



## **Comparative study of quality changes in Lowland Transgenic RNAiACO1(T2) Tomato Fruit during Storage at Ambient and Low Temperature**

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**Abstract :** Tomatoes (*Solanum lycopersicum* L.) are sensitive to the low temperature and easy to be subjected to chilling injury, which causes fruit ripening disorder. This study aimed to investigate the effects of the low temperature on development internal CI symptoms of the wild type and transgenic RNAi ACO1-21 tomato fruit during prolonged storage. This study focuses on the efficacy down regulating ACO1 gene tomato on its quality during the cold storage as comparison to wild type tomato fruit. Tomato fruit was firstly stored at 10° C for 28 days, followed by ripening at 28°C for 10 and 18 days in non-transgenic and lowland transgenic tomato fruit respectively. Chilling injury symptoms such as pitting was observed with more sheet pitting and deeper peel depression in non-transgenic tomato fruit after transferred to ambient temperature (28°C). However, no significant injury and cells were a normal appearance of transgenic RNAi ACO1-21 tomato fruit at the same storage conditions. The results also showed that fruit of lowland transgenic RNAiACO1-21 tomato experienced a significant delayed in skin color development, weight loss and reduced firmness loss compared to the non-transgenic tomato fruit. Furthermore, the transgenic RNAiACO1-21 tomato fruit stored at 10 °C retain shelf life for 18 days at ambient temperature without any spoilage from chilling injury (CI). While, non-transgenic retain shelf life only 4 days. Thus it may be concluded that the Lowland transgenic RNAiACO1-21 able to delay the softening process and further storage at low temperature extended the postharvest life and maintained the quality of the tomato fruit.

**Keywords :** Transgenic tomato, Storage conditions, Physical quality, Ripening.

### **Introduction**

Low temperature storage used widely as a postharvest technology to prolong the postharvest life of fruits and vegetables and allow the preservation of fruit and vegetables quality after harvest; because low

temperature reduces the speed of cell metabolism and delay plant senescence and fruit ripening (23). Tomatoes (*Solanum lycopersicum* L.) are usually stored at low temperature to delay ripening and extend shelf life, but the fruit is susceptible to chilling injury (CI) when continuously exposed to temperatures less than 12 °C but above freezing (28). Although incipient CI in the tomatoes is not generally apparent during storage at low temperatures, visible symptoms of CI, such as failure to ripen normally, surface lesion or indentations, discoloration, and increased water loss and decay, develop following transfer to warmer temperatures (14). The result of these changes, quality and consumer acceptability decrease which, leading to the substantial economic losses (15).

In addition, the temperature is the most important environmental factor has a significant impact on the rate of biological processes, including the development of red color and softening of the fruit (25). In the order to extend tomato commercial life, it is usually harvested at the unripe mature stages and stored at low temperatures (11; 21). Damage of plasma membranes is considered to be one of the most common primary causes for CI in fruit because membrane damage can, in turn, cause secondary reactions, including increases in ethylene production and respiration, interference with energy production, enzymatic browning, and the accumulation of toxic compounds (22). The increment of ethylene is associated with an induction of ACO and ACS gene expression during cold storage (18), while temperature conditioning treatments down-regulated expression of ACO gene (17). The objective of current study was to determine effects low temperature on development internal CI symptoms of the wild type and transgenic RNAi *ACO1-21* tomato fruit during prolonged storage. The aim of this study focused on efficacy down regulating *ACO1* gene tomato on its quality during cold storage as comparison to wild type tomato fruit.

## Materials and Methods

### 1. Materials

Tomato seeds of transgenic RNAi *ACO1-21*(T<sub>2</sub>) and non-transgenic used in this experiment were obtained from UKM Experimental Plots. Tomatoes were grown under the same field conditions. Fruits of transgenic RNAi *ACO1* and wild type generation harvested at the mature green stage (fully developed fruit with thick pericarp and dark green skin) for low temperature treatment.

