Berita Biologi
LIPI
Jurnal ilmu-ilmu hayati

Berita Biologi 25(1): 63–78 (2026)
https://ejournal.brin.go.id/berita_biologi ISSN 2077 7019
DOI: 10.55981/berita_biologi.2026.11373

P-ISSN 0126-1754
E-ISSN 2337-8751

ARTICLE

IN VITRO SHOOT INDUCTION IN STRAWBERRY (*Fragaria* × *ananassa* Duch.) CLONE BAT 1 USING VARIOUS TYPES AND CONCENTRATIONS OF CYTOKININS

[*Induksi Pembentukan Tunas secara In Vitro pada Stroberi (Fragaria × ananassa Duch.) Klon BAT 1 dengan Berbagai Jenis dan Konsentrasi Sitokinin*]

Pauline Elisabeth[1], Anne Nuraini[2*], Murgayanti[2], Muhammadiyah[1]

[1]Department of Biotechnology, School of Life Sciences and Technology, Institut Teknologi Bandung, Jl. Ganesha No. 10, Lebak Siliwangi, Coblong District, Bandung City, West Java 40132, Indonesia
[2]Department of Agrotechnology, Faculty of Agriculture, Universitas Padjadjaran, Jl. Raya Bandung-Sumedang Km. 21 Jatinangor, Sumedang 45363, Indonesia

ABSTRACT

Strawberry *(Fragaria* × *ananassa* Duch.) is a high-value subtropical horticultural crop. One promising local variety under development is clone BAT 1. To accelerate propagation, tissue culture techniques are needed, especially during bud initiation, which is influenced by cytokinin type and concentration. This study used a Completely Randomized Design (CRD) with 7 treatments and 4 replications, using Murashige and Skoog (MS) medium supplemented with Thidiazuron (TDZ) and 2-Isopentyl adenine (2-iP) at concentrations of 0; 0.50; 0.75; 1 ppm TDZ and 1; 2; 3 ppm 2-iP. The procedures included explant preparation, sterilization of equipment and area, stock solution preparation, media formulation and sterilization, explant sterilization, and planting into treatment media. Results showed that cytokinins at 0.50–1 ppm significantly enhanced bud number, leaf number, culture height, and fresh weight. The most effective treatments were 1 ppm 2-iP (5.24 buds; 5.90 leaves; 2.23 cm height) and 0.50 ppm TDZ (1.36 g fresh weight), indicating their potential for improving in-vitro propagation of clone BAT 1.

Keywords: *Fragaria* × *ananassa*, bud initiation, *in vitro* culture, cytokinin, TDZ, 2-iP

ABSTRAK

Stroberi (Fragaria × ananassa Duch.) merupakan komoditas hortikultura subtropis bernilai ekonomi tinggi. Salah satu varietas unggul lokal yang tengah dikembangkan adalah klon BAT 1. Untuk mempercepat perbanyakan tanaman ini, teknik kultur jaringan diperlukan, terutama pada tahap inisiasi tunas yang dipengaruhi oleh jenis dan konsentrasi sitokinin. Penelitian ini menggunakan Rancangan Acak Lengkap (RAL) dengan 7 perlakuan dan 4 ulangan, pada media MS (Murashige and Skoog) yang diberi Thidiazuron (TDZ) dan 2-Isopentyl adenine (2-iP) dengan konsentrasi 0; 0,50; 0,75; 1 ppm TDZ dan 1; 2; 3 ppm 2-iP. Prosedur penelitian meliputi penyiapan eksplan dari lapangan, sterilisasi alat dan ruang, pembuatan larutan stok, pembuatan dan sterilisasi media, sterilisasi eksplan, serta penanaman ke media perlakuan. Perlakuan yang paling efektif adalah 1 ppm 2-iP (5,24 tunas; 5,90 daun; 2,23 cm tinggi) dan 0,50 ppm TDZ (1,36 g bobot segar), yang menunjukkan potensinya dalam meningkatkan perbanyakan in vitro klon BAT 1.

Kata kunci: *Fragaria × ananassa, inisiasi tunas, kultur in vitro, sitokinin, TDZ, 2-iP*

Submited: 16 May 2025; **Revision:** 31 October 2025; **Accepted:** 22 December 2025
***Corresponding author:** anne.nuraini@unpad.ac.id

INTRODUCTION

Strawberry (*Fragaria × ananassa* Duch.) is a globally popular subtropical fruit crop, highly valued for its distinctive sweet-sour flavor, appealing red color, and high nutritional content, particularly vitamin C. Strawberries are commonly consumed fresh as dessert or processed into a variety of products such as jams, juices, and pastries (Sukasih & Setyadjit, 2019). According to USDA (2016), strawberries contain 58.8 mg of vitamin C per 100 g. This antioxidant accumulates in white blood cells and contributes to immune responses, offering protective effects against infectious diseases and reducing complications during viral outbreaks, such as COVID-19 (Setiawan et al., 2016; Setyoningsih, 2021).

Strawberry cultivation holds significant potential in Indonesia, particularly in highland regions. The most commonly cultivated species is *Fragaria × ananassa*, a hybrid valued for its favorable agronomic traits (Budiman, 2005). Despite this potential, national strawberry production has declined in recent years. In 2020, Indonesia produced 8,350 tons, reflecting a 2.12% decrease compared to 2018. West Java remains the leading production area, contributing 5,955 tons in 2020, down from 6,296 tons in 2018 (BPS, 2020). Lembang, located in West Bandung Regency, is among the most prominent strawberry-producing regions and a notable center for agritourism.

Strawberries are traditionally propagated via seeds or runners; however, these methods are labor-intensive, time-consuming, and often result in variable plant quality. Moreover, strawberry plants are highly sensitive to fluctuations in temperature and humidity (Sukasih & Setyadjit, 2019). Micropropagation provides a reliable alternative for producing uniform, high-quality, and disease-free planting material. According to Kikas et al. (2006), *in vitro*-derived strawberry plants generated more runners, exhibited higher survival rates, and achieved yield increases of up to 24% compared to conventionally propagated counterparts.

The availability of healthy and uniform explants is critical to the success of *in vitro* propagation. An efficient regeneration system significantly enhances the potential for large-scale micropropagation (Haddadi et al., 2013). Common explants used for strawberry tissue culture include runners, shoot tips, nodal segments, and leaf segments (Thi et al., 2018).

