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Physicochemical properties in NOR tomato line MA 131-6-3 after treated with ethephon and calcium carbide induced ripening

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Abstract. *Khairi A, Murti RH, Irwan SNR, Putra ETS. 2023. Physicochemical properties in NOR tomato line MA 131-6-3 after treated with ethephon and calcium carbide induced ripening. Biodiversitas 24: 3029-3037.* Tomato fruit Postharvest Losses (PHL) from rotting, Physiological Weight Loss (PWL), inadequate nutrition, and other factors can lower fruit quality. Tomato is included as climacteric fruit which shows a significant increase in respiration and ethylene. The use of tomato with an extended shelf life due to genetic engineering, such as a Non-Ripening (NOR) tomato, can lower PHL. This experiment was carried out to evaluate physicochemical characteristics of NOR tomato fruit (line MA 131-6-3 as result of selection F6) during postharvest after being treated by Ethephon (ET) (375, 750, 1,125, and 1,500 ppm), calcium carbide (CaC_2) (5, 10, 15, and 20 g kg^{-1}), and control. The experimental design was arranged in Randomized Completely Block Design with three replications. The ambient temperature was 28.30±1.75°C and relative humidity of 60.69±2.33%. The results showed the fruit ripening and physicochemical altered with CaC_2 treatment, then followed by yellow but did not alter to the red color for all treatments. The value of reduction sugar, respiration, ethylene, PWL, and pH increased, meanwhile the fruit firmness decreased caused by CaC_2. Furthermore, ET could increase rotting during storage.

Keywords: Calcium carbide, ethephon, non-ripening, postharvest, tomato

INTRODUCTION

Fruit ripening is the series of processes that take place from the fruit's later growth and development stages until it is ready for consumption. Fruit quality changes as the fruit ripens. Usually, the fruit flesh's stiffness softens, the amount of sugar increases, and the acidity decreases (Hewajulige and Premaseela 2020). Along with other hormones and signals, the gaseous ethylene is crucial in triggering the ripening of many fruits. Typically, an immature fruit has little ethylene present. Ethylene is released as the fruit ages as a signal to cause fruit ripening. After harvest, ethylene production keeps rising, which shortens fruit's shelf life, reduces its ability to be stored, and makes it more vulnerable to pathogen attacks (Iqbal et al. 2017).

At the same time as tomato (*Solanum lycopersicum* L.) fruit is marketed before it reaches customers, Postharvest Losses (PHL) at that fruit become a significant issue. The quality of tomato fruit can be affected by rotting, Physiological Weight Loss (PWL), loss of nutrients, and other causes. The tomato belongs to the same category as climacteric fruits such as apples, melons, pears, and bananas. At the start of ripening, climacteric fruits exhibit a characteristic increase in respiration and a noticeable increase in ethylene production (Li et al. 2020). When harvested, some tomato cultivars are challenging to ripen. This is due to the discovery of single gene mutations that prevent fruit from ripening normally, such as Ripening Inhibitor (RIN), NOR, and Colorless Non-Ripening (CNR) (Siddiqui et al. 2013). The genetic alteration significantly extended the tomato shelf life (Siddiqui et al 2013). Utilizing ripening mutants and associated alleles with extended shelf life was the breeding approach used to increase the tomato fruit shelf life (Roohanitaziani et al. 2022). Although compared to a normal tomato, the NOR tomato has a longer shelf life and can be used for postharvest handling tasks including long-distance delivery and fruit resistance to rotting.

PHL is the loss of food throughout the food supply chain from harvest to consumption. It is very important to know the source cause of PHL, especially for tomato fruit (Kumar and Kalita 2017). Poor postharvest handling of tomato can cause PHL of 25-50% and even more (Tiwari et al. 2020). Fruit ripeness and quality can be assessed from four components (i) color and visual, (ii) taste and aroma, (iii) texture, and (iv) nutrient (Maduwanthi and Marapana 2019). Several technologies are used to reduce PHL such as control atmosphere or modified atmosphere, ripening inhibitor materials, and genetics. Among them, genetics was considered the best in reducing PHL in tomato fruit during storage. The NOR tomato mutant generates a truncated 186-amino-acid protein (NOR186) and has been demonstrated previously to be a gain-of-function mutant. The NOR mutant was considered to be due to loss of function of the NAC-NOR gene, and NAC-NOR was considered to be a core transcription factor regulating the initiation of tomato fruit ripening (Gao et al. 2020). The solution that can be used to reduce PHL is to use the

tomato genetics such as NOR tomato line MA 131-6-3, because it can be a long-time to store. NOR tomato line MA 131-6-3 is a tomato line as result of conventional breeding (pedigree selection) from plant breeding program at Universitas Gadjah Mada (UGM) that produces plants with high yields but is difficult to ripen (Murti et al. 2022).

The NOR tomato was tested for physicochemical alters using Artificial Ripening (AR) such as Ethephon (ET) and Calcium Carbide (CaC_2), because it is easy to obtain and its application does not require additional materials or instruments such as the application of exogenous ethylene. This work has been investigated by Khairi et al. (2022) used the tomato seeds of NOR tomato line MA 131-6-3 and the same treatments (ET and CaC_2). The result in ET can increase fruit rotting, CaC_2 can accelerate metabolism which has an impact on PWL, and the color of tomato fruit cannot turn red during postharvest. The application of exogenous ethylene was in a storage room or ripening chamber. Appropriate compounds used to produce exogenous ethylene are ET and CaC_2. Dhall and Singh (2013) reported that application of 1,500 ppm ET on hybrid tomato can increase fruit ripening and lycopene content. Meanwhile, 20 g kg^{-1} CaC_2 increased sweetness and aroma in banana fruit based on the study by Nura et al. (2018). The present work aimed to evaluate physicochemical characteristics of NOR tomato line MA 131-6-3 during postharvest treated with ET and CaC_2.