### 2. Methods

#### 2.1 Fruit sampling and postharvest treatments

Mature-green (MG) tomato fruit (transgenic RNAi *ACO1-21* and wild type) of uniform shape, size and free from fungal infection were selected. Fruit washed in tap water and air-dried at room temperature. Half of transgenic and wild type fruits were stored at 10 °C for 28 days, while remaining fruit kept at 28 °C for ripening. After low temperature storage, fruits removed from storage and kept at 28 °C until the ripening process was completed. Samples of fruits were taken on different days of storage. Each data recording was of six replicates of tomato fruit. The symptoms of cold injury, skin discoloration, firmness, and weight loss were determined in transgenic RNAi *ACO1-21* and wild type tomato fruits at different days. In summary, the experiment was composed of the following treatments:

- a. Fruit not stored at 10 °C and kept at 28 °C (RT).
- b. Fruit stored at 10 °C for 28 days (LT), transferred and kept at 28 °C (10 °C/RT).

#### 2.2 Evaluation of chilling and ripening characteristics

The method of (16) used to evaluate chilling injury during the ripening period after fruit were removed from cold storage. The method is based on a rating scale of surface browning intensity (0, sound fruit; 1, less than 10%; 2, 10 to 20%; 3, 30 to 40% and 4, more than 50% pitting). The chilling injury index (CI) that expresses the severity of cold damage, was calculated by adding the products the number of fruit in each category by the value assigned to this category of the rating scale and dividing the sum by the total number of fruit evaluated.

Chilling injury index (CI) =  $(\sum (\text{CI level}) \times (\text{Number of fruit at the CI level})) / (\text{Total number of fruit in the treatment})$

### 2.3 Weight loss measurements

Weight loss of wild type and transgenic RNAi *ACO1-21* tomatoes fruit used in this experiment recorded and measured at different days of ripening. The rate of water loss fruits was calculated according to (16) method. Results were expressed as a percent weight loss from the fruits, using the equation:

$$WL\% = [W_0 - W_f / W_0] \times 100$$

Where:  $W_0$  = weight tomatoes at mature green stage (0 day),  $W_f$  = weight tomatoes at different days of ripening during storage.

### 2.4 Changes skin colour fruit

The color of the fruit was evaluated using reflectance meter (Minolta Chromameter, Japan) and color at the same location was recorded every other day as  $a^*$ ,  $L^*$  and hue angle value. The chroma meter was calibrated with a white standard tile. The color was recorded as numerical values of  $a^*$ , indicating a color range from green to red, whose values are  $-60$  to  $+60$ . Fruits were harvested in mature green stage (MG), at  $a^*$  value of around  $-17$ . Breaker (BR) fruits had  $a^*$  value of about  $-12$ , orange (OR) fruits a value of  $20$ . Measurement was continued until the fruits reached the red ripe (RR) stage, with a value of  $37$  (10). The results are presented as lightness ( $L^*$ ), and hue angle ( $H^\circ$ ).

The hue angle was calculated from the measured  $a^*$  and  $b^*$  values using the formulas

$$H^\circ = \text{arc tangent } (b^*/a^*) \quad (19).$$

### 2.5 Fruit firmness determination

Fruit firmness was determined by measuring the amount of force (N) 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).

