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Total soluble solid and titratable acidity in different fruit maturation stages of *Solanum lycopersicum cv*. micro-tom and its mutant *iaa9-3* and *iaa9-5*

Abstract. Fruit development influences the metabolite contents and then its biological activity; however, such report is still limited in tomato *IAA9* mutants. This study aims to evaluate total soluble solid and titratable acidity in several stages of fruit maturation of the mutant micro-tom tomato. The experimental method used is the t-test method with three replications and followed by correlation and principal component analysis. The tested genotype were *iaa9-3* and *iaa9-5* mutants against *WT-MT*. Pearson correlation analysis showed that *iaa9-3* and *iaa9-5* produced higher levels of total soluble solid and titratable acidity in different fruit maturity levels; and the increase of flowering age and all fruit maturity ages, except for the breaker age that was similar to *WT-MT* tomato.

Keywords: Tomatoes · Fruit maturity level · *iaa9* · Fruit quality

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Introduction

Tomato *(Solanum lycopersicum)* provides a vitamin containing food, carotenoid and phenolic compounds (Marti et al., 2016). Growing environmental factors may affect the number, size and quality of fruit (Albert et al., 2016). The ambient growth of plants with high temperatures can lead to the failure of tomato formation (Hoshikawa, 2017). High temperature responses can be overcome by a tomato metabolic change (Albert et al., 2016).

Metabolic changes can be done with molecular technologies such as the use of gene mutations (Saito et al., 2011). Mutation in the 'Hong Anliu' orange bud can increase monosaccharides and but decrease the levels of organic acid (Pan et al., 2013). Arabidopsis of mutants *iaa-5, iaa-6,* and *iaa-19* planted at heat stress can reduce 45–50% of the primary root length and fresh weight, while control plants have dead plant conditions (Shani et al., 2017). Tomato var. *Ailsa Craig* granted for exogenous auxin at 10^{-5} is needed to delay maturing in mature green and ten days before the phase breaker while the red phase has increased its ripening rate (Cohen et al., 1996).

Fruit development is a multiphase process that requires close coordination between molecular, biochemical, and structural elements. DNA modification leads to metabolic properties resulting from the differences in metabolic pathways. A nutrient quality metabolic capacity results in differences in fruit growth and maturation (Zhang et al., 2010). Studies of the mutant effect in the *IAA9* genes for changes in metabolic properties and fruit maturity age in tomatoes have not been made, thus present study aimed to evaluate total soluble solid and titratable acidity in several stages of fruit maturation of the mutant micro-tom tomato.

Materials and Methods

Study Site. The study was conducted for three months in the hydroponic garden of Bale Tatanen Faculty of Agriculture, Universitas Padjadjaran, with an altitude of ± 750 meters above sea level. The post-harvest quality test was conducted for three months in the Horticulture Laboratory, Faculty of Agriculture Universitas Padjadjaran.

Materials. Plant materials used are the Micro-Tom (MT) tomato seeds, namely wild type of MT (WT-MT), *iaa9-3,* and *iaa9-5* mutants. Other materials were AB mix hydroponic nutrition, charcoal chaff, cocopeat, compost, and insecticide. The materials used in the tomato nutrition analysis are fresh tomato samples, NaOH, and aqua dest.

The items needed in the laboratory were analytical balances, pH meters, 100 mL and 50 mL cups, micro pipets, blender, aluminium foil, plastic wrap, spectrophotometer UV-vis Orion AquaMate 8000 (Thermo Scientific, USA), refractometer PAL-J (Atago, Tokyo, Japan), refrigerator, analytic scale, micro tube, mortars, and aquadest. The needed tools in the field were pots and watering cans.

Preparation of plant. Mutant Micro- Tom tomatoes namely *iaa9-3* and *iaa9-5*; and its *WT-MT were* tested by two testing stages of generic growth and fruit quality. The experimental method used was the t-test which was followed by a principal component analysis.

Generative Growth

The Flowering Age. Flowering age is measured by counting the number of days after seedling until the first flower in one flowering plant.

