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Variability of fruit shelf-life of commercial tomatoes and their hybrids crossing with insensitivity ethylene mutant tomato *Sletr1-2*

Gungun Wiguna¹, Nedya Putri Bachtiar², Anas³, Noladhi Wicaksana³, Syariful Mubarok³, and Imas Rita Saadah^{1*}

¹Research Center for Horticultural and Estate Crops, Research Organization of Agriculture and Food, National Research and Innovation Agency (BRIN)

Jl. Raya Jakarta – Bogor, Cibinong, Kabupaten Bogor 16915, Indonesia

²Graduates of the Department of Crop Science, Faculty of Agriculture, Universitas Padjadjaran

Jl. Raya Bandung – Sumedang KM 21, Jatinangor, Kabupaten Bandung 45363, Indonesia

³Department of Crop Science, Faculty of Agriculture, Universitas Padjadjaran

Jl. Raya Bandung – Sumedang KM 21, Jatinangor, Kabupaten Sumedang 45363, Indonesia

*Corresponding author: imas005@brin.go.id

Article Info	Abstract
Received : 3 rd March 2023 Revised : 14 th July 2023 Accepted: 10 th January 2024	Tomatoes are climacteric fruits that experience a surge in respiration rate and ethylene production. This condition affects the fruits' physiological deterioration, shortens their shelf-life, and degrades the quality of tomatoes. The study aimed to enhance
Keywords : Fruit ripening, genetic, phenotypes, shelf-life, <i>Sletr1-2</i>	the shelf-life of commercial tomatoes with different genetic backgrounds by utilizing the <i>Sletr1-2</i> mutant, which has a lower sensitivity to ethylene. A randomized block design (RBD) was applied to achieve this goal, where 12 different plant genotypes were considered as treatments, and each treatment was replicated four times. The results showed that fruit shelf-life was extended by crossing commercial tomatoes with <i>Sletr1-2</i> mutant tomatoes. Genetic variability for all shelf-life characteristics were narrow. In contrast, there were broad phenotypic variability for fruit hardness and weight loss characteristics.

INTRODUCTION

Tomatoes are climacteric fruits, meaning that during the ripening process, there is a surge in respiration rate (Paul and Pandey, 2014; Colombié et al., 2017) and ethylene production (Liu et al., 2015; Li et al., 2020; Iqbal et al., 2017). Increased respiration rate can impact physiological damage where aging or decay occurs, decreasing shelf-life and maturation. At the same time, increasing ethylene affects its nutrient content and can accelerate fruit ripening. Both processes also impact tomatoes' quality during storage (Wicaksana, Wijaya, and Soeparjono, 2019).

Additionally, the biochemical and physiological changes during the ripening process will cause changes in the fruit's taste, color, flavor, and texture (Wang et al., 2018; Quinet et al., 2019), thereby reducing its

quality. Short shelf-life in open or unpacked conditions is also one of the causes of the low quality of tomatoes. Therefore, proper handling to suppress the rate of respiration and ethylene needs to be done to extend shelf-life and produce quality tomatoes (Deepika and Rex, 2020; Pholoma, 2020).

The Indonesian Vegetable Research Institute has released several high-yielding tomato varieties that have become popular for public consumption, such as Intan, Mirah, Ratna, and Mutiara varieties. Even though these four varieties are superior and widely used by farmers, they have disadvantages because of their short shelf-life. Tomato fruit has a short shelf-life of 1 to 2 weeks after harvest. In some commercially advanced varieties released and circulated in Indonesia, the most extended shelf-life of fruit at room temperature may be up to 20 days, with a mean

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of about seven days (Wicaksana et al., 2019). Even in a single hybrid variety, the shelf-life is only eight days (Rachma, 2013). The short shelf-life of tomatoes is because, since harvest, tomatoes will continue to undergo physiological processes that result in several important biochemical reactions, such as changes in metabolites, color, texture, and taste (Amin et al., 2012). This reaction results in a decrease in both the quantity and quality of fruit. Due to these issues, it is necessary to improve commercial tomato varieties to prolong their shelf-life and preserve the quality of the fruit, one of which is through breeding programs.