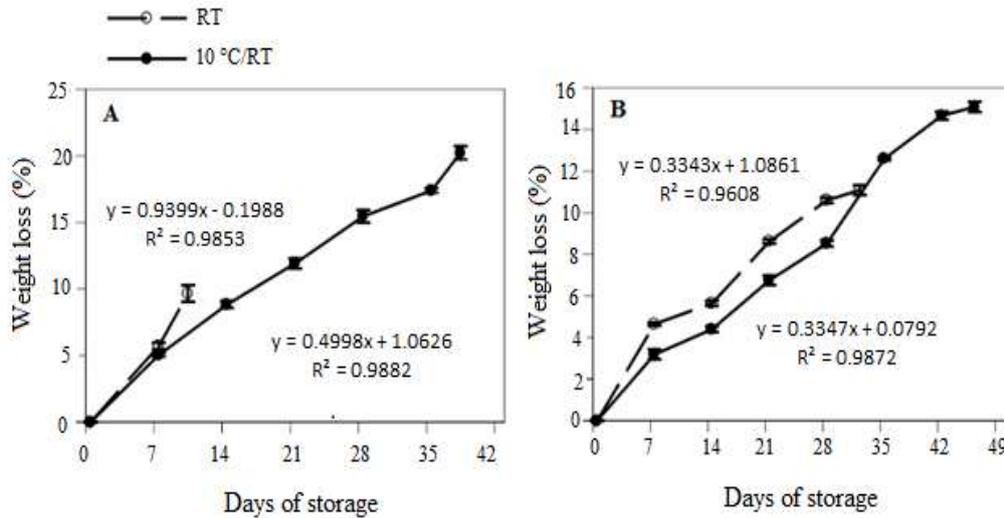
### 2.6 Statistical analysis

All readings are taken from six replicates samples of tissue and expressed as mean  $\pm$  standard error ( $n = 6$ ). The data obtained for physical qualities (weight, color and firmness). All Analysis of variance (ANOVA) was determined, using the Tukey method of the General Linear Model procedure of SPSS statistical package (version 20; SPSS Inc., Chicago, IL, USA). Regression analysis between transgenic RNAi *ACO1-21*(T2) and wild type tomato, and its correlation coefficient were then determined using Excel software.

## 3. Results and Discussion

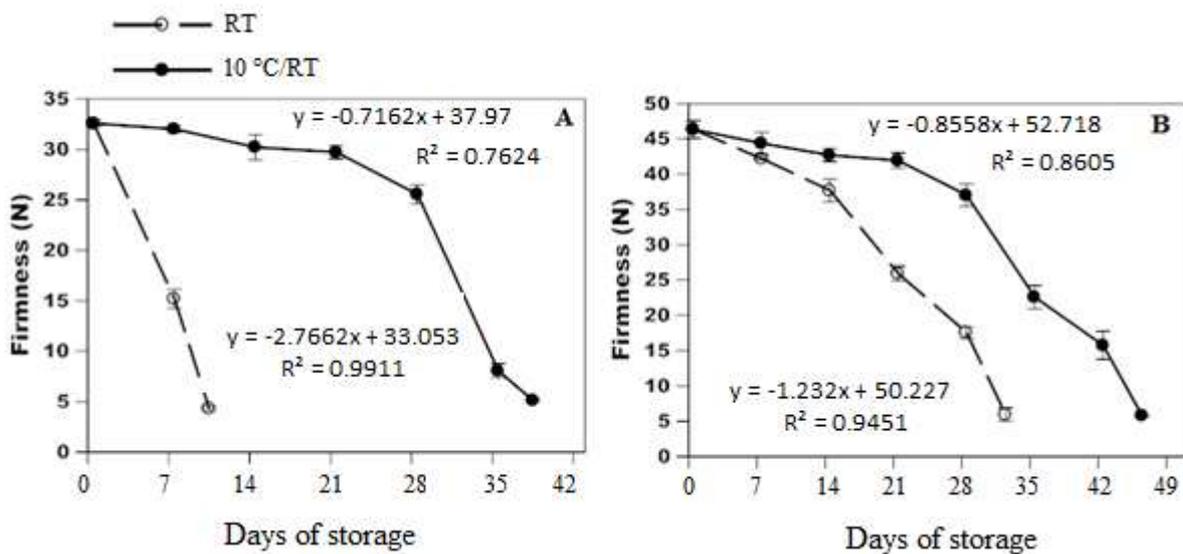
### 3.1 Effects of storage temperature on weight loss percentage.