Figure 1. Fruit maturation stage of Micro Tom and its mutant

Fruit Maturation Stage. The fruit maturation stage is varied, i.e., the green, mature green, breaker, pink and red. Harvesting is done when the fruit meets the harvest criteria. The harvest criteria used in present experiment were as follows (Mubarok et al., 2019) :

- Green (G) (Flowering +20 day): The color of the fruit are showing green.
- Mature Green (MG) :
  The color of the fruit is bright green normally called mature green.
- Breaker (Br):
  The discolored condition of the fruit indicates fragmentation in green with yellow or pink at the base of the fruit about 10%.

Jurnal Kultivasi Vol. 22 (1) April 2023
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- Pink (P): The color of the fruit are showing pink with age (Br+3).
- Red (R): The color of the fruit indicating a deep red to the entire surface of the fruit (Br + 7).

Fruit Quality. The fruit quality test criteria consist of three phases of maturation: breaker, pink dan red.

- **Total Soluble Solid (TSS)**
  The mutant *iaa9-3* tomato and *iaa9-5* are prepared in microtubes with a weight of fruit juice of about 5 g. Microtube was then centrifuged at 1000 rpm. Supernatan 1000 rpm was collected using a micropipette and transferred to the refractometer lens (Majidi et al., 2011).
- **Titratable Acidity (TA)**
  Titratable acidity measurements are done using 5 mL of tomato juice that was titrated by NaOH 0,1 N (Tilahun, 2013).

Result and Discussions

The Flowering Age (DAP). The results of the flowering age analysis can be shown in Figure 2.

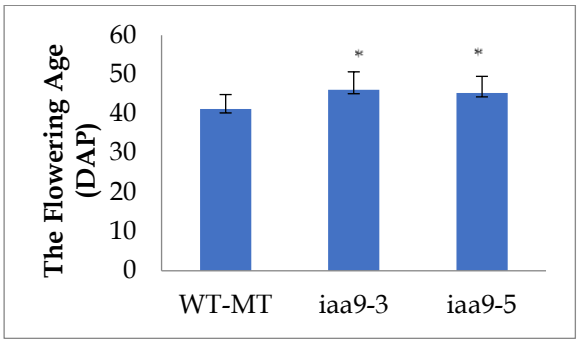

Figure 2. The flowering age of *iaa9-3*, *iaa9-5* and *WT-MT*
Average value ± Standard Error (SE) (n=30) followed by star (*) shows a significant different compared with control (*WT-MT*) according to Student's T-Test in p< 0.05

Mutants *iaa9-3* and *iaa9-5* had significantly slower flowering age than the *WT-MT* (Figure 2). Mutant *iaa9-3* and *iaa9-5* have a longer life span of 46 DAP and 45 DAP than the *WT-MT* of 41 DAP. A mutation effect on the *IAA9* gene can induce early ovarian growth before the flower blooms (Kim et al., 2020). The effects of mutations in the genes *IAA9*, causes cell expansion in the thickness of the ovarian wall (Kim et al., 2020). The thickening of the ovarian wall result in increased cell size, the number of cell layers and the area of mesocarp (Kim et al., 2020).

The success of cell division and expansion in flowers is the beginning of fruit growth (Gillaspy et al., 1993). Ovula embryo bags containing vascular ovaries and micropylar poles are where auxin accumulates (Pattison and Catala, 2012). Auxin was accumulated in ovula embryo six days before anthesis (Pattison and Catala, 2012). In auxin mutant eggplant *parental advice-1 pad-1*, IAA levels rise just when the flowers bloom, while the *WT-MT* decreases and remains low at the right time (Matsuo et al., 2020). The affects the rise in flower formation, the increase in the formation of fruits and the formation of partensian fruits, which results in much more desirable results (Matsuo et al., 2020).

The Age of Green Fruit Maturity Stage (DAP). Research indicates that the green phase age of the mutants was *iaa9-3* and *iaa9-5* significantly from statistics compared with the *WT-MT* (Figure 3). Mutants *iaa9-3* and *iaa9-5* had the slower green phases of 66 DAP and 65 DAP than *WT-MT* of 61 DAP (Figure 3). The slower life span resulting from a mutation influence in the *IAA9* gene (Figure 2), affected the green harvests lifespan compared with the *WT-MT* (Figure 3).