Breeding programs that can be carried out are hybridization techniques to combine the good qualities derived from two or more parents to obtain superior offspring (Sharma et al., 2019). The existence of crosses with genetic backgrounds of different parents can produce diverse offspring. Several researches have been done to generate tomatoes with a long shelf-life resulting from mutation (Casals et al., 2012; Yogendra and Gowda, 2013). However, many studies have also reported that tomato varieties from mutations or mutant tomatoes are generally less tasty than local varieties. An example of a mutant tomato is Sletr1-2 tomatoes produced from the Micro-Tom library, which can delay the normal ripening process (Okabe et al., 2012) and decrease the susceptibility to ethylene (Mubarok et al., 2019). Sletr1-1 along with Sletr1-2, are the novel mutant alleles which are isolated from an ethylene receptor gene, Solanum lycopersicum ETHYLENE RESPONSE 1 (SIETR1), taken from a Micro-Tom Library mutant population (Okabe et al., 2011).

The appearance of a plant is the result from interactions between plant genotypes and the environment in which they grow. Crowder (1993) states that appearance can be different or change due to the interaction between one gene and another. The four genotypes used, namely Intan, Mirah, Ratna, and Mutiara, have different genetic backgrounds. The

Intan tomato was resulted from a cross between Nagcarlan and Anahu (introduced by AVRDC Taiwan). The Mirah variety was generated from Malang (tomatoes PB). Ratna was resulted from a cross between Nagcarlan and Anahu (introduction from BPI Philippines). Meanwhile, Mutiara tomato was generated from a cross between Monaibo and Venus. Since genetic background plays a crucial role in determining the appearance of tomatoes, this study aimed to investigate the variability in the formation of commercial tomatoes and their F1 hybrids resulting from crosses with Sletr1-2 mutant tomatoes. Information on alteration and variability in fruit shelf-life characteristics due to the introduction of the *Sletr1-2* mutant is currently lacking in Indonesia. Meanwhile, this is needed as very valuable and helpful information for future tomato breeding programs.

MATERIALS AND METHODS

The equipment used in this study includes calipers, ruler, digital scales, erlenmeyer, magnetic stirrer, pH meter, spectrophotometer, penetrometer, refractometer, measuring cup, pipette, test tube, volumetric flask, color chart, and knife. Meanwhile, the materials used in this study were four commercial parents (Intan, Mirah Ratna, and Mutiara) and 8 F1 plants as progeny between commercial tomato varieties as female parents and tomato mutants (*Sletr1-2* and wild-type) as male parents. Other materials used are distilled water, 1% indicator Phenolphthalein (PP), and 0.1N NaOH.

The experimental design used was a randomized block design (RBD) with a factor in genotype (G). The experiment consisted of 12 treatments, each with four replications. The effect of each treatment was analyzed using Analysis of Variance (ANOVA). Data showing significant differents between treatments were then analyzed using the Scott-Knott post-hoc

Table 1. Main fruit characteristics of four commercial tomatoes

Genotype	Fruit shape	Number of locules	Weight per fruit (g)	Fruit length (cm)	Fruit diameter (cm)	Pericarp thickness (cm)
Intan	Oblong	3 – 9	40 - 65	3.2 – 4.9	3.8 - 5.0	0.3 - 0.4
Mirah	Oblate	3 – 7	20-40	2.1 - 4.1	3.0 - 4.0	0.2 - 0.3
Ratna	Oblong	4 – 8	45 – 85	3.5 – 5.7	5.0 - 6.0	0.3 - 0.4
Mutiara	Oval	3 – 6	35 – 50	3.0 - 4.4	3.5 – 4.5	0.3 – 0.5

Remarks: The data are summarized from variety descriptions (Anas et al., 2022).

comparison.