Fruit weight losses are known for the most significant physiological disorder during the postharvest life. Weight change was related to temperature and the storage time. Post-harvest weight change in the vegetables is usually due to loss of water through transpiration. In this study, there is a strong positive relationship between days of storage and water loss in control group and treated with low temperature of wild type tomato fruit ( $R^2 = 0.98$  and  $R^2 = 0.98$ , respectively) (Fig. 1 A). Water loss was increased with increasing duration of storage. There was also a positive correlation between water loss and time of storage, in both groups (control fruit and treated) of transgenic RNAi *ACO1-21* ( $R^2 = 0.96$  and  $R^2 = 0.99$ , respectively). However, the weight loss was significantly higher in the wild type tomato fruit than transgenic RNAi *ACO1-21* stored at low and room temperature (Fig.1 A and B).



**Fig.1. Changes in the weight loss of wild type tomato (A) and lowland transgenic RNAiACO1-21 tomato (B) during storage at room temperature 28 °C as control (○), and low temperature stored tomatoes for 28 d at 10 °C followed by ripening days at room temperature 28°C (●).The values are the means of six replicate samples, and their S.E.s are indicated.**

Data presented in Figure1 A and B showed that, weight loss significantly reduced of transgenic RNAi *ACO1-21* tomato fruits during storage time under room temperature after storage at fridge conditions (10 °C). Therefore, the best reduction of weight loss was about 8.5 % at 10 °C after 4 weeks of storage and 15% after 18 days storage at 28 °C. Whereas, wild type tomato fruit produced the highest values of weight loss under low temperature storage and after transferred the fruit to room temperature conditions (16 and 20 %), respectively. Similar results observed by (24) after 35 days of stored tomato at 4 and 10 °C. These differences could be attributed to the fact that loss of water caused by transpiration and respiration processes (29). In addition, the reduction of weight loss rate in transgenic RNAi *ACO1-21* tomato fruits may be attributed to reducing the respiration process rates during postharvest storage, this agreement with (8). Therefore, this reduction is important for transgenic RNAi *ACO1-21* tomato fruit storage and transports to consumer markets.



**Fig.2. Changes in the firmness (N) of non-transgenic tomato (A) and lowland transgenic RNAiACO1-21 tomato (B) during storage at room temperature 28°C as control (○),and low temperature stored tomatoes for 28 d at 10 °C followed by ripening days at room temperature 28 °C (●).The values are the means of six replicate samples, and their S.E.s are indicated.**

### 3.2. Effects of storage temperature on firmness

Besides weight loss, fruit texture as characterized in tissue firmness, also changed with ripening. As shown in Figure 2 A and B, there is negative correlation between firmness (N) and the duration of storages on the wild type tomato fruit stored as control ( $R^2 = 0.76$ ) and treated group of wild type tomato with low temperature ( $R^2 = 0.99$ ), as well as between transgenic RNAi *ACO1-21* and time of storage ( $R^2 = 0.86$  and  $R^2=0.94$ ). The relationship was better in transgenic tomato fruit than that of wild type tomato fruit. Fruit softening greatly reduced in transgenic RNAi *ACO1-21*. The wild type fruit lost their textural integrity faster than the transgenic fruits. The maintenance of firmness in transgenic fruits is suggested to be due to their higher reducing respiration during storage as suggested by (4). The decrease in fruit firmness delayed by four weeks storage at 10 °C in both the transgenic RNAi *ACO1-21* and wild type tomato fruit. However, they showed a sharp decrease in firmness after transferred the fruit to room temperature 28 °C (Fig.2 A and B). This result may be related to low temperature storage that led to delay softening pitaya fruit during storage (20).

In wild type tomato fruit, the rate of softening was higher than in transgenic RNAi *ACO1-21* tomato fruit during storage and ripening at room temperature. These results may be related to the pectin solubility and the depolymerization, which agreement with (6), who reported that, fruit firmness was, associated an increase of the pectin solubility and depolymerization of the matrix polysaccharides which was believed to be a major contributor to reduce rigidity of cell walls that lead to fruit softening. While, transgenic RNAi *ACO1-21* tomato fruit showed a significant increase in firmness with an extended shelf life (Fig.2 B). However, significant residual softening still persists indicating the presence of an ethylene-independent component in flesh softening. On other hand, it is possible that the level of inhibition of ethylene biosynthesis in transgenic RNAi *ACO1-21* was not enough to avoid the trigger action of ethylene, as observed for respiratory climacteric in melon by (3).

