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High flowering frequency of in vitro tomato using 6-Benzylaminopurine (BA) and uniform light

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ABSTRACT

Light is essential for plant morphogenesis and flowering induction. Therefore, maintaining uniform light conditions across all cultures may enhance the success of in vitro tomato flower production. Nevertheless, limited information is available on how specific and uniform light conditions can affect in vitro flower production. Thus, the current research aimed to determine how uniform light influences in vitro flowering induction and flower production in tomato plantlets. Murashige and Skoog (MS) medium, MS medium supplemented with 1 ppm 6-Benzylaminopurine (BA), and Murashige Modified Shoot Multiplication medium were used. The effects of uniform light intensities of 1,300, 2,000, and 3,000 lux generated by white light-emitting diode grow lamps were also investigated to determine the optimum condition for flowering induction. The results showed that 1 ppm BA combined with 2,000 lux uniform light produced 100% in vitro flowers in the tomato variety Tymoti. The uniform 2,000 lux light intensity significantly improved in vitro flowering frequency. This study is the first to report on the effect of uniform light on in vitro flower induction in tomato plantlets. The results of this study will contribute to the improvement and innovations of tomato plant breeding.

Keywords: *6-Benzylaminopurine; in vitro flowers; tomato; Tymoti; uniform light*

INTRODUCTION

Plant tissue culture (PTC) is a modern method of propagation aimed to enhance the quantity and quality of plants. This method can be used in plant experiments for various purposes, such as multiplication, genetic engineering, and production of pathogen-free plants (Algophisi *et al.*, 2025; Park, 2021; Roy, 2024). PTC increases plant production rapidly while maintaining quality compared to traditional methods (Park, 2021). Plant propagation using this method requires a suitable medium and a controlled sterile environment (Rizvi *et al.*, 2025). PTC relies on the totipotency of plant cells, whereby a single cell can regenerate into a complete plant. Plants cultured with the PTC method can also reach the generative phase, in which plants can produce flowers and fruits under in vitro conditions (Baday, 2018).

In vitro flowering provides a powerful approach to overcome biological and environmental constraints associated with conventional plant development, particularly in species with long juvenile phases such as orchids, geophytes, and many perennial plants. By cultivating plants under controlled *in vitro* conditions, the juvenile period can be significantly shortened, enabling earlier floral induction than under natural conditions (Guillén-Rodríguez *et al.*, 2022; Ziv & Naor, 2006). This accelerated alteration from vegetative to reproductive phase is highly advantageous for ornamental, food, medicinal, and craft plants, as it reduces production time and enhances research efficiency.

Another major benefit of *in vitro* flowering lies in the ability to precisely control and manipulate environmental and physiological factors that regulate floral development. Parameters such as light intensity, photoperiod, temperature, and nutrient composition can be systematically adjusted, allowing detailed investigation of the physiological, genetic, and molecular systems underlying flowering. Moreover, the controlled regulation of plant growth regulators within the culture medium provides valuable insights into hormonal interactions that influence floral initiation and organ development, making *in vitro* systems ideal experimental platforms for flowering studies (Gonçalves *et al.*, 2018; Guillén-Rodríguez *et al*., 2022; Nadal *et al*., 2023).

In vitro flowering also plays an essential function in plant breeding, genetic improvement, and commercial production. By shortening plant life cycles, it accelerates breeding programs and enables early identification and selection of desirable traits, thereby improving breeding efficiency (Nadal *et al*., 2023). From a commercial perspective, *in vitro* flowering allows year-round production independent of seasonal constraints and supports the large-scale propagation of genetically uniform plants (Mobini *et al.*, 2015; Nadal *et al.*, 2023; Ziv & Naor, 2006). Additionally, *in vitro* floral organs can serve as sources of valuable secondary metabolites, further enhancing the economic potential of cultivated species. Collectively, these advantages establish *in vitro* flowering as an essential tool for advancing plant science, breeding innovation, and sustainable commercial production.

In vitro flowering represents a promising approach for accelerating breeding and genetic improvement in tomato plants (*Lycopersicon esculentum*) by inducing reproductive development under controlled conditions. Although *in vitro* flowering has been extensively investigated in various plant species, studies focusing specifically on tomato remain relatively limited. Available research indicates that the induction of flowering in tomato is highly dependent on precise manipulation of environmental and nutritional factors, underscoring the need for optimized protocols tailored to different tomato genotypes (Dewi *et al.*, 2022).