Genetic variability ($\sigma^2 g$) and phenotypic variability ($\sigma^2 f$) can be obtained based on the analysis of variance. Determination of criteria for genetic and phenotypic variability is done by comparing genetic and phenotypic variability value if the value is equal to or twice the genetic standard deviation ($\sigma^2 g \ge 2\sigma_{\sigma^2 g}$). The same applies to phenotypic variability. Phenotypic variability is broad if the phenotype values are the same or greater than twice the standard deviation ($\sigma^2 f \ge 2\sigma_{\sigma^2 f}$). The standard deviation of genetic variance and phenotype is assumed by the following formulas (Anderson and Bancroft, 1952):

Remarks:

 $\sigma_{\sigma^2 g}$ = standard deviation of genetic variance $\sigma_{\sigma^2 f}$ = standard deviation of the phenotypic variance

MSg = genetic mean square

MSe = error mean square

 df_q = genetic degrees of freedom

r = replication

The experiment was carried out using tomatoes planted in the Indonesian Vegetable Research Institute Experimental Field at an altitude of 1,250 m asl with andosol soil types. Planting was done in an area of 100 m². Tomato plants were grown on land with genotype as factor consisting of 12 treatments and four replications. Each replication in one treatment consisted of five plants, resulting in a total of 240 experimental units.

Yields from planting were used as testing samples. The fruit was harvested when the maturation stage is Br+6 (Mubarok et al., 2015) to determine the character of the shelf-life of each treatment. Character analysis was carried out at the Physiology of Postharvest Laboratory, the Indonesian Vegetable Research Institute, Lembang. Observations were made by observing and analyzing the character of the storability of tomatoes for a shelf-life of 0 days, a shelf-life of 10 days, and a shelf-life of 20 days. The sampling method for one replication in one genotype was by combining the yields in each replication, and then ten pieces were taken for the needs of the entire quality analysis. The treatment in one observation was repeated three times.

Determining long shelf-life involved monitoring the fruit from the start of storage until its quality deteriorates, indicated by the emergence of black spots or wrinkles on the fruit surface. Measurement of fruit hardness was done using a penetrometer. The sample used in each genotype is four pieces to get ten stabbing times. The way to do this is to put tomatoes just below the penetrometer piercing needle. The tomatoes were stabbed at three points on their surface (tip, middle, and base), and the duration required to exert maximum pressure on the tomatoes was measured using a stopwatch for 10 seconds. A ballast with a weight of 50 grams was used during the test. The calculation results were the average numbers obtained from the measurements. The units used are millimeters (mm) per 10 seconds with a specific load weight expressed in grams or mm/second/gram.

Weight loss during storage was measured on the ten days post storage (DPS) and 20 DPS during storage by weighing tomatoes. The level of water loss indicates the weight loss from tomatoes during the storage period because only the water can evaporate during the storage process. The formula used is as follows:

Loss of water = $\frac{(a-b)}{a} \times 100\%$ (3)

Remarks:

a = weight before storage or initial weight b = weight after being stored

RESULTS AND DISCUSSION

Fruit freshness

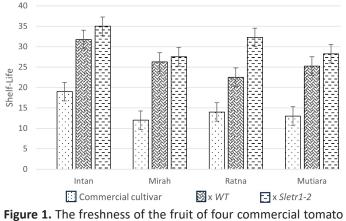
The analysis results showed that Intan, Mirah, Ratna, and Mutiara's four commercial parents have a shorter shelf-life than the tomatoes from crossing with the *Sletr1-2* mutant (Figure 1). This result is in accordance with Afifah et al. (2021), comparing some commercial tomato varieties, including Intan and Ratna varieties, with a mutant breeding line, where this line showed a better performance in shelf-life. According to the findings, Intan x *Slert1-2* hybrids have the longest average shelf-life (35 days), while Mirah, Ratna, and Mutiara hybrids have the shortest average shelf lives (12, 14, and 13 days, respectively). The results of crosses between commercial tomatoes and mutant *Sletr1-2* tomatoes differ from the commercial parents. This result shows that the *Sletr1-2* mutant allele influences the shelf-life of the crosses from tomatoes. The effect given by the mutant allele on tomatoes from crosses is a positive influence that can increase the shelf-life of tomatoes. Hiwasa-Tanase (2016) states that *Sletr1-2* can extend tomatoes' fruit shelf-life. *Sletr1-2* can maintain fruit surface intact even 60 days after harvest. The ability of mutant alleles to increase shelf-life is due to the *SlETR1* gene mutation, which can inhibit the normal ripening process in the fruit because it can reduce sensitivity to ethylene (Mubarok et al., 2015).

