C) and hue angle (h°) (D). Data are the mean ± S.E (n=6). Data were analysed by 2-way ANOVA.

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1.2. Skin colour

The visual appearance of fresh fruit and vegetables is used as a quality indicator by consumers. Fig. 2 shows the values for the quality characteristics of the transgenic RNAi ACO1-21 and non-transgenic tomato fruit at different days of ripening. The colour value a* which characterised the red colouring of the fruit [5], was examined at different days of ripening fruit and presented in Fig. 2 A. As the storage of the fruit continued, the green colour began to fade more in the non-transgenic fruit, as seen by their faster increases in a* values. In these fruit, the BR stage was observed on day 2 (−12.03), the OR stage on day 6 (23.8) and the RR stage on day 10 with an a* value of 37.8. These stages occurred in 8 days, whereas with the transgenic RNAi ACO1-21, the 25% and 75% stages were delayed to day 8 and 16 of ripening respectively (Fig. 2 A). The differences observed between non-transgenic and transgenic fruit in any particular ripening stage (MG, BR, OR, and RR) were not statistically significant ($P > 0.05$).

During tomato ripening the ratio of white to black colour decreased (Fig. 2 B), and this indicates darkening of the fruit. The lowest colour lightness L* value (47.03) was recorded in the completely ripened fruits on day 22, and the highest L* value (67.45) was observed at the onset of ripening on day 1 of transgenic RNAi ACO1-21 tomato fruit, whereas the non-transgenic fruit had the lowest colour L* on day 10. Additionally, the difference in the L* values obtained for the transgenic RNAi ACO1-21and non-transgenic fruit during ripening were not statistically significant. According to a previous study[43], the loss of luminance is usually the result of normal ripening. In our study, the colour lightness (L*) decreased, indicating a darkening of the tomato red colour. This observation is consistent with a previous study[30].

The chroma results (Fig. 2 C) demonstrated that colour vividness was not significantly lower at the beginning of normal ripening for transgenic RNAi ACO1-21 fruit (24.78) than was observed in the non-transgenic fruit (29.66). Furthermore, the highest chroma value (39.40) was recorded amongst the non-transgenic tomatoes at the end of normal ripening. Chroma values obtained for both transgenic and non-transgenic tomato fruit increased during normal ripening days.

The external colour, expressed in terms of hue angle, is considered the most important measure of tomato quality because it is perceived by the human eye[30]. Consistent with a* values results, the hue angle data (tan^{-1} b/a) declined as the degree of greenness decreased and the degree of yellowing increased. For the non-transgenic tomato fruit (day 1), the initial hue angle values averaged 125° as shown in (Fig. 2 D). The hue angle of the transgenic RNAiACO1-21 fruit was highest at the most days of ripening, and it decreased from 134 degrees in green fruits to 19 degrees in fully ripened fruits. The non-transgenic fruit had the lowest hue angle value at only 125 degrees in green fruit; however, it was the highest in fully ripened fruits (22 degrees).

In this study, the development of skin colour was delayed in transgenic RNAiACO1-21 tomato fruit, and these results suggest that the activities of the enzymes involved in ripening processes are regulated by ethylene. The retardation of colour development in transgenic tomato fruit may be attributed to the low ethylene production and the delay in ethylene production reaching the threshold concentration for colour development[41].

2. Bioactive compounds of transgenic RNAi ACO1-21 tomato fruit

2.1. Lycopene and β-carotene contents

The carotenoid contents of transgenic tomato fruit were measured at different days of ripening. Based on the analyses of the lycopene levels, no significant differences were detectable between the lowland transgenic RNAi ACO1 line-21 and the non-transgenic fruit at the onset of the maturation stage. However, lycopene levels increased significantly ($p < 0.05$), reaching 1.6 fold at day 22 compared to the non-transgenic fruit at day 8 (Fig. 3). The analyses of β-carotene in tomato fruit at different days of normal ripening showed that the content increased significantly (Fig. 4). In transgenic RNAi ACO1-21 fruit, the β-carotene content increased 3.0 fold by the final days of normal ripening, whereas the β-carotene content only increased 2.2 fold in non-transgenic tomato fruit by the final days of normal ripening.