Several studies have demonstrated the importance of light intensity, culture media composition, and plant growth regulators in promoting *in vitro* flowering and associated developmental processes in tomato, highlighting the synergistic role of light and hormonal regulation in floral initiation (Dewi *et al.*, 2022). In addition, the interaction between light intensity and photoperiods is critical for flowering development. Although such studies have not yet been conducted using *in vitro* plantlets, experiments performed under greenhouse conditions have successfully produced uniform flower development in strawberry. The results showed that high light intensity promoted uniform flower development by reducing variability in flowering time and bud number (Wang *et al.*, 2020). In contrast, shading or low light intensity prolongs the vegetative phase and delays floral induction, as seen in *Ptilotus nobilis* and other species (Orzek *et al.*, 2009). Photoperiod is also a major environmental cue regulating floral induction. Depending on the plant species, short-day or long-day conditions may either promote or inhibit flowering. For instance, in *Viola philippica*, long photoperiods inhibited petal and stamen development, resulting in the formation of cleistogamous (self-pollinating) flowers, while short photoperiods promoted complete floral organ development (Li *et al*., 2016; Li *et al*., 2017).

BA alone has been widely used to induce flowering *in vitro*. For example, in *Bletia urbana*, higher concentrations of BA (4.5 – 6.8 ppm) significantly increased the frequency of flowering, although they also reduced shoot and leaf production and overall plant growth (Guillén-Rodríguez *et al.*, 2022). Similarly, in *Kniphofia leucocephala*, flowering was achieved on media supplemented with 2.5 ppm BA combined with 6% fructose (Taylor *et al*., 2007). This finding indicates that the effectiveness of BA in promoting flowering is highly dependent on its concentration and interaction with other medium components. For instance, in *Rosa hybrida*, optimal flowering was obtained using 1 ppm BA combined with 0.0001% $AgNO_3$ and 4% sucrose (Tran, 2025).

While BA is a potent inducer of flowering, its effects are often modulated by light conditions. For example, in *Rosa hybrida*, flowering was induced only when BA was combined with an 8/16 light/dark photoperiod at a light intensity of 2,220 lux. Likewise, in *Oldendandia umbellate*, BA-induced flowering was enhanced by a specific light intensities (3,330 lux) and a particular photoperiod (Behera *et al.*, 2018). While in *Bletia urbana*, BA was used to induce *in*

vitro flowering in combination with a light intensity of 4,440 lux and a 12 h/12 h photoperiod (Guillén-Rodríguez *et al.*, 2022).

Studies of tomato *in vitro* flowering have been conducted by some scientists (Dewi *et al.*, 2022; Kaur-Sawhney *et al.*, 1996; Mamidala & Nanna, 2009; Rastogi & Sawhney, 1986; Savitri *et al.*, 2019; Sheeja & Mandal, 2003). But, the information regarding var. Tymoti is still limited. Tomato cv. Tymoti was selected for this study because of its superior agronomic characteristics, including resistance to Gemini Virus, blossom-end rot, and bacterial wilt caused by *Ralstonia solanacearum*. In addition, this cultivar exhibits a relatively short harvesting period and good tolerance to high-temperature conditions. These traits make Tymoti a commercially valuable cultivar and a suitable experimental material for evaluating *in vitro* flowering responses under different culture conditions. Based on the previous report, *in vitro* flowering of the Tymoti variety was low (2%) in the culture media MS + 1 ppm BA (Savitri *et al*., 2019). However, there have been no reports on the application of specific and uniform light conditions influencing flowering induction in *in vitro* tomato cv. Tymoti culture. Therefore, this experiment assessed the effects of growth media, PGR, and uniform light to develop and optimize a protocol for *in vitro* flowering of tomato var. Tymoti.

MATERIALS & METHODS

The experiments were conducted at the Plant Biotechnology Laboratory, Faculty of Biotechnology, University of Surabaya from November 2022 to April 2023.

Plant material

This study used commercial Tymoti® seeds for germination and single nodes were used for multiplication after the plantlets had been produced. Surface sterilization was applied to the seeds according to the protocol by Savitri *et al.* (2019).