The success of *in vitro* culture is influenced by the composition of the culture medium, particularly the incorporation of plant growth regulators (PGRs). Media supplemented with cytokinins have been shown to stimulate shoot development and plant morphogenesis, which are essential for modern plant biotechnology (Rahmawati; Murvanidze et al., 2021). Cytokinins such as Thidiazuron (TDZ), 2-Isopentenyl adenine (2-iP), kinetin, 6-benzylaminopurine (BAP), and zeatin are commonly utilized in plant tissue culture (Erawati et al., 2020). TDZ, in particular, has been demonstrated to promote cell division and adventitious shoot formation more effectively than natural cytokinins (Li et al., 2021), while also enhancing runner production (Cappelletti et al., 2016). In contrast, 2-iP has been associated with increased chloroplast formation, enhanced leaf surface area, and the promotion of lateral shoot development, primarily due to its role in stimulating cell division (Nurana et al., 2017).

Conventional propagation methods are often limited in efficiency, whereas cytokinins have been shown to enhance micropropagation. This study aimed to evaluate the effects of different types and concentrations of cytokinins specifically Thidiazuron (TDZ) and 2-Isopentenyl adenine (2-iP) on *in vitro* shoot initiation of the BAT 1 strawberry clone. Optimizing cytokinin treatment could facilitate large-scale propagation of elite strawberry clones under controlled conditions.

MATERIALS AND METHODS

Materials and Equipment

The experiment was conducted from January to April 2022 at the Tissue Culture Laboratory of Seed Technology, Faculty of Agriculture, Universitas Padjadjaran, location in Jatinangor, Sumedang Regency, Indonesia. The equipment required for media preparation includes an analytical balance, beakers (1 L, 250 mL, and 50 mL), micropipettes, pH meter, hot plate, magnetic stirrer, spatula, 100 mL culture bottles, heat-resistant plastic, labels, pencils, rubber bands, and a mobile phone camera. Tools used for explant planting include a size 11 scalpel blade, 25 cm and 10 cm

tweezers, petri dishes, sterile paper, jam jars, flame sterilizer, and plastic wrap. Sterilization of tools and media was performed using an autoclave and oven. Planting was carried out in a laminar air flow (LAF) cabinet, and the culture bottles were stored on a culture rack equipped with 16-watt fluorescent lamps, air conditioning and a thermohygrometer. Additional supporting equipment includes a stopwatch, tissue, scissors, sponge, filter, and brush.

The plant material used in this experiment was strawberry runners of the Bumi Agro Tech 1 (BAT 1) clone, approximately 1.5 cm in length, with the description of this clone provided in Appendix 1. The media was prepared using Murashige and Skoog (MS) instant powder, with the composition listed in Appendix 3, supplemented with plant growth regulators including cytokinins: TDZ (0.50 mg/L, 0.75 mg/L, 1.0 mg/L) and 2-iP (1.0 mg/L, 2.0 mg/L, 3.0 mg/L), 2 g/L agar gelzan, 30 g/L sucrose, aquadest, 0.1 N NaOH, and 0.1 N HCl. The sterilization agents for explants included aquadest, detergent, the fungicide Propineb, the bactericide Agrept 20 WP, bayclin bleach, and $HgCl_2$. Additional supporting materials included antibacterial soap (Mr. Muscle), spirit, 70% alcohol, and 90% alcohol.

Experimental Design

This study employed an experimental method with a Completely Randomized Design (CRD), consisting of 7 treatment factors, each repeated 4 times, resulting in 28 experimental units. The experiment was conducted with two samples per treatment to anticipate contamination factors. The treatments are outlined in Table 1.

Table 1. Experimental treatments (*perlakuan percobaan*).

Treatment Code (*Kode perlakuan*)	**Treatment** (*Perlakuan*)
A	Control (no cytokinin)
B	0.50 ppm TDZ (Thidiazuron)
C	0.75 ppm TDZ (Thidiazuron)
D	1.0 ppm TDZ (Thidiazuron)
E	1.0 ppm 2-iP (2-Isopentenyl adenine)
F	2.0 ppm 2-iP (2-Isopentenyl adenine)
G	3.0 ppm 2-iP (2-Isopentenyl adenine)

Preparation of Explant Sources from the Field

The experiment was carried out in several stages, including the preparation of explant sources from the field, sterilization of tools and the workspace, preparation of stock solutions, media preparation and sterilization, explant sterilization, and explant planting.

The explants used in this experiment were strawberry runners of the Bumi Agro Tech 1 (BAT 1) clone, approximately 1.5 cm in length, obtained from the CV. Bumi Agrotech farm located in Kertawangi, Cisarua District, West Bandung Regency.

Sterilization of Tools and Workspace

The tools for media preparation and explant planting were washed with antibacterial detergent, sponges, and brushes, then rinsed and dried. The tools were sterilized in an autoclave at 17.5 psi and 121°C for 20 minutes. The workspace was sterilized aseptically in the LAF by spraying with 70% alcohol and wiping with dry tissue. The UV light was turned on for 60 minutes to sterilize the LAF and tools, and after the UV was turned off, the blower and lights were switched on.

Preparation of Cytokinin Stock Solution

The 100 ppm cytokinin stock solution was prepared by weighing 0.01 g of cytokinin, dissolving it in 1 N HCl, and diluting with distilled water to a final volume of 100 mL. The solution was then stored in a labeled bottle at 4°C.

Preparation of Treatment Media

The treatment media were prepared by mixing 2.48 g of instant MS powder, 16.8 g of sucrose, and 1.12 g of gelzan in 250 mL of distilled water. The mixture was stirred using a magnetic stirrer until homogeneous and then divided into seven 250 mL beakers (40 mL each) according to treatment groups. Cytokinin stock solutions (TDZ and 2-iP) were added using a micropipette according to the designated concentrations, and the volume was adjusted to 80 mL with distilled water. The pH was adjusted to 5.6–5.8 using either 0.1 N NaOH or HCl if needed. Gelzan was added to each solution, and the media were heated to boiling until fully dissolved and homogeneous. A total of 56 culture bottles (100 mL size) were prepared, each filled with 10 mL of media and sealed with heat-resistant plastic and rubber bands. The media were sterilized using an autoclave at 121°C and 17.5 psi for 20 minutes, then stored for one week to check for contamination before use.

Explant Sterilization

The explant sterilization procedure was adapted from Raisya et al. (2020). Runners of BAT 1 clone were trimmed to ±1.5 cm, with roots and soil residues removed. Explants were washed twice under running tap water and shaken in distilled water for 15 minutes, repeated twice. The explants were then soaked in a detergent solution (0.2 g in 200 mL distilled water) for 10 minutes and rinsed twice until no foam or odor remained. A sterilization solution containing 1 g bactericide and 0.3 g fungicide in 100 mL distilled water was applied for 15 minutes, followed by rinsing. Further sterilization was performed using 1% Clorox (20 mL Clorox in 80 mL distilled water) for 10 minutes, followed by rinsing, and repeated using 10% Clorox (10 mL in 90 mL distilled water) for 10 minutes, followed by 4–5 rinses. Finally, explants were immersed in 0.1 g $HgCl_2$ dissolved in 100 mL distilled water for 5 minutes, drained without rinsing, and air-dried on Petri dishes in a laminar airflow cabinet.