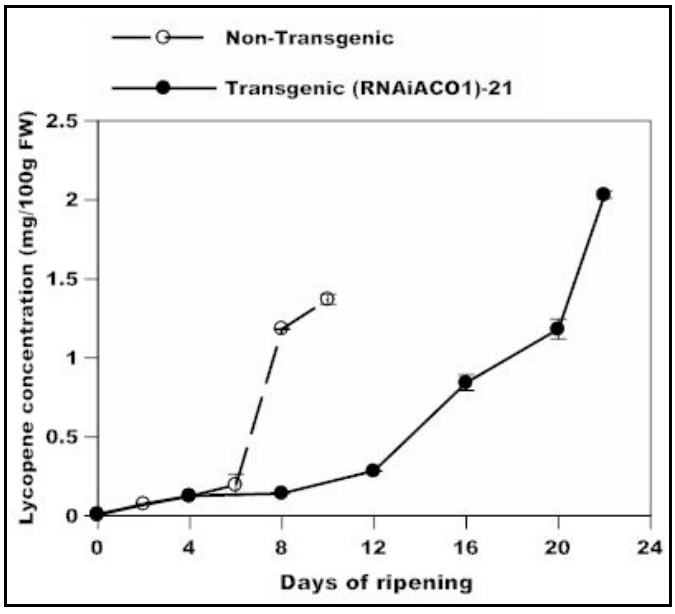

Fig.3. Changes in lycopene concentrations of the transgenic RNAiACO1-21 and non-transgenic tomato fruit during days of naturally ripening .Data are the mean ± S.E (n=6) Data were analysed by 2-way ANOVA.

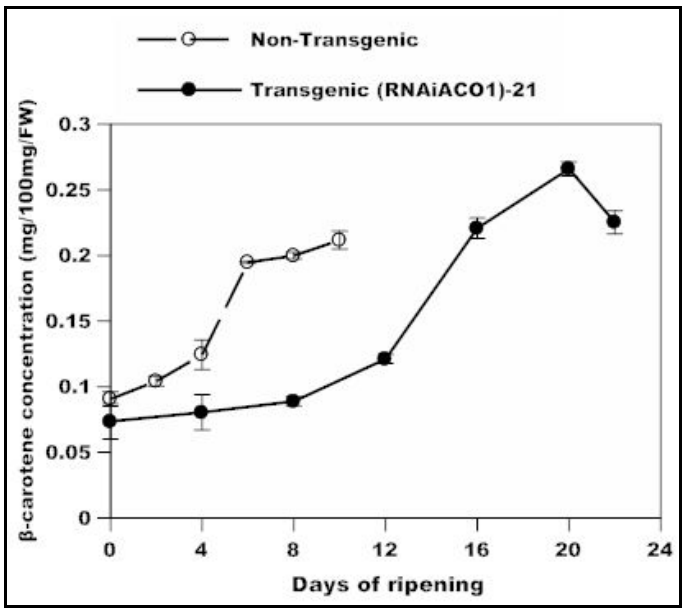

Fig.4. Changes in β-carotene concentrations of the transgenic RNAiACO1-21 and non-transgenic tomato fruit during days of naturally ripening .Data are the mean ± S.E (n=6) Data were analysed by 2-way ANOVA.

In our study, the lycopene and β-carotene concentrations increased in fully ripened fruits from the transgenic and non-transgenic tomatoes (Fig. 3 and 4), which is consistent with a previous study[31]. During tomato fruit ripening, the carotenoid concentrations change constantly, which is related to chlorophyll degradation and carotenoid synthesis, where chloroplasts are synthesized into chromoplasts[4]. In addition, an increase in lycopene content in transgenic tomatoes may be attributed to the conversion of other carotenoids such as phytoene, z-carotene, phytofluene and neurosporene into lycopene through desaturation, isomerization and cyclization[9]. Regarding the role of ethylene in the maturation of tomatoes, transcriptome / metabolite analysis indicates that ethylene is involved in several carotenoid biosynthetic processes, affecting their qualitative and quantitative deposition[2].

