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Effect of oxalic acid on postharvest life of tomato modified with the TomLoxB gene in antisense

Efecto del ácido oxálico en la vida poscosecha del tomate modificado con el gen TomLoxB en antisentido

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ABSTRACT

Oxalic acid is an organic compound found in green leafy vegetables, which has proven to be effective in delaying ripening by inhibiting ethylene synthesis in fruits such as banana, mango, peach, tomato, plum, and others. In this study, the response to oxalic acid application on postharvest physiology was evaluated in transgenic tomatoes (*Solanum lycopersicum*) variety TA234 with the *TomLoxB* gene insertion in antisense, at two concentrations of oxalic acid: 3 and 10 mM, during 30 d of storage at 25 ± 1 ºC and a relative humidity of 65 - 70 %. The fruits were harvested at break stage and immersed for 10 minutes in the oxalic acid solution, which was maintained at 25 ºC. Upon treatment, less weight loss, better retention of lightness, delay in the decrease of firmness and hue angle, decrease in lipoxygenase activity, low electrolyte leakage and increase in total phenolics content were observed. The most effective oxalic acid concentration was 3 mM, that extended postharvest life by up to 30 d and reduced deterioration of the genetically modified (GM) tomatoes. In turn, the untreated GM tomatoes showed an acceptable appearance up to day 24 of storage, while the wild type fruits were kept satisfactorily for 15 d.

Keywords: *Solanum lycopersicum*; lipoxygenase B; electrolyte leakage; total phenolic content.

RESUMEN

El ácido oxálico es un compuesto orgánico que se encuentra en vegetales de hoja verde y que ha demostrado ser eficaz para retrasar la maduración mediante la inhibición de la síntesis de etileno en frutos. En este estudio se evaluó el efecto de la aplicación de ácido oxálico sobre la fisiología poscosecha en frutos de tomate (*Solanum lycopersicum*) variedad TA234 genéticamente modificados (GM) con la inserción del gen *TomLoxB* en antisentido, a dos concentraciones de ácido oxálico (3 y 10 mM) durante 30 d de almacenamiento a 25 ± 1 ºC y una humedad relativa del 65 - 70 %. Los frutos en estado *break* se sumergieron durante 10 min en la solución de ácido oxálico. Se observó una menor pérdida de peso, mejor retención de la luminosidad, un retraso en la disminución de la firmeza y del ángulo hue, disminución de la actividad lipoxigenasa, baja pérdida de electrolitos y un aumento en el contenido de fenoles totales. La concentración más eficaz fue de 3 mM, que prolongó la vida poscosecha hasta 30 d y redujo el deterioro del tomate modificado, mientras los frutos GM testigos tuvieron una vida postcosecha de 24 d y los frutos silvestres testigo se mantuvieron satisfactoriamente por 15 d.

Palabras clave: *Solanum lycopersicum*; lipoxigenasa B; fuga de electrolitos; fenoles totales.

INTRODUCTION

Due to the commercial and nutritional importance of tomato (*Solanum lycopersicum*) around the world, extensive research has been carried out to extend its shelf life and counteract postharvest losses for several years, finding solutions only to certain problems. With the advances in genetic engineering techniques, effective procedures have been developed to improve their properties during postharvest life and extend their shelf life by slowing their ripening rate (Isack and Lyimo, 2015; Asrey *et al*., 2021).

Among the chemical treatments applied to fruits intended to extend postharvest life is oxalic acid, which is an organic acid widely distributed in plants. Oxalic acid plays an important role in stress response and redox homeostasis in plant as well as exerts antisenescence effects. The presence of oxalic acid increases the activity of antioxidant enzymes such as superoxide dismutase, ascorbate peroxidase among others, and promotes the accumulation of antioxidants such as flavonoids, total phenolic compounds and carotenoids. Its functions include acting as an acid catalyst, metal ion chelator, as well as an electron donor (Shimada *et al*., 1997). It has been shown to delay the ripening process in fruits such as mango (Zheng *et al*., 2007a; Razzaq *et al*., 2015), banana (Huang *et al*., 2013), tomato (Kant *et al*., 2013; Li *et al*., 2016), pear (Tarabih, 2014), peach (Zheng *et al*., 2007b; Razavi *et al*., 2017), sweet cherry (Valero *et al*., 2011) and plum (Wu *et al*., 2011). These authors used oxalic acid solutions at milimolar concentrations, reporting favorable effects on the preservation of fruit attributes.