MATERIALS AND METHODS

Experimental procedure

The tomato fruit was produced in Hargobinangun Village, Pakem Sub-district, Sleman District, Yogyakarta Province, Indonesia with 715 m above sea level (asl) (7°36'52.7"S 110°25'43.3"E) from May to September 2021. Location has temperature of 25.08±2.11°C, Relative Humidity (RH) of 75.48±3.89%, sunlight intensity of 33407.31±1505.48 lx, and wind speed of 3.49±0.24 m s^{-1}. Soil contained nitrogen total of 0.32±0.00%, phosphate availability of 11.08±0.00 ppm, potassium exchange of 0.15±0.00 me%, calcium exchange of 3.97±0.00 me%, magnesium exchange of 0.66±0.00 me%, and pH of 5.70±0.85. The location of storage and application of treatment was made in Sidoarum Village, Godean Sub-district, Sleman District, Yogyakarta Province, Indonesia with 125 m asl (7°46'50.6"S 110°19'01.9"E), temperature of 28.30±1.75°C and RH of 60.69±2.33%.

The tomato seeds of NOR tomato line MA 131-6-3 were obtained from Plant Breeding Laboratory, Department of Agronomy, UGM. Tomato seeds were sown and then grown at 21 Days After Seedling (DAS) into a 40 cm × 40 cm polybag individually. The growing media used were regosol soil, compost, and rice husk ash (3:1:1). Compost given was 2 ton ha^{-1} before planting, while 15 g $plant^{-1}$ NPK Mutiara (N 16%, P 16%, K 16%) fertilizer was applied five times 10, 20, 30, 40, and 50 Days After Planting (DAP), just as Kalsi gro 98 15 g $plant^{-1}$ was applied once time at 60 DAP. Watering on plants was carried out for two to three days. The pest and disease control are carried out once a week with foliar spray Dithane M-45 80 WP and Curacron.

Experimental design

The treatments consisted of ET (375, 750, 1,125, and 1,500 ppm), CaC_2 (5, 10, 15, and 20 g kg^{-1}), and control. This research follows the preliminary study from Khairi et al. (2022) with 1,500 ppm ET, 20 g kg^{-1} CaC_2, and control. The design for experimental was arranged in Randomized Completely Block Design with three replications. Fruit storage is placed at ambient temperature. The treatments were applied once when the fruit had been harvested at the age of 80, 85, and 90 DAP (age of harvesting as block). ET was applied by mixing ET at various treatment concentrations in 1 L of water in a bucket. The fruits were dipped for 15 minutes (Dhall and Singh 2013). While the CaC_2 with different dose were placed in each jar. The 3 L of jar were used to store 27 fruit jar^{-1}. The characteristics of the fruit used in this study were fruit clean, non-cracking fruit, not infected with pathogens, and fruit weight of 40±20 g $fruit^{-1}$.

Physicochemical analyses of tomato

Fruit firmness

The instrument used was Penetrometer Bareiss Prüfgerätebau GmbH type BS 61 II. Four fruit samples were used to measure the fruit firmness expressed in Newton (N).

Fruit color

Fruit color was determined using Chromameter CR-400 and the color coordinates L*, a*, and b* were measured on all five sides of the fruit surface. The measurements are also carried out at the fruit surface top, middle, and bottom. Fruit color is expressed as a color value by the Commission International de L'Eclairage with L*, a*, and b* coordinates (Khan et al. 2016). L* is brightness level, with a larger range of 0-100 indicating a brighter level. a* is range -128-127, where value of a*(-) indicates sample is getting greener, value a*(+) indicates sample is getting redder. b* has a range of -128-127, where values of b*(-) indicate sample is getting bluer, values of b*(+) indicate sample is getting yellow.

pH, physiological weight loss (PWL), and rotting

pH testing using pH Meter Apera Instruments PH700 Benchtop. The next time, PWL and rotting are expressed in percentage, the formula for calculating these variables was as follow (Dhall and Singh 2013):

$$\text{PWL (\%)} = \frac{A-B}{A} \times 100 \quad \text{and} \quad \text{Rotting (\%)} = \frac{R}{T} \times 100$$

Where A is the fruit weight at 0 day after treatment, B is the fruit weight at each sampling time, R is number of rotting, and T is number of fruit.

Lycopene content and sugar reduction

Lycopene content was analyzed by following the method of Suwanaruang (2016) which has been modified, with the following formula:

$$\text{Lycopene (mg kg}^{-1}) = \frac{\text{Absorbance 503 nm} \times 537 \times 8 \times 0.55}{0.1 \times 172}$$

Where: 537 g mole^{-1} is molecular weight of lycopene content, 8 mL is volume of mixed solvent, 0.55 is the ratio of the volume of the top layer to the mixed solvent, 0.1 g is weight of added tomato, and 172 mM^{-1} is coefficient of lycopene loss in hexane.

