

Effect of 6-Benzylaminopurine (BA) and Paclobutrazol (PBZ) with Light Intensity Variations for Cherry Tomatoes *In vitro* Flowering

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Abstract. Utilization of *Solanum lycopersicum* L. and its varieties contribute to plant biotechnology to study the effect of genes and flower transition events. This study aims to optimize media and light intensity for *in vitro* flowering of a cherry tomato variety. Induction of flowering was carried out using seven types of media combined with 3 light intensities, followed by feeding by adding liquid medium MS+PBZ 1 mg/L. A positive response is shown at the light intensity of 2,600 lux; there are 5 explants that have flower buds. The percentage of flower buds for each treatment combination was 50% from two replications. The first shoots occurred at 142 days after planting (DAP) on explants grown on media containing 0.5 mg/L BA, 0.75 mg/L PBZ, with feeding treatment. It can also be assumed that the emergence of flower buds occurred not because of the PGR induction treatment, but because at that time the explants had entered the generative phase, considering that the plantlets had been growing for a long time. Although the effects of BA and PBZ have not been confirmed, the use of 2,600 lux light appears to be the best condition for cherry tomato *in vitro* flowering induction.

Key words: cherry tomato, feeding treatment, plant growth regulator

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INTRODUCTION

Solanum lycopersicum L. or tomato is one of the most widely used horticultural crops. Tomato itself is the result of domestication to obtain several varieties and sub varieties that exist today. To obtain superior seeds, plant breeding is generally carried out both conventionally (crosses) and modern (genetic engineering). One of the premium tomato varieties favored by the public is *Solanum lycopersicum* var. *cerasiforme* or cherry tomatoes. Cherry tomatoes themselves have many types, for example the Ruby variety which has red fruit and Golden Sweet which has yellow fruit. Tomato plants are one of the popular foodstuffs because they are known to have content that is beneficial to health, namely vitamin E, ascorbic acid, and lycopene which can act as antioxidants. Apart from being used as food, as an edible vaccine candidate, tomato plants are also used for scientific purposes (Concha et al., 2017; Gill and Kaur, 2019; Iswari

& Susanti, 2016; Paduchuri et al., 2010; Raiola et al., 2014; Setiaji et al., 2020; Wu et al., 2011).

Utilization of tomatoes for scientific purposes is done *in vitro*. The *in vitro* method or plant tissue culture is carried out because it is superior and makes it easier for researchers because of several advantages, namely explants can be grown at any time without being influenced by season and weather, it is saving the land, it is not susceptible to disease transmission, the environmental conditions and growth media can be optimized so that growth becomes more optimum. In addition, other factors that can interfere or are not known can be suppressed or eliminated so that unbiased and purposeful results are obtained, and observations will be easier (Baday, 2018). However, this is still limited to the vegetative phase because generally, acclimatization is carried out before the plants flower and bear fruit *ex vitro*. Therefore, an *in vitro* flowering method was developed. So that studies on tomato plants *in vitro* can be carried out

until flowers and fruit appear in the bottle. The development of *in vitro* flowering methods is also carried out because it can be used to preserve rare plants that are difficult to flower *ex vitro*, to study flower transition events, and others.

The *in vitro* flowering method itself has been used in several studies, especially for ornamental plants (orchid, baby's breath), traditional medicinal plants (rungia, swertia), and also for tomatoes (Bhowmik and Rahman, 2017; Dielen et al., 2001; Kanchanapoom et al., 2011; Mamidala and Swamy Nanna, 2009; Savitri et al., 2018; Sharma et al., 2014; Sheeja and Mandal, 2003; Shekhawat et al., 2016). Not enough information can be obtained about *in vitro* flowering for tomato plants. Based on the existing literature, it is known that there are two factors that influence the success of flowering, namely genetic factors and environmental factors. Genetic factors affect the response of plants to the given stimuli, while environmental factors can be in the form of temperature, photoperiod, light intensity, and growth regulators (PGR). Based on research on *in vitro* flowering that has been done previously, the intensity of light used is quite diverse, namely in the range of 162.8–12,580 lux. For PGR, 6-benzylaminopurine (BA) group cytokinin are used with varying concentrations (Dielen et al., 2001; Mamidala and Swamy Nanna, 2009; Savitri et al., 2018; Sheeja and Mandal, 2003). In one study (Savitri et al., 2018) it was also stated that paclobutrazol (PBZ) could be used to induce flowering. However, the optimum concentration is still unknown. Flowering time occurs in the range of 2 to 6 months depending on the variety of tomatoes used.