An unexplored field is the application of oxalic acid on

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GM fruits, and its putative effect on their postharvest physiology. There have been work about fruit softening such as: pectinmethyl esterase (PME) and polygalacturonase (PG) silencing (Pear *et al*., 1993), β-Galactosidase silencing (Smith *et al*., 2002), *TomLoxA* and *B* gene silencing (Griffiths *et al*., 1999; Bo *et al*., 2008; Hu *et al*., 2014).

León-García *et al*. (2017) silenced the *TomLoxB* gene in tomato (*Solanum lycopersicum*) fruits variety TA234, that resulted in increased firmness, reduced lipoxygenase activity and extended shelf life compared to control fruits. Studies have been conducted to learn about the physiology of the modified tomatoes (Velázquez-López *et al*., 2020; Mazón-Abarca *et al*., 2022a; Mazón-Abarca *et al*., 2022b); however, more work remains to be pursued. The objective of this study was to evaluate the response of tomato (*Solanum lycopersicum*) variety TA234 with silenced *TomLoxB* gene after the application of oxalic acid during ripening. Overall physiology, lipoxygenase activity, electrolyte leakage and total phenolics content were monitored.

MATERIALS AND METHODS

Plant material

In a prior investigation, León-García *et al*. (2017) conducted a study focused on the production of transgenic tomato plants with antisense constructs targeting tomato lipoxygenase (*TomloxB*) to carry out the genetic modification. The researchers employed *Rhizobium radiobacter* strain LBA4404 (previously known as *Agrobacterium tumefaciens*), containing the binary vector pCAMBIA 2301, which carried the CaMV 35S promoter. To effectively monitor and assess the success of the transformation, two specific genes were employed: neomycin phosphotransferase (NPTII) as a selection marker, and β-glucuronidase (UIDA) as a reporter gene. The plasmid used in the study contained the *TomloxB* sequence, which was 2745 base pairs long. This specific sequence, with its corresponding GenBank accession number U09025, was inserted into the plasmid in the antisense direction. Genetically modified (GM) tomatoes from the F1 lineage and unmodified or wild-type (WT) fruits from the same TA234 variety were included as a basis of comparison. Located at the Instituto Tecnológico de Veracruz , we find the postharvest handling laboratory, in whose greenhouse both, wild type and transgenic tomato fruits, were planted, grown and harvested. Four hundred and ninety-five tomatoes weighing between 18 and 20 g, at break maturity stage with no apparent skin damage were chosen. Subsequently, they were washed by immersion in a 500 mg/L sodium hypochlorite solution for 2 m and placed on a grid to remove surface water.

Chemical preparation and treatments

For the preparation of the oxalic acid (OA) solutions (Sigma-Aldrich®, Merck, St. Louis, MO) applied to tomatoes selecting different concentrations according to previous studies (Kant *et al*., 2013; Li *et al*., 2016); two concentrations were selected: 3 and 10 mM to carry out the study. Tomatoes were immersed in the respective solutions for 10 min at 25 °C. After treatment, they were placed in a plastic container for 3 h to remove the solution from the surface and subsequently stored at 25 °C with a relative humidity of 65-70 %.

Postharvest monitoring

Weight loss

Tomato weight was measured using an electronic balance (Sartorius, model BL 2100, Germany). Percent weight loss over time was reported.

External color

With the use of a Hunter lab colorimeter (model 4500L, Reston, VA), Lighness and Hue angle were measured at three different spots on the equatorial zone of the fruit (Kalantari *et al*., 2015).

Firmness

The force needed to puncture the tomato fruit was calculated in three equatorial spots with a fruit texture analyzer (model GS25, Geneq Inc., Montreal, Canada) fitted with an 8 mm diameter stainless steel cylindrical probe.