Explant Inoculation onto Treatment Media

Explant inoculation was conducted under aseptic conditions within a laminar airflow cabinet sterilized with 70% ethanol and exposed to UV light for 20 minutes. Planting tools were disinfected by immersion in 90% ethanol, flame-sterilized over a spirit burner, and stored in sterile jars. Sterilized runners were placed on sterile, paper-lined Petri dishes, and browned tips were removed using sterile scalpels. Single explants (~1.5 cm) were transferred to culture bottles using sterile forceps and inserted into the respective treatment media. Bottles were sealed with heat-resistant plastic and rubber bands, wrapped with plastic film, and sprayed externally with 70% ethanol. Cultures were arranged on racks using a completely randomized design (Appendix 2) and incubated under 16-watt fluorescent lighting. The culture room was maintained at 20–26 °C with 60–70% relative humidity, a 16/8 h light/dark photoperiod, and a light intensity of 40.5 $\mu mol\ m^{-2}\ s^{-1}$ (Erawati et al., 2020).

Observation

Observations were conducted on weekdays (Monday to Friday) until the end of the experimental period at week 11. The observations included both supporting and main parameters. Main observations were statistically analyzed, while supporting observations provided additional context regarding culture conditions.

Supporting Observations

Culture room temperature and relative humidity were monitored using a thermohygrometer five times per week for 12 weeks. The visual appearance of explants was observed weekly to assess morphological development. Shoot initiation time was recorded weekly based on the first visible shoot per treatment. At the end of the experiment, the following parameters were evaluated:

percentage of viable explants, browning, physiological death, contamination, and plantlet formation. Each parameter was calculated using the formula:

$$\text{Percentage} = \frac{\Sigma \text{ number of specific explants}}{\Sigma \text{ total number of explants}} \times 100\%$$

Main Observations

The number of shoots and leaves (including fully developed ones) was recorded biweekly at 2, 4, 6, 8, 10, and 11 weeks after planting (WAP). Plant height was measured at week 11 from the shoot tip to the stem base, excluding the initial explant and roots. Fresh weight was determined at final harvest (week 11) by weighing each culture using an analytical balance.

Data Analysis Design

Quantitative data were square root-transformed to satisfy normality assumptions prior to analysis of variance (ANOVA). Data were analyzed using ANOVA followed by Duncan's Multiple Range Test (DMRT) at a 5% significance level. Statistical analyses were conducted using IBM SPSS Statistics (version 25). Qualitative data, including visual assessments, were analyzed descriptively.

RESULTS

Supporting Observations

Culture room temperature and relative humidity, critical environmental factors for plant growth in tissue culture, were recorded monthly. The average culture room temperature was 23.33°C (Month 1), 22.70°C (Month 2), and 22.44°C (Month 3), with corresponding relative humidity values of 62.35%, 57.05%, and 62.53%. Overall, the daily averages were 22.82°C (Figure 1) and 60.64% humidity (Figure 2).

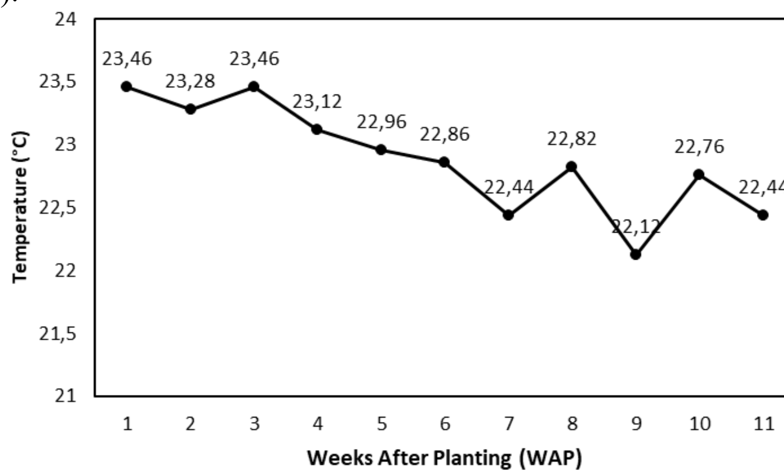

Figure 1. Culture room temperature (*suhu ruang kultur*).

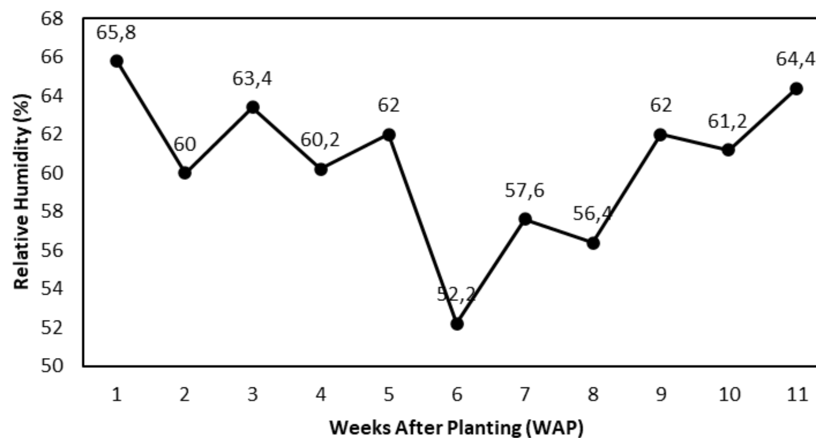

Figure 2. Culture room relative humidity (*kelembapan relatif ruang kultur*).

The visual appearance of the explants was observed and documented weekly to monitor growth and morphological development. Observations were conducted from the initial planting until the emergence of organs such as shoots, leaves, callus, and roots. Each treatment showed distinct visual differences due to the varying concentrations of TDZ and 2-iP. The described appearances represent the best replication from each treatment.

Treatment A1 (No Cytokinin)

The first shoot emerged at 1 week after planting (WAP), followed by leaf formation, although leaves were not fully expanded. By 11 WAP, five shots and 15 leaves had formed. Shoots grew upright with dark green trifoliate leaves and serrated margins. No rooting was observed at the end of the experiment, and no plantlet formation occurred.

Figure 3. 1 WAP (*1 minggu setelah tanam*).

Figure 4. 11 WAP (*11 minggu setelah tanam*).

Treatment B1 (0.5 ppm TDZ)

Shoots, leaves, stems, and roots emerged at 1 WAP. Organ formation increased weekly, with compact and pale shoots and leaves. This treatment showed optimal regeneration, successfully producing complete plantlets.

Figure 5. 1 WAP (*1 minggu setelah tanam*).