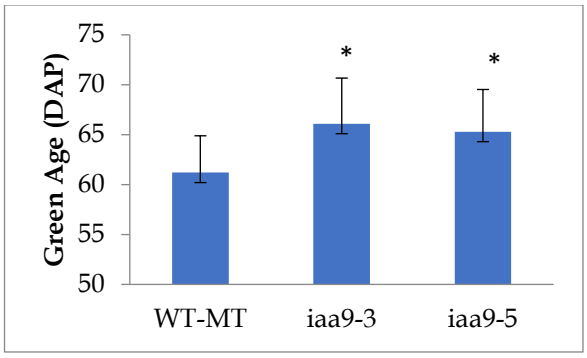

Figure 3. The age of green fruit maturity stage in *iaa9-3*, *iaa9-5* and *WT-MT*
Average value ± Standard Error (SE) (n=30) followed by star (*) shows a significant different compared with control (*WT-MT*) according to Student's T-Test in p< 0.05

The tomato is climacteric, which means that at the beginning of the fruit growth, the automatic de-blocking phase of "system 1" is called (Kumar et al., 2014). The ripening of the

fruit of the "system 1" has a reduced maturing response a level of accumulated basic ethic and ethylene sensitivity (Kumar et al., 2014). The maturation of "system 2" takes a place at the beginning of the auto catalitic phase (the rapid rise of ethylene) so that ethylene is called the fruit development transition (Giovannoni et al., 2021).

IAA can change regulation from system 1 to system 2 by pressing ethylene and abscisic acid (ABA) as a result of crosstalk auxin and GA while beginning fruit development (Pattison et al., 2015). The regulation of transportation for auxin on the maturation of the fruit can present a high degree of ethylene sensitivity (Shin et al., 2019). This led to the significant slower of green age of mutant *iaa9-3* and *iaa9-5* fruit than its WT-MT. This is supported in the study of *Ailsa Craig* tomatoes provided with auxin exogen 10^{-5} and a got a delay of maturation green stage and 10 days before phase breaker (Cohen et al., 1996).

The Age of Mature Green Fruit Maturity Stage (DAP). Research shows that the mature green age of mutant *iaa9-3* and *iaa9-5* is significantly different to *WT-MT*. Mutants *iaa9-3* and *iaa9-5* are slower mature green of 75 DAP and 74 DAP compared to the *WT-MT* of 70 DAP (Figure 4). The slower age of the green in the *IAA9* (Figure 3) gene mutation of the tomato, affected the lagging of the mature green phase fruit harvest compared with the *WT-MT*.

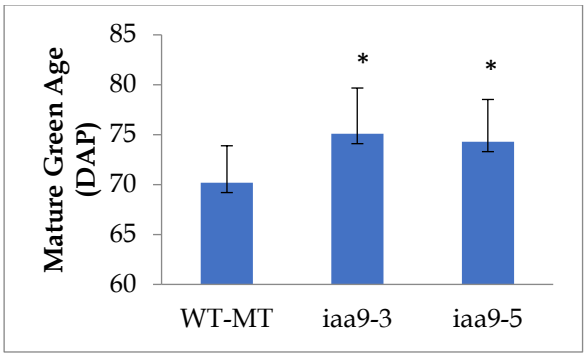

Figure 4. The age of mature green fruit maturity stage in *iaa9-3*, *iaa9-5* and *WT-MT*
Average value ± Standard Error (SE) (n=30) followed by star (*) shows a significant different compared with control (*WT-MT*) according to Student's T-Test in $p < 0.05$

Auxin can control cell division and differentiate into stomata (Balcerowicz and Hoecker, 2014). Temperature 30-35 °C mutant *iaa9-5* and *iaa9-3* have a higher number of stomata of about 160 stomata mm^{2-} and 156 stomata mm^{2-}, respectively, compared with *WT-MT* of about 146 stomata mm^{2-} (Mubarok et al., 2020). The formation and distribution of higher stomata are affected by the auxin pathways (Balcerowicz dan Hoecker, 2014).

The increase in the number of stomata in the tomato plays a key role in improving photosynthesis, thus creating fruit growth (Wang et al., 2009). Increased photoactivity in plants can increase the accumulation of starch and the metabolism of sugars in the fruit (Pattison et al., 2015). Photosynthate is transported to the fruit in order to maximize the growth of the fruit of mature green phase (Wu and Kubota, 2008). The increased fruit reached its maximum size on the mature green stage and at the breaker stage the fruit size remained virtually unchanged (Wu and Kubota, 2008). The mutations in *iaa9-3* and *iaa9-5* have a distinct mature green age slower than the *WT-MT*.

