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REVIEW PAPER

REVIEW OF IN VITRO FLOWERING METHOD FOR TOMATO (SOLANUM LYCOPERSICUM L.)

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Abstract

Tomatoes are one of the most popular vegetable plants globally because of the many benefits that can be obtained from tomatoes. However, its availability is still insufficient for market demand. Therefore, researchers began to develop tissue culture methods to cope with market demand. Although there have been several studies on tomato flowering in vitro, information about this method is minimal and needs further development. In vitro flowering is a technique in tissue culture for flowering plants in vitro. One of the benefits is cultivating plants in a relatively short time under controlled conditions to prevent disease transmission between plants. Therefore, we conducted a literature study to compare the results of these studies to know an effective and efficient method of factors that increase the in vitro flowering plants of tomatoes. In vitro flowering methods generally focus on flowering media, especially cytokinin types such as 6-Benzylaminopurine (BAP) and kinetin, and the concentration of those hormones, also light intensity used to induce flowering of tomato plants. Factors that affect in vitro flowering of tomato plants are tomato plant varieties, hormones, and light intensity.

KEYWORDS:

BAP, In Vitro Flowering, Kinetin, Light Intensity, Tomato

1 | INTRODUCTION

Solanum Lycopersicum L., commonly known as tomato, is one of the most popular vegetable crops in the world. One example of the use of tomatoes in the health sector is their transformation into edible vaccine products[1]. In molecular biology, tomatoes are used as plant models for introducing essential genes in dicotyledonous plants[2]. In addition, the tomato is an important climacteric fruit, as it contains many compounds that promote health, such as carotenoids, flavonoids, tocopherols, and vitamins[3, 4]. Tomatoes are suitable for cultivation in temperate and tropical climates[4], including Indonesia. However, tomatoes are native to Central and South America[3].

A lot of research on tomatoes has been done. However, it is still necessary to develop methods to accelerate the growth of tomato plants so that they are more effective and efficient both in vitro and in vitro because in vitro propagation with tissue culture provides more benefits in several aspects[5, 6]. Because of these advantages, researchers are doing more research on the optimization and modification of factors that affect the growth of tomato plants in vitro. Time is one aspect that gets more attention because the development will be faster, and the plant can be commercialized. Therefore, research on how to shorten the vegetative period so that flowering and fruiting are more rapid has begun to receive special attention. Two factors influence the growth and development of tomato plants, namely genetic and environmental factors (temperature, additional nutrients, light intensity, and other factors). Differences in species and variations of tomato plants cause differences in flowering time because the number of leaves that must be reached to reach flowering time is different, followed by environmental conditions that will affect the activity of these plants[7].

The in vitro flowering method is known to have many benefits, including obtaining genetic diversity in tomato plants so that superior seeds can be obtained, developing self-fertilization, even preventing plant disease transmission, and others[8]. However, there is not enough research on in vitro flowering in tomatoes[8–11], so information on the in vitro flowering of tomato plants is minimal. The methods used for culturing vary due to differences in explants. This is because there is no patent protocol regarding the general in vitro flowering method for tomato plants. Based on the results of several studies that have been carried out, it is known that several important factors that affect in vitro flowering are hormones and light intensity. Hormones that have a positive impact are cytokinins whose optimum concentrations depend on the species or varieties of tomato plants used.

Therefore, this literature study will discuss the in vitro flowering method and its factors, especially regarding hormones and good light conditions for tomato flowering. In addition, it will also discuss the importance of subculture and other supporting factors. The results of these studies will be compared to know what elements need to be considered to increase the success of in vitro flowering for tomato plants. This is done so that in future research, the flowering time of tomatoes in vitro can be faster.

