ISSN: 1412-4718, eISSN: 2581-138x

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The effect of fruit maturation stage on seed germination of *iaa9-3* and *iaa9-5* tomato mutants

Abstract. One of the internal factors that play an important role in seed quality is the level of the phytohormone auxin. Efforts to increase auxin in seeds include developing new varieties with increased indigenous auxin, such as those found in mutant tomatoes from Micro-Tom, namely *iaa9-3* and *iaa9-5*. This research was conducted to determine the germination response of *iaa9-3* and *iaa9-5* mutant tomato seeds at different levels of the fruit maturation stage. The research was carried out at the Seed Technology Laboratory, Faculty of Agriculture, Padjadjaran University, and the Seed Testing Laboratory of the Center for Standard Testing of Vegetable Plant Instruments. The response design used was a completely randomized factorial design consisting of two factors and repeated three times. The first factor was the *iaa9-3, iaa9-5*, and Wild-Type Micro-Tom (WT-MT) mutants as controls. The second factor is the fruit maturation stage, which consists of Breaker, Pink, and Red. The research results showed that there was an interaction between genotype and fruit maturation stage on the parameters of germination, growth simultaneity, and hypocotyl length. The harvest stage for tomatoes to produce normal strong seed germination was the pink stage in all tomato genotypes tested. The best germination rate was shown by WTMT seeds at the pink stage, genotype *iaa9-3* at the red stage, and genotype *iaa9-5* at the red or pink stage. The effect of fruit maturity stage on the synchronization of sprout growth was relatively not significantly different in mutant tomatoes, but it had an effect in WTMT tomatoes, namely the best pink stage.

Keywords: Auxin · Breaker · Germination rate · Micro-tom

Submitted: 22 July 2023, Accepted: 28 April 2024, Published: 30 April 2024
DOI: 10.24198/kultivasi.v23i1.40859

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Aufa EMIA, Mubarok S, Nuraini A, Budiarto R, Waluyo N, Ezura H. 2024. The effect of fruit maturation stage on seed germination of *iaa9-3* and *iaa9-5* tomato mutants. Jurnal Kultivasi, 23(1): 101-107

ISSN: 1412-4718, eISSN: 2581-138x

Introduction

Optimal plant growth and development is a success in the cultivation process. Among the factors that are very important in the process of accelerating plant growth and development are phytohormones. Phytohormones function to stimulate growth and regulate physiological processes that occur in plants (Sukmadi, 2013). One of the phytohormones that play an important role in the process of plant growth and development is auxin.

Auxin has an important role in improving seed quality. Auxin acts as a key regulator to modulate cellular processes in seed development (Cao et al., 2020). According to Zhao & Zhong (2013), exogenous administration of indole acetic acid (IAA) affects the early stages of seed germination and stimulates seed germination. This is thought to be related to the repair of membranes and seed respiration that occurs due to the administration of exogenous auxin.

iaa9-3 and *iaa9-5* tomatoes are two mutant tomatoes, from the clay parent Micro-Tom (WTMT) which experienced mutations due to the mutagen of ethyl methyl sulfonate (Saito et al., 2011). *IAA9* is a family of Aux/IAA genes that plays a role in suppressing transcription of the endogenous auxin signal pathway, i.e., IAA (Guilfoyle & Hagen, 2007). The effect of mutations in *IAA9* will trigger a different auxin response from its parents (Wang et al., 2005).

Increasing seed quality with the presence of auxin is also influenced by the physiological ripening conditions of the seeds. According to Rusmin et al., (2007) improving the quality of seeds is influenced by the physiological maturation conditions of the seeds at the right time of harvest. Appropriate physiological ripening conditions result in maximum dry weight and seed vigor. So if harvesting is not in the right conditions, the seeds produced tend to be of suboptimal quality. The stage of fruit maturation greatly influences seed vigor. When fruit is harvested too young, the quality of the seeds produced tends to have low physiological potential. This condition is related to immature seeds due to suboptimal seed filling in the fruit (Tetteh et al., 2018).