The Age of Breaker Fruit Maturity Stage (DAP). Research shows that the age of breaker in mutants *iaa9-3* and *iaa9-5* are not significantly different than the *WT-MT*. Mutants *iaa9-3* and *iaa9-5* have a timeless age breaker which is 81 DAP and 80 DAP compared to the *WT-MT* which is 82 DAP (Figure 5). Auxin can control crucial processes in the development of the fruit (Teale et al., 2006). Auxin can encourage the initiation of fruit formation by stimulating the appearance of the hormone gibberellin (Serrani et al., 2008). The metabolism of GA can cause the parthenocarpy occurance in Arabidopsis of auxin mutants. Parthenocarpy occurs because pollen fails to fertilize ovula, producing several signals to encourage fruit initiation (Molesini et al., 2020).

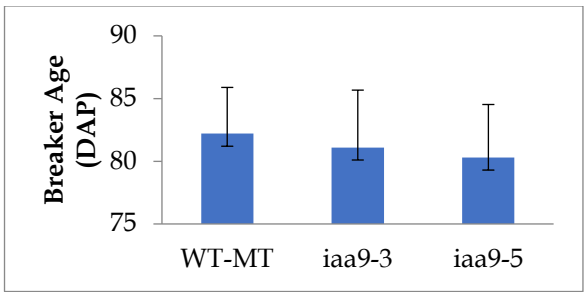

Figure 5. The age of breaker fruit maturity stage in *iaa9-3*, *iaa9-5* and *WT-MT*
Average value ± Standard Error (SE) (n=30) followed by star (*) shows a significant different compared with control (*WT-MT*) according to Student's T-Test in $p < 0.05$

Jurnal Kultivasi Vol. 22 (1) April 2023
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The Age of Pink Fruit Maturity Stage (DAP) Research has shown that the pink phase ages on mutants *iaa9-3* and *iaa9-5* are not significantly different according to statistics compared with the *WT-MT*. Mutants *iaa9-3* and *iaa9-5* have a faster pink phase age and are not significantly 84 DAP and 83 DAP than *WT-MT* is 85 DAP. The increase in the number of chlorophyll can increase photosynthesis in plants, in effect can increase the accumulation of starch and metabolism in fruit (Pattison et al., 2015).

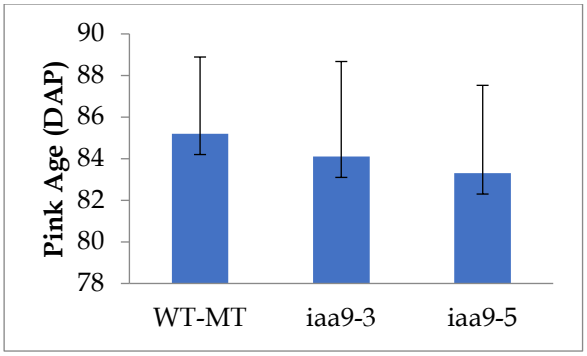

Figure 6. The age of pink fruit maturity stage in *iaa9-3*, *iaa9-5* and *WT-MT*
Average value ± Standard Error (SE) (n=30) followed by star (*) shows a significant different compared with control (*WT-MT*) according to Student's T-Test in p< 0.05

The Age of Red Fruit Maturity Stage (DAP). Research shows that the longevity of red phase in mutant *iaa9-3* and *iaa9-5* is not significantly different to *WT-MT* (Figure 7). Mutants *iaa9-3* and *iaa9-5* have a faster and surer years of the red phase, which is 88 DAP and 87 DAP compared to *WT-MT* which is 89 DAP.