2 | PREVIOUS RESEARCHES

Our previous research[8] investigated a flowering strategy in tomatoes by using different kinds of cytokinin, auxin, plant growth inhibitors, gibberellin, and various concentration of KH_2PO_4. The flowers and fruits occurrences were quite well. However, they needed a very long time to produce. Other experiments by using different intensities of lights were also done. We used different lighting strengths in the growth chamber to observe the in vitro tomato's growth and floral transition. All of the last two attempts were without success. The vegetative phase of the in vitro tomato did not go well when it was grown inside the growth chamber. Previous experiments from researchers concluded that various cytokinins and auxins were responsible for flowering and fruiting promotion in tomato plantlets, depending on the varieties[9–11]. Moreover, photoperiod[12] and light intensity[10] were known to be the 'generative-phase' inductor in tomatoes. However, there was still a lack of information about the same range for both factors in tomato plantlets.

3 | MATERIAL AND METHOD

This article review used the literature research method. The pieces of literature were found by doing a scientific search on Google. The keywords used included "tomato, in vitro flowering." The initial investigation found about 4,670,000 results. We only could find seven specific journal articles. The other references were complementary to the review that explains the science of light intensity, flowering signaling, tomato as sample plants in plant research, etc.

4 | RESULTS AND DISCUSSION

4.1 | Findings

References on in vitro flowering tomatoes are still very minimal and specific, so it is still challenging to develop because there is no general protocol for this method. The following is a description of the methods and results from several journals. According to Dielen et al.[9] research, auxin does not affect flowering even though it has a role in regulating plant cell activity because several sources state that flowering can be induced by reducing the presence of auxin. Sucrose levels that are too high cause

TABLE 1 List of media compositions for in vitro flowering method of tomatoes proposed by Sheeja & Mandal (2003).

Medium Code	Composition
T1	2 ppm BAP
T2	2 ppm BAP + 0.5 ppm GA + 0.5 ppm IAA
T3	2 ppm BAP + 1 ppm GA + 0.5 ppm IAA
T4	2 ppm BAP + 1.5 ppm GA + 0.5 ppm IAA
T5	2 ppm BAP + 2 ppm GA + 0.5 ppm IAA
T6	2 ppm BAP + 1 ppm ABA + 0.5 ppm IAA
T7	2 ppm BAP + 2 ppm ABA + 0.5 ppm IAA
T8	2 ppm BAP + 10 µM $AgNO_3$
T9	2 ppm BAP + 20 µM $AgNO_3$

disruption of leaf and root growth, possibly due to osmosis events that cause cell damage because the cells become hypertonic. The sterilization method was carried out by washing the 7-day-old uniflora mutant tomato node explants using 3% calcium hypochlorite with 0.1% Tween 20 added for 5 minutes and rinsing with distilled water four times. This study examines the effect of several growth regulators, namely BAP, GA, BAP+GA, kinetin, zeatin, and IPA. The experimental results showed that 0.58 M BAP affects flowering by 63.2% and can cause callus formation even though it inhibits root growth. GA did not affect flowering and was even antagonistic to BAP and kinetin when combined in one medium. Zeatin, IPA, and kinetin were each tested for their effects using the same concentration of 3.5 M. The study results showed that kinetin had the best effect on flowering, 66.7%.

All types of cytokinins used cause callus formation at the base of the plant. Kinetin and IPA are also known to cause higher root growth than BAP. The effect of variations in nitrogen concentration is also discussed in the journal, where when the attention is lowered, plant growth also decreases and causes chlorosis and unstable root growth. However, flowering occurred optimally (100%) in N with a concentration of 8 g/L. In conclusion, the best flowering medium was the macro-microelement media composition of MS media, vitamins from the composition of B5 media, 3% sucrose, 8 ppm N, and 3.5 M kinetin. The environmental conditions used were pH 5.7-5.8, a temperature of 23.5°C, 16 hours of light, and 12,580 lux of light intensity. Each treatment used ten replications, and the experiment was carried out twice.