Until now, there is still no information about the *in vitro* flowering of cherry tomatoes. Even though it is possible that cherry tomatoes will also be used to explore knowledge in determining the physiological response of plants to stimuli. This information can be used by researchers and plant breeders (especially tomatoes) to carry out plant breeding or mutation and obtain superior seeds, for example to increase nutritional value, yield, and plant resistance to stress (Arie et al., 2006; Ranjan et al., 2012; The Tomato Genome Consortium, 2012; Shikata and Ezura, 2016; Quinet et al., 2019). Therefore, this study was conducted to determine the optimum light intensity and PGR to induce flowering of cherry tomato plants.

METHODS

One milligram per liter BA solution was used for germination with a pH of 5.8, while the media for multiplication used MS0 media. Flower induction media used MS0 media with BA and PBZ with a concentration of 0 mg/L (control); BA 0.5 mg/L; BA 1 mg/L; BA 2 mg/L; BA 0.5 mg/L + PBZ 0.75 mg/L; BA 1 mg/L + PBZ 0.75 mg/L; BA 2 mg/L + PBZ 0.75 mg/L. BA 1 mg/L solution was prepared by diluting a concentrated BA solution (100 mg/L) as much as 10 mL for 1 L of water. The 10 mL BA solution was poured into a culture bottle that already contained tissue paper. MS media (Phytotech) was prepared by weighing 4.43 g for 1 L of media, added with 30 g/L sugar, 7 g/L agar, and PGR according to the provisions (except for treatment control media; no PGR was used). Before adding agar, the pH was adjusted at 5.8. Then, the media was heated to boiling and poured into culture bottles of 30 mL each. For the second flowering induction, liquid MS media with 1 mg/L PBZ was used, the pH was adjusted to 5.8. Next, the liquid medium was put into a 15 mL centrifugation tube with 10 mL per tube. All media were sterilized by autoclave at 121°C, 1 atm, for 20 minutes.

Cherry tomato seeds var. Golden Sweet was obtained from Known-You Seed Co., Ltd. Sterilization was initiated by washing the tomato seeds using sodium hypochlorite 1.8% (v/v) solution containing Tween 20 (one drop per 100 mL) for 20 minutes then rinsed with sterile distilled water twice. Then, a second sterilization was carried out with sodium hypochlorite 2.6% (v/v) solution containing Tween 20 (one drop per 100 mL) for 15 minutes and then rinsed with sterile distilled water three times. The sterile seed explants were then planted in the germination medium. Each bottle was planted with 10 explants and grown for 8 days. Explants aged 8 days from germination were cut aseptically to leave the cotyledons and hypocotyls, with a height of about ± 1 –1.15 cm. Then planted on MS0 media to induce shoots. Each bottle was planted with a maximum of 4 nodes and grown for 16 days.

After 16 days of shoot induction, shoots were subcultured to flowering induction medium, namely MS0 (control); BA 0.5 mg/L; BA 1 mg/L; BA 2 mg/L; BA 0.5 mg/L + PBZ 0.75 mg/L; BA 1 mg/L + PBZ 0.75 mg/L; BA 2 mg/L + PBZ 0.75

Table 1 Combination treatment at flower induction first stage

Group	Treatment combination		
	Light intensity (Lux)	BA (mg/L)	PBZ (mg/L)
L1K	3,600	–	–
L1B05	3,600	0.5	–
L1B1	3,600	1	–
L1B2	3,600	2	–
L1B05p	3,600	0.5	0.75
L1B1p	3,600	1	0.75
L1B2p	3,600	2	0.75
L2K	2,300	–	–
L2B05	2,300	0.5	–
L2B1	2,300	1	–
L2B2	2,300	2	–
L2B05p	2,300	0.5	0.75
L2B1p	2,300	1	0.75
L2B2p	2,300	2	0.75
L3K	2,600	–	–
L3B05	2,600	0.5	–
L3B1	2,600	1	–
L3B2	2,600	2	–
L3B05p	2,600	0.5	0.75
L3B1p	2,600	1	0.75
L3B2p	2,600	2	0.75

Note: The code with the letters L1–L3 indicates the light intensity; codes B05, B1, and B2 indicate the concentration of BA; the code letter “p” indicates the presence of 0.75 mg/L PBZ in the media, while the code letter K indicates the control treatment (MS0 without PGR) in each light group. Each treatment used 4 replications.

mg/L (four replications for each treatment). Each treatment of hormonal variation and control was given light intensity treatment at 2,300 lux; 2,600 lux; and 3,600 lux. After a sufficient number of shoots appeared, the nodes were subcultured again and planted on the same treatment medium. The complete combination treatment is as shown on **Table 1**.