Media preparation

This experiment used three different culture media (Table 1), namely Murashige & Skoog (MS) (Phytotech®; M519) and Murashige Modified Shoot Multiplication Medium (Phytotech®; M491). MS supplemented with 1 ppm BA (Phytotech®, B151) and Murashige Modified Multiplication Medium (containing 170 ppm Sodium Phosphate monobasic, 80 ppm Adenine Hemisulfate, 30 ppm 2iP, 0.3 ppm Indole-3-acetic acid (IAA), and FeNa-EDTA) were used for flowering induction. One liter of multiplication medium was prepared by adding 4.43 g of MS powder and 30 g sugar to 900 mL distilled water. This was followed by the addition of BA, stirring, and the addition of distilled water until the volume reached 995 mL. The pH was adjusted to 5.8 and distilled water was added until the volume reached 1000 mL. Subsequently, 7 g agar was added, followed by boiling and stirring until the solution was homogeneous. The same medium was also used for flowering induction. The previous protocol was also applied to the germination medium, without BA being added to the medium (MS0). Murashige modified multiplication medium (flowering induction) was also prepared similarly to the MS0 medium, but the powder used for 1-liter medium was 4.41 g.

After homogenization, the solutions were dispensed to culture bottles and then sterilized. The autoclave was set at 121°C, 1.5 atm, for 20 minutes. Sterilized media were stored in the storage room for two days and used thereafter.

Table 1. Media composition used for the *in vitro* tomato cv. Tymoti

No.	Name	Medium	Plant Growth Regulator
1	MS0	Murashige & Skoog	-
2	MS + 1 ppm BA	Murashige & Skoog	1 ppm 6-Benzylaminopurine (BA)
3	MMSMM*	Murashige modified multiplication medium	30 ppm 6-(γ,γ-Dimethylallylamino) purine (2iP) + 0.3 ppm Indole-3-acetic acid (IAA)

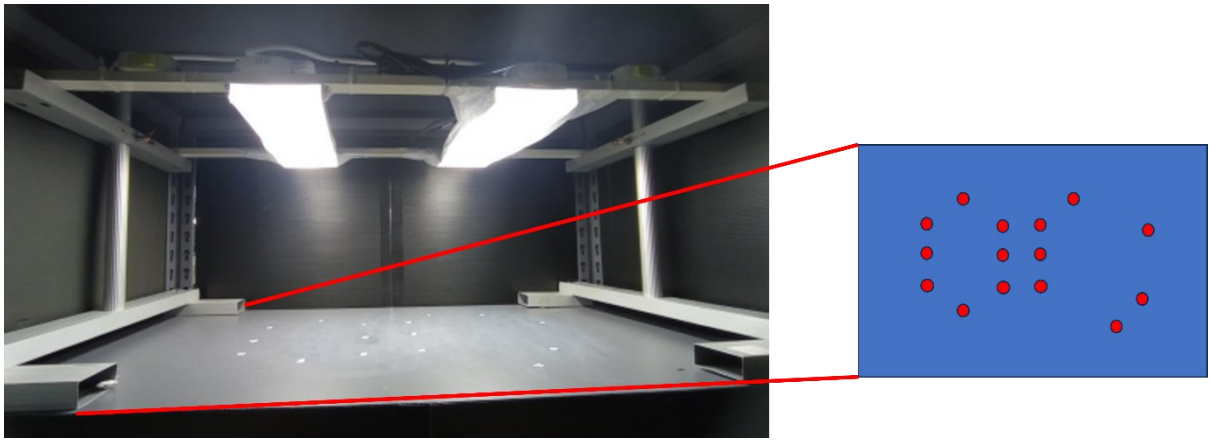

Figure 1. A sample of a closed culture rack showing LED grow light and marks on the base based on precisely measured 3,000 lux.

Culture room condition

The sterilized cultures were incubated in a culture room with environmental conditions of 20 ± 23°C and 70–80% relative humidity, at 16/8 h day/night photoperiod. Light intensity for the germination and multiplication stage was set at 2,000 lux, while for flowering induction, three light intensities were applied, 1,300, 2,000, and 3,000 lux. To ensure the uniformity of light intensity for each culture bottle, marks were made on the of the culture racks based on the measured light intensity using a lux meter (Sanwa® LX2), as shown in Figure 1.

Parameters

The parameters assessed in this study included plant height (cm), number of leaves per plantlet, flower bud formation (%), number of inflorescences, and plantlet age at first flowering (days). In addition, some images of *in vitro* flower buds, fruit, and seeds were captured using a stereomicroscope (Olympus SZ51) at a magnifications of 40x.