Another study conducted by Sheeja and Mandal[10] used seven varieties of tomatoes (Pant11, PP2, Pant 5, KS118, No: 342, Cl9d 0-0-6-3, Le3704, and Le79) which are commonly found in India. They list the media composition as shown in Table 1 . The research was started by optimizing the flowering media for the Pant11 variety tomato; then, the optimum medium was tested on other varieties. The sterilization method was carried out by soaking the seeds into a 2% Bavistin solution to which 5% Teepol had been added for 15 minutes, then washed with 0.1% HgCl2 solution for 5 minutes and rinsed with sterile distilled water three times. Germination media used MS medium with 3% sucrose and pH 5.8. After 15 days, the leaves (about 1 cm2 were subcultured into media that had added 2 ppm BAP to induce callus. Callus (0.5 g) aged 30 days were used as explants to test the effect of several combinations of growth regulators added to the plant. The following is a table of the types of media used and the results of experiments for optimization of flowering media using tomato explants of the Pant11 variety.

T1 and T6 media better affected overall plantlet growth and flowering. However, the addition of ABA decreased the number of flower buds, so it could be that ABA has an antagonistic effect on BAP, such as the GA hormone. In this study, GA has an excellent impact on producing good quality callus and inhibiting flowering, which supports the statement of previous research journals[9]. This study also shows that decreasing light intensity from 2,908 lux to 162.8 lux can help flowers bloom fully within four days of moving under 24-hour light conditions. Experiments with 16-hour light conditions led to the death of flower buds. This could be due to changes in photosynthetic reactions when plantlets flower. The KS118 variety blooms the fastest in 58 days (about eight weeks).

In conclusion, the best medium for flowering is MS0 + BAP 2 ppm. The environmental conditions used were 16 hours of light, and the light intensity was at 2,908 lux and then reduced to 162.8 lux. Each treatment used a minimum of 15 replications, and the experiment was carried out three times. Mamidala and Nanna[11] conducted another study using cv dwarf tomato explants. Micro-Msk is a hybrid of the Micro-Tom mutant tomato and the wild-type MsK[13]. Seed sterilization begins with water overnight, then washing with 10% NaOCl, adding 2% SDS for 3 minutes, and rinsing with sterile distilled water three times. Seeds were planted on MS medium containing 300 mg/L Amp and grown for three weeks. Then, the leaves from the plantlets were cut 1 cm2 and subcultured into several media variations for shoot induction. The following are variations of the media used.

TABLE 2 List of media compositions for in vitro flowering method of tomatoes proposed by Mamidala and Nanna (2009.

Medium Code	Composition	Medium Code	Composition
SIM 1	1 ppm BAP	SIM 8	2 ppm BAP + 0.1 ppm NAA
SIM 2	2 ppm BAP	SIM 9	1 ppm BAP + 0.1 ppm IAA
SIM 3	3 ppm BAP	SIM 10	2 ppm BAP + 0.1 ppm IAA
SIM 4	1 ppm Zeatin	SIM 11	1 ppm Zeatin + 0.1 ppm NAA
SIM 5	2 ppm Zeatin	SIM 12	2 ppm Zeatin + 0.1 ppm NAA
SIM 6	3 ppm Zeatin	SIM 13	1 ppm Zeatin + 0.1 ppm IAA
SIM 7	1 ppm BAP + 0.1 ppm NAA	SIM 14	2 ppm Zeatin + 0.1 ppm IAA

TABLE 3 List of media compositions for in vitro flowering method of tomatoes proposed by Savitri et al. (2009).