Lipoxygenase activity

Lipoxygenase (LOX) activity was assayed according to Velázquez-López *et al*. (2020), using linoleic acid as substrate. Tomato juice was extracted and centrifuged for 5 min at 8765xg in an Eppendorf centrifuge model 5415R (Hamburg, Germany). The supernatant was filtered with a 0.45 µm pore diameter Millipore membrane disc filters. Forty µL of the sample were taken, mixed with 1360 µL of 0.2 M sodium phosphate buffer and 2.64 mL of substrate consisting of 42 µL of linoleic acid (Sigma-Aldrich ®), 39 µL of Tween 20 (Sigma-Aldrich ®) and 250 µL of 1 N NAOH (Golden Bell®); subsequently the volume was made up to 100 mL with sodium phosphate buffer and kept at 37 ºC for 10 m. To stop the reaction, the samples were cooled in ice water. A UV-Vis spectrophotometer with diode array (Agilent Technologies®, model 8453, Waldbronn, Germany) was used to measure the absorbance at 234 nm.

Electrolyte leakage

Electrolyte leakage was estimated according to the method of Li *et al*. (2016) with some changes. Two 6-mm diameter discs per tomato were removed with a scalpel from the equatorial and opposite region of the fruit. Five tomatoes were used for each treatment. Fruits were then immersed in 40 mL of distilled water and incubated for 3.5 h at 25 ºC. Electrical conductivity was measured using a benchtop conductivity meter (Thermo Scientific Orion 1114000-WA, 3star, Madrid, Spain), then the samples were placed on a stirring hot plate (Corning PC-420D, Somerville, MA) for 20 m at 230 ºC and incubated for 3.5 h at 25 ºC. Electrical conductivity was measured after the incubation time was completed. The percentage of total electrolyte leakage was defined as the ratio between the initial and final conductivity, multiplied by 100.

Total phenolics content

Total phenolics content was analyzed according to the method reported by Domínguez *et al*. (2016): 200 µL filtered tomato juice was blended with 1 mL of Folin-Ciocalteau reagent (Sigma-Aldrich®), shaken vigorously and after 3 min, 1.5 mL of 7 % sodium carbonate (J.T. Baker, Xalostoc, Mexico) and 800 µL of Milli-Q water were added, then incubated for 1 h at 25 °C. The absorbance was measured at 765 nm using a UV-Vis diode array spectrophotometer (Agilent Technologies, model 8453, Waldbronn, Germany). Total phenolics were reported as mg gallic acid equivalent (GAE) per 100 g FW (Fresh Weight). Based on a calibration curve with different concentrations of gallic acid and performed in duplicate, with a R^2 of 0.9997.

Experimental design and statistical analysis

A completely randomized design (CRD) was used. Analysis of variance (one-way ANOVA) and Tukey's rank test ($p < 0.05$) were employed to investigate the difference between treatments. The analyses were computed in Minitab v. 18 (LLC, State College, PA). All treatments were made in triplicate.

RESULTS

Weight loss

The data obtained for weight loss were as follows: GM-3mM tomatoes showed a significantly ($p < 0.05$) smaller weight loss of 8.51 ± 0.30 % compared to the rest of the treated tomatoes during the entire 30-days storage period (Figure 1). WT-C tomatoes showed the highest weight loss (19.57 ± 0.39 %) during the same period. The 3 mM concentration allowed lower weight losses than the 10 mM treatment. Likewise, GM fruits were superior in response to wild-type fruits.

Lightness

Lightness in GM-3 mM tomatoes, recorded a significantly lower decrease of 45.07 ± 0.30 L* ($p < 0.05$), compared to GM-C treatments with 42.92 ± 0.83 L* and GM-10 mM with 38.96 ± 0.59 L*. WT-3 mM tomatoes recorded values of 40.47 ± 0.68 L*, being significantly higher ($p < 0.05$) than WT-C tomatoes with 36.51 ± 0.53 L* and WT-10 mM tomatoes with 37.61 ± 0.31 L* at 15 days of storage; with GM-3 mM tomatoes having the highest values, among all treatments (Figure 2).