The reduction sugar content was analyzed using a UV-Visible Spectrophotometer with a wavelength of 540 nm and the blank was filled with distilled water. Connecting the regression of glucose concentration with absorbance obtained Y = 7.8618X + 0.0034. The sample concentration was calculated following the glucose standard curve regression equation. Reduction sugar was analyzed by following the method of Romadhoni et al. (2017) which has been modified, with the following formula:

$$\text{Reduction Sugar (\%)} = \frac{C \times DF}{g} \times 100$$

Where Y is Spectrophotometer value, X is glucose concentration, C is sample concentration (X value), DF is dilution factor, and g is weight of the sample used

Respiration and ethylene

The method of respiration using titration from da Costa Nascimento et al. (2019) has been modified. The formula for calculating respiration was as follow:

$$\text{Respiration (mg kg}^{-1}\text{ h}^{-1}) = \frac{11\,(X-Y)}{24}$$

Where X is the volume of HCl without fruit and Y is volume of HCl with fruit.

Endogenous ethylene analysis method with Gas Chromatography-Mass Spectrometry (GC-MS). The fruit samples were put into the jar (750 mL) has been modified and one sample only consisted of one fruit. Then in a vacuum and incubated for 1 h using acetylene gas. Fill the incubation gas with as much as 10% of the jar volume. After 1 h, 1 mL of GC-MS was injected. After that, the result is a chromatogram.

Statistical analysis of data

The method of statistical analysis used is Analysis of Variance (ANOVA). Before ANOVA testing was carried out with the assumption of a normal distribution and homogeneity. If there is a significant difference, then a post-hoc test with Tukey's Honestly Significant Difference (HSD) $\alpha = 0.05$. Software is used for data analysis are JMP v.16 and SAS® OnDemand for Academics (ODA) via a web browser (https://welcome.oda.sas.com/login). Data shown in the figures and tables are mean ± Standard Deviation (SD).

RESULTS AND DISCUSSION

Lycopene content, reduction sugar, respiration, and ethylene

All treatments given to NOR tomato had no effect to lycopene content of fruit at 5 Days After Treatment (DAT) (Table 1). As consequence, it is no change color fruit from green, then the lycopene content is 0 mg kg^{-1} at all treatment and control. AR treatments (ET and CaC_2) were able to increase the reduction of sugar. Reduction sugar content of the fruit treated by 20 g kg^{-1} CaC_2 was significantly higher than control at 5 DAT. The treatment of 20 g kg^{-1} CaC_2 response at 5 DAT was also significantly higher in respiration. AR treatments to NOR tomato gave a positive response to the increase in ethylene in all observations, except 375 ppm ET. Furthermore, 15 g kg^{-1} CaC_2 was significantly higher than other treatments. At the beginning of the observation (5 DAT), the response of 375 ppm ET was lower than control, suggesting an adjustment of the ethylene formation mechanism in the fruit.

Fruit firmness

All treated and control experienced a gradual decrease in fruit firmness and did not show significant differences between treatments until the third observation (15 days after treatment) (Figure 1). The mean of all treatments (ET and CaC_2) for fruit firmness treated at 5 to 25 DAT were 29.23, 21.04, 15.23, 9.61, and 3.72 N. At 20 DAT, the fruit firmness of control and treatment 1,125 ppm ET of 18.07 and 16.58 N respectively were significantly higher than 10 and 15 g kg^{-1} CaC_2 (lowest of 3.75 and 1.63 N) (Figure 1). Before 20 DAT, the fruit firmness was comparable between treated fruit as well as control. NOR tomato fruit can be stored for a long-time postharvest more than 15 DAT.

Table 1. Lycopene content, reduction sugar, respiration, and ethylene of NOR tomato fruit influenced by different concentrations of ET or doses of CaC_2 at 5 DAT

Treatments	Lycopene content (mg kg^{-1})	Reduction sugar (%)	Respiration (mg kg^{-1} h^{-1})	Ethylene (µmole g^{-1} h^{-1})
Control	0.00 ± 0.00	0.84 ± 0.01 b	0.46 ± 0.00 b	0.0044 ± 0.0000 h
Ethephon				
375 ppm	0.00 ± 0.00	0.91 ± 0.01 b	0.46 ± 0.00 b	0.0038 ± 0.0000 i
750 ppm	0.00 ± 0.00	0.93 ± 0.00 ab	0.46 ± 0.00 b	0.0909 ± 0.0000 c
1,125 ppm	0.00 ± 0.00	1.07 ± 0.30 ab	0.46 ± 0.00 b	0.1011 ± 0.0000 b
1,500 ppm	0.00 ± 0.00	1.15 ± 0.04 ab	0.46 ± 0.00 b	0.0272 ± 0.0000 g
Calcium Carbide				
5 g kg^{-1}	0.00 ± 0.00	0.95 ± 0.07 ab	0.46 ± 0.00 b	0.0342 ± 0.0000 f
10 g kg^{-1}	0.00 ± 0.00	0.97 ± 0.05 ab	0.46 ± 0.00 b	0.0349 ± 0.0000 e
15 g kg^{-1}	0.00 ± 0.00	1.00 ± 0.08 ab	0.46 ± 0.00 b	0.2055 ± 0.0000 a
20 g kg^{-1}	0.00 ± 0.00	1.23 ± 0.05 a	0.50 ± 0.00 a	0.0438 ± 0.0000 d

Note: The number followed by the same letter on the variables has no significant difference based on Tukey's HSD test $\alpha = 0.05$ (n = 3)

Fruit color

Color and visuals of tomato that were given different treatments resulted in different ripeness. At 5 DAT, all fruit treated by ET and CaC_2 started to turn turning from a dark green color. In the range of 5-25 DAT, the control fruit showed insignificant color changes. At 10 DAT, the exogenous ethylene treated fruits were more responsive to the formation of color indicating a turning was more dominant than the control. All treatments applied to NOR tomato fruit had no effect on formation to the red or yellow color as commonly ripe tomato color. Visually, the condition of color development is quite homogeneous between concentrations of ET (375, 1,125, and 1,500 ppm) and doses of CaC_2 (15 and 20 g kg^{-1}). These five treatments were able to provide more attractive fruit color development, especially at the end of the observation (25 DAT) whereas compared to other treatments.