Medium Code	Growth Hormone Composition (ppm)
MS0	None
B1	1 BAP
I0.5+G1.5	0.5 IAA + 1.5 GA3
I1+G1	1 IAA + 1 GA3
I1.5+G0.5	1.5 IAA + 0.5 GA3
I0.5+G1.5+K0.5	0.5 IAA + 1 GA3 + 0.5 KH_2PO_4
I1+G1+K0.5	1 IAA + 1 GA3 + 0.5 KH_2PO_4
I1+G0.5+K0.5	1 IAA + 0.5 GA3 + 0.5 KH_2PO_4
I1.5+G0.5+B1+K1	1.5 IAA + 0.5 GA3 + 1 BAP + 1 KH_2PO_4
I0.5+G0.5+B1.5+K1	0.5 IAA + 0.5 GA3 + 1.5 BAP + 1 KH_2PO_4
I0.5+G0.5+B2+K1	0.5 IAA + 0.5 GA3 + 2 BAP + 1 KH_2PO_4
I0.5+B1	0.5 IAA + 1 BAP
I0.5+B1.5	0.5 IAA + 1.5 BAP
I0.5+B2	0.5 IAA + 2 BAP
G0.5+B1	1 GA3 + 1 BAP
G0.5+B1.5	1 GA3 + 1.5 BAP
G0.5+B2	1 GA3 + 2 BAP

Based on these data in Table 2 , the best medium to induce shoots was SIM 14. Then, SIM 9, 10, 13, and 14 were retested as a flowering medium. Flower buds appeared in the sixth week after subculture or when the plantlets were nine weeks old. However, flowering became faster when the medium was added with Timentin 300 mg/L, where flower buds appeared at the age of seven weeks. Timentin is one type of antibiotic that is used to induce the growth of plant shoots. The environmental conditions were pH 5.8, a temperature of 25°C, 60-70% RH, 16 hours of light, and a light intensity of 4,440 lux. The experiment was carried out at least twice, with each treatment having ten replications.

Recent research on the in vitro flowering of tomatoes was conducted by Savitri et al.[8]. The explants used were tomato seeds of the Tymoti variety, one of Indonesia's most popular tomato varieties. The seeds were rinsed with water first, then washed with 2.6% NaOCl solution for 5 minutes, washed again with 1.8% NaOCl solution for 15 minutes, then rinsed with sterile distilled water three times. The seeds were then grown on cotton media moistened with 10 mL of 1 ppm BAP solution with a pH of 5.6. After two weeks, the nodes were subcultured into several media variations, as shown in Table 3 . Using BAP with a high enough concentration caused the appearance of callus. However, all of these media were good for germination with a percentage of 80%. However, media with a more economical composition were chosen, namely MS and MS0 + 1 ppm BAP. The next stage uses MS0 + BAP 1 ppm media because this media has a more significant potential to affect the plantlet flowering process. BAP is a cytokinin known to stimulate cell growth, budding, and flowering. Using a concentration of 1 ppm aims to suppress the formation of callus. This study also provided information that additional phosphate compounds (KH_2PO_4), ancymidol retardant, and paclobutrazol retardant did not affect the flowering of Tymoti tomatoes. Flower buds are formed when the plantlets are six months and six days old. The environmental conditions used were pH 5.8, temperature 20°C, and 16 hours of light.

Based on these sources in Table 4 , it is known that the BAP hormone has a good effect on in vitro flowering. Not only for tomatoes but also the in vitro flowering of some other plants. However, due to several factors (genetic/variety, additional compounds, and light intensity), the optimal concentration of BAP hormone varies. The hormone kinetin is used less frequently for research on tomatoes because BAP is more economical than kinetin. In the studies carried out, the best BAP concentrations are 1 ppm and 2 ppm[8, 10]. Because in the reference journal, the explants used are explants of mutant plants that grow to a smaller size when mature (for research purposes only). The concentration of growth regulators is also minimal (3.5 M), so it will not be

TABLE 4 Cytokinins that affects in vitro flowering of tomatoes.

Variety	Explant Source	Cytokinin	Concentration	Flowering (%)	Reference
Mutant uniflora (uf)	Node	Kinetin	3.5 µM (N 8 g/L)	66.7	Dielen et al.[9]
Pant11, PP2, Pant5, KS118, No: 342, Cl9d 0-0-6-3, Le3704, & Le79	Seed, leaf, callus	BAP	2 ppm	100	Sheeja and Mandal[10]
Micro-MsK	Seed, leaf	BAP & Zeatin	1-2 ppm	unknown	Mamidala and Nanna[11]
Tymoti	Seed	BAP	1 ppm	80	Savitri et al.[8]
Sheeja and Mandal[10]					

TABLE 5 Effect of light on in vitro flowering of tomatoes.