Hue angle

The Hue angle value for the tomatoes analyzed at the break stage was 88.76 ± 1.90 °; when they reached the red color, an indication of fruit ripening; their values were 41.32 ± 1.68 h^o. All the treated tomatoes gradually decreased the Hue angle values throughout the storage period. GM-3 mM tomatoes showed a significantly ($p < 0.05$) lower decrease in Hue angle with a value of 43 ± 1.38 °, compared to GM-C tomatoes with 39 ± 1.53 ° and GM-10 mM tomatoes with 37 ± 1.39 °, even to WT-3 mM tomatoes with 37 ± 1.38 °, and to both WT-10 mM and WT-C tomatoes with a value of 35 ± 1.99 ° on day 24 of storage (Figure 3).

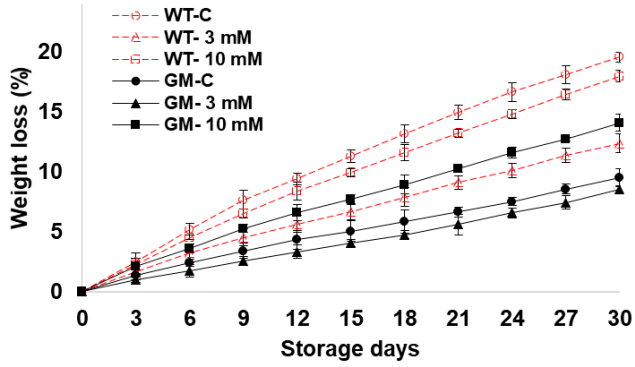

Figura 1. Efecto del tratamiento con ácido oxálico a 3 y 10 mM sobre la pérdida de peso en tomates silvestres (WT) y modificados genéticamente (GM) mantenidos a 25 ± 1 °C durante 30 d. Los tomates testigo de ambas líneas se designan con la letra C.

Figure 1. Effect of oxalic acid treatment at 3 and 10 mM, on weight loss in wild type (WT) and genetically modified (GM) tomatoes stored at 25 ± 1 °C for 30 d. The control tomatoes of both lines are designated with the letter C.

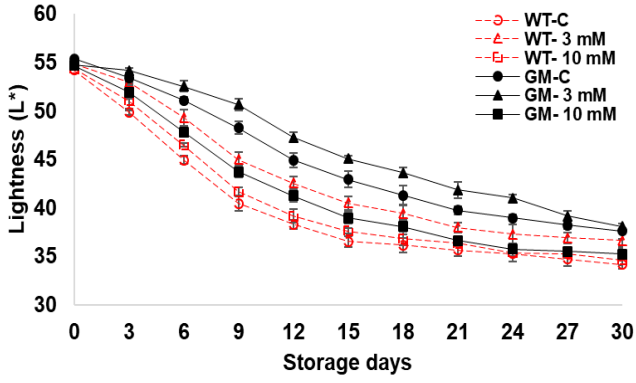

Figura 2. Efecto del tratamiento con ácido oxálico a 3 y 10 mM sobre la luminosidad en tomates silvestres (WT) y modificados genéticamente (GM) mantenidos a 25 ± 1 °C durante 30 d. Los tomates testigo de ambas líneas se designan con la letra C.

Figure 2. Effect of oxalic acid treatment at 3 and 10 mM, on lightness in wild type (WT) and genetically modified (GM) tomatoes stored at 25 ± 1 °C for 30 d. The control tomatoes of both lines are designated with the letter C.

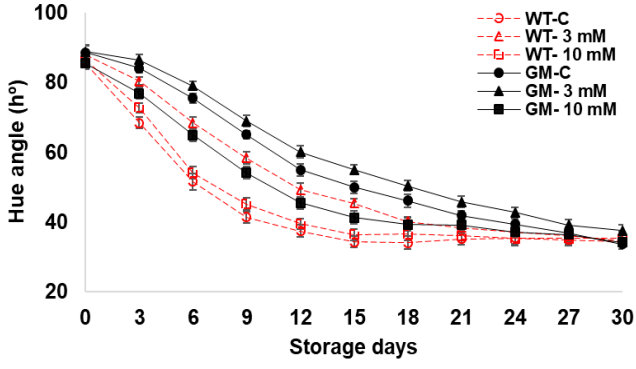

Figura 3. Efecto del tratamiento con ácido oxálico a 3 y 10 mM sobre el ángulo Hue en tomates silvestres (WT) y modificados genéticamente (GM) mantenidos a 25 ± 1 °C durante 30 d. Los tomates testigo de ambas líneas se designan con la letra C.