Fruit color is determined by L*, a*, and b*. In L*, tomato fruit was given with 10 g kg^{-1} CaC_2 resulted in the lowest at 10 DAT (60.22) and the treatment of 750 ppm ET resulted in the higher at 20 DAT (70.81) (Figure 3A). All treatments showed a significant difference at 10-20 DAT. ET concentrations (375 and 750 ppm) respond to an increase in vivid color at 10-20 DAT. NOR tomato can be increased vivid color if it starts at 10 DAT and continues to give a positive response up to 20 DAT. After that, AR response did not occur significantly.

The a* indicated redness (+) to greenness (-). All fruit showed a* negative value that implied all fruits were green. In a*, tomato fruit treated with 10 g kg^{-1} CaC_2 was significantly lowest (greenest) at 10 DAT and 375 ppm ET was significantly higher at 15 DAT (Figure 3B). Concentration 375 ppm ET always caused the highest response, meanwhile 10 g kg^{-1} CaC_2 was the lowest (negative) in the formation of red color in NOR tomato. which took place at 10-15 DAT. Based on this result of a*, there was no change in the fruit color from green to red. The response was seen significantly during 10 DAT and ended up giving a response 15 DAT.

In b* (yellow (+) to blue (-)), the value all of fruit increased gradually from 5-25 DAT. It implied the color was changing to yellow. The control fruit was significantly lowest at 15 DAT of 33.57 and contrary in 375 ppm ET was significantly highest at 25 DAT of 61.17 (Figure 3C). At 20 and 25 DAT, 750 ppm ET gave significantly different responses to the control.

Physiological Weight Loss (PWL)

PWL in all of the control and treated fruit increased from 5-25 DAT (Table 2). The dose of 10 g kg^{-1} CaC_2 always produced the highest response compared to 1,125 ppm ET.

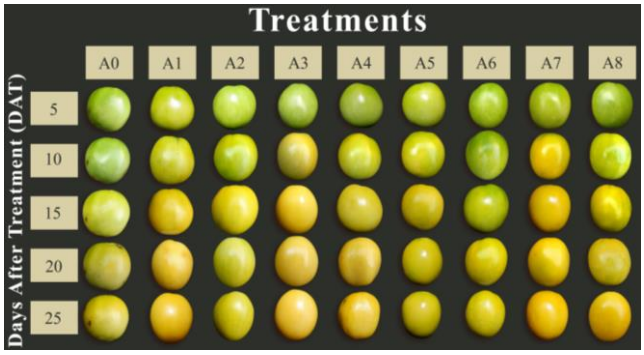

Figure 2. Effect of different concentrations of ET or doses of CaC_2 on fruit color development and ripening at ambient temperature. Note: A0: control, A1-A4: 375, 750, 1,125, and 1,500 ppm ET, A5-A8: 5, 10, 15, and 20 g kg^{-1} CaC_2

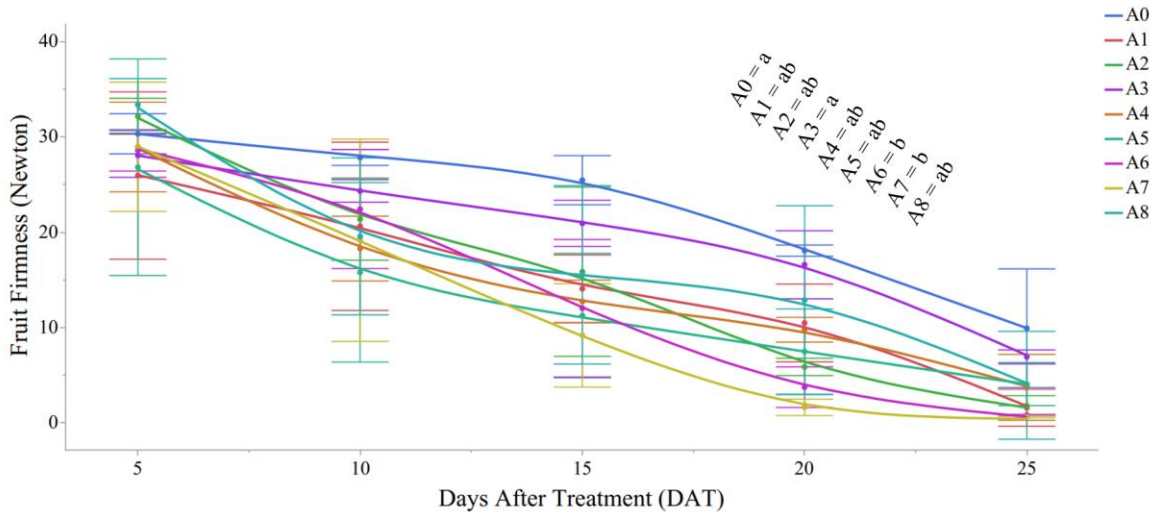

Figure 1. Fruit firmness of NOR tomato fruit influenced by different concentrations of ET or doses of CaC_2 observed at 5-25 DAT. Note: A0: control, A1-A4: 375, 750, 1,125, and 1,500 ppm ET, A5-A8: 5, 10, 15, and 20 g kg^{-1} CaC_2, lines graph followed by the same letter on the same Days After Treatment (DAT) have no significant difference based on Tukey's HSD test $\alpha = 0.05$ (n = 3)