Data analysis

Analysis of variance (ANOVA) was used to analyze plant height and leaf number data followed by Duncan's test with α = 0.05% (IBM SPSS Statistics 26). Ten samples were used for each treatment.

RESULTS AND DISCUSSION

Sterilized seeds cultivated on Murashige and Skoog basal medium (MS0) had robust germination after 7 days. The germination success rate was 100% when using the current surface sterilization protocol. Approximately 20 sterilized seeds were cultured in MS0, and all successfully germinated after 7–14 days. Table 2 presents the effects of different light intensities (1,300, 2,000, and 3,000 lux) and culture media on the growth and flowering characteristics of plantlets. Three culture media were evaluated at each light intensity level: basal Murashige and Skoog medium (MS0), MS supplemented with 1 ppm BA, and a modified Murashige shoot-multiplication medium.

The interaction between light intensity and culture medium significantly influenced the plant height. The greatest plant height was observed in plantlets grown on MS0 medium, with the tallest plants recorded at 3,000 lux (12.42 cm), followed by 2,000 lux (11.71 cm) and 1,300 lux (10.42 cm). In contrast, supplementation with 1 ppm BA resulted in shorter plants across all light intensities, with plant heights of 8.84, 7.54, and 8.54 cm under increasing light intensities, indicating that cytokinin application suppressed stem elongation. Plantlets cultured on the modified shoot-multiplication medium exhibited the shortest height regardless of light intensity level, reflecting its function in promoting growth rather than elongation. Overall, increased light intensity enhanced plant height only in the absence of cytokinin supplementation.

The number of leaves was also affected by both light intensity and culture medium. Similar to plant height, plantlets grown on MS0 medium produced the highest number of leaves across all light levels, with maximum leaf development observed at 1,300 and 3,000 lux (8.4 and 7.5, respectively). Conversely, the addition of 1 ppm BA resulted in a reduction in leaf number, particularly under higher light intensities (7.6, 6, and 6.7, respectively). The modified shoot-

multiplication medium yielded the lowest number of leaves at all light intensity levels, with the lowest number of leaves was 2.8 at 3,000 lux. These results indicate that leaf development is adversely influenced by cytokinin supplementation and modified shoot-multiplication medium, especially under high-light conditions.

Similarly, the interaction between light intensity and culture medium impacted flowering response. The highest flowering percentage (100%) was achieved in plantlets cultured on MS medium supplemented with 1 ppm BA under 2,000 lux. In addition, the highest percentage of flower bud formation under 1,300 (by MS0 and MS + 1 ppm BA) and 3,000 lux (by MS + 1 ppm BA) were 70%. Plantlets grown on MS0 medium exhibited moderate flowering rates, ranging from 70% to 90% depending on light intensity. On the other hand, the modified shoot-multiplication medium resulted in very low flowering percentages and completely inhibited inflorescence formation at all light levels. These findings demonstrate that cytokinin application, when combined with moderate light intensity, plays a critical role in promoting floral induction in plantlets.

The type of culture medium had a stronger influence on the number of inflorescences than light intensity. Plantlets grown on MS0 and MS medium supplemented with 1 ppm BA produced one inflorescence per plantlet across all light levels. However, plantlets cultured on the modified shoot-multiplication medium failed to produce any inflorescences regardless of light intensity. These results suggest that modified shoot-multiplication medium preferentially promotes vegetative growth while strongly inhibiting reproductive development.

The age at first flowering was affected by both culture medium and light intensity. The earliest flowering was observed in plantlets grown on MS medium supplemented with 1 ppm BA under 2,000 lux, with flowering was initiated at 140 days. In comparison, plantlets cultured on MS0 medium exhibited delayed flowering, occurring between 169 and 170 days, while those grown on the modified shoot-multiplication medium showed the greatest delay, with flowering occurring at up to 172 days. These results signify that cytokinin supplementation under moderate light intensity markedly accelerates floral initiation in plantlets.