### 3.3. Effects of storage temperature on color changes

Color Measurements is important for classifying raw materials and knowing how technological processes affect the stored fruits. Storage temperature could significantly affect the color properties of tomato fruits, except low temperature storage (Fig.3, 4and 5A, B).

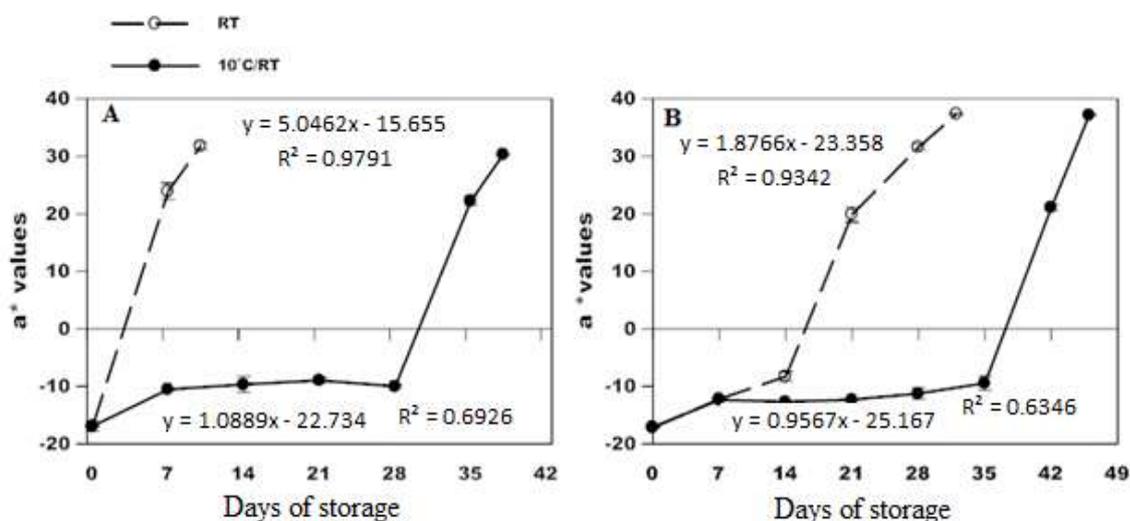
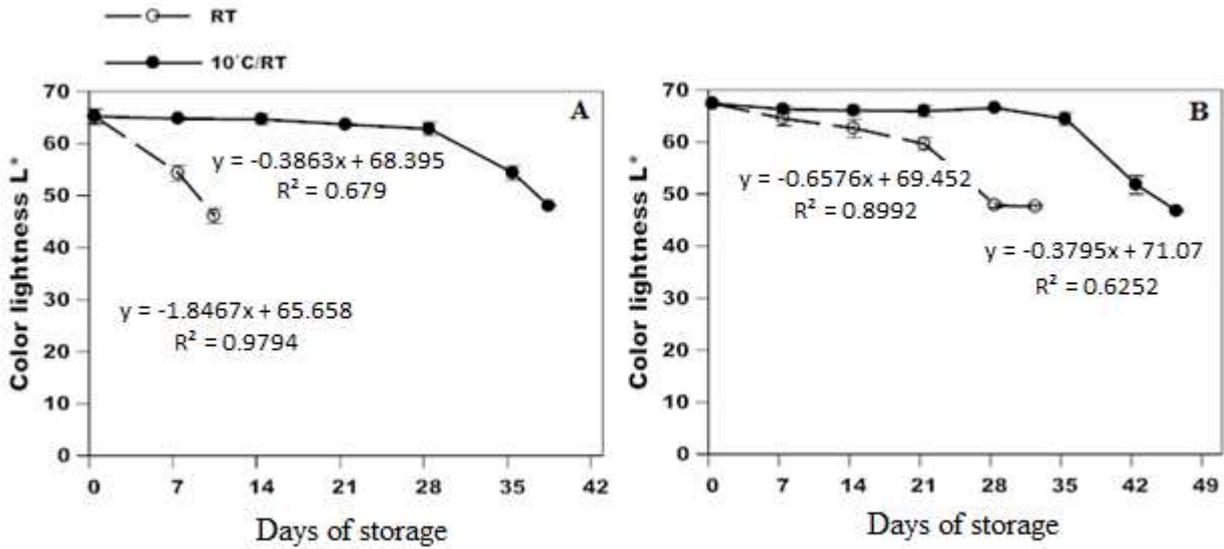
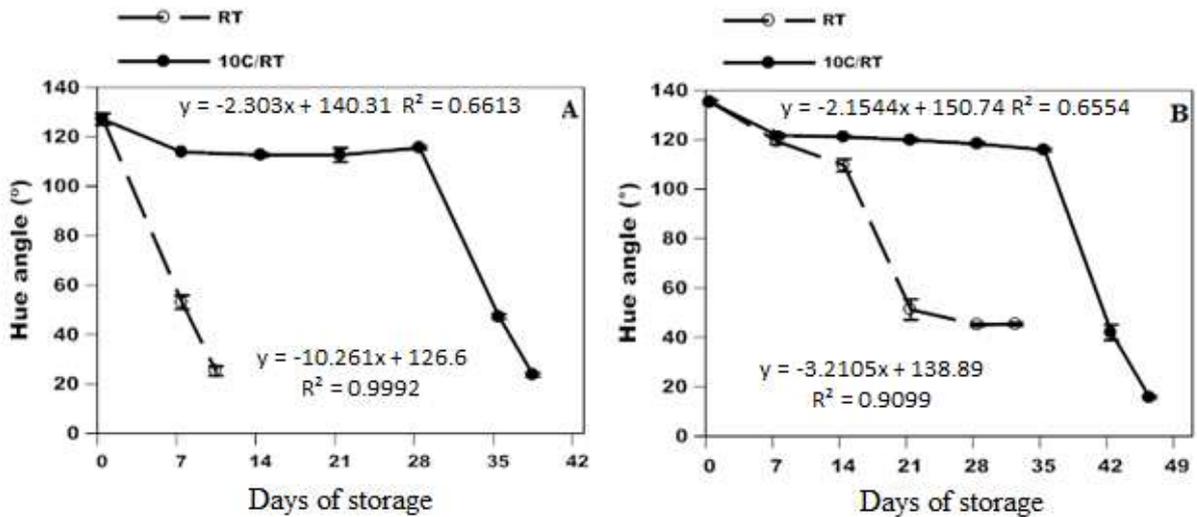