Fruit hardness

Loss of fruit hardness is one of the significant changes during fruit ripening (Ghaiet al., 2016). The

hardness of fruit between commercial tomatoes and the results of their crosses is generally in a different average group. The results indicated significant differences between the two groups. Nevertheless, at 0 DPS, Mirah x *Sletr1-2*, at 10 DPS and 20 DPS, Intan x *Sletr1-2*, and at 20 DPS, Mutiara x *Slert1-2*, exhibited fruit hardness levels that were comparable to their commercial parents. However, the hardness values tended to be slightly lower (Table 2). The lower hardness value suggests that the fruit texture is firmer.

In the fruit from the cross between commercial tomatoes and *Sletr1-2* mutants, the fruit hardness is more potent than commercial tomatoes. The fruit firmness of commercial tomatoes is softer than that of tomatoes crossed with the *Sletr1-2* mutant, probably due to a higher respiration rate. It is well known that



cultivars and the results of the crossing with tomatoes WT and *Sletr1-2* mutants

Constune		Fruit hardness (mm/10 sec/50 g)					
Genotype	0 DPS		10 DP	10 DPS		5	
Intan	17.09	а	17.34	b	17.43	b	
Intan x WT	16.99	а	17.32	b	17.97	b	
Intan x Sletr1-2	15.35	b	15.90	b	16.65	b	
Mirah	17.76	а	18.42	а	19.29	а	
Mirah x WT	17.78	а	17.92	а	17.99	b	
Mirah x Sletr1-2	16.74	а	16.96	b	17.24	b	
Ratna	17.28	а	18.42	а	20.40	а	
Ratna x WT	16.74	а	18.38	а	20.02	а	
Ratna x Sletr1-2	14.36	b	16.32	b	18.56	b	
Mutiara	16.95	а	17.46	b	19.11	а	
Mutiara x WT	15.81	b	17.33	b	18.61	b	
Mutiara x Sletr1-2	15.74	b	17.01	b	18.03	b	

Table 2. The Scott-Knott test results on fruit hardness of 12 tomato genotypes

Remarks: Means followed by the same lowercase letters in the same column are not significantly different based on the Scott-Knott test at the level of α =5 %.

respiration can reorganize carbohydrate polymers in cell walls, particularly pectin and cellulose. As a result, the wall strength and bonding cohesion may decrease, softening the texture of the fruit (Wills et al., 1981). Crosses between commercial tomatoes and *Sletr1-2* mutants in tomatoes can prevent cell wall breakdown. The *SlETR1* gene mutation found in these crosses decreases ethylene sensitivity, avoiding the respiration rate that initiates the process. This state is in line with the research of Sholiha (2018), reporting that the treatment of 1-MCP concentrations of 30.625 mg/100 ml showed a maturity level of 1, which describes the textures of the fruit in harsh conditions.