Table 2. Effects of different media and (uniform) light intensity on plantlet growth and flower formation

Light intensity (lux)	Medium	Plant height (cm ± SD)	No. of leaves ± SD	Flower buds (%)	No. of inflorescence (rate)	Plantlets age (rate) at the first flowering (days)**
1,300	MS0	10.42^{cd} ± 2.2	8.4^{e} ± 1.4	70	1	170
	MS + 1 ppm BA	8.84^{bc} ± 2.7	7.6^{de} ± 1.6	70	1	161
	MMSMM*	2.23^{a} ± 0.7	0.9^{a} ± 0.8	10	0	172
2,000	MS0	11.71^{de} ± 3.1	7.5^{de} ± 2.1	90	1	170
	MS + 1 ppm BA	7.54^{b} ± 1.4	6^{cd} ± 1.6	100	1	140
	MMSMM	3.56^{a} ± 1.3	4.3^{bc} ± 3.6	40	0	164
3,000	MS0	12.42^{e} ± 1.4	8.8^{e} ± 1.7	40	1	169
	MS + 1 ppm BA	8.54^{b} ± 1.8	6.7^{de} ± 2	70	1	158
	MMSMM	3.13^{a} ± 1.2	2.8^{ab} ± 2.2	20	0	172

* MMSMM: Murashige modified shoot multiplication medium

** Plantlet age was determined since the day of explant transfer from *in vitro* seedlings to vegetative culture media.

Note: Distinct letters within each column denote significant differences by Duncan's test at α = 0.05. Flowering percentage was determined based on the number of plantlets that produced flower buds in each treatment group (10 replications each).

Based on Table 2, the optimum combination of light intensity and medium for inducing *in vitro* tomato flowering was 2,000 lux using MS medium supplemented with 1 ppm BA. For plant height and number of leaves, MS0 was superior among all media used. Plant height generally increased with increasing of light intensity. The parameters of plant height and number of leaves represent vegetative growth, while the flower bud percentage represents generative growth of *in vitro* tomato.

The highest *in vitro* flower production in this experiment was 100% (50 times higher than in the previous experiment using non-uniform light, No. 5 in Table 3) using a combination of MS medium supplemented with 1 ppm BA under uniform light at 2,000 lux. Uniform light plays a crucial role in promoting consistent and high-frequency flowering by ensuring stable photosynthetic activity across plant individuals (Balázs *et al*., 2023). Light intensity directly regulates photosynthesis (Wu *et al*., 2025), uniform exposure enables balanced energy production and physiological development, which are essential for floral initiation and progression (Trouwborst *et al.*, 2010). Consistent light conditions reduce variability in plant responses, thereby enhancing synchronization and efficiency of flowering within a population (Wang *et al.*, 2020).

Photosynthetic Photon Flux Density (PPFD) is a key determinant of photosynthetic rate and plays a central role in regulating plant growth and flowering. Higher and consistently applied PPFD values enhances photosynthetic efficiency, thereby supporting uniform assimilate production required for synchronized floral development (Coêlho *et al*., 2023). Empirical evidence, including studies on strawberry plants, demonstrates that high light intensity provided by LED sources promotes more uniform flowering, whereas low or inconsistent PPFD leads to variability in flowering time and growth responses (Wang *et al*., 2020). These findings emphasize the significance of maintaining adequate and uniform PPFD to achieve consistent and high-quality floral production.

Light uniformity is essential for achieving synchronized plant growth and flowering, as equal light distribution ensures that all plant tissues receive comparable photonic input. Such uniformity can be optimized through appropriate arrangement and density of LED lamps, as well as by enhancing the reflectivity of growth chamber inner walls. Simulation studies have demonstrated that inner wall reflectivity exerts the strongest influence on light uniformity, followed by the divergence angle of LED lamps, highlighting the importance of chamber design in maintaining consistent light environments for uniform plant development (Lai & Zhuang, 2022).

Light quality and direction are important determinants of flowering responses in plants, as they influence both photosynthetic efficiency and developmental signaling (Yang *et al.*, 2022). The spectral composition of light regulates specific physiological pathways, with red light playing a particularly critical role in initiating and completing flowering due to its high quantum yield in photosynthesis (Cerdán & Chory, 2003). In addition, lighting direction affects canopy light interception; for instance, combined top and side lighting has been shown to enhance branch formation and accelerate flowering in chrysanthemum plants compared with single-direction lighting (Yang *et al*., 2022). These findings highlight the integrated roles of light spectrum and spatial distribution in optimizing flowering performance.

In this study, MS medium supplemented with BA, particularly in combination with specific light intensity, promoted a higher frequency of flower bud formation. These findings are consistent with several previous studies. For example, in *Oldenlandia umbellata*, *in vitro* flowering was successfully induced using 1.5 ppm BA combined with a low concentration of auxin under a light intensity of 3,330 lux (Behera *et al*., 2018). Similarly, in *Portulaca pilosa*, the application of 0.23 ppm BA in combination with a lower auxin concentration resulted in successful *in vitro* flowering induction a t light intensity of 7,400 lux (Xiong *et al.*, 2021). In tomato, previous studies have reported that BA alone, at concentrations ranging between 1 – 2 ppm, in combination with relatively higher light intensities (2,600 to 3,700 lux) than those used in the present study, effectively induced *in vitro* flowering (Dewi *et al*., 2022; Rastogi & Sawhney, 1986; Sheeja & Mandal, 2003).