Rotting

Rotting of NOR tomato increased by time in all treatments of ET or doses of CaC_2. Treatment of 1,125 ppm ET resulted in the significantly highest increased rotting response (Table 3) at the beginning of the observation (5 DAT). In fact, the dose of CaC_2 resulted in the lowest value at increasing rotting. Significantly different increase in rotting took place at the beginning of the observation only, and thereafter was no significant. Until the last observation at 25 DAT, the fruit of control had the lowest rooting fruit. The treatment of control had a significantly lower effect rotting compared to 1,125 ppm ET, and then the fruit damage that occurred to the control was less. The NOR tomato fruit is suitable as immature fruit for vegetable or raw tomato sauce which is trending in recently.

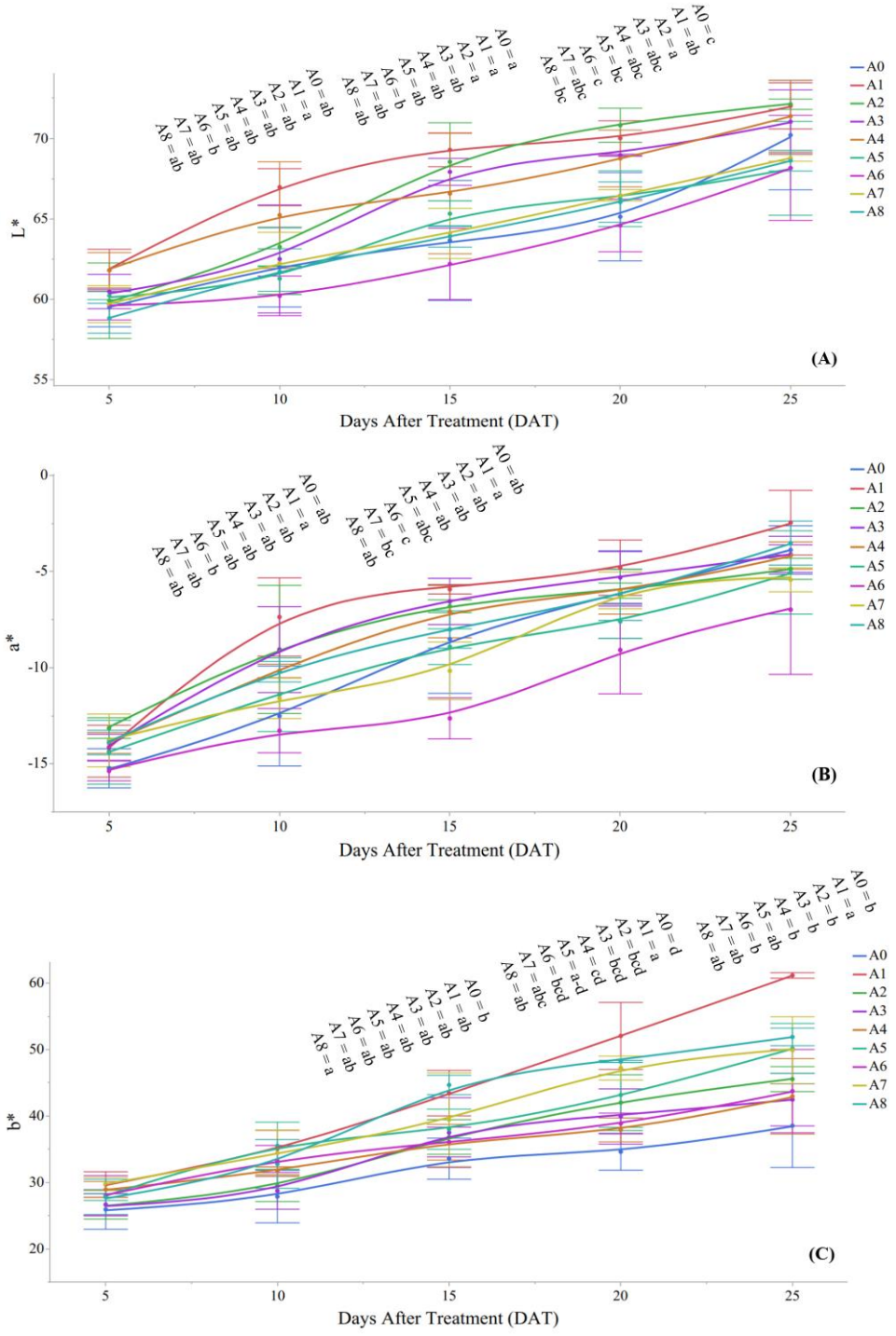

Figure 3. NOR tomato fruit influenced by different concentrations of ET or doses of CaC2 at L* (Figure 3A), a* (Figure 3B), b* (Figure 3C). Note: A0: control, A1-A4: 375, 750, 1,125, and 1,500 ppm ET, A5-A8: 5, 10, 15, and 20 g kg^{-1} CaC_2, lines graph followed by the same letter n the same Days After Treatment (DAT) have no significant difference based on Tukey's HSD test $\alpha = 0.05$ (n = 3)