At the age of 120 days, the light treatment group with complete explants was selected and then divided into two groups. The first group was the group that was not given additional induction media and the second group was the group that was given additional induction media. The second flowering induction used liquid MS0 media containing 1 mg/L PBZ 10 mL. The addition of media was carried out by a feeding technique where liquid media was added to solid media so that the media in the bottle became two layers, the bottom layer was the first stage of flowering induction media according to the treatment code and the top layer was an additional liquid medium to induce further flowering.

Data on plant height and number of leaves in each treatment were processed with K-independent sample – Kruskal Wallis and

Pairwise comparison using SPSS software (version 26.0; SPSS Inc.; Chicago, IL, USA). The provisions used are as follows: H0 means that there is no difference for each sample variation, while H1 means that there is a difference for each sample variation. Reject H0 if $p < 0.005$.

RESULTS AND DISCUSSION

Germination was carried out using liquid media in the form of 1 mg/L BA solution as in the study of Savitri et al. (2018). Germination occurred 8 days after planting (DAPs) with an average plant height of 1,4 cm as shown at **Fig. 1**. Then the explants were sub-cultured to shoot multiplication media (MS0) until they were high enough and produced a large number of leaves as shown in **Fig. 2**. Explants resulting from shoot multiplication aged 24 DAPs were then sub-cultured on the nodes to the first phase of flowering induction media.

BA is used to induce flowering because it is known to have a good role in regenerating tomato plants by inducing more shoots. In addition, when compared to other PGR cytokinin, BA is known to be more resistant to enzymatic degradation by

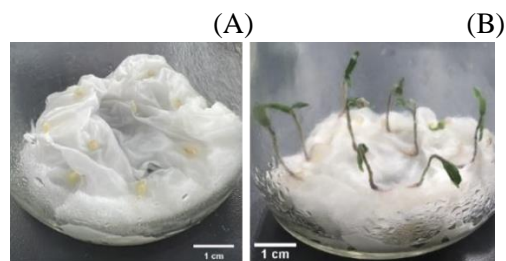


Figure 1. Growth of Golden Sweet cherry tomatoes at the germination stage: (A) 0 DAP; (B) 8 DAPs

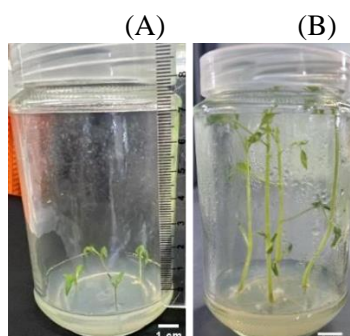


Figure 2. Growth of Golden Sweet cherry tomatoes at the shoot multiplication stage: (A) 9 DAPs; (B) 26 DAPs

plants, more stable in media, has more free form molecules so that it is easier to use directly by plants (Podwyszynska, 2003). However, the most available information is regeneration of cherry tomatoes from callus explants (Gerszberg et al., 2016; Mamidala and Swamy Nanna, 2011; Otroshy et al., 2013). The concentration used is in the range of 0.5 mg/L to 4 mg/L where BA 3 and 4 mg/L show negative effects because they are toxic (Farouk Omar, 2019). In addition, it is known that the concentration of BA that is too high can cause callus growth more dominant that can inhibit the growth of shoots and leaves. Until now, there is still no research or reference that states the optimum concentration of BA for the *in vitro* flowering of cherry tomatoes. Based on these references, this experiment was conducted using a BA concentration of 0.5 mg/L; 1 mg/L; and 2 mg/L.

Besides BA, PBZ is also known to induce flowering in plants (Demmassabu et al., n.d.; Dewir et al., 2007). PBZ can block the formation of kaurenoic acid which is a precursor of the endogenous hormone gibberellin which inhibits flower bud differentiation (Desta and Amare, 2021). The role of PBZ as a retardant is in contrast

to BA where BA induces growth and PBZ inhibits growth. BA is expected to induce more shoots, while PBZ is expected to inhibit plant growth in the vegetative phase so that the plant transition period is expected to occur more quickly. In one study (Savitri et al., 2018), it was found that PBZ with a concentration of 1 mg/L was still too high to induce flowering because the plantlets height decreased by 62% compared to control, yet there was still no flower produced. Also, in other experiments related to *Dendrobium* (Te-chato et al., 2009), they used lower concentration of PBZ but successfully produced flowers and fruits. Therefore, in this experiment 0.75 mg/L PBZ was used and was given from the beginning of the induction.