Figure 6. 11 WAP *(11 minggu setelah tanam*).

Treatment C2 (0.75 ppm TDZ)

The first shoot emerged at 1 WAP with incompletely expanded leaves. By 7 WAP, a thickened stem and surrounding callus were observed.

Figure 7. 1 WAP (*1 minggu setelah tanam*).

Figure 8. 7 WAP (*7 minggu setelah tanam*).

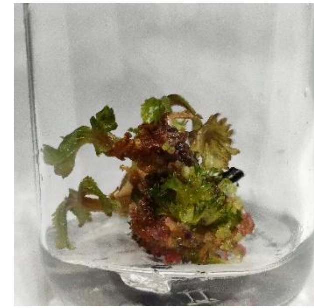

Figure 9. 11 WAP (*11 minggu setelah tanam*)

Treatment D3 (1 ppm TDZ)

Callus formation occurred, likely induced by the presence of plant growth regulators (PGRs) in the medium and originating from sterilized plant tissues such as roots, stems, or leaves.

Figure 10. 1 WAP (*1 minggu setelah tanam*).

Figure 11. 11 WAP (*11 minggu setelah tanam*).

Treatment E1 (1 ppm 2-iP)

Shoots emerged at 1 WAP, followed by fully expanded leaves and new shoot formation by 2 WAP. Stem thickening was visible in subsequent weeks, indicating potential for further shoot and leaf growth. Root formation occurred by 4 WAP, possibly enhancing nutrient uptake. By 8 WAP, shoots and leaves became densely packed and difficult to count.

Figure 12. 1 WAP *(1 minggu setelah tanam*).

Figure 13. 2 WAP (*2 minggu setelah tanam*).

Figure 14. 4 WAP (*4 minggu setelah tanam*).

Figure 15. 8 WAP (*8 minggu setelah tanam*).

Figure 16. 11 WAP (*11 minggu setelah tanam*).

Treatment F2 (2 ppm 2-iP)

Shoots emerged at 1 WAP, along with stem, leaf, and callus formation. No rooting occurred during the 11-week period. Callus formation was first observed at 3 WAP, likely due to the cytokinin effect in the medium.

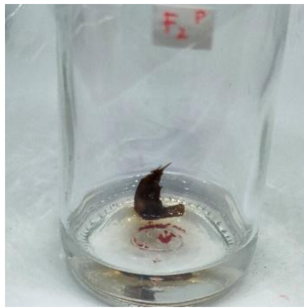

Figure 17. 1 WAP (*1 minggu setelah tanam*).

Figure 18. 3 WAP (*3 minggu setelah tanam*).

Figure 19. 11 WAP (*11 minggu setelah tanam*).

Treatment G2 (3 ppm 2-iP)

This treatment exhibited the slowest growth response, with the first shoot emerging only at 7 WAP. By 11 WAP, shoots and leaves were present but appeared short and small, indicating stress.

Figure 20. 7 WAP (*7 minggu setelah tanam*).

Figure 21. 11 WAP (*11 minggu setelah tanam*).

Shoot Emergence Time

The emergence of shoots from newly planted explants, indicated by the appearance of new shoot organs, was monitored weekly (weeks after planting; WAP). The fastest shoot emergence occurred in treatment B (0.5 ppm TDZ), with an average of 1.50, while the slowest emergence was observed in treatment G, with an average of 3.87. The application of TDZ or 2-iP at specific concentrations influenced the timing of initial shoot formation, with higher concentrations of 2-iP tending to delay shoot emergence (Table 2.). The variation in shoot emergence times among treatments was likely attributable to differences in cytokinin type and concentration. These findings

suggest that cytokinin-supplemented media, with optimized concentrations, can accelerate shoot induction.

Table 2. Average time of shoot emergence (*rata-rata waktu muncul tunas*).

Treatment (*Perlakuan*)	**Average time of shoot emergence** (*rata-rata waktu muncul tunas*)
A (Control)	2.71 ± 1.28
B (0.5 ppm TDZ)	1.50 ± 1.00
C (0.75 ppm TDZ)	2.96 ± 2.08
D (1 ppm TDZ)	1.96 ± 0.90
E (1 ppm 2-iP)	2.00 ± 1.83
F (2 ppm 2-iP)	2.50 ± 1.29
G (3 ppm 2-iP)	3.87 ± 1.97

Percentage of Viable Explants, Browning Explants, Physiologically Dead Explants, Contaminated Explants, and Explants Forming Plantlets

This supporting observation was conducted to assess the success of shoot initiation in *Fragaria × ananassa* clone BAT 1 through the application of different types and concentrations of cytokinins. The results are presented as percentages of viable explants, browning explants, physiologically dead explants, contaminated explants, and explants forming plantlets (Table 2).

Table 2. Percentage of Living Explants, Browning, Physiological Death, Contamination, and Planlet Formation in Response to Different Types and Concentrations of Cytokinins for In Vitro Strawberry Clon BAT 1 (*Persentase Eksplan Hidup, Eksplan Browning, Kematian Fisiologis, Kontaminasi, dan Pembentukan Planlet sebagai Respons terhadap Berbagai Jenis dan Konsentrasi Sitokinin pada Kultur In Vitro Stroberi Klon BAT 1*).

Treatment (*Perlakuan*)	**Viabel explant** (*Eksplan viable*) **(%)**	**Browning explant** (*Eksplan kecoklatan*) **(%)**	**Physiologically dead explant** (*Eksplan mati fisiologis*) **(%)**	**Contaminated explan** (*Eksplan terkontaminasi*) **(%)**	**Explant forming planlet** (*Eksplan membentuk planlet*) **(%)**
A	75	25	25	0	25
B	100	0	0	0	75
C	75	25	25	0	0
D	75	25	25	0	0
E	100	0	0	0	100
F	100	0	0	0	0
G	25	75	0	0	0
Total	78.57	21.42	10.71	0	28.57

Percentage of Viable Explants (%)

Viable explants were defined as those that showed no signs of physiological death, browning, or contamination by the 11th week after culture (WAC), and exhibited growth and organogenesis (shoots, stems, leaves, roots, or callus). The average viability rate was 78.57%, with the highest (100%) recorded in treatments B (0.5 ppm TDZ), E (1 ppm 2-iP), and F (2 ppm 2-iP), while the lowest (25%) occurred in treatment G (3 ppm 2-iP). Viable explants were characterized by growth in height and organ development, indicating successful regeneration.

Percentage of Browning Explants (%)

Browning was observed in treatments A2 (control), C1 (0.75 ppm TDZ), and D2 (1 ppm TDZ), likely due to chemical toxicity during explant sterilization. The highest browning percentage (75%) was recorded in treatments G1, G3, and G4 (Table 3). Browning symptoms appeared as early as 2 WAP, starting around the explants and spreading throughout the media by 4 to 11 WAP.