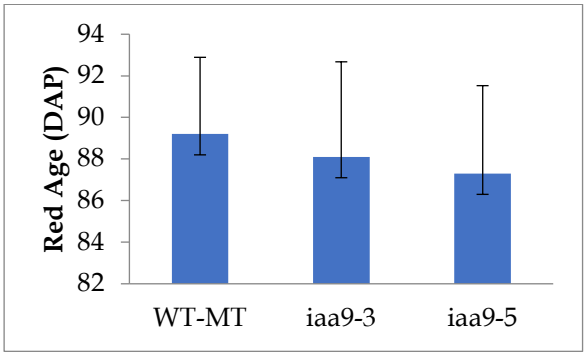

Figure 7. The age of red fruit maturity stage in *iaa9-3*, *iaa9-5* and *WT-MT*
Average value ± Standard Error (SE) (n=30) followed by star (*) shows a significant different compared with control (*WT-MT*) according to Student's T-Test in p< 0.05

Mutants *iaa9-3* and *iaa9-5* have a significant TSS and TA value higher on breaker, pink and red fruit maturity than the *WT-MT* (Figure 8 and Figure 9). This leads to the longevity of the mutants *iaa9-3* and *iaa9-5* at red maturity levels precisely despite the slowdown in the maturity stages of *green* dan *mature green*. The hormone auxin affects proper and efficient fruit growth and thus can coordinate normal tomato growth (Gorguet et al., 2005).

Total Soluble Solid (TSS). Mutants *iaa9-3* and *iaa9-5* have significantly higher TSS values in breaker, pink and red fruit maturity than in *WT-MT* (Figure 8). Mutants *iaa9-3* and *iaa9-5* at the breaker maturity age have higher TSS value of 4.933 and 5.133 °Brix compared to *WT-MT* which is 4.133° Brix (Figure 8). Mutants *iaa9-3* and *iaa9-5* with pink maturity has a higher TSS value of 6.467 and 6.767 °Brix than the *WT-MT* of 6.133 °Brix (Figure 8). Mutants *iaa9-3* and *iaa9-5* at red maturity level have a higher TSS rate of 6.767 and 7.2 °Brix compared to *WT-MT* which is 6.567 °Brix (Figure 8). High quality tomatoes score for TSS in breaker phase and red phase were breaker 4.47 °Brix and 6,57 °Brix, respectively (Campos et al., 2006).

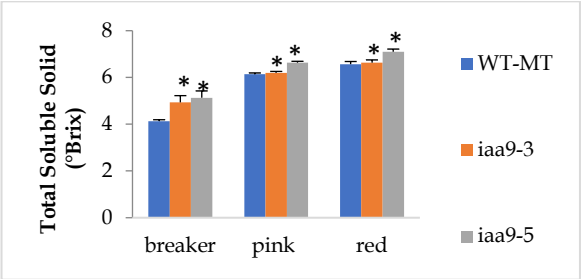

Figure 8. Total Soluble Solids of *iaa9-3*, *iaa9-5* and *WT-MT*
Average value ± Standard Error (SE) (n=30) followed by star (*) shows a significant different compared with control (*WT-MT*) according to Student's T-Test in p< 0.05

Auxin appears as a negative regulator of SlGLK2 which can boost cytokinin response (Quinet et al., 2019). The interaction of cytokinin with auxin can affect the allocation of root biomass, promoting cell growth and root growth (Sachs, 2005). In the tobacco plant increased partition resulting from changes the CWIN in root and leaf activity (Werner *et al* 2008). This enzyme regulates the flow of sucrose by controlling the apoplastic removal of the floem

(Roitsch et al., 2003). Induction CWIN at the root increases the strength of cytokinin to build sink activity (Roitsch et al., 2000).

IAA appears to induce the activities of sink (sucrose allocation) and CWIN on young leaves and roots (Roitsch et al., 2003) This enzyme regulate the transport of sucrose by regulating the apoplastic degradation of floem (Roitsch et al., 2003). Total soluble solids is increasing at amylase stimulation and can interfere the quality of fruit during ripening (Quinet et al., 2019).

Titratable Acidity (TA). Mutant *iaa9-3* and *iaa9-5* have significantly higher TA values in breaker, pink and red fruit maturity than the *WT-MT* (Figure 9). Mutants *iaa9-3* and *iaa9-5* with breaker maturity levels have a higher TA rate of 0.49 and 0.494% compared to *WT-MT* 0.321% (Figure 9). Mutant *iaa9-3* and *iaa9-5* with a pink maturity rate have a higher 0.619 and 0.625% compared with *WT-MT* 0.564% (Figure 9). Mutant *iaa9-3* and *iaa9-5* at red maturity rate have a higher value of TA 0.702 and 0.756% compared with *WT-MT* 0.612% (Figure 9).