Fig.3. Changes in the colour index  $a^*$  of non-transgenic tomato (A) and lowland transgenic RNAiACO1-21 tomato (B) during storage at room temperature 28°C as control (○), and low temperature stored tomatoes for 28 d at 10°C followed by ripening days at room temperature 28°C (●). The values are the means of six replicate samples, and their S.E.s are indicated.



**Fig.4.** Changes in the colour lightness  $L^*$  of non-transgenic tomato (A) and lowland transgenic RNAiACO1-21 tomato (B) during storage at room temperature  $28^{\circ}\text{C}$  as control ( $\circ$ ), and low temperature stored tomatoes for 28 d at  $10^{\circ}\text{C}$  followed by a ripening days at room temperature  $28^{\circ}\text{C}$  ( $\bullet$ ).The values are the means of six replicate samples, and their S.E.s are indicated.



**Fig.5.**Changes in the Hue angle ( $^{\circ}$ ) value of non-transgenic tomato (A) and lowland transgenic RNAiACO1-21 tomato (B) during storage at room temperature  $28^{\circ}\text{C}$  as control ( $\circ$ ), and low temperature stored tomatoes for 28 d at  $10^{\circ}\text{C}$  followed by a ripening days at room temperature  $28^{\circ}\text{C}$  ( $\bullet$ ).The values are the means of six replicate samples, and their S.E.s are indicated.