Percentage of Physiologically Dead Explants (%)

Physiological death was observed in treatments A2, C1, and D2, each with a percentage of 25% (Table 3).

Percentage of Contaminated Explants (%)

Contamination, caused by the unintended presence of microorganisms in the culture, was not observed in any treatment, resulting in a contamination percentage of 0% (Table 3).

Percentage of Explants Forming Plantlets (%)

The highest percentage of explants forming complete plantlets was observed in treatment E (1 ppm 2-iP) at 100%, followed by treatment B (0.5 ppm TDZ) at 75% (Table 3).

Main Observations

Main observations were conducted at 11 weeks after planting (WAP), including number of shoots, number of leaves, plant height, and fresh weight, as presented in Table 3. The results indicated that both TDZ and 2-iP at specific concentrations (0.5–1 ppm) significantly increased the observed parameters compared to the control treatment (A, without cytokinin).

Table 3. Number of shoots, number of leaves, plant height, and fresh weight of cultures under different types and concentrations of cytokinins *in vitro* culture of strawberry clone BAT 1 (*jumlah tunas, jumlah daun, tingi kultur, bobot basah kultur pada pemberian berbagai jenis dan konsentrasi sitokinin stroberi klon BAT 1 in vitro*).

Treatments (*Perlakuan*)	**Number of shoots** (*Jumlah tunas*) **(buds)** (*tunas*)	**Number of leaves** (*jumlah daun*) **(leaves)** (daun)	**Culture height** (*Tinggi kultur*) **(cm)**	**Fresh Culture Weight** (*Berat kultur segar*) **(g)**
A	1.74 ± 0.75^{ab}	2.75 ± 1.46^{ab}	1.90 ± 0.86^{b}	0.98 ± 0.25^{ab}
B	**5.16 ± 0.35^{c}**	**5.51 ± 0.26^{cd}**	**1.95 ± 0.11^{b}**	**1.36 ± 0.12^{b}**
C	3.67 ± 1.95^{bc}	3.95 ± 2.12^{bcd}	1.66 ± 0.66^{ab}	1.32 ± 0.48^{b}
D	3.35 ± 1.95^{bc}	3.64 ± 1.94^{bc}	1.40 ± 0.49^{a}	1.00 ± 0.26^{ab}
E	**5.24 ± 1.63^{c}**	**5.90 ± 0.91^{d}**	**2.23 ± 0.27^{b}**	**1.30 ± 0.41^{b}**
F	3.41 ± 0.36^{bc}	4.3 ± 0.48^{bcd}	2.02 ± 0.42^{b}	1.05 ± 0.21^{ab}
G	1.11 ± 0.74^{a}	1.21 ± 0.95^{a}	0.98 ± 0.53^{a}	0.75 ± 0.10^{a}

Description: Numbers followed by the same letter within each column are not significantly different based on DMRT at the 5% significance level (*Deskripsi: Angka yang diikuti dengan huruf yang sama dalam setiap kolom tidak berbeda secara signifikan berdasarkan DMRT pada tingkat signifikansi 5%*).

Number of Shoots

Analysis of variance followed by post-hoc tests revealed that the highest number of shoots was observed in treatment E (1 ppm 2-iP) with an average of 5.24 shoots. Among the TDZ treatments, treatment B (0.5 ppm TDZ) produced the most shoots with 5.16. Both types of cytokinin significantly outperformed the control in stimulating shoot formation. The lowest shoot number was recorded in treatment G (3 ppm 2-iP) with an average of 1.11, likely due to stress caused by high cytokinin concentration that inhibited shoot development.

Number of Leaves

The number of leaves was counted based on the presence of fully expanded leaves. Treatment E (1 ppm 2-iP) resulted in the highest leaf number with an average of 5.90, and was not significantly different from treatment B (0.5 ppm TDZ) which had 5.51 leaves. Increasing 2-iP concentration beyond 1 ppm (e.g., treatments F and G) significantly reduced leaf number, indicating that optimal cytokinin concentration is crucial. Treatment G (3 ppm 2-iP) again resulted in the lowest value (1.21 leaves).

Plant Height

The tallest cultures were found in treatment E (1 ppm 2-iP) with an average height of 2.23 cm, followed by treatment B (0.5 ppm TDZ) at 1.95 cm. These concentrations supported optimal plant organ development. Although the control (A) did not differ significantly in height from E and B, it exhibited weak growth with fewer and thinner leaves and shoots. Treatment G (3 ppm 2-iP) produced the shortest plants (0.98 cm), likely due to inhibitory effects at higher concentrations.

Fresh Weight of Culture

Analysis of variance showed no significant differences in fresh weight among treatments; therefore, no post-hoc test was conducted. However, descriptive analysis showed that treatment B (0.5 ppm TDZ) had the highest average fresh weight, followed closely by treatment E (1 ppm 2-iP). The lowest fresh weight was recorded in treatment G (0.75 g), corresponding with its poor morphological appearance and limited growth. Despite the reduced weight, all cultures in this treatment remained viable.

DISCUSSION

Supporting Observations

Culture Room Temperature and Relative Humidity

Each plant species requires a specific temperature range to support optimal growth. Temperature and relative humidity were recorded at 3 PM daily. According to Rahmawati (2021), temperature strongly influences physiological responses and the rate of growth, particularly during the early stages. The optimal temperature for *in vitro* culture conditions is between 22–25°C. During this study, the average temperature recorded was 22.82°C, which falls within the optimal range for culture growth and development.

The ideal relative humidity for plant tissue culture rooms is approximately 70% (Basri, 2016). In this experiment, the average humidity recorded was 60.64%, which is slightly below the optimal level and thus considered suboptimal. Lower humidity can increase evapotranspiration, leading to faster nutrient depletion from the medium (Basri, 2016). However, reduced humidity may also help lower the risk of contamination by fungi or bacteria, as reported by Chen (2004), by slowing down microbial growth rates.