In WTMT tomatoes themselves, there are several stages of fruit development. According to Wang et al., (2005), the stages of development of Micro-Tom tomato fruit are divided into; early light green (EIM), light green (IM), dark green (MG), breaker (BR), orange (OR), early red (ER), red (R), and mature red (RR). Unfortunately, there has been no study of differences in fruit maturation stages on the quality of WTMT mutant tomato seeds. This research aimed to determine the germination response of iaa9-3 and iaa9-5 mutant tomato seeds at different levels of fruit maturation.

Materials and Methods

This research was conducted at (i) the Seed Laboratory of the Faculty of Agriculture, Universitas Padjadjaran, located in Jatinangor, Sumedang Regency, West Java, and (ii) the Seed Testing Laboratory of the Vegetable Crops Research Institute in Lembang, Bandung Regency, West Java.

The experiments utilized tomato planting materials, namely *iaa9-3, iaa9-5*, and their respective wild parents (WT-MT) as controls. The experimental design employed a factorial completely randomized design with two treatment factors. The first factor comprised the mutant lines, i.e., *iaa9-3, iaa9-5*, and WTMT (control), while the second factor involved the fruit maturation levels of the breaker, pink, and red-stage seeds (Mubarok et al., 2023).

Seeding Preparation. Seeding was conducted using a husk charcoal and cocopeat planting medium in a 1:1 ratio in a seed tray. Subsequently, tomato seeds that were 15-20 days old or had formed 4-5 leaves were transferred to planting pots. Plant maintenance included replanting, watering, fertilizing, and controlling pests and diseases.

Seed Quality Testing. Tomato fruits are harvested at maturation stages including breaker, pink, and red. Subsequently, the seeds undergo a seed quality test consisting of the following procedures:

(a) Calculation of the percentage of normal, abnormal, and dead sprouts from the beginning to the end of a 14-day observation period.
(b) Measurement of hypocotyl length using a ruler, from the base to the point of the cotyledon stalk, expressed in centimeters (cm).
(c) Assessment of germination capacity by observing normal tomato germination.

Normal sprouts exhibit essential structures such as primary roots, coleoptiles, and well-developed plumules. The calculation of normal germination can be performed using the formula provided by Sutopo (2010).

(d) Assessment of the simultaneity of seed growing used to determine the percentage of seeds capable of normal germination under optimal conditions by the 7th day (first-day count).

Data analysis. Data were analyzed using a two-way analysis of variance (ANOVA). Further tests were carried out using the Duncan test at a significance level of 5%.

Results And Discussion

Percentage of Strong Normal Sprouts, Weak Normal Sprouts, Abnormal, and Dead Sprouts. The results of the observations showed that there were seeds that experienced normal strong, normal weak, abnormal, and dead germination. Normal strong sprouts are characterized by good root and hypocotyl growth, and the seeds can separate from the cotyledons. Meanwhile, normal weak sprouts are not separated from the cotyledons, and root and hypocotyl growth tends to be suboptimal (Nuraini et al., 2024). Abnormal sprouts show stunted and unbalanced growth between the roots and hypocotyl. Moreover, the seeds are not able to stand up straight because they are too small. Dead sprouts are caused by fungi and bacteria, which cause the seeds to die and not grow at all.

Table 1 shows the percentage of strong normal sprouts, weak normal sprouts, abnormal sprouts, and dead sprouts. During the 14-day germination process, the best percentage of normal strong sprouts was shown in the *iaa9-5* treatment at the maturation level of pink fruit, with an average of 7 sprouts. On average, strong normal sprout development occurred in the pink phase, both in the *iaa9-3, iaa9-5*, or WTMT lines. This condition shows that fruit maturation at the pink stage is the optimal phase for seed quality, as at this stage, the seeds are neither too young nor too old. Conversely, in the breaker phase, the seeds are still too young, and in the red phase, the seeds are too old.

This condition aligns with research conducted by Tetteh et al., (2018) regarding the influence of seed maturation level on seed quality, which indicates that seeds that are too young seed derived from green fruit stage had a low germination rate. Moreover, the *iaa9-3* and *iaa9-5* mutants showed different germination results. Even though they were harvested in the breaker and red phases, they still exhibited good germination. This condition may be attributed to the presence of auxin in mutant plants, which affects the ability of seeds to germinate. According to Maemunah & Adelina (2009), in the process of seed metabolism, auxin acts as the main compound. The presence of auxin causes cell division and stimulates the formation of young roots. When auxin is in optimal conditions in the plant, it facilitates the utilization of growth factors such as water, nutrients, and food reserves in the cotyledons by the seeds. The optimal presence of auxin corresponds to the high seed-growing performances.