Based on **Table 2**, it is known that BA and PBZ affect the growth of plant height and number of leaves. The best plant height occurred in the control, namely MS0 media with a light intensity of 2,600 lux, while the best number of leaves was on MS + BA 2 mg/L + PBZ 0.75 mg/L with 2,300 lux light. Likewise, the effect of PBZ which is also in accordance with the literature where the growth of plant height becomes shorter compared to media without PBZ but does not affect the

Table 2. Effect of BA, PBZ, and light intensity on the average plant height and the average number of leaves on the Golden Sweet variety

Group code	Height (cm)	Number of leaves	Survival Rate (%)
L1K	5.71 ^{cd} ± 5.17	4.3 ^{ab} ± 3	75
L1B05	2.73 ^{abc} ± 1.66	3.8 ^{ab} ± 1	100
L1B1	2.52 ^{abc} ± 0.23	8.5 ^a ± 8.3	0
L1B2	4.07 ^{abc} ± 0.8	8.3 ^{abc} ± 3	75
L1B05p	1.51 ^a ± 0.79	8.5 ^a ± 8.5	75
L1B1p	1.81 ^{ab} ± 1.42	10.3 ^{bc} ± 7.4	50
L1B2p	0.98 ^a ± 0.92	8.3 ^{abc} ± 4.6	50
L2K	7.70 ^d ± 4.51	7.5 ^{abc} ± 6	100
L2B05	2.42 ^{abc} ± 2.27	3.3 ^a ± 2.1	100
L2B1	2.72 ^{abc} ± 2.01	10 ^{abc} ± 7.4	100
L2B2	3.79 ^{abc} ± 1.87	16.5 ^c ± 15.8	100
L2B05p	1.47 ^a ± 1.17	8 ^{abc} ± 4.3	50
L2B1p	2.04 ^{abc} ± 1.01	14 ^{abc} ± 2.2	100
L2B2p	1.43 ^a ± 0.75	25.5 ^d ± 17.1	100
L3K	8.01 ^d ± 1.75	6.8 ^{abc} ± 1.7	100
L3B05	5.65 ^{bcd} ± 4.12	4.8 ^{ab} ± 3.9	100
L3B1	3.76 ^{abc} ± 3.21	10.8 ^{ab} ± 10	100
L3B2	2.19 ^{abc} ± 1.66	10.8 ^{abc} ± 1.5	100
L3B05p	1.29 ^a ± 0.5	7.8 ^{abc} ± 5	100
L3B1p	2.16 ^{abc} ± 0.72	8.8 ^{abc} ± 1.7	100
L3B2p	1.07 ^a ± 0.88	10.7 ^{abc} ± 6.1	100

Note: Data analysis used non-parametric statistical test Kruskal Wallis and Pairwise Comparison with $p = 0.05$. The same letter notation on the growth rate data and number of leaves indicated that there was no significant difference. Group L3K (MS0; 2,600 lux) had the best average plant growth, group L2B2p (MS + BA 2 mg/L + PBZ 0.75 mg/L; 2,300 lux) had the best mean number of leaves, while the light group was 2,600 lux (group L3K–L3B2p) had the best survival rate of up to 120 DAPs.

number of leaves. The light intensity of 2,600 lux is also thought to affect the survival rate of the explants. The explant group grown in 3,600 lux light had the lowest percentage of survival rate, followed by 2,300 lux light, while in 2,600 lux light, no explants died. Allegations that light intensity affects the growth of Golden Sweet varieties can be caused by genetic factors. In addition, according to the statement of Cioć et al., (2019). The good light intensity for most plants is in the range of 30–40 mol.m⁻².s⁻¹ or equivalent to 2,220–2,960 lux. Other studies on tomato regeneration *in vitro* also generally use a light intensity of no more than 2,960 lux (Jamous and Abu–Qaoud, n.d.; Raza et al., 2020). Based on the results of survival rate data, it is suspected that 2,600 lux light can be used to induce flowering of cherry tomatoes of the Golden Sweet variety. At this stage of the experiment, the 2,600 light group had the highest survival rate where all explants were still alive up to 120 DAPs. Therefore, the explant group was used for the next step.