Variety	Photoperiodism	Light intensity (lux)	Reference
Mutant uniflora (uf)	16 h light	12,580	Dielen et al.[9]
Pant11, PP2, Pant 5, KS118, No: 342, Cl9d 0-0-6-3, Le3704, & Le79		2,908 & 162.8	Sheeja and Mandal[10]
Micro-MsK		4,440	Mamidala and Nanna[11]
Tymoti		unknown	Savitri et al.[8]

TABLE 6 Flowering time of several tomato varieties in vitro.

Variety	Time of Flower Initiation	Reference
Mutant uniflora (uf)	unknown	Dielen et al.[9]
Pant11, PP2, Pant 5, KS118, No: 342, Cl9d 0-0-6-3, Le3704, dan Le79	8 weeks	Sheeja and Mandal[10]
Micro-MsK	7 weeks	Mamidala and Nanna[11]
Tymoti	6 months & 6 days	Savitri et al.[8]

suitable for varieties commonly used for mass production. There is still no reference to directly compare the effects of the two types of cytokinins without adding auxin hormones or antibiotic compounds.

Other vital factors are environmental factors, namely, media pH, temperature, photoperiod, and light intensity. The optimum condition of the media was at pH 5.8 and 16 hours photoperiod as reference. In several previous studies, the range of light intensity used was extensive enough that it needed to be tested more specifically. Table 5 are the results of a literature study on photoperiod and light intensity from previous studies. Table 6 shows that the flowering time of tomatoes in vitro is different based on the experimental results of some of these references. In conclusion, genetic factors of tomato varieties, type and concentration of hormones (cytokinins), and light intensity affect the in vitro flowering of tomato plants. The following are some of the flowering results from a literature study.

4.2 | In Vitro Flowering Method for Tomatoes

Plant tissue culture is a technique for growing plant cells or tissues in a container with a growth medium containing the nutritional components needed by the plant to grow under controlled and sterile environmental conditions[14]. This technique requires a small part of the plant, either from the leaves, stems, or roots, to grow new individual plants by utilizing the totipotency properties of plant cells generally located in meristem cells. Controlled environmental conditions (temperature, light, humidity, etc.) and sterile processing and storage provide several advantages: germplasm preservation and plant maintenance, production of pathogen-free plants, production of drugs or secondary metabolites, and fast propagation. Using tissue culture techniques makes it possible to reproduce homogeneous plants shorter than conventional techniques, multiplying hybrid plants or even re-cultivating endangered plants that are difficult to cultivate conventionally by considering the media used and optimized environmental conditions. One approach in tissue culture is in vitro flowering or in vitro flowering. Flowering plants with tissue culture techniques has several benefits, namely as a solution for cultivating plants that produce seeds naturally, especially for

threatened plants. Because it is carried out in vitro, the spread of disease or pest disturbances does not occur or does not spread. It can also be used for physiological studies and flowering in plants.

Light affects plant physiological activities in photosynthesis, morphogenesis, metabolism, gene expression, and others. The light received by plants will be converted into chemical energy and used for physiological processes, and some of it is lost as heat. Three kinds of light effects affect plant growth and development: photoperiodism, light intensity, and the type of light used. Light requirements for in vitro plants are lower than those for ex vitro plants. This is because in vitro plants grow in a sufficiently controlled environment, and light is conditioned to be evenly distributed throughout the culture room. The energy from light received by plants in the culture room is relatively the same. In contrast to ex vitro plants which require more light because other plants block sunlight, there is competition for light, or it could be because the light is covered by clouds (especially during the rainy season) so that the energy from the light is not entirely accepted by plants and other conditions that can block sunlight from reaching the plant[12, 15].