Based on Figure 2a, the first *in vitro* flower buds were observed on 130 d. The earliest *in vitro* tomato flower was very small and could only be captured using a stereo microscope (Fig. 2a and 2b). *In vitro* tomato needed 172 d to develop (Fig. 2c) and 77 more days to reach maturity (Fig. 2e.).

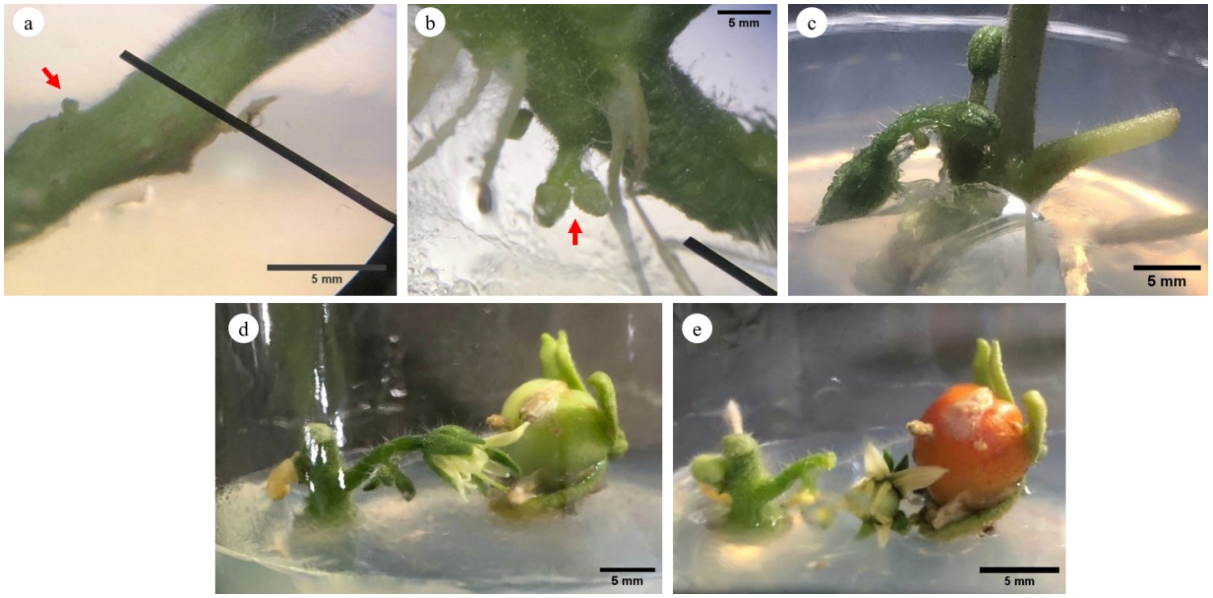

Figure 2. *In vitro* tomato cv. Tymoti development after treatment with MS + 1 ppm BA and 2,000 lux. (a) 130 days old. (b) 140 days old. (c) 172 days old. (d) 249 days old. (e) 256 days old. The red arrows show the flower bud(s) captured under the stereomicroscope at 40x magnification.

This study contributes to ongoing investigations of in vitro flower and fruit development in tomatoes, as shown in Table 2. The majority of previous studies used BA at concentrations ranging from 0.5 to 2 ppm to induce flowering and fruiting in tomato explants. Zeatin was used at low concentrations of 0.66 and 2 ppm. Some studies included small amounts of auxins (Aux), such as Isatin and Indole-3-acetic acid (IAA) (Kaur-Sawhney *et al*., 1996; Mamidala & Nanna, 2009), although these compounds were not applied alone. Timentin was relatively rarely used to induce tomato flowering, but a previous study incorporated the antibiotic along with other cytokinins and Aux for *in vitro* floral induction (Mamidala & Nanna, 2009). BA in combination with paclobutrazol (PBZ) was reported as a plant growth inhibitor that induced flower bud formation in cherry tomatoes (Dewi *et al*., 2022). In general, low concentrations of cytokinins were crucial for inducing flowers and fruits in *in vitro* tomato.