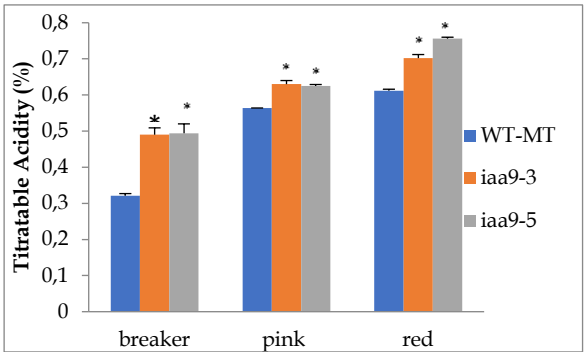

Figure 9. Titratable acidity of *iaa9-3*, *iaa9-5* and *WT-MT*

Average value ± Standard Error (SE) (n=30) followed by star (*) shows a significant different compared with control (*WT-MT*) according to Student's T-Test in p< 0.05

Previous styudy reported a positive correlated sugar content with titratable acidity (Georgelis, 2002). Positive correlation between sugar and titratable acidity suggests that plants with high sugar generally have more free organic acid and less concentration of hydrogen ions (Georgelis, 2002). The acidity of the higher fruit has the advantage of the yeast infection (Mohammed et al., 1999).

The Correlation of TSS, TA and the Ages of Fruit Maturation Stage in WTMT and its mutants

Table 1. Pearson correlation of TSS, TA and the Ages of Fruit Maturation Stage in WTMT and its mutants

Variable	B	G	MG	BR	P	R	TSS
G	1 *						
MG	1*	1*					
BR	(-) 0.83	(-) 0.83	(-) 0.83				
P	(-) 0.83	(-) 0.83	(-) 0.83	1*			
R	(-) 0.83	(-) 0.83	(-) 0.83	1*	1*		
TSS	0.63	0.63	0.63	(-) 0.9*	(-) 0.9*	(-) 0.9*	
TA	0.86	0.86	0.86	(-) 0.9*	(-) 0.9*	(-) 0.9*	0.9

Description: B- The flowering Age; G- green fruit maturity; MG- mature green fruit maturity; BR- breaker fruit maturity; P- pink fruit maturity; R- red fruit maturity; TSS- Total Soluble Solid; TA- Titratable Acidity

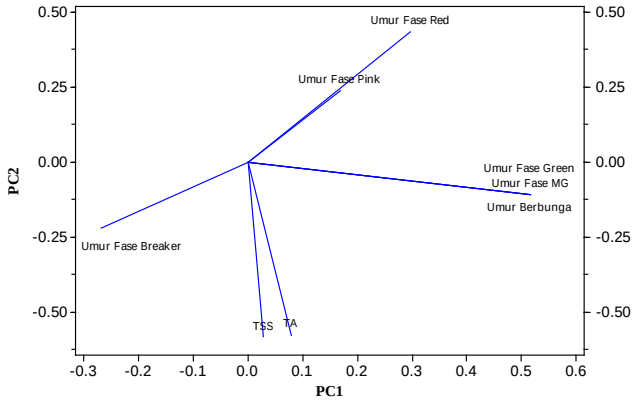

Figure 10. Biplot of the principal component analysis of total soluble solid, titratable acidity and age of fruit maturation stage

1. The biplot of principal component analysis is a 2-dimensional form of the combined main component 1 or PC1 and the main component 2 or PC2.
2. Biplot can explain 76,6% of the variety in the data population because it has a cumulative proportion of 0.766. This cumulative proportion was the accumulation of proportion on the PC1 of about 0.446 and PC2 of about 0.321.

Jurnal Kultivasi Vol. 22 (1) April 2023
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3. On PC1, the flower age, green age phase, mature green age phase, pink age phase, red age phase, TSS and TA has the same direction, while they had an opposite of the age of breaker maturation age (Figure 10). PC1 value is positive, except the age of breaker.

Conclusion

Mutation tomatoes in the *IAA9* genes produced an increased TSS and TA content at all fruit maturity levels, along with increased flowering age and all of the age of fruit maturity phases except the breaker that similar to WT-MT tomato.

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