The liquid medium used was MS0 media containing 1 mg/L PBZ. MS0 media was used to support the nutrients needed by plants to grow

during observations while 1 mg/L PBZ was used to induce further flowering because PBZ is known to suppress vegetative growth to trigger the plant transition to the generative phase. PBZ is also known to increase endogenous cytokinin in plants, because the previous media already contained BA. Therefore, the addition of this PGR was not carried out to prevent the accumulation of excess cytokinin which would have a negative impact on explants (Desta and Amare, 2021; Tesfahun, 2018). The addition of PBZ (by feeding technique) was used to observe the further effect on the tomato *in vitro* flowering. In addition, it is beneficial to maintain the plant growth so that the plant height can be reduced. Based on the observations, it is known that the feeding technique has an effect on plant height, occurrence of callus, and the production of flower buds. The plantlets given the feeding technique were relatively shorter than the control, had larger callus sizes, and could induce flowering in one of the plantlets. These results are in line with the references used regarding the effect of PBZ on plant growth and development (Desta and Amare, 2021; Tesfahun, 2018).

Table 3. Percentage of flowering (bud appearance) of the Golden Sweet variety at a light intensity of 2,600 lux

PGR (mg/L)	MS + PBZ 1 (mg/L) liquid	Flowering (%)	Flowering time (DAPs)
Control (MS0)	No	50	169
BA 0.5	Yes	50	157
BA 0.5 + PBZ 0.75	Yes	50	142
BA 2 + PBZ 0.75	No	50	160
BA 2 + PBZ 0.75	Yes	50	166

Note: Each treatment was carried out with 2 replications. “No” means explants were not given feeding treatment, whereas “Yes” means explants were given feeding treatment. Percentage of flowering was calculated based on the number of flower clusters (raceme) that appeared divided by the number of replications for each treatment (2 replications).

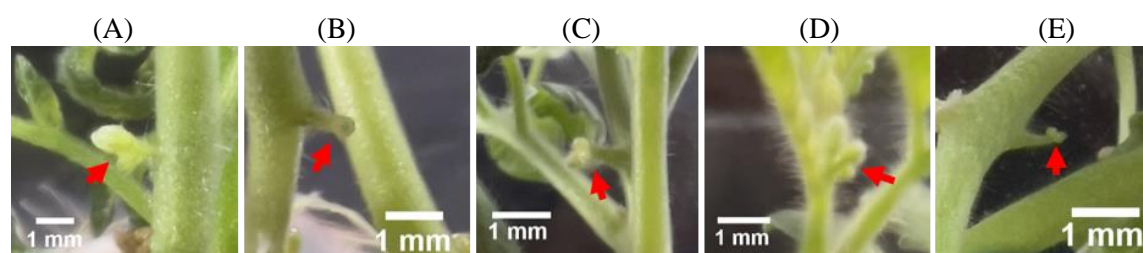


Figure 3. Flower buds appearing on cherry tomato explants var. Golden Sweet grown at a light intensity of 2,600 lux on media: (A) MS + BA 0.5 mg/L + PBZ 0.75 mg/L + feeding treatment at 142 DAPs; (B) MS + BA 0.5 mg/L + feeding treatment at 157 DAPs; (C) MS + BA 2 mg/L + PBZ 0.75 mg/L without feeding treatment at 160 DAPs; (D) MS + BA 2 mg/L + PBZ 0.75 mg/L + feeding treatment at 166 DAPs; (E) MS0 (Control, without feeding treatment) at 169 DAPs.

The flower buds first appeared at the age of 4 months 22 DAPs, which was longer than the *ex vitro* flowering period (between 2–3 months). This could be due to nutrients, PGR, and environmental conditions that are still not optimal for the Golden Sweet cherry tomatoes grown *in vitro*. Based on this information, it is also possible to suspect that the emergence of flower buds occurred not due to the induction of PGR or light intensity. Rather, at that time the explants had already transitioned to the generative phase, considering that the explants had been grown for quite a long time. Flower buds (**Fig. 3**) appear at different times in different treatments. However, the percentage of flowering was the same for all treatments, namely 50%, where flower buds appeared only on one explant in each treatment according to the information in **Table 3** (there were 2 replications for each treatment combination).

Based on the results of the study, it is known that BA and PBZ affected the plant height and number of leaves on cherry tomato plant explants of Golden Sweet variety. The combination of PGR, namely BA and PBZ (below 1 mg/L),

followed by feeding treatment gave more flowering results even though buds appeared at different times. In addition, based on survival rate data and flowering data, the better light intensity is at 2,600 lux. This information is expected to help further research in optimizing PGR on media and light intensity for *in vitro* flowering cherry tomatoes.

CONCLUSION

In this experiment the effect of BA alone or in combination with 0.75 mg/L PBZ and also PBZ enriched in feeding treatment are still not known to induce *in vitro* flowering on cherry tomatoes. However, the light intensity at 2,600 lux is recommended for floral induction.

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