Table 1. The mean of strong normal, weak normal, abnormal, and dead sprouts in three tomatoes obtained from different fruit maturation stages

No	Treatment		Mean of Sprouts			
	Genotype	Fruit Maturation Stage	Strong Normal	Weak Normal	Abnormal	Dead
1	WTMT	Breaker	1.67	2.00	1.33	4.00
2	WTMT	Pink	7.33	5.67	2.67	0.00
3	WTMT	Red	1.33	0.67	0.00	2.67
4	*iaa9-3*	Breaker	1.67	1.00	1.00	3.67
5	*iaa9-3*	Pink	5.33	5.33	0.67	3.00
6	*iaa9-3*	Red	4.33	9.00	0.67	2.00
7	*iaa9-5*	Breaker	3.33	5.33	1.00	6.33
8	*iaa9-5*	Pink	7.00	6.00	0.33	3.67
9	*iaa9-5*	Red	5.00	4.67	0.67	4.33

Jurnal Kultivasi Vol. 23 (1) April 2024
ISSN: 1412-4718, eISSN: 2581-138x

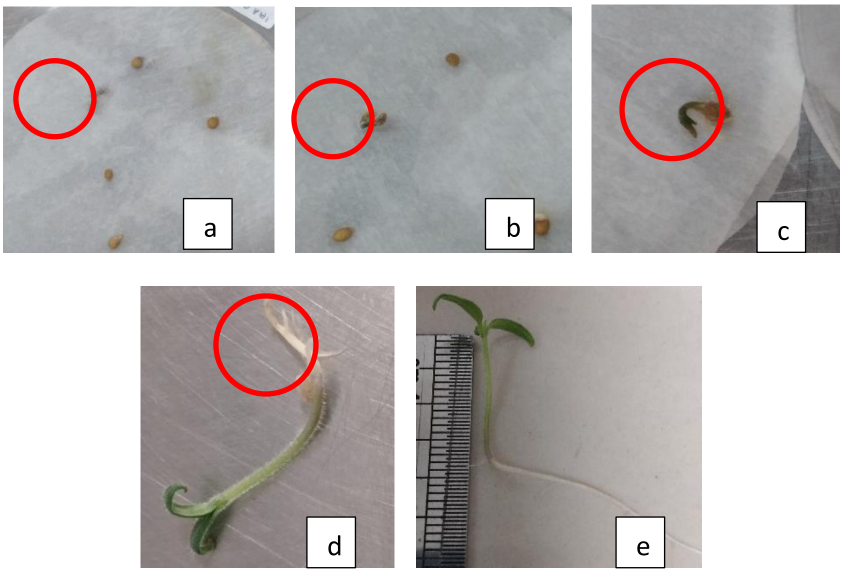

Figure 1. Morphological appearance of sprouts: (a) dead sprout as infected by fungus, (b) dead sprout as infected by bacterial pathogen, (c) abnormal sprout, (d) weak normal sprout, (e) strong normal sprout

These germination conditions indicate an influence between genotype and fruit maturation level. The pink stage is the optimal maturation level because it is neither too young nor too old. Meanwhile, the genotype factor shows an increase in germination ability at the breaker or red stage.

Figure 1 provides the differences between normal, weak, strong, and abnormal sprouts. Weak normal sprouts are characterized by unbalanced growth between the roots and the hypocotyl. Meanwhile, abnormal sprouts tend to be stunted and fail to develop. On the other hand, in normal sprouts, strong root growth and hypocotyl development occur in a balanced manner.

The dead seeds in this study were affected by bacteria and fungi. Seeds affected by fungus can be identified by the fine white threads surrounding them (Figure 1a). In contrast, seeds affected by bacteria show softness and destruction (Figure 1b).