In vitro flowers and fruits usually take longer to form than tomato plants grown in soil. Nonetheless, in two studies where *ex vitro* floral parts were used as an explant source, the time required for flower formation was shorter (9 d in cv. Pixie Hybrid II and 35 d in cv. Early Cahatam). Flowering started between 65 and 142 days in cultures derived from *in vitro* seeds.

All reviewed studies commonly used a photoperiod of 16/8 hours of light and dark. However, there were differences in light intensity used, ranging from 1,226 to 4,440 lux. Although applied to different tomato cultivars, light intensities ranging from 2,000 to 3,700 lux produced 50–100% flowering (Dewi *et al*., 2022; Rastogi & Sawhney, 1986; Sheeja & Mandal, 2003). Light uniformity is a decisive factor in improving flowering efficiency. Table 3 shows that the the best in vitro flowering result occurred only when light is uniform and the conditions are balanced. Our results showed an increase in *in vitro* flower production from 2% to 100% compared to previous studies (Savitri *et al*., 2019). Uniform light is important for producing a uniform plant yield meaning that light is evenly distributed to the growing plants (Runkle, 2017).

Further studies have shown that *in vitro* flowers exhibit normal morphology, including structures such as the pedicel, sepals, petals, stamens, and carpel (Fig. 3a). *In vitro* fruits also show normal morphology, although some features can only be observed under a stereomicroscope. A longitudinal section of *in vitro* fruit showed the presence of the outer pericarp, internal pericarp (radial), and placenta (Ramesh *et al*., 2021). However, the funiculus and locular gel were not visible, as shown in Figure 3d. The outer pericarp consists of three layers, the exocarp, mesocarp, and endocarp (Ramesh *et al*., 2021). Upon further magnification, *in vitro* seeds were found to be very small, less than 0.1 mm (Fig. 3e).

Table 3. List of experiments and successes in tomato *in vitro* flowering

No.	Tomato cultivar	Explant source	Plant growth regulator	Concentration (ppm)	Light intensity (lux)	Light uniformity in each culture	Photoperiod (light/dark) (hours)	Flowering (%)	Explant age at flowering (days)	Reference
1	cv. Early Chatam	floral buds with sepal primordia (ex vitro explant brought into in vitro)	BA	1	3,700	no	16/8	60 – 70	35	(Rastogi & Sawhney,1986)
2	cv. Pixie Hybrid II	pedicel (ex vitro explant brought into in vitro)	Isatin	1.47	1,226	no	16/8	10	9	(Kaur-Sawhney et al., 1996)
			Zeatin	0.66						
3	cv. Pant 11	callus, in vitro leaf	BA	2	2,908 decreased to 163 after flowering	no	16/8	n.a.	65	(Sheeja & Mandal, 2003)
4	cv. Micro-MsK	in vitro leaf	Zeatin	2	4,440	no	16/8	n.a.	>120	(Mamidala & Nanna, 2009)
			IAA	0.1						
			Timentin	300						
5	cv. Tymoti	in vitro node	BA	1	n.a.	no	n.a.	2	n.a.	(Savitri et al., 2019)
6	cv. Golden Sweet	in vitro node	BA	0.5	2,600	no	n.a.	50	142	(Dewi et al., 2022)
			PBZ	0.75						
			PBZ solution	1						
7	cv. Tymoti	in vitro node	BA	1	2,000	yes	16/8	100	140	Current experiment