At room temperature (RT), transgenic RNAi *ACO1-21* and wild type tomato fruit showed lower lightness values as compared to low temperature (LT). However, higher redness and hue angle values observed in the same condition as compared to those at low temperature storage (Fig.3, 4 and 5 A, B). Both parameters represent tomato color development, from green to red, during ripening. That was an indicator for red color development and the degree of ripening in tomato (13). There is a positive correlation between  $a^*$  values and the duration of storage on the wild type tomato fruit stored as control ( $R^2 = 0.98$ ) and in the treated fruit with low temperature ( $R^2 = 0.98$ ). Similar relationship was found in transgenic RNAi *ACO1-21* tomato fruit stored as control ( $R^2=0.93$ ) and treated with low temperature ( $R^2=0.64$ ) as shown in Fig.3 A and B.

On the other hand, a negative correlation is seen between the lightness values and duration of the storage in both wild type tomato fruit stored as the control and treated with the low temperature ( $R^2 = 0.98$  and  $R^2 = 0.68$ ) respectively (see Fig.4 A & B). Similar correlation was observed in the transgenic RNAi *ACO1-21*

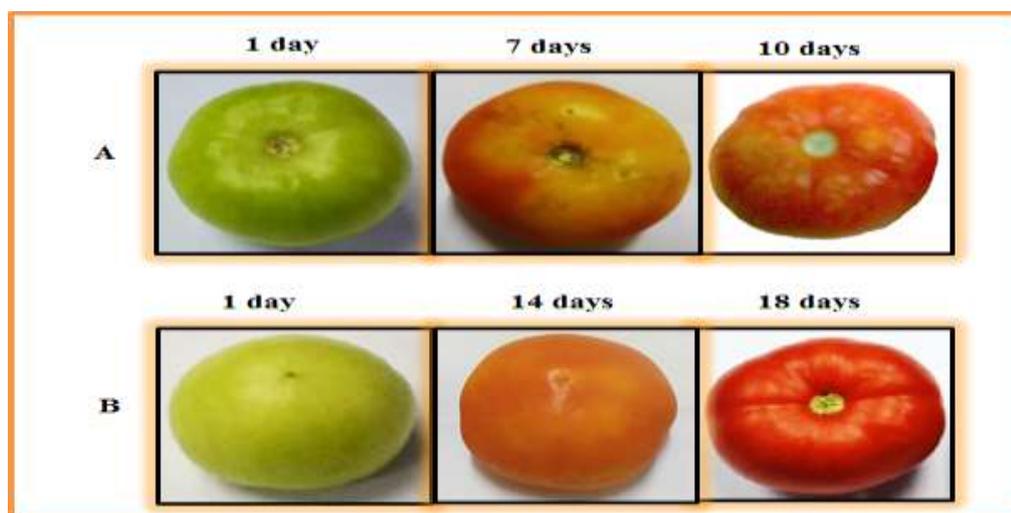
which was ( $R^2=0.90$ ) in the control group and ( $R^2=0.63$ ) in the treated fruit. However, the values were higher during storage at low temperature than that stored at room temperature. The lightness related to moisture content. As shown in figure 4 A and B, water content decreased with storage time. Thus, the decreasing in  $L^*$  values during storage maybe related to the water content of fruit. Furthermore, lightness values significantly decreased in wild type and transgenic RNAi *ACO1-21* tomato fruit that stored at room temperature, which maybe resulted by carotenoid synthesis (27).