Table 2. PWL of NOR tomato fruit influenced by different concentrations of ET or doses of CaC_2

Treatments	Physiological Weight Loss (%)				
	5 DAT	10 DAT	15 DAT	20 DAT	25 DAT
Control	0.26±0.03 ab	0.66±0.06 ab	1.05±0.10 abc	1.32±0.11 ab	1.55±0.13 abc
Ethephon					
375 ppm	0.21±0.04 ab	0.60±0.06 ab	0.98±0.07 abc	1.21±0.13 ab	1.42±0.11 bc
750 ppm	0.21±0.05 ab	0.62±0.10 ab	1.01±0.16 abc	1.25±0.11 ab	1.53±0.09 abc
1,125 ppm	0.13±0.03 b	0.45±0.05 b	0.77±0.11 c	1.04±0.10 b	1.35±0.06 c
1,500 ppm	0.14±0.04 b	0.49±0.07 b	0.79±0.05 bc	1.15±0.15 ab	1.47±0.14 bc
Calcium carbide					
5 g kg^{-1}	0.20±0.05 ab	0.61±0.11 ab	0.92±0.12 abc	1.20±0.07 ab	1.40±0.07 bc
10 g kg^{-1}	0.37±0.07 a	0.95±0.15 a	1.32±0.20 a	1.56±0.27 a	1.94±0.33 a
15 g kg^{-1}	0.34±0.03 a	0.87±0.05 a	1.23±0.07 ab	1.63±0.09 a	1.92±0.06 a
20 g kg^{-1}	0.23±0.13 ab	0.67±0.26 ab	1.14±0.29 abc	1.48±0.26 ab	1.80±0.15 ab

Note: The number followed by the same letter on the same days after treatment (DAT) has no significant difference based on Tukey's HSD test $\alpha = 0.05$ (n = 3)

Table 3. Rotting of NOR tomato fruit influenced by different concentrations of ET or doses of CaC_2

Treatments	Rotting (%)				
	5 DAT	10 DAT	15 DAT	20 DAT	25 DAT
Control	2.43±2.12 ab	6.10±7.37	7.29±9.37	7.29±9.37	7.29±9.37
Ethephon					
375 ppm	3.66±0.08 ab	10.21±5.95	15.29±7.84	31.77±8.49	44.35±3.13
750 ppm	2.22±3.85 ab	13.81±11.98	28.00±22.40	38.25±8.53	45.76±5.70
1,125 ppm	12.09±8.78 a	28.50±17.06	36.50±19.40	40.30±18.90	48.06±10.19
1,500 ppm	1.19±2.06 b	6.36±8.03	11.40±10.28	21.46±14.84	36.32±12.26
Calcium carbide					
5 g kg^{-1}	0.00±0.00 b	15.02±15.40	22.25±19.30	24.64±21.50	31.78±16.26
10 g kg^{-1}	1.33±2.31 b	1.39±2.41	3.77±3.59	7.34±9.34	21.82±30.90
15 g kg^{-1}	1.11±1.92 b	6.09±7.75	22.29±17.13	30.94±22.90	37.30±24.80
20 g kg^{-1}	0.00±0.00 b	0.00±0.00	4.89±2.18	8.47±5.37	19.22±20.80

Note: The number followed by the same letter on the same days after treatment (DAT) has no significant difference based on Tukey's HSD test $\alpha = 0.05$ (n = 3)

Table 4. pH of NOR tomato fruit influenced by different concentrations of ET or doses of CaC_2

Treatments	pH				
	5 DAT	10 DAT	15 DAT	20 DAT	25 DAT
Control	3.30±0.00 b	3.47±0.06 b	3.90±0.00 b	4.17±0.06 b	4.27±0.21 b
Ethephon					
375 ppm	3.50±0.00 ab	3.70±0.00 ab	4.13±0.06 ab	4.33±0.06 ab	4.53±0.06 a
750 ppm	3.50±0.00 ab	3.57±0.06 ab	4.13±0.06 ab	4.37±0.06 a	4.53±0.06 a
1,125 ppm	3.53±0.06 ab	3.60±0.00 ab	4.13±0.06 ab	4.33±0.06 ab	4.50±0.00 ab
1,500 ppm	3.60±0.10 a	3.73±0.06 a	4.13±0.06 ab	4.37±0.12 a	4.50±0.00 ab
Calcium carbide					
5 g kg^{-1}	3.50±0.10 ab	3.57±0.06 ab	4.03±0.06 ab	4.30±0.00 ab	4.50±0.00 ab
10 g kg^{-1}	3.57±0.06 ab	3.67±0.15 ab	4.17±0.15 a	4.40±0.00 a	4.57±0.06 a
15 g kg^{-1}	3.57±0.12 ab	3.80±0.17 a	4.17±0.15 a	4.37±0.06 a	4.57±0.06 a
20 g kg^{-1}	3.63±0.21 a	3.80±0.00 a	4.27±0.06 a	4.47±0.12 a	4.60±0.00 a

Note: The number followed by the same letter on the same Days After Treatment (DAT) has no significant difference based on Tukey's HSD test $\alpha = 0.05$ (n = 3)

pH

Treatment of 20 g kg^{-1} CaC_2 resulted in the significantly highest value of fruit pH during DAT compared to control at NOR tomato (Table 4). Increasing the pH can be applied to the 20 g kg^{-1} CaC_2 treatment during postharvest. Almost all days of observation given AR showed the best response, however 20 g kg^{-1} CaC_2 persisted.