Figure 3. Effect of oxalic acid treatment at 3 and 10 mM, on Hue angle in wild type (WT) and genetically modified (GM) tomatoes stored at 25 ± 1 °C for 30 d. The control tomatoes of both lines are designated with the letter C.

Firmness

A decrease in firmness was observed during storage for both types of tomatoes (Figure 4). No significant differences ($p < 0.05$) in firmness were found between GM-C (6.3 ± 0.25 N) and GM-3 mM (6.7 ± 0.24 N) tomatoes in the first 6 days. From day 30, the firmness of GM-3 mM (3.9 ± 0.23 N) tomatoes maintained higher values than the rest of the treatments, especially WT-C (2.9 ± 0.17 N) tomatoes at day 30 (Figure 4).

LOX activity

WT-C tomatoes showed a maximum peak of LOX activity with 1433 ± 57.1 U/mg protein on day 12, whereas WT-3 mM tomatoes recorded the maximum activity of 1254 ± 38.72 U/mg protein on day 18. Conversely, both GM-C tomatoes with an activity of 1122 ± 32.83 U/mg protein and GM-3 mM tomatoes with 935 ± 36.37 U/mg protein showed the highest peak until day 24; the latter having significantly lower LOX activity ($p < 0.05$) among all treatments (Figure 5).

Electrolyte leakage

Electrolyte leakage gradually increased during ripening in storage. Transgenic fruits manifested a better response measured in lower electrolyte leakage than wild-type fruits. GM-3 mM tomatoes showed a significantly lower electrolyte leakage rate of 62.48 ± 3.30 % ($p < 0.05$) with respect to WT-C tomatoes with 92 ± 3.39 % at day 30 of storage. In turn, GM-C tomatoes recorded an electrolyte leakage of 69 ± 3.80 % and for WT-3 mM tomatoes it was 77 ± 3.77 %, finding no significant differences (Figure 6).

Total phenolics content

Regarding the total phenolics content in both, transgenic and wild-type fruits, this value increased during the entire storage time. By means of a two-way ANOVA, it was determined that time had a significant effect on phenolics content, as well as the different factors (*fruits*:WT or GM and *oxalic acid concentration*: 0, 3 and 10 mM) over time ($p < 0.05$).

Specifically in GM-3 mM tomatoes, it was significantly higher ($p < 0.05$) with a value of 11.76 ± 0.13 mg GAE/100 g FW, compared to WT-C tomatoes with 9.85 ± 0.14 mg GAE/100 g FW, to WT-3 mM tomatoes with 10.5 ± 0.18 mg GAE/100 g FW, to GM-C tomatoes with 11.0 ± 0.18 mg GAE/100 g FW at day 30 of storage. (Figure 7).

DISCUSSION

The data show a positive effect during the ripening of GM fruits and the application of OA. By analyzing the mechanism of action of each of the factors (oxalic acid and silencing of *TomloxB* gene) we could argue that there was an additive effect, but also a synergistic effect. It is an additive effect, since both promote a different mechanism by which senescence in the fruit is delayed. Silencing of the *TomloxB* gene delays the metabolic and biochemical processes that lead to senescence. Furthermore, OA promotes the accumulation of antioxidant molecules and enzymes (ascorbate peroxidase, catalase, glutation reductase and others) which help to neu-