Visual Appearance of Explants

The treatment without cytokinins was unexpectedly able to regenerate the explants by inducing shoot and leaf development. This is supported by the findings of Bhatt and Dhar (2000), who reported that young plant explants, particularly shoot or leaf tissues, often contain sufficient concentrations of endogenous hormones such as cytokinins and auxins to promote cell division and shoot formation naturally, without the need for exogenous hormonal treatments. Treatment B1 (0.5 ppm TDZ) demonstrated highly optimal morphological responses, with explants successfully regenerating into complete plantlets. This aligns with findings by Raisya *et al*. (2020), who reported that TDZ supplementation promoted dense and compact shoot formation, making individual shoots difficult to count. Treatment C2 (0.75 ppm TDZ) exhibited visual characteristics associated with low-concentration TDZ (0.08–1 ppm), which enhances auxin activity (NAA and 2,4-D) and stimulates callus formation (Pramanik & Rachmawati, 2010). Treatment D3 (1 ppm TDZ) induced callus formation, particularly on cambium-containing explants, which can form callus even in the absence

of exogenous plant growth regulators (Gunawan, 1992). The treatment also produced short, curled, and densely packed shoots, consistent with Ghasemi *et al*. (2015), who reported that high TDZ concentrations may negatively affect shoot morphology. Treatment E1 (1 ppm 2-iP) showed vigorous root development, indicating efficient nutrient uptake and healthy *in vitro* growth. Rooting is a critical phase for confirming the success of *in vitro* propagation (Immalasari, 2018). Treatment F2 (2 ppm 2-iP) resulted in shoot growth without roots after 11 weeks and the presence of callus. This is supported by Wardani *et al*. (2004), who stated that lignification often occurs in callus tissue, possibly due to cytokinin-induced nutrient transport. Furthermore, 2-iP stimulates cell division and shoot regeneration via callus differentiation (George, 1984). Treatment G2 (3 ppm 2-iP) showed the slowest growth among all treatments. The high concentration may have induced stress, resulting in suppressed development, although the explants remained viable.

The application of 3 ppm 2-iP resulted in delayed growth, with the first shoot emerging at 7 WAP. By 11 WAP, the shoots and leaves remained small and stunted, indicating stress. This aligns with Werner *et al*. (2003), who reported that high cytokinin concentrations, including 2-iP, can reduce shoot regeneration, induce abnormal tissue formation, and inhibit growth.

Shoot Emergence Time

The emergence of shoots from newly cultured explants was marked by the development of new organs, which were monitored weekly. According to Lestari (2011), the addition of cytokinins to the culture medium can elevate endogenous plant growth regulator levels, thereby stimulating tissue growth and development. The rapid and abundant shoot formation observed is likely due to the application of exogenous cytokinins. To initiate shoot induction, supplementation with appropriate concentrations of cytokinins has been recommended (Poonsapaya *et al*., 1989).

Percentage of Viable Explants, Browning Explants, Physiologically Dead Explants, Contaminated Explants, and Explants Forming Plantlets

The percentage of surviving explants represents those capable of continued growth, indicated by increased height, organ development, and successful regeneration until the end of observation. One of the main factors contributing to explant survival is the sterilization process. Gajbhiye *et al*. (2011) demonstrated that sterilizing explants with 0.1% $HgCl_2$ for 5 minutes significantly increased survival rates by reducing contamination and physiological death.

Explant browning was observed in some treatments, characterized by the medium turning brown after planting. This phenomenon can inhibit growth and result in tissue necrosis (Hutami, 2008). Browning is often linked to chemical toxicity from sterilizing agents, as also noted by Insiana *et al*. (2017), who reported that poor responses during culture were often associated with harsh sterilization treatments. Browning typically results from the oxidation of phenolic compounds released by the explant, which disrupts further shoot formation (Immalasari, 2018). In treatment G (3 ppm 2-iP), the high cytokinin concentration likely stressed the explants, impeding normal development. This is in line with Zulkarnain (2009), who emphasized that not all plants tolerate high hormone concentrations.

Stagnation, defined as the absence of growth despite explant viability, was observed in this treatment and is likely related to the medium's cytokinin concentration (Syabana *et al*., 2017). Similarly, Arimarsetiowati (2012) noted that specific media compositions can cause stagnation by limiting cell division. Physiological death was also noted in several treatments, likely due to sterilization-induced chemical toxicity. Denish (2007) stated that prolonged browning impairs nutrient uptake and ultimately leads to physiological death. Another contributing factor may be the explant's age-very young explants often contain high levels of phenolic compounds, increasing susceptibility to browning and death (Basri, 2016). Conversely, older explants may exhibit reduced totipotency and limited cell division potential.

No contamination (0%) was observed across all treatments, indicating successful sterilization and maintenance of aseptic conditions throughout the experiment. The use of 0.1% $HgCl_2$ was effective in eliminating field-derived microorganisms, supporting findings by Gajbhiye *et al*. (2011).

Hg^{2+} ions from $HgCl_2$ disrupt microbial enzyme and protein function, particularly at the cell membrane and cytoplasmic levels (Sundram *et al*., 2012).

Plantlet formation was observed in treatments E and B. Not all treatments resulted in complete plantlets, as this depends on the type and concentration of cytokinin applied to the strawberry explants. Successful regeneration into complete plantlets (with shoots, stems, leaves, and roots) is influenced by both environmental (e.g., water content, temperature, storage conditions) and genetic factors (Devy, 2006; Basri, 2016). A balanced cytokinin concentration may promote plantlet development, while endogenous factors, such as high internal auxin levels, can stimulate root growth (Haddadi *et al*., 2013). Increased root length and number can enhance nutrient uptake, ultimately supporting overall plantlet development.

Main Observations

Shoot Number

Cytokinins such as 2-iP and TDZ are known to promote shoot formation *in vitro* cultures. Vejsadová (2008) reported that 2-iP significantly induced organogenesis from meristematic tissues, enhancing shoot regeneration in strawberry cultivars such as 'Rebe' and 'Van Werden Poelman'. Similarly, Procházka *et al*. (1989) found that shoot multiplication improved markedly when 2-iP was applied at higher concentrations (8–10 ppm) in combination with IAA. On the other hand, TDZ has been demonstrated to stimulate both adventitious and axillary shoot formation (Immalasari, 2018). TDZ has also been highlighted as an effective cytokinin, either alone or in combination, for enhancing the number and frequency of shoots in strawberry explants (Haddadi *et al*., 2010).

However, high cytokinin concentrations can induce stress in plantlets, potentially inhibiting shoot proliferation. Murthy *et al*. (1998) noted that excessive levels of cytokinins may disrupt mineral or metabolite accumulation, reducing regeneration capacity. Hormonal balance is essential for optimal plantlet development, and excessive cytokinin levels may disturb this balance, leading to growth inhibition (Haddadi *et al*., 2013). Sihotang (2010) emphasized that shoot induction can be enhanced by adding exogenous cytokinins, which complement the endogenous plant hormones already present in the explants.

Leaf Number

The role of cytokinins in leaf development is closely tied to their involvement in cell division, nucleic acid metabolism, and protein synthesis (Adds *et al*., 2004). Cytokinin-induced leaf growth also contributes to increased photosynthetic capacity, which directly influences overall plant vigor (Awanda, 2015). Nonetheless, cytokinin concentration is a key factor: while moderate concentrations can promote leaf formation, higher levels may have the opposite effect. Murthy *et al*. (1998) observed that TDZ concentrations >0.5 ppm may impair explant tissues if not regularly subcultured, ultimately hampering shoot and leaf formation. Moreover, Insiana *et al*. (2017) noted that appropriate cytokinin balance is essential to stimulate nodular responses that initiate leaf development in axenic cultures.