Photoperiodism is the response of plants to the length of light and dark periods. The light and dark periods will affect the activity of phytochromes (blue-green pigments) for the germination process until the formation of flowers and seeds. Light periods that are too long can interfere with growth and cause plants to become abnormal. For example, the leaves become smaller, thicker, and have chlorosis, the stems cannot grow tall, and the root system is not sound. Optimum light and dark periods for each plant are different, so optimization needs to be done. For example, in tomato plants, it is known that a good photoperiod is between 12 hours of light to 18 hours of the morning[16, 17]. In addition to photoperiodism, light intensity also needs to be considered because it affects the growth and formation of plant shoots. This is related to plants' amount of light energy to achieve optimal photosynthetic photon flux density (PPDF). The optimal PPDF of each plant is different in a specific range, so it is necessary to optimize it so that the given light intensity does not cause the plant to overheat and cause cell damage.

Another light effect is the type of light or the color of the morning. Red light (610 nm – 750 nm) and blue light (400 nm – 520 nm) individually have different plant benefits. Red light plays a role in the vegetative period and the flowering process of plants, while blue light plays a role in maintaining the rate of plant growth. However, if one type of light is given in excess, then plant growth becomes abnormal. Therefore, the provision of light also needs to be considered because plants need all the light spectrum. The combination of red and blue light at a specific ratio is known to have a better effect on the growth of certain plants. In addition, it is also known that light stress treatment can trigger plant hormone activity, so the interaction between light factors and plant hormones can trigger different plant growth responses[15].

LED lamps have several advantages compared to other lights (natural daylight or fluorescent) commonly used in culture rooms. LED has wavelength specificity, durability, and long operating life. It is also relatively cold or emits less heat. LED's photon output is proportional to the electrical input. Lastly, its spectral composition can be adjusted[17].

4.3 | Factors Affecting In Vitro Flowering for Tomatoes

Based on the literature study that has been carried out, we obtained some information about in vitro flowering for tomato plants. Several factors affect the flowering of tomato plants. First, the source and type of explant used will impact the in vitro flowering method because of the genetic characteristic of the tomato plant. Genetic factors cause differences in the sensitivity of explants to stimuli from the environment in which they grow. Therefore, optimization is necessary to find the most appropriate method for an explant. To meet the needs or market demand for tomatoes, choosing tomatoes with superior quality, high yields, and easily obtained seeds is necessary. The ease of getting seeds is related to research to optimize the methods used for in vitro multiplication. One of the excellent explant candidates is the Tymoti tomato variety because it is proven to have several advantages and is quite popular among the Indonesian people. In addition to the source of the explant, the type of explant also needs to be considered. Second, the size of the explant will affect the optimal sterilization method. Surface sterilization methods are needed to remove dirt, be it dust, preservatives, residual pesticides, and even pathogens (bacteria, fungi, and others) attached to the explants. The most commonly used sterilization for in vitro flowering tomatoes is chemical. Chemical sterilization uses chemical compounds that contain detergents. A joint and readily available detergent compound is sodium hypochlorite (NaOCl). 5.25% NaOCl is found in Bayclin's commercial products. Based on the research done by Savitri et al.[8], simple surface sterilization can be done by washing seed explants using 2.6% NaOCl for 5 minutes, then rinsing.

Furthermore, it was rewashed using 1.8% NaOCl for 15 minutes, then rinsed three times. Because the NaOCl contained in Baylin is 5.25%, while the concentration is lower, it is necessary to dilute it. Rinsing was carried out using sterile distilled water to

remove chemical residues still attached to the seeds. Too long exposure to these compounds can cause cell death in the seeds, which cannot grow properly.