Discussion

The significant increase in respiration occurs at the beginning of storage (5 DAT). Treatment of 20 g kg^{-1} CaC_2 can increase up to 8.70% compared to control. The tomato fruit reaction to 20 g kg^{-1} CaC_2 and ambient temperature support increased cellular respiration, while ET was not affected respiration. This result is not in line with studies of

Li et al. (2014) indicated ET can increase respiration. The study of Li et al. (2014) reported that Ailsa Craig (AC) control compared AC with 100 µL L^{-1} ET, in respiration of 5.83 and 32.27 mg kg^{-1} h^{-1}, respectively. Increasing respiration with ET treatment is effective for tomato fruit ripening. In addition, several methods can be used to inhibit respiration with 1-Methylcyclopropene (MCP), in addition to the use of NOR or RIN tomato as reported by Xu et al. (2016). The study of Xu et al. (2016) reported that 1.2 µL L^{-1} 1-MCP treatment could inhibit the increase in respiration by 51% compared to control in hybrid tomato. The study is in line with the study conducted by da Costa Nascimento et al. (2019) on banana fruit 50 g CaC_2, which showed the highest value compared to control at 85.4 mg kg^{-1} h^{-1} in respiration. Starch degradation and hexose oxidation produce energy (ATP) for the respiration process in climacteric fruit caused by phosphofructokinase activity (da Costa Nascimento et al. 2019). CaC_2 produces exogenous ethylene which causes an increase in the activity of the enzyme synthase and oxidase of ACS and ACO, accordingly that it can induce respiration in NOR tomato.

The increased of flavor and pigmentation during ripening of tomato fruit is caused by Lipoxygenase C (LOXC) and Phytoene Synthase1 (PSY1), in this pathway encoding enzymes that catalyze the production of polyunsaturated fatty acids and lycopene precursors (Wang et al. 2020b). NOR tomato have not been able to synthesize lycopene at harvest up to 5 DAT. ET and CaC_2 cannot generate the lycopene (0 mg kg^{-1}) in all fruit as shown in Table 1. Siddiqui et al. (2016) reported that lycopene content in RIN tomato peel (BCT-11 genotype) of 0.26 mg/100 g. The results of these studies were reported to be lower than the administration of CaC_2 in NOR tomato. Dhall and Singh (2013) reported that application of 1,500 ppm ET on hybrid tomato can increase lycopene compared to control. The correlation of lycopene with the red color of tomato is usually very strong, but NOR tomato do not show the strength of the relationship. NOR tomato did not indicate a red color change and when viewed from the lycopene content (Table 1) at 5 DAT, it was not detected. The reason is that the carotenoid biosynthetic pathway that produces lycopene is inhibited due to a mutation in the PSY1 gene. At the moment, lycopene synthesis will not process normally and there will be an accumulation of phytoene precursors which will eventually turn the color of the tomato into turning, and not turn red (Chattopadhyay et al. 2021). The color of the tomato at the end of storage was turning, as in therefore the result of flavonoid accumulation was suspected (Orsi et al. 2021).

Treatment of 20 g kg^{-1} CaC_2 increased in reduction sugar compared to control at 5 DAT (46.43%). CaC_2 contributed significantly to increase the reduction of sugar at the time storage of NOR tomato fruit. The correlation data between reduction sugar and pH at 5 DAT shows that the relationship is very strong, with a value of 81% (data not shown). The very strong correlation of these variables was due to the response of 20 g kg^{-1} CaC_2 in the physicochemical alter of the fruit at the beginning of the observation. The study reported by Ibrahim et al. (2017) that tomato cv. Bizly of 1.55%. This result is higher than the control at 5 DAT of 0.84%.

Ethylene is a plant hormone that regulates the ripening process in fruit and is synthesized from its precursor is 1-Aminocyclopropane-1-Carboxylic Acid (ACC) which is produced from S-Adenosyl-L-Methionine (SAM), furthermore catalyzed by ACC Oxidase (ACO) and ACC Synthase (ACS) (Wang et al. 2020b). Ethylene increase occurred in 15 g kg^{-1} CaC_2 by 97.86 at 5 DAT compared to control. CaC_2 can induce an increase in endogenous ethylene in NOR tomato. The results of this study are in line with the research conducted by da Costa Nascimento et al. (2019) on banana fruit 50 g CaC_2, which showed the highest value compared to the control at 61.9 ppm in ethylene production. Several studies using RIN tomato, such as Li et al. (2014) reported that ethylene was significantly lowest at 0.80 nL g^{-1} h^{-1} at 0 DAT. Xu et al. (2016) reported that the treatment of 1.2 µL L^{-1} 1-MCP could inhibit the action of ethylene formation by 62% compared to control in hybrid tomato. The increase in tomato fruit pigmentation during storage was caused by ethylene activity through induction of the carotenoid pathway and chlorophyll degradation (Wang et al. 2017; Li et al. 2020), accordingly that the green color of the NOR tomato will degradation (Figure 2). CaC_2 can alter fruit quality (aroma, color, taste, and texture), chlorophyll degradation, increase cell wall enzyme degradation activity, conversion of starch to sugar, carotenoid synthesis, increase respiration process, and endogenous ethylene synthesis (da Costa Nascimento et al. 2019).