Germination Rate. The results of the analysis revealed significant differences and interactions between tomato genotype and fruit maturation stage. In WTMT tomatoes, seed germination at the green or red fruit maturation stage demonstrates a low percentage. This finding corresponds with research by Pradnyawati et al. (2019) on the influence of harvest age on long bean seeds. Seeds harvested too young tend to exhibit suboptimal seed quality due to many seeds not yet being physiologically mature. Conversely, late harvest diminishes seed quality.

Referring to Table 2, the *iaa9-3* and *iaa9-5* mutant tomatoes exhibit robust germination at both green and red harvest times. This phenomenon may be attributed to an increase in auxin in mutant tomatoes, which affects seed germination. This aligns with research by Adnan et al. (2017) on the application of Auxin to enhance the viability of aged watermelon seeds, resulting in an increased germination percentage. Auxin treatment accelerates sprout growth, leading to the production of vigorous seedlings.

Table 2. The interaction effect of tomato genotypes and fruit maturation stages on the seed germination rate

Fruit maturation stages	Genotype		
	WTMT	*iaa9-3*	*iaa9-5*
Breaker	6.67a A	5.00a A	11.67a A
Pink	28.33b B	8.33a A	25.00b B
Red	14.33a A	11.33b A	21.67b B

Notes: Means followed by the same letter in the same column and factor is not significantly different based on Duncan's Multiple Range Test at 5% significance level. Lowercase letters are read vertically and capital letters are read horizontally.

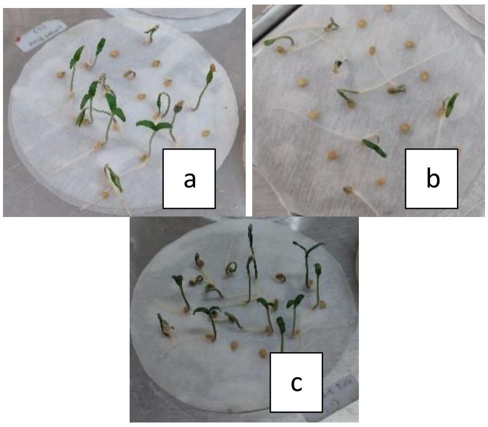

Figure 2. Morphological appearance of sprouts at 7 days after germination, obtained from seed in pink fruit maturation stages: (a) WTMT, (b) *iaa9-3*, (c) *iaa9-5*.

In Figure 2, it can be seen that the germination capacity of the seed obtained from the pink fruit maturation stage at 7 days after sowing was quite good. Germination conditions are much better than the breaker or red phase. This can be seen in Figure 3.

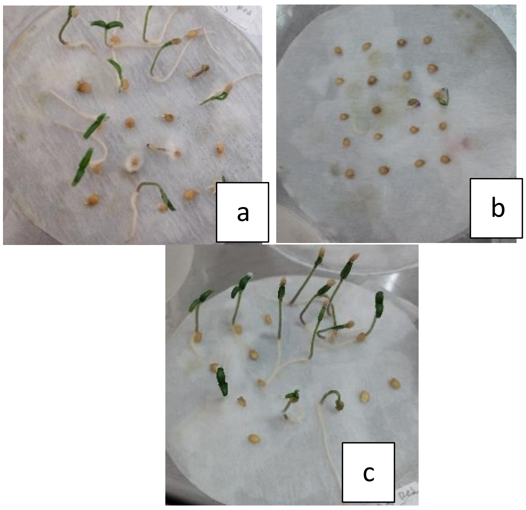

Figure 3. Morphological appearance of sprouts at 7 days after germination, obtained from seed in red fruit maturation stages: (a) *iaa9-3*, (b) WTMT, and (c) *iaa9-5*.

The effect of auxin on germination can be seen in Figure 3. Seeds from the WTMT line at the red fruit maturation stage showed low germination, and did not even germinate. However, seeds from genotypes *iaa9-3* and *iaa9-5* showed good germination even though they were taken from red stage fruit.

The Simultaneity of Seed Growing. The results of the seed simultaneity test show that there is an interaction between genotype and the level of seed maturation. Based on Table 3, the harvest age of the pink stage in the WTMT line has a fairly good level of seed growth synchrony, followed by the *iaa9-5* line. These results show that the pink stage is the best seed maturation level to produce quality seeds. In the breaker or red stages, results tend to be not very good. This condition can occur due to the existence of optimum food reserves at the maturation level of seeds that are not too young, but also not too old. Because the condition of the seeds is too young, the food reserves in the seeds are not yet optimal. On the other hand, at the maturation level of seeds that are too old, the food reserves contained in the seeds decrease.