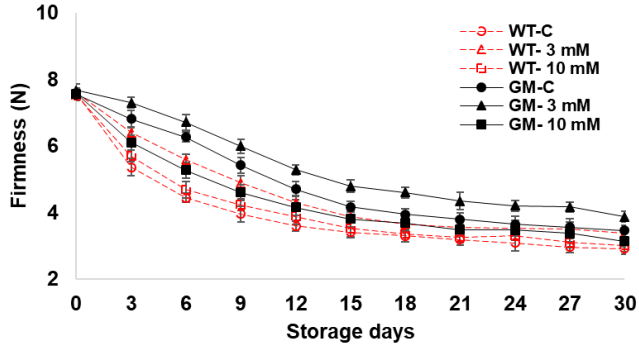

Figura 4. Efecto del tratamiento con ácido oxálico a 3 y 10 mM sobre la firmeza en tomates silvestres (WT) y modificados genéticamente (GM) mantenidos a 25 ± 1 °C durante 30 d. Los tomates testigo de ambas líneas se designan con la letra C.
Figure 4. Effect of oxalic acid treatment at 3 and 10 mM, on firmness in wild type (WT) and genetically modified (GM) tomatoes stored at 25 ± 1 °C for 30 d. The control tomatoes of both lines are designated with the letter C.

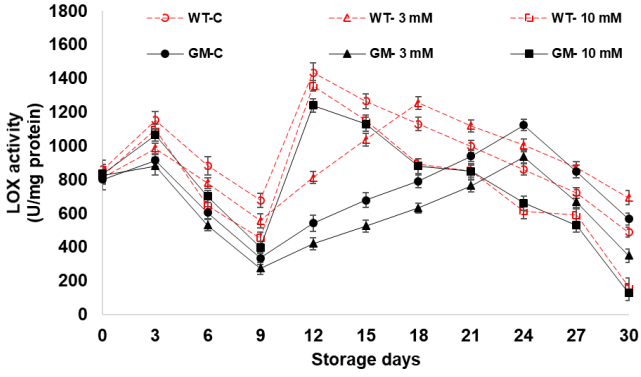

Figura 5. Efecto del tratamiento con ácido oxálico a 3 y 10 mM sobre la actividad de LOX en tomates silvestres (WT) y modificados genéticamente (GM) mantenidos a 25 ± 1 °C durante 30 d. Los tomates testigo de ambas líneas se designan con la letra C.
Figure 5. Effect of oxalic acid treatment at 3 and 10 mM, on LOX activity in wild type (WT) and genetically modified (GM) tomatoes stored at 25 ± 1 °C for 30 d. The control tomatoes of both lines are designated with the letter C.

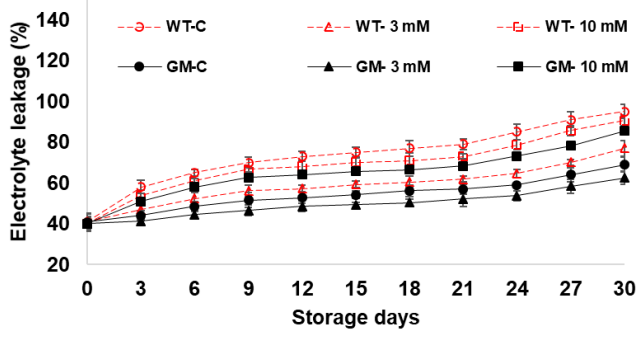

Figura 6. Efecto del tratamiento con ácido oxálico a 3 y 10 mM sobre la fuga de electrolitos en tomates silvestres (WT) y modificados genéticamente (GM) mantenidos a 25 ± 1 °C durante 30 d. Los tomates testigo de ambas líneas se designan con la letra C.
Figure 6. Effect of oxalic acid treatment at 3 and 10 mM, on electrolyte leakage in wild type (WT) and genetically modified (GM) tomatoes stored at 25 ± 1 °C for 30 d. The control tomatoes of both lines are designated with the letter C.

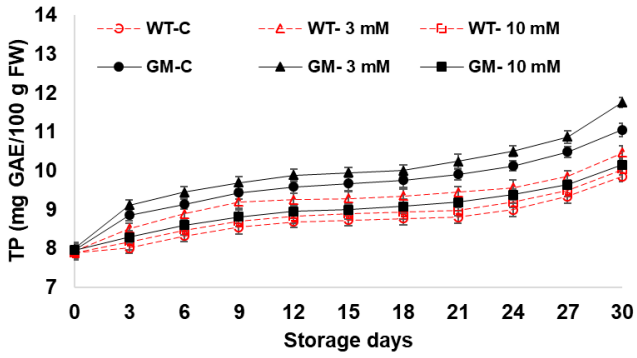

Figura 7. Efecto del tratamiento con ácido oxálico a 3 y 10 mM sobre el contenido de fenoles totales (TP) en tomates silvestres (WT) y modificados genéticamente (GM) mantenidos a 25 ± 1 °C durante 30 d. Los tomates testigo de ambas líneas se designan con la letra C.