Fruit ripening of NOR tomato

Tomato fruit ripeness can change the aroma, color, taste, and texture. Observation variables that describe fruit ripeness are fruit firmness and fruit color. Treatments of 10 and 15 g kg^{-1} CaC_2 were able to soften the fruit at 20 DAT. CaC_2 is more effective in causing softening of tomato fruit at ambient temperature than ET. Cell wall degradation causes a decrease in fruit firmness during storage caused by the breakdown of insoluble protopectin into soluble pectin or by cellular disintegration leading to membrane permeability. Furthermore, the fruit ripening process can also cause loss of pectin in the middle lamellae of the cell wall, resulting in fruit softening (Dhall and Singh 2016). The study by Li et al. (2014) reported AC control produces fruit firmness of 83.20 g force (0.82 N) at 42 DAT. RIN tomato control produces a significant fruit firmness of 222.54 g force (2.18 N) at 0 DAT. AC tomato fruit with 100 µL L^{-1} ET produces a significantly lowest fruit firmness of 53.66 g force (0.53 N) at 42 DAT. The next time, Xu et al. (2016) reported that the treatment of 1.2 µL L^{-1} 1-Methylcyclopropene (MCP) could significantly inhibit fruit softening by 33% compared to control on hybrid tomato, which was observed 20 DAT. Fruit firmness and susceptibility to pathogen attack are important factors in maintaining fruit shelf life during storage from the aspect of tomato breeding. Several important genes involved in cell wall modification that affect susceptibility to pathogen attack and fruit firmness during fruit storage are Polygalacturonase (PG), Pectate Lyase (PL), and

Cellulase2 (CELL2) (Uluisik et al. 2016; Wang et al. 2020b).

The increase of carotenoid synthesis and decreased chlorophyll were associated with color development in fruit. AR treatments can increase carotenoid synthesis and chlorophyll degradation by inducing metabolism of chlorophyllase enzyme synthesis which is responsible for alpha carotene pigment expression and chlorophyll degradation in fruit. The results of the study reported that it did not show a color alter to the red stage. The expressed color is turning in all treatments. But 15 g kg^{-1} CaC_2 showed a significant difference in turning (Figure 2). CaC_2 treatment is known to accelerate the degradation of chlorophyll or carotenoid synthesis by stimulating hydrolase enzyme, thereby producing lycopene content. There is no alteration in the color of the fruit to the red but only alters to turning from green. According to Wang et al. (2020a), the inhibiting action of the new protein on ethylene signal transduction is extremely strong that cannot be overcome by ET and CaC_2 and a new protein acts as a repressor. Khan et al. (2016) reported that the treatment given 1-Hexylcyclopropene (HCP) was brighter on hybrid tomato than the control, with a value of 51.89. 1-HCP is a ripening inhibitor compound similar to 1-MCP. The performance of ethylene in fruit can control gene expression by regulating the Ethylene Receptor (ETR) and a series of signal transduction that causes an increase in ripeness. Ethylene binds to the receptor resulting in inactivation of Constitutive Triple Response1 (CTR1). Inactivation releases repression of the ethylene response gene, leading to the ethylene response. Inhibitors inhibit ethylene performance by binding to ethylene receptors which act as negative regulators in ethylene signal transduction (Dhall and Singh 2016).

Physicochemical of NOR tomato

CaC_2 can increase PWL throughout storage of NOR tomato. The response given is more effective than ET. 10 g kg^{-1} CaC_2 (1.94%) and 15 g kg^{-1} CaC_2 (1.92%) were significantly the highest value (Table 2). Several cases also mention that PWL can be suppressed by using 1-MCP derived compounds as reported by Khan et al. (2016). The results reported that 500 nL L^{-1} 1-HCP could inhibit PWL of 9.85% in hybrid tomato fruit. Several cases of postharvest research with tomato were studied from the acceleration or inhibition of ripening with different responses. At that moment, some authors also use tomato that is difficult to ripen. Tomato fruit has a high content of water, as in therefore the transpiration process is strongly correlated with PWL during storage (Orsi et al. 2021).

The ET response in increasing the rotting of NOR tomato was significant at 5 DAT only. The reaction produced by ET and water will cause an increase in rotting caused by pathogenic activity. The highest rotting in ET was probably due to direct contact of fruits with water consequently that some invisible cuts and bruises on surface of fruit could absorb water during immersion which became entry point for pathogen infections by *Botrytis cinerea* (Akter et al. 2019).

pH increased throughout postharvest with NOR tomato. Significant improvement was found in AR treatments compared to control on all observation days (DAT). Adding AR can cause an increase in pH in NOR tomato and change the sour taste to neutral or sweet. This study did not examine the organoleptic properties of NOR tomato, accordingly the taste and aroma of the fruit could not be specifically assessed. However, according to Xi et al. (2016) almost all organic acids will decrease in the fruit ripening process consequently that the sensory attributes (sweetness, sourness, aroma, flavor, and acceptability) become better. The results of this study are not in line with the research conducted by da Costa Nascimento et al. (2019) on banana fruit is 50 g CaC_2, showed the lowest value compared to the control at 4.5 in pH.

In conclusion, ET and CaC2 cannot fruit color altered NOR tomato from green to red during postharvest. The CaC_2 only increased to physicochemical of tomato fruits such as pH, respiration, ethylene, and others. The genetics of NOR tomato did not show alter from green to red stage, but only turning for all treatments.

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