In vegetables, the hue angle ( $h^\circ$ ) is used to express color variation. It assumes  $0^\circ$  for the color red,  $90^\circ$  for yellow and  $180^\circ$  for green (9). There is a negative relationship between hue angle and duration of storage under treatment temperatures (Fig.5 A and B), hue angle decreased significantly ( $p < 0.05$ ) during storage for both transgenic RNAi *ACO1-21* and wild type tomato fruit, which stored at room temperature ( $R^2=0.91$  and  $R^2=0.99$  respectively). However, no significant change in hue angle values at LT ( $R^2=0.661$  and  $R^2=0.655$  respectively). These results agree with (1). For both transgenic and wild type, during cold storage ( $10^\circ\text{C}$ ), no color changes were observed until the fruit transferred to room storage temperature  $28^\circ\text{C}$ . Many authors have reported that, the skin color remained green until 15 day, and then the  $h^\circ$  values reached values similar to those in the control groups at the end of the storage period (9) similar results found by (26) cold storage affected color and expansion gene expression in banana fruit.

It is evident from the data that color skin of tomato affected by RNAi *ACO1* silencing technique during storage and the color development was significantly slower in transgenic fruits (Fig. 3,4 and 5 A B). These results suggest that the activity of enzymes involved in these processes is somehow related to ethylene. The retardation of color development in transgenic RNAi *ACO1-21* tomato fruit could attribute to the low ethylene production and delayed increase in ethylene production to reach the threshold concentration for indication of color development (4). According to the results obtained, the storage temperature ( $10^\circ\text{C}$ ) preserved the better transgenic RNAi *ACO1-21* tomato quality, avoiding chilling injuries and prolongation its shelf life.

### 3.4 Chilling injury index

Chilling injury symptoms include failure to ripen and develop full color and flavor, irregular color development, excessive softening, surface pitting, and increased decay (7). Fig 6 showed the CI index of transgenic RNAi *ACO1-21* and non-transgenic tomato fruits exposure to  $10^\circ\text{C}$  and transferred to room temperature  $28^\circ\text{C}$  for storage.



**Fig.6. Development of chilling injury and changes color of non-transgenic tomato fruit (A) and transgenic RNAi *ACO1-21* tomato fruit (B) after storage at  $10^\circ\text{C}$  for 28 days.**

Transgenic RNAi *ACO1-21* tomato fruit kept at ambient temperature had a delay in skin color development when compared to the non-transgenic tomato fruit. The non-transgenic tomato fruit reached 100 % red skin in about 10 days compared to 32 days for transgenic RNAi *ACO1-21* tomato fruit (Fig.6). Non-transgenic tomato fruit kept at low temperature (LT) reached to the complete ripening stage (100 % red skin) in 10 days at ambient temperature. In contrast, the transgenic RNAi *ACO1-21* reached to the full ripening stage

during 18 days of storage at ambient temperature after low temperature treatment (Fig 6). This provides additional evidence that color development is less ethylene dependent (5) than softening once low storage temperature has affected ethylene production at 10 °C.

Chilling injury (CI) symptoms, expressed as surface lesions and mesocarp discoloration started to appear in fruit kept at LT storage (data not shown). Non-transgenic tomato fruit at LT exhibited CI earlier on the second at ambient and all fruit developed decay rapidly in the lesions despite being relatively firmer on the sixth day at ambient. The CI symptoms were noticeable in transgenic RNAi*ACO1*-21 tomato fruit on day 14 at ambient and the CI was less pronounced (less than 5%) as compared to non-transgenic tomato fruit. These physiological disorders might be indicative of subtle damages to membrane systems (2). For 'transgenic RNAi*ACO1*-21 tomato, CI symptoms were noticed less in fruit than that in non-transgenic tomato fruit the CI symptoms which were more pronounced throughout the ripening stages. According to (12), in banana CI caused the membrane of certain protein such as ethylene receptor losses its function thus resulting in failure to ripening normally.

## Conclusions

Low temperature treatment has been shown to give a very good impression in keeping and maintaining the level of quality changes during storage ripening. However, this treatment also accompanied by other side effects, the chilling injury (CI). The main CI symptom of wild type tomato fruit was uneven ripening and color development, followed by pitting and decay. In contrast, postharvest transgenic RNAi *ACO1*-21 tomato fruit has the potential of improving tolerance to cold thereby reducing the incidence of CI symptoms. Thus, low temperature (10°C) was the best treatment combination for storage the transgenic RNAi *ACO1*-21 tomato fruit.

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