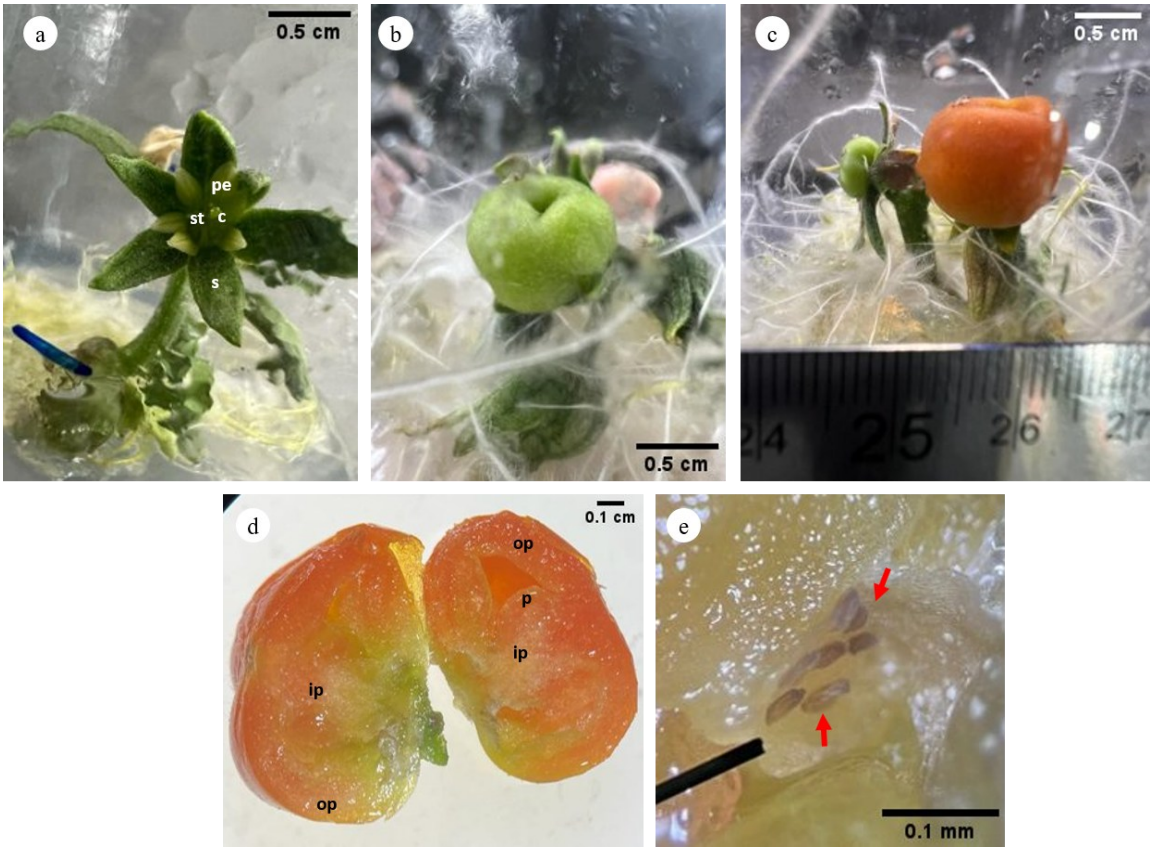

Figure 3. Further investigation of tomato cv. Tymoti flowers and fruits. (a) mature *in vitro* flowers. (b) immature *in vitro* fruit. (c) mature *in vitro* fruit. (d) longitudinal section of *in vitro* fruit under the stereomicroscope (40x magnification). (e) microscopic *in vitro* seeds (40x magnification). The red arrows show *in vitro* seeds. s: sepal; pe: petal; st: stamen; c: carpel; op: outer pericarp; ip: internal pericarp; p: placenta.

In the future, studies should aim to overcome the prolonged duration of *in vitro* flowering observed in tomato cultivar Tymoti, for instance, by initiating cultures from *ex vitro*-derived plants. In addition, further investigations are needed to evaluate fruit formation and production efficiency, as well as to assess the viability of seeds produced under *in vitro* conditions.

CONCLUSION

In conclusion, the results demonstrate that the effects of light intensity on plant growth and flowering are strongly dependent on the culture medium. Optimal flowering was achieved under a combination of uniform light at 2,000 lux and MS medium supplemented with 1 ppm BA, which resulted in 100% flowering, with the earliest flowering occurring at 140 days. In contrast, MS0 medium primarily promoted vegetative growth, as indicated by increased plant height and leaf number, but did not maximize flowering. The modified shoot-multiplication medium completely suppressed floral development irrespective of light intensity, indicating that it is suitable only for vegetative propagation. Furthermore, the higher light intensity (3,000 lux) did not enhance flowering performance and, in some cases, reduced leaf development and flowering efficiency.

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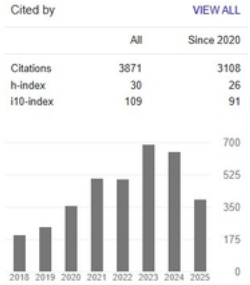

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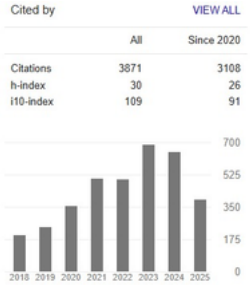

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