Fruit weight loss

Regarding weight loss, commercial tomatoes and their progeny results are generally in the same average group. The findings indicated that the *Sletr1-2* mutant allele on crossed tomatoes had no significant effect. However, there was a significant effect on weight loss

Conotypo		Weight loss (%)				
Genotype	0 DPS	5	20 DPS			
Intan	4.28	b	4.15 b			
Intan x WT	5.31	а	6.85 a			
Intan x Sletr1-2	4.33	b	4.88 b			
Mirah	4.18	b	6.68 a			
Mirah x WT	4.20	b	6.57 a			
Mirah x Sletr1-2	4.42	b	5.03 b			
Ratna	3.90	b	5.17 b			
Ratna x WT	6.03	а	7.17 a			
Ratna x Sletr1-2	5.29	а	6.93 a			
Mutiara	4.03	b	7.95 a			
Mutiara x WT	3.87	b	6.67 a			
Mutiara x Sletr1-2	4.06	b	7.72 a			

Table 3. The Scott-Knott test results on fruit	weight loss of
12 tomato genotypes	

Remarks: Means followed by the same lowercase letters in the same column are not significantly different based on the Scott-Knott test at the level of α =5 %.

Character	Days after	(Genetic variabilit	y
	storage	σ²g	$2\sigma_{\sigma^2 g}$	Gloss
Fruit freshness	1–36	61.340	96.730	narrow
Fruit hardness	0	0.803	1.267	narrow
	10	0.450	0.777	narrow
	20	1.051	1.491	narrow
Fruit weight loss	10	0.325	0.720	narrow
	20	1.271	1.674	narrow

Table 5. The phenotypic variability values of the character of the storability of 12 tomato genotypes

Character	Days after	Р	henotype variabilit	У
Character	storage	σ²g	$2\sigma_{\sigma^2 g}$	Gloss
Fruit freshness	1–36	62.593	96.729	narrow
	0	1.574	1.247	broad
Fruit hardness	10	1.034	0.758	broad
	20	1.672	1.480	broad
Fruit weight loss	10	0.833	0.709	broad
	20	1.714	1.670	broad

Vol. 9 No. 1, April 2024

for Ratna x *Sletr1-2* at 10 DPS and Mirah x *Sletr1-2* at 20 DPS (Table 3). The increase in weight loss in tomatoes resulted from crossing with the *Sletr1-2* mutant allele is thought to be because the effect of the mutation on the *SlETR1* gene has been lost. As a result, it cannot inhibit ethylene sensitivity.

According to Okabe et al. (2012), mutations in *SIETR1* (*Solanum lycopersicum* ETHYLENE RESPONSE 1) produce *SIetr1-1* and *SIetr1-2* mutants that experience inhibition of gene activity as indicated by an unresponsive reaction to exogenous ethylene to a concentration of 10 ppm. The disappearance of the effect of the *SIETR1* gene causes the process of respiration and transpiration in the fruit to function normally, so the weight loss that occurs can be equal to or greater than the commercial parent.

Variability of shelf-life appearance

The value of genetic variability for all observed characters is narrow (Table 4). This result indicates that the tested plant population tends to be homogenous. Furthermore, it should be noted that the sampled population is the F1 generation, which does not undergo segregation. This limited genetic variability must be enhanced to succeed in breeding programs. As highlighted by Poehlman and Sleper (1995), plants cannot be improved without genetic variability, and one approach is crossing with distant relatives. Additionally, Crowder (1993) suggested that plants in the F2 generation exhibit high variability due to segregation under Mendel's law.

The variation in phenotype for fruit freshness is narrow (Table 5), which suggests that only some individuals can be chosen. Conversely, the characteristics of fruit hardness and weight loss display a broad phenotypic variability. Austiet al. (2014) indicated that the broad phenotypic variability could be attributed to extensive environmental variations. Furthermore, plant varieties grown under the same conditions may exhibit distinct phenotypic responses.

According to Murti et al. (2002), different genotypes have different abilities in absorbing nutrients even though the environment used is relatively the same, so it can cause a variety of phenotypic appearances from each genotype planted. The high and low variability of the phenotype illustrates the appearance of genotypes tested in the field.

CONCLUSIONS

All hybrid *Sletr1-2* mutants have a more excellent freshness than their commercial parents. Intan x *Sletr1-2* shows the best shelf-life, with a shelf-life of 35 days. All characters show narrow genetic variability. The broad variability of phenotypes is found in the character of fruit hardness and fruit weight loss.

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