2.2. Ascorbic acid (AsA) content

Ascorbic acid is often used as an indicator for the nutritional quality of normally ripening fruit and vegetables. In the present study, ascorbic acid (AsA) levels changed significantly during the ripening of transgenic RNAi ACO1-21 and non-transgenic tomato fruit (Fig. 5). The ascorbic acid in the non-transgenic fruit increased to a maximum of 51 mg /g FW after day 10, whereas in transgenic RNAi ACO1-21 fruit, it took 22 days to reach a maximum of 49 mg mg/g FW. However, these differences were not statistically significant, and the transgenic RNAi ACO1-21 plants only showed delayed increases in ascorbic acid content without altering its level.

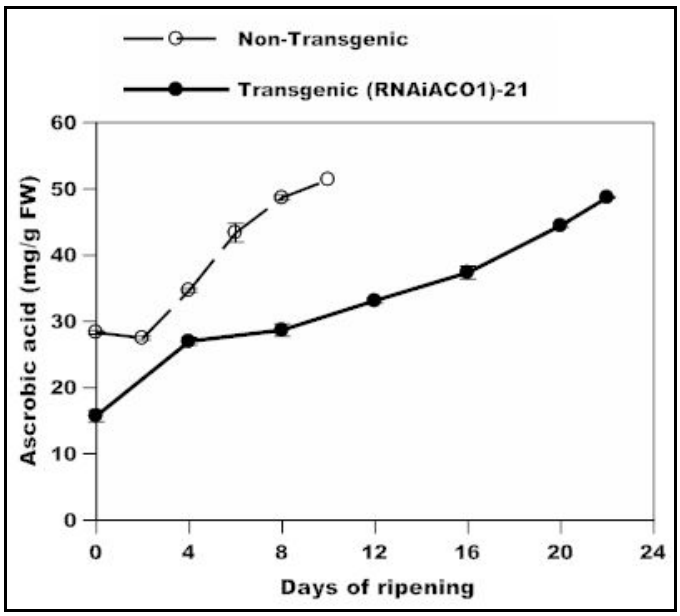

Fig.5. Changes in ascorbic acid content (AsA) of the transgenic RNAi ACO1-21 and non-transgenic tomato fruit during days of naturally ripening .Data are the mean ± S.E (n=6). Data were analysed by 2-way ANOVA.

According to these results, the ascorbic acid levels increased significantly ($P < 0.05$) during the ripening process. This large increase suggests that ascorbic acid may be actively involved in the removal of H_2O_2 in response to elevated oxidative stress[12]. This result was consistent with other reports that demonstrated an increase in ascorbate levels during the ripening of pepper fruit [28]. In addition, the delayed increase in ascorbic acid in transgenic fruit[8] may be due to the decrease in respiration and other metabolic activities, which may have also slowed the ascorbic acid synthesis. A similar finding was previously observed in tomato[20]. Additionally, 1-MCP treatment is reported to maintain the quality of minimally processed pineapple fruits, at least partially, by reducing the hydrolysis of endogenous ascorbic acid[11].

Conclusions

Consumer awareness about biotechnology and genetically modified crops has increased, yet confusion remains. Therefore, the results of this study contribute to understanding the development of transgenic food crops without compromising fruit quality. Experiments presented here investigated changes in colour and firmness, where the transgenic fruit exhibited reduced rates of firmness loss, which can be associated with a decrease in ethylene production. Moreover, the fruit from the transgenic RNAi ACO1-21 plants had a late ripening time with elevated lycopene and β-carotene levels and unaltered vitamin C concentrations, suggesting their high nutritional quality. Thus, tomato fruit from transgenic plants could effectively be harvested at commercial levels to help meet the goal of food security by ensuring that the tomato fruit from transgenic RNAi ACO1-21 plants is as safe as that from non-transgenic tomatoes. The results of this study would be useful in integrating the proper timing of harvest and handling to extend tomato fruit shelf life and nutritional quality.

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