Figure 7. Effect of oxalic acid treatment at 3 and 10 mM, on total phenol content (TP) in wild type (WT) and genetically modified (GM) tomatoes stored at 25 ± 1 °C for 30 d. The control tomatoes of both lines are designated with the letter C.

tralize free radicals generated during the decompartmentalization of the cell membrane, maintaining textural quality and lowering softening during storage, and therefore contributes to the delay in the processes of oxidative deterioration in this membrane, preserving its integrity for a longer time and thus contribute to the delay of senescence (Hasan *et al*., 2023; Ali *et al*., 2021; Wu *et al*., 2011; Wang *et al*., 2009).

On the other hand, it could also be assumed that there was a synergistic effect in the fruits modified with the silencing of the *TomloxB* gene and the application of OA. There is a positive regulation between the TomloxB enzyme and ethylene, by decreasing the activity of the enzyme, attributed to the effect of silencing; ethylene production decreased due to a reduction in the activity of the enzyme ACC Oxidase (1-aminocyclopropane-1-carboxylic acid oxidase) at the *Break* stage of ripening (Velazquez-López *et al*., 2019), an enzyme required for the last step of ethylene production from methionine. In addition to this, OA also affects ethylene production by decreasing the activity of a key enzyme in the production pathway of this hormone from methionine, ACC synthase (1-aminocyclopropane-1-carboxylic acid synthase), and since ethylene is the key hormone in ripening, this process is also affected (Wu *et al*., 2011; Wang *et al*., 2009).

The treatments (WT and GM) with application of OA at a concentration of 3 mM showed the best results compared to their respective controls. Weight loss was lower, due to a mesocarp wall strengthening and a softening slowdown, caused by the formation of oxalate-pectin complex as a result of the application of OA (Martínez-Esplá *et al*., 2019). In this experiment, a smaller weight loss of 8.5 % was obtained in GM fruits, with 3 mM at day 30. Here the synergistic effect is shown, between the afore mentioned mechanisms of action of OA and the silencing of *TomLoxB* gene, which decreased the production and expression of the same enzyme, preventing the fatty acids that constitute the membrane from being oxidized by the enzyme (Velázquez-López *et al*., 2020; León-García *et al*., 2017). This also contributed to maintaining lightness (L*) longer in GM-C (18 %) and GM-3mM (23 %) tomatoes at day 15 and GM-C (10 %) and GM-3mM (12 %) at day 30, compared to WT-C control fruits (36 L*, day 15; 34 L*, day 30). The same trend was observed for color, measured as Hue angle, where a gradual decrease was recorded for GM-C, GM-3 mM and WT-3 mM tomatoes. Therefore, we can infer that the slow decrease in Hue angle may be attributed to a reduction in ethylene production, which promotes carotenoid biosynthesis (Sun *et al*., 2020). In this case, the decrease in ethylene production is a result of the synergy between OA and *TomLoxB* gene silencing.

GM fruits had higher firmness than WT fruits, and the GM-3 mM treatment had the highest firmness retention. The application of OA decreased the fruit softening process by the formation of an oxalate-pectin complex that strengthens the mesocarp wall, conferring greater firmness to the fruit (Martínez-Esplá *et al*., 2019). Kant *et al*. (2013) showed that tomatoes treated with 3 mM OA recorded an improvement in tomato firmness for up to 21 d compared to the control that lasted 15 d, attributing this fact to a low solubilization of pectin, which in turn improved water holding capacity and decreased cell wall-degrading enzymes activity. While the effect of OA on WT tomato has shown to have an impact on cell wall degrading enzymes, for GM tomato the antisense insertion of the *TomLoxB* gene confers an additional effect, which bestows integrity to the membrane by decreasing the expression of lipoxygenase. This maintains intact the fatty acids of the membrane longer, thus preserving its integrity (León-García *et al*., 2017).