Table 3. The interaction effect of tomato genotypes and fruit maturation stages on the simultaneity of seed growing

Fruit Maturation Stage	Genotype		
	WTMT	***iaa9-3***	***iaa9-5***
Breaker	0.05 a A	0.05 a A	0.08 a A
Pink	0.20 b B	0.03 a A	0.13 a B
Red	0.02 a A	0.03 a A	0.13 a B

Notes: Means followed by the same letter in the same column and factor is not significantly different based on Duncan's Multiple Range Test at 5% significance level. Lowercase letters are read vertically and capital letters are read horizontally.

The influence of fruit maturation level is in line with research conducted by Wulananggraeni et al. (2016) regarding the effect of differences in fruit maturation levels in 3 genotypes of cucumber on seed quality. Cucumber seeds that are harvested 18 days after flowering have a good effect on the simultaneous growth of the seeds. Wulananggaraeni et al. (2016) explained that this situation was caused by the condition of the seeds being ripe and not too ripe when harvested. The food reserves contained in the seeds are in optimum condition and come from food deposits resulting from photosynthesis. The influence of maturation level on seed quality was also revealed by Early harvest, before physiological maturation, which results in many seeds not being fully formed and filled so it will produce seeds of low quality because many of the seeds are wrinkled. Meanwhile, harvesting after physiological maturation will most likely experience harsh conditions on the seeds, resulting in many seeds experiencing a decline in quality before harvest.

Hypocotyl Length. The results of the analysis for hypocotyl length at 14 days after sowing showed that there was an interaction between genotype and seed harvest age. The response shown was that the hypocotyl length at the breaker or red harvest age still showed good growth. In contrast, the WT-MT control plants only grew optimally at the pink harvest age (Table 4).

Table 4. The interaction effect of tomato genotypes and fruit maturation stages on the length of seed hypocotyl

Fruit Maturation Stage	Genotype		
	WT-MT	*iaa9-3*	*iaa-9-5*
Breaker	0.57b	0.79a	1.04a
	A	B	C
Pink	1.62c	1.39b	1.58c
	C	A	B
Red	0.25a	1.46c	1.17b
	A	C	B

Notes: Means followed by the same letter in the same column and factor is not significantly different based on Duncan's Multiple Range Test at 5% significance level. Lowercase letters are read vertically and capital letters are read horizontally.

At the breaker stage, the germinated seeds tend to be dwarfed. This situation can be caused by the harvest being too young so that the seeds are not yet at the physiological maturation phase. According to Singkaew et al., (2017) at the green harvest age, the condition of the seed pericarp has not developed optimally and the process of translocating nutrients from fruit to seed does not run optimally. This condition causes too-young harvested seeds are not develop well.

Apart from that, WT-MT tomatoes whose seeds were harvested at the green or breaker harvest age did not germinate optimally and did not even grow. Meanwhile, the *iaa9-3* or *iaa9-5* mutagens showed signs of growth even though they were harvested at different stages. As can be seen in Figure 3 which shows the condition of the hypocotyl length at the breaker stage. In *iaa9-3* mutants and *iaa9-5* mutants, the hypocotyl length is better and more upright, even at the breaker stage. Meanwhile, as seen in Figure 3, the length of the hypocotyl in WTMT is shorter and tends to be less upright.

Figure 3. Hypocotyl length of seed obtained from breaker fruit maturation stages: (a) WTMT, (b) iaa9-5, (c) iaa9-3.

Conclusion

There was an interaction between genotype and fruit maturation stage on the parameters of germination, growth simultaneity, and hypocotyl length. The harvest stage for tomatoes to produce normal strong seed germination was the pink stage in all tomato genotypes tested. The best germination rate was shown by WTMT seeds at the pink stage, genotype *iaa9-3* at the red stage, and genotype *iaa9-5* at the red or pink stage. The effect of the fruit maturity stage on the simultaneity of seed growing was relatively not significantly different in mutant tomatoes, but it had an effect in WTMT tomatoes, namely the best pink stage.

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