Furthermore, in the germination stage, a simple, effective method was obtained from the research of Savitri et al.[8]. Sterile seed explants were planted in culture bottles filled with sufficient cotton media moistened with 10 mL of 1 ppm BAP solution. BAP is a type of synthetic cytokinin. Cytokinin compounds can stimulate seed growth, so the seeds are expected to grow within 14 days or faster. In line with the above result, the use of cytokinins as a priming agent has also been reported by Nawaz et al.[18]. Their results showed that the germination percentage of two tomato cultivars, Nagina and Pakit, increased after the cytokinins treatment. It is also known that cytokinins, auxins, gibberellins, ethylene, and abscisic acid, are essential in seed maturation and germination[19, 20].

In addition, using BAP at the beginning also aims to adapt tomato plants to the early addition of cytokinin compounds. In one bottle, the number of seeds planted is limited to 8-10 seeds to avoid stunting the growth of each seed due to lack of space to grow, especially for the roots, or due to competition for nutrients. After the plant is tall enough and the cotyledons are large enough, it is continued with subculture using flowering media.

During the ex vitro growth process of tomato plants, fertilizer is generally added to add nutrients in the media so that growth becomes faster, especially for roots, height gain, and the number of leaves to shorten the plant's vegetative period and move faster its generative phase. It can be adapted for tomato growth in vitro. Three important elements that contribute to the growth of tomato plants are N, P, and K. To support growth in the vegetative period, more N is required. The N is needed to grow leaves and allow chlorophyll to increase the photosynthesis rate optimally. This is related to the number of leaves that must be achieved to reach the transition period (from the vegetative phase to the generative phase) so that flowering can occur [7]. However, when growing plants in vitro, there will be a limited area because the plants are grown using culture bottles, so it is necessary to subculture periodically. It is well known that the number of subcultures can affect the multiplication potential of a plant, so the effect of adding fertilizer and the number of subcultures may be interrelated for the growth of tomato plants in vitro in the vegetative phase.

Light is essential because the amount of energy obtained and the amount of energy used affect plant physiological processes (photosynthesis, metabolism, and others). The light intensity for plant growth in vitro is lower than that for plants grown in ex vitro. However, as already discussed, the energy requirements of light for each plant are different, so it is necessary to optimize to find the right light intensity so that plants are not exposed to too hot light and cause damage to plant cells[15]. Therefore, it is necessary to research the optimal light intensity range to accelerate tomato plants' flowering. A light intensity of 32 Watt/2,880 lux at the germination stage is generally used (fluorescent lamp). This type of lamp is commonly used in the culture room, while at the flowering stage, there is a theory that a decrease in light intensity can stimulate the flowering of tomato plants more quickly[10]. LED lamps to have a better impact on the plant growing environment, one of which is that they produce or emit less heat. However, there has been no research on the optimum light intensity for tomato flowering. In addition, the saturation light limit is also not known for sure. No study describes the saturation light limit for tomato plants, specifically for tomato plant varieties in Indonesia.

In addition, light also influences plant morphogenesis. Phytochromes (blue and green pigments of plants that receive light) are influenced by photoperiod[12]. The photoperiod in the study used was at 16 hours of light and 8 hours of darkness because exposure of tomato plants to too long light (more than 18 hours of light) can cause a decrease in the rate of photosynthesis due to lack of chlorophyll, so that plants experience chlorosis and do not grow normally (short stems), leaves are smaller, thicker, and yellowed)[16, 17].

5 | CONCLUSION

In vitro flowering for Solanum Lycopersicum L. is carried out by optimizing the flowering media using cytokinin hormones or a combination of cytokinin and auxin hormones (higher cytokinin concentrations) grown under the optimal light intensity. The factors that affect in vitro flowering are varieties of explants, hormones in the media, and the intensity of light used.