The 3 mM OA concentration (WT-3 mM and GM 3 mM) had a delaying effect on LOX activity and therefore the fruits showed a delay in senescence. Ding *et al*. (2007) also showed low LOX activity in mangoes treated with OA for up to 30 days of storage, attributing this effect to the ability of OA to inhibit peroxide accumulation. Therefore, the decrease in LOX activity in tomato is attributed to the action of OA, which induced resistance to the peroxidation of fatty acids present in the membrane and, together with the low expression of lipoxygenase in the tomato modified by the insertion of the *TomLoxB* gene in antisense, decreased the accumulation of hydroperoxides responsible for causing damage to the membrane and thus preserve its integrity, thereby extending its shelf life.

It is reported that OA can increase the activity of antioxidant enzymes such as catalase, ascorbate peroxidase, superoxide dismutase, among others. Application of 3 mM OA in tomato was effective in reducing electrolyte leakage in WT (61 %) and GM (52 %) fruits on day 21, compared to their WT-C (79 %) and GM-C (59 %) controls. At the same concentration, Kant *et al*. (2013) reported values of 45.66 % at 21 d of storage, while for the control fruit was 71.66 %. Similar results were obtained by Li *et al*. (2016), who observed that electrolyte leakage in tomatoes treated with OA was significantly smaller by 20 % compared to the control fruits, after been stored between 20 and 32 d. It is reported that OA can increase the activity of antioxidant enzymes, which act

on free radicals released during the ripening and softening process of the fruit, relaxing membrane permeability. Electrolyte loss reflects membrane permeability and, indirectly, the effect of oxalic acid application (Nyanjage *et al*., 1999; Zheng *et al*., 2007c; Razzavi *et al*., 2017) which together with the silencing the *TomLoxB* gene, decreases the expression of lipoxygenase and thus the decompartmentalization and loss of tissue structure in tomato, providing a synergistic effect to extend the postharvest life of the fruit.

OA promotes the accumulation of total phenolic compounds, which favors the protective action against reactive oxygen species (ROS) involved in membrane destabilization. Its positive effect has been proven in peaches (Razavi *et al*., 2017) and mangoes (Razzaq *et al*., 2015), where a significant trend of increased total phenolics content was observed from day 7 to day 21 of storage compared to untreated fruit. It has also been reported that with reduced ethylene levels, there is an increase in phenolic content in tomatoes (Dominguez *et al*.**,** 2016). In this experiment, the 3 mM concentration of OA in GM fruit was the treatment that showed the highest results of total phenolics over time ($p < 0.05$).

The effects of antisenescence and antistress might be associated with ethylene signaling regulated by OA. It has been demonstrated that OA delayed the ethylene climacteric peak and exhibited 2-fold lower rates of ethylene production (Wu *et al*., 2011). Wang *et al*. (2009) reported that reduced ethylene production in OA-treated fruit might be ascribed to the reduced ACC synthase activity. By virtue of the regulation of OA on ethylene, via ACC synthase, and knowing that ethylene is an indispensable and key hormone during the ripening process, it can be assumed that the 10 mM concentration of OA strongly blocked ACCS enzyme production and thus ethylene synthesis, and because of the above, the ripening processes were affected.

In turn, the 3 mM concentration was adequate to partially inhibit the activity of the ACC synthase enzyme, still allowing the production of the hormone, which is necessary to trigger those metabolic and biochemical processes involved in ripening.

CONCLUSIONS

Of the two concentrations of OA applied to WT and GM tomatoes, the 3 mM OA solution, was the one that showed more favorable effects on the physiology of GM tomatoes, producing greater lightness retention, delay in the appearance of red color, greater firmness and total phenolics content, lower weight loss, lipoxygenase activity and electrolyte leakage. All of these resulted in an extension of shelf life of 30 d. In turn, the untreated GM tomatoes showed an appropriate appearance up to day 24 of storage. However, increasing the concentration to 10 mM showed adverse effects on the physiology of both GM and WT tomatoes, that resulted in irregularities in the development of treated fruit such as premature shriveling and uneven coloration, with a shelf life of only 18 d, while the WT control tomatoes had a postharvest shelf life of 15 d.

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CONFLICTS OF INTEREST

The authors report there are no competing interests to declare.

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