Plantlet Height

Cytokinins are crucial in stimulating organ development such as shoots, leaves, and nodes during *in vitro* culture, as long as the concentration is optimal (Gunawan, 1992). Among various types, 2-iP is widely used in plant tissue culture due to its natural origin and effectiveness (Warseno, 2018). However, excessive cytokinin levels may result in growth suppression, leading to stunted and poorly developed plantlets. Prasiwi (2018) reported that high TDZ concentrations can impede metabolic activity and reduce explant growth, emphasizing the importance of proper cytokinin dosage in regulating plantlet elongation.

Fresh Weight

Fresh weight is influenced by the accumulation of plant organs such as shoots, leaves, roots, and callus tissues. Even though no significant differences may be statistically detected among treatments, variation in tissue proliferation and organogenesis can lead to differences in fresh

biomass. A balanced cytokinin concentration contributes to optimal organ formation, which in turn affects plantlet mass. Conversely, cultures under hormonal stress, particularly at high cytokinin concentrations, may exhibit limited development, reduced leaf and shoot number, and ultimately lower fresh weight. Zulkarnain (2009) noted that *in vitro* cultures are highly sensitive to environmental and hormonal conditions, which directly affect cellular and tissue development.

CONCLUSION

The application of various types of cytokinins at concentrations of 0.50–1 ppm significantly influenced shoot number, leaf number, plant height, and fresh weight of *Fragaria × ananassa* Duch. clone BAT 1 explants grown *in vitro*. Among the treatments, 1 ppm 2-iP showed the best performance by producing the highest average shoot number (5.24), leaf number (5.90), and plant height (2.23 cm), while 0.50 ppm TDZ resulted in the highest fresh weight (1.36 g). These findings suggest that appropriate types and concentrations of cytokinins can enhance *in vitro* growth and development of strawberry explants.

ACKNOWLEDGMENT

We gratefully acknowledge Universitas Padjadjaran for the research funding and access to the Plant Cultivation Laboratory facilities, which were essential to this study. We also sincerely thank Bumi Agro Technology (BAT) for providing the explants used in this research.

AUTHOR CONTRIBUTIONS

PE: collecting research data, drafting the article, final revision of the manuscript; AN: Creating research concept, final revision of manuscript; MU: Create research concepts, revise manuscripts; MH : drafting the article, revise manuscripts.

REFERENCES

Adds, J., Larkcom, E., & Miller, R. 2004. Genetics, evolution and biodiversity (Rev. ed.). In M. Furness-Smith (Ed.), *Nelson Advanced Science*. United Kingdom: Nelson Thornes.

Arimarsetiowati, R. 2012. *Kultur jaringan tanaman kopi*. Pusat Penelitian Kopi dan Kakao Indonesia. 24(2), pp.13-17.

Awanda, R. K. 2015. Inisiasi tunas sengon (*Paraserianthes falcataria*) secara *in vitro* dengan BAP dan GA3. Skripsi. Fakultas Pertanian, Universitas Muhammadiyah Jember, Jember.

Badan Pusat Statistik. 2020. *Produksi Tanaman Buah-buahan 2021*. Retrieved from https://www.bps.go.id/indicator/55/62/1/produksi-tanaman-buah-buahan.html

Basri, A. H. H. 2016. Kajian pemanfaatan kultur jaringan dalam perbanyakan tanaman bebas virus. *Agrica Ekstensia*, 10(1), pp.64-73.

Bhatt, I. D., & Dhar, U. 2000. Micropropagation of Indian wild strawberry (*Fragaria indica*). *Scientia Horticulturae*, 85(3) pp.357–364.

Budiman, S., & D. Saraswati. 2005. Berkebun Stroberi Secara Komersial. Jakarta: Penebar Swadaya.

Cappelletti, R., S. Sabbadini., & B. Mezzetti. 2016. The use of TDZ for the efficient *in vitro* regeneration and organogenesis of strawberry and blueberry cultivars. *Journal Scientia Horticulturae*, 207, pp.117–124.

Chen, C. 2004. Humidity in plant tissue culture vessels. *Biosys Engin,* 88(2), pp.231- 241.

Denish, A. 2007. *Percobaan perbanyakan vegetatif kematian (Lunasia amara Blanco.) melalui kultur jaringan*. Skripsi. Departemen Konservasi Sumberdaya Hutan dan Ekowisata, Fakultas Kehutanan, Institut Pertanian Bogor.

Devy, L., & D. R. Sastra. 2006. Pengaruh radiasi sinar gamma terhadap kultur *in vitro* tanaman jahe. *Jurnal Sains dan Teknologi Indonesia*, 8(1), pp.7-14.

Erawati, D. N., U. Fisdiana, & M. Kadafi. 2020. View of respon eksplan vanili (*Vanilla planifolia*) dengan stimulasi BAP dan NAA melalui teknik mikropropagasi. *Journal of Applied Agricultural Sciences,* 4(2), pp.146-153.

Gajbhiye, S. S., M.K Tripathi., V. Shankar, M. Singh, M. Baghel, & B. S. Tiwari. 2011. Direct shoot organogenesis from cultured stem disc explants of tuberose (*Polianthes tuberosa* Linn.). *Journal of Agricultural Technology*, 7(3), pp.695-709.

George, E. F., & Sherrington, P. D. 1984. *Plant Propagation by Tissue Culture: Handbook and Directory of Commercial Laboratories.* Exegetics Ltd., Eversley, Basingstoke.

Gunawan, L. 1992. *Bioteknologi Tanaman*. Bogor: PAU Bioteknologi IPB.

Haddadi, F., M. A. Aziz, G. Saleh, A. A. Rashid, & H. Kamaladini. 2010. Micropropagation of strawberry cv. Camarosa: prolific shoot regeneration from *in vitro* shoot tips using thidiazuron with N6-benzylamino-purine. *Jurnal HortScience,* 45(3), pp.453–456.

Haddadi, F., M. A. Aziz, H. Kamaladini, & S. A. Ravanfar. 2013. Thidiazuron and zeatin induced high frequency shoot regeneration from leaf and shoot tip explants of strawberry. *Hort Technology*, 23(3), pp.276-281.

Hutami, S. 2008. Masalah pencoklatan pada kultur jaringan. *Jurnal AgroBiogen*, 4(2), pp.83-88.

Immalasari, N. R. 2018. *Variasi media dan konsentrasi zat pengatur tumbuh TDZ pada induksi kalus pucuk jati (Tectona grandis) secara in vitro*. Skripsi. Fakultas Kehutanan, Universitas Hasanuddin, Makassar.

Insiana, A., T. Herawan., & L. Haryjanto. (2017). Pengaruh teknik sterilisasi eksplan terhadap tingkat perolehan kultur jaringan aksenik ramin (*Gonystylus bancanus). Jurnal Pemuliaan Tanaman Hutan,* 11(2), pp.131-138.

Kikas, A., A. Libek., & V. Vasar. 2006. Influence of micropropagation on the production of strawberry runner plants, yield and quality. *Journal Acta Horticultura*, 5(708), pp.241-244.

Lestari, E. G. 2011. Peranan zat pengatur tumbuh dalam perbanyakan tanaman melalui kultur jaringan. *Jurnal AgroBiogen*, 7(1), pp.63-68.

Li, Y., J. Hu., J. Xiao., G. Guo., & B. R. Jeong. 2021. Foliar thidiazuron promotes the growth of axillary buds in strawberry. *Journal Agronomy*, 11(3), p.594.

Murthy, B. N. S., S. J. Murch., & P. K. Saxena. 1998. Thidiazuron: a potent regulator of *in vitro* plant morphogenesis. *Journal In Vitro Cellular and Developmental Biology-Plant,* 34(4), pp.267-275.

Murvanidze, N., Nisler, J., Leroux, O., & Werbrouck, S.P.O. 2021. Cytokinin oxidase/dehydrogenase inhibitors stimulate 2iP to induce direct somatic embryogenesis in *Coffea arabica*. *Journal Plant Growth Regulation*, 94(2), pp.195-200.

Nurana, A. R., W. Gede, & R. Dwiyani. 2017. Pengaruh 2-iP dan NAA terhadap pertumbuhan plantlet anggrek dendrobium hibrida pada tahap subkultur. *Agrotrop: Journal on Agriculture Science*, 7(2), pp.139-146.

Poonsapaya, P. M. W., Nabors., W. Kersi., & M. Vajrabhaya. 1989. A comparison of methods for callus culture and plant regeneration of RD25 rice (*Oryza sativa* L.) in two laboratories. *Plant Cell Tiss Org Cult,* 16, pp.175-186.

Pramanik, D., & F. Rachmawati. 2010. Pengaruh jenis media kultur *in vitro* dan jenis eksplan terhadap morfogenesis Lili oriental. *Journal Hort*, 20(2), pp.111- 119.

Prasiwi, I. D., & Wardiyati. (2018). Pengaruh pemberian thidiazuron (TDZ) terhadap pertumbuhan tunas nanas (*Ananassa comosus* (L.) Merr.) cv. "Smooth Cayyene" asal mahkota buah. *Jurnal Produksi Tanam*, 6(1). pp.9-15.

Procházka, S., Macháčková L., Krekule J., *et al*. 1989. *Fyziologie rostlin* [*Plant Physiology*]. Praha: Academia.

Rahmawati, D. 2021. *Panduan Praktik Teknologi Kultur Jaringan di Laboratorium Bioteknologi*. Bogor : SEAMEO BIOTROP.

Raisya, E., D. S. Sobarna., A. Nuraini., S. Mubarok., E. Suminar., & M. Akutsu. 2020. Multiplikasi *in vitro* stroberi kultivar Tochiotome dengan penambahan jenis dan konsentrasi sitokinin untuk perbanyakan bibit. *Jurnal Kultivasi*, 19(3), pp.1189-1195.

Setiawan, G. W., D. Pangemanan., & P. Hedison. 2016. Pengaruh pemberian vitamin C terhadap kadar neutrofil setelah latihan fisik. *Jurnal e-Biomedik,* 4(1), pp.136-139.

Setyoningsih. 2021. Penggunaan vitamin untuk meningkatkan imunitas tubuh di masa pandemi. *Jurnal Pengabdian Kesehatan*, 4(2), pp.136-150.
Sihotang, N. 2010. Kultur meristem pisang baranangsiang (*Musa paradisiaca* L.) pada media MS dengan beberapa komposisi zat pengatur tumbuh NAA, IBA, BAP dan kinetin. *Jurnal Ilmu Pertanian*, 3(2), pp.19-25.
Sukasih, E., & Setyadjit. 2019. Teknologi penanganan buah segar stroberi untuk mempertahankan mutu. *Jurnal Litbang Pertanian*, 38(1), pp.47-54.
Sundram, T. C., M. Suffian., M. Annuar., & N. Khalid. 2012. Optimization of culture condition for callus induction from shoot buds for establishment of rapid growing cell suspension cultures of Mango ginger (*Curcuma mangga*). *Australian Journal of Crop Science,* 6(7), pp.1139-1146.
Syabana, M. A., P. Marianingsih., N. Hermita., & I. Rohimah. 2017. Induksi dan pertumbuhan kalus tanaman Stevia (Stevia rebaudiana Bertoni M.) dengan perbedaan konsentrasi PEG (Polyethylene Glycol) pada kondisi pencahayaan secara *in vitro*. *Jurnal Biodidaktika*, 12(2), pp.57-68.
Thi, K., K. Swar., & Y. Mon. 2018. Establishment of efficient surface sterilization protocol on different types of field grown strawberry explants (*Fragaria* × *ananassa* Duch.). *Journal of Scientific and Innovative Research*, 7(3), pp.70-74.
USDA (United States Department of Agriculture National Nutrient Database). 2016. *Strawberry*. National Agricultural Library.
Vejsadová, H. (2008). Growth regulator effect on *in vitro* regeneration of rhododendron cultivars. *Horticultural Science*, 35(2), pp.90-94.
Wardani, D. P., Solichatun, & A. D Setyawan. 2004. Pertumbuhan dan produksi saponin kultur kalus *Talinum paniculatum* gaertn pada variasi penambahan asam 2,4 diklorofenoksi asetat (2,4-D) dan kinetin. *Biofarmasi,* 2(1), pp.35-43.
Warseno, T., & D. Meiningsasi. 2018. Multiplikasi tunas dan induksi perakaran pada perbanyakan rhododendron radians J.J.Sm (*Ericaceae*) secara *in vitro*. *Jurnal Hort*, 28(1), pp.51-58.
Werner, T., Motyka, V., Laucou, V., Smets, R., Van Onckelen, H., & Schmülling, T. 2003. Cytokinin-deficient transgenic Arabidopsis plants show multiple developmental alterations indicating opposite functions of cytokinins in the regulation of shoot and root meristem activity. *The Plant Cell*, *15*(11), pp.2532-2550.
Zulkarnain, Z. 2009. *Kultur Jaringan Tanaman: Solusi Perbanyakan Tanaman Budidaya*. Jakarta: Bumi Aksara.