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References

1. Concha C, Canas R, Macuer J, Torres MJ, Herrada AA, Jamett F, et al. Disease prevention: An opportunity to expand edible plant-based vaccines? Vaccines 2017 6;5:4.
2. Paduchuri P, Bindu Thamke SG, Subha M. Transgenic tomatoes-a review. International Journal of Advanced Biotechnology and Research 2011;1:69–72. http://www.bipublication.com.
3. Quinet M, Angosto T, Yuste-Lisbona FJ, Blanchard-Gros R, Bigot S, Martinez JP, et al. Tomato fruit development and metabolism. Frontiers in Plant Science 2019;10:1–23.
4. Piscitelli C, Lavorgna M, Prisco RD, Coppola E, Grilli E, Russo C, et al. Tomato plants (Solanum lycopersicum L.) grown in experimental contaminated soil: Bioconcentration of potentially toxic elements and free radical scavenging evaluation. PloS one 2020;15:1–14.
5. Saeed W, Naseem S, Gohar D, Ali Z. Efficient and reproducible somatic embryogenesis and micropropagation in tomato via novel structures-Rhizoid Tubers. PloS One 2019;14.
6. Gerszberg A, Grzegorczyk-Karolak I. Influence of selected antibiotics on the tomato regeneration in in vitro cultures. Notulae Botanicae Horti Agrobotanici Cluj-Napoca 2019;47:558–564.
7. Samach A, Lotan H. The transition to flowering in tomato. Plant Biotechnology 2007;24:71–82.
8. Savitri WD, Nurtyandi FWN, Hardjo PH. Induction of Flowering and Fruiting in Plantlets of Tomato (Lycopersicon esculentum Mill.). Media Pharmaceutica Indonesiana 2018;2:57–63.
9. Dielen V, Lecouvet V, Dupont S, Kinet JM. In vitro control of floral transition in tomato (Lycopersicon esculentum Mill.), the model for autonomously flowering plants, using the late flowering uniflora mutant. Journal of Experimental Botany 2001;52:715–723.
10. Sheeja TE, Mandal AB. In Vitro flowering and fruiting in tomato (Lycopersicum esculentum Mill.). Asia Pacific Journal of Molecular Biology & Biotechnology 2003;2:98–102.
11. Mamidala P, Nanna RS. Efficient in vitro plant regeneration, flowering and fruiting of dwarf Tomato cv. micro-msk. Plant Omics Journal 2009;2:98–102. www.pomics.com.
12. Sandag AR, D, Rawung HL. Pemberian cahaya tambahan dengan lampu HID dan LED untuk merespon waktu pembungaan tomat cherry (Solanum lycopersicum var cerasiforme) di dalam rumah tanaman. Cocos, E-Journal Unstrat 2017;1.
13. Lima JE, Carvalho RF, Neto AT, Figueira A, Peres LEP. Micro-MsK: A tomato genotype with miniature size, short life cycle, and improved in vitro shoot regeneration. Plant Science 2004 10;167:753–757.
14. Sathyanarayana BN, Varghese DB. Plant Tissue Culture: Practice and New Experimental Protocols. I.K. International Publishing House Pvt. Ltd; 2007.

15. Cioc M, Kalisz A, Zupnik M, zena Pawłowska B. Different LED light intensities and 6-benzyladenine concentrations in relation to shoot development, leaf architecture, and photosynthetic pigments of gerbera jamesonii bolus in vitro. Agronomy 2019;9:358.

16. Withrow AP, Withrow RB. Photoperiodic chlorosis in tomato. Plant Physiology 1949;24:675–663.

17. Dorais M, Yelle S, Gosselin A. Influence of extended photoperiod on photosynthate partitioning and export in tomato and pepper plants. New Zealand Journal of Crop and Horticultural Science 2010;24:29–37.

18. Nawaz A, Amjad M, Khan SM, Afzal I, Ahmed T, Iqbal Q, et al. Tomato seed invigoration with cytokinins. Journal of Animal and Plant Sciences 2012;23:121–128.

19. Bewley JD, Black M. Seed: Physiology of Development and Germination. 2 ed. Plenum Press; 1994.

20. Liu Y. Hormones and tomato seed germination. PhD thesis, Wageningen Agricultural University; 1996.

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