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The Establishment of Disease-Free Planting Material of *Ananas comosus* Cv. MD2 (JengkaPine) via Direct Organogenesis Using Sucker Explants

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ABSTRACT

JengkaPine is an MD2 pineapple brand exclusive to the Universiti Teknologi MARA (UiTM) Pahang Branch Jengka Campus. Like other pineapple varieties, JengkaPine has issues with bacterial and Phytophthora heart and root rot diseases, resulting in significant yield losses. One option for completely overcoming this problem is to eliminate the pathogens at the earliest propagation stage using plant tissue culture technology. This paper describes a plant tissue culture procedure through direct organogenesis to obtain disease-free planting materials for JengkaPine using sucker explants. Direct organogenesis was obtained through the culture of explants on modified MS media supplemented with 40.0 g/L sucrose and different concentrations and combinations of plant growth regulators such as BAP, NAA, myo-inositol, kinetin, and IAA. The organogenesis of MD2 JengkaPine was investigated in three phases: initiation, multiplication, and rooting. The optimum shoot initiation was observed on MS + 2.0 g/L BAP and MS + 3.0 mg/L BAP after 12 weeks with a total of 5–6 shoots per culture, while clonal shoot multiplication was found optimal on MS medium added with 3.0 mg/L BAP with a total of 12 shoots. MS + 3.0 mg/L IAA was ideal for rooting MD2 JengkaPine clonal shoots, giving 28 roots after 12 weeks. Improvement in Phytophtora-reduced contamination during surface sterilization was also investigated by applying 3% hydrogen peroxide. Plantlets obtained will be acclimatized and subjected to further research under greenhouse and field planting.

INTRODUCTION

Pineapple (*Ananas comosus* L.) is considered one of the world's most popular exotic fruits, ranking third to bananas and mangoes [1]. In Malaysia, pineapple ranks fourth (6.3%) in agro-food usage, trailing after durian (41.3%), banana (18.0%), and rambutan (9.8%). Among the prominent varieties commercially farmed in the country are Morris, Josapine, Yankee, Gandul, and N36. However, the MD2 variety is the most popular among Malaysians in the market for its great taste and quality [2]. JengkaPine

is an MD2 pineapple brand developed exclusively by Universiti Teknologi MARA (UiTM) Pahang Branch Jengka Campus in 2020 and was formally patented in August 2021. When JengkaPine first began, it was intended to serve as a teaching and learning project for the completion of practical training for the Diploma in Plantation and Technology Management (AT110) course. This initial 2-acre project was carried out at the Fruit Farm, UiTM Pahang, under the direction of the Farm Management Unit (UPL) and the Faculty of Plantation and Agrotechnology. The project is also receiving strong support and full collaboration from the top management of UiTM Pahang, particularly Prof. Ts. Dr. Mohd Ilham Adenan, who is acting as Rector, as well as the Malaysian Pineapple Industry Board (LPNM), Pahang. LPNM Pahang has monitored the crop plots numerous times, and the organization has also offered guidance on planting and disease prevention.

In addition to being used as a teaching and learning initiative, the UPL Revenue Sales Unit sells JengkaPine products throughout Malaysia in retail and wholesale. Jengkapine products are sold as seedlings and cost between RM 1.20 and RM 1.50 per sucker. In order to give clients more options for purchasing, the fruits are currently being sold for RM5 per kilogram, in addition to cutting fruits and pineapple juice. JengkaPine was sold in bulk for 2 tonnes during the COVID-19 pandemic, demonstrating its commercial value [3]. JengkaPine is susceptible to disease outbreaks and attacks, much like any other variety of pineapple (Figure 1). Fungi, bacteria, nematodes, and viruses are the principal causes of pineapple diseases. These disease infections could destroy the entire pineapple plant or significant portions of it. Bacterial heart rot, phytophthora heart rot, and root rot diseases are among JengkaPine's main health concerns. Due to the formality, as the demand for JengkaPine grows, UPL has needed help meeting the demand for planting materials and fruits.

Figure 1. Heart rot incidence of JengkaPine in The Farm Management Unit, Faculty of Plantation and Agrotechnology, UiTM Pahang Branch Jengka Campus.

Bacterial heart rot is a bacterial infection caused by a soft rot bacterium, *Erwinia chrysanthemi*, while Phytophthora heart rot and root rot diseases are caused by Phytophthora and Pythium species (soilborne fungi). This disease can attack various plant components at various growth stages. Among the general symptoms of heart rot diseases are soft rot in ripe fruit and blistering of the leaves, which damages the tissues of the basal leaves and can potentially cause fruit rot. Meanwhile, root rot disease attacked and destroyed the root system of pineapples. Root rot symptoms include a decrease in plant growth, the emergence of reddish-colored leaves, the browning of the leaf margins, and the decline and eventual death of the plant. Each symptom may only appear in fruit two to three weeks before ripening, and if left untreated, they can result in lower crop yields or crop loss altogether [4,5]. The disease-causing pathogens mentioned above are well known for flourishing in damp settings. Phytophthora heart can spread more quickly during the rainy season.

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According to the senior assistant manager of UPL [6], attacks on JengkaPine have been observed to have occurred during the early monsoon season 2022. When the diseases invade the UiTM farm, the pineapple trees are about 150 days old after being sown. The attacks began from the pineapple plot on the upper hillside of the farm, which later spread to the other plots situated at the foothills by rainwater runoff. Most diseased pineapple plants have been severely damaged, with symptoms such as rotten smell, water-drenched basal white leaf tissue, wilting, and plant death in the worst-case scenario. The disease has been recorded to have infected more than 4000 plants from the total of 34000 pineapple trees being planted. Inadequate drainage systems on the farm could also contribute to the rapid spread of diseases. The rigorous removal and burning of sick plants are the primary defence against disease outbreaks at the JengkaPine planting site. Additionally, fungicides were used to reduce the rate of infection. Even though several agronomic practices may initially reduce the symptoms, this disease regrettably persists in the soil for a very long period. It causes in-borne disease in pineapple seedlings, ensuring its recurrence. These major issues can be easily overcome using modern breeding technologies.

Plant tissue culture is one of the most well-known and commonly practiced breeding technologies. Plant tissue culture, known as micropropagation or *in vitro* breeding, is a method of growing plants on synthetic media using a minimal amount of plant tissue called explants. It is one of the technologies that can be utilized as an alternative to creating disease-free plantlets by eradicating the cause of disease at the earliest stage [7]. The advantages of plant tissue culture technology include the quick availability of plants and uniform growth, which can be conducted around the year, irrespective of the growing season. The controlled growing environment of plant tissue culture also protected plants from pests, pathogens, and vagaries of nature [8]. Commonly practiced plant tissue culture techniques include direct organ development from explants, known as organogenesis. Organogenesis is defined as initiating organs such as roots, shoots, leaves, or flowers directly from organ or callus culture. Organogenesis is among the best plant tissue culture techniques for producing disease-free plantlets that are uniform and genetically identical to the parent plants for quick application [9]. Thus, this paper described the organogenesis of JengkaPine using plant tissue culture. The objective of the research is to establish a direct organogenesis protocol from sucker explants to obtain high-frequency clonal shoot regeneration from the pineapple cultivar MD2 JengkaPine.

EXPERIMENTAL

Preparation of Plant Materials

Planting materials for the MD2 JengkaPine pineapple cultivar were freshly collected from the UiTM Pahang Branch Jengka Campus pineapple farm. The explants used for the study were the scions (suckers) of the plant at the age of 3 months after initiation, with about 15 leaves and a length of 30 cm. Before the surface sterilization method, the plant materials were washed under running tap water, and the outer leaves were cut in half of their initial length and treated with fungicide for an hour.

Surface Sterilization of Plant Materials

The outer leaves of fungicide-treated plant materials were removed completely. During this stage, surface sterilization methods were investigated to reduce the potential attacks of heart rot bacteria or pathogens. Some plant materials are exposed to soaking with 3% hydrogen peroxide, while others are not. Later, the plant materials and surface were sterilized in 70% – 98% ethyl ethanol for 5 minutes, followed by soaking in 50% – 95% sodium hypochlorite with a few drops of Tween 20 for 20 minutes. The surface sterilized plant materials were later rinsed with double-sterile distilled water, giving three consequent changes before drying in a laminar airflow chamber. Then, the plant materials were excised to a size of 1-2 cm to facilitate culture (Figure 2a).

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Preparation of Culture Medium

MS (Murashige and Skoog) culture medium containing 4.41 mg/L MS powder, 2.5 g/L gelrite, and 40 mg/L sucrose were prepared. Plant growth regulators were added accordingly to achieve direct organogenesis and rooting of explants. The pH of the medium was adjusted to 5.8 with 1M sodium hydroxide (NaOH) and 1M hydrochloric acid (HCl) prior to autoclaving at 121ºC for 20 minutes at a pressure of 1.1 kg cm^2. Explants cultured on MS medium without growth regulators served as controls.

Direct Organogenesis and Maintenance

The organogenesis of explants was determined by culturing a single explant in each culture vessel. Plant growth regulators (PGRs) such as myo-inositol (0.1 g/L), benzylaminopurine (BAP) [1.0–5.0 mg/L], naphthaleneacetic acid (NAA) [1.0–4.0 mg/L], and kinetin (2.0–6.0 mg/L) were added to the culture medium in combinations to achieve organogenesis initiation and clonal shoot multiplication of pineapple cultivar MD2 JengkaPine. The PGR dosage utilized in the experiment is determined and adjusted by the efficacy of previous investigations conducted on the MD2 pineapple variety [12,15]. All cultures were later maintained in an aseptic environment adjusted to a temperature of 25±2ºC and illuminated with continuous white-fluorescent light controlled at a 16-hour photoperiod. Subcultures were carried out at monthly intervals.

In vitro rooting of Explants

The rooting phase of culture was established on MS medium supplemented with NAA (1.0–4.0 mg/L), 1.0 mg/L Indole-3-butyric acid (IBA), and 1.0–3.0 mg/L Indole-3-acetic acid (IAA).

Statistical Analysis

All data and variables of the study were laid out using a randomized complete block design (RCBD). Each treatment was replicated at least ten times. Significant differences among used treatments were analyzed via analysis of variance (ANOVA) and a Tukey post-hoc test using SPSS software. Each mean value was expressed as the mean ± standard error (SE) at $P < 0.05$. Organogenesis induction frequency (%), a mean number of clonal shoots per culture, mean clonal shoot length (cm) and mean root numbers were monitored as development parameters.

RESULTS AND DISCUSSION

In vitro surface sterilization

Potential attacks of heart rot pathogens on the in vitro growth of JengkaPine were reduced using varying concentrations of ethyl alcohol (70–98%) and sodium hypochlorite (50–98%) with and without exposure to 3% hydrogen peroxide. After the investigation, it was discovered that increased concentrations of surface sterilizing agents (98%) combined with sufficient exposure to (3%) hydrogen peroxide resulted in less than 10% contamination of the culture. Repeated processes with improved operator handling decreased contamination even further. When lower concentrations of surface sterilization chemical agents were used simultaneously, almost 60% of the culture was contaminated by pathogens. While other plant species cultures might work well with lower concentrations of surface sterilization chemical agents, pineapple is not one of them because it is planted at the ground level and is prone to diseases that come from the soil. *In vitro* sterilization is a fundamental component of plant tissue culture, and the effectiveness of sterilization directly impacts the culture's final outcomes [10]. The effectiveness of using higher concentrations of surface sterilization agents and hydrogen peroxide to reduce bacterial contamination in plant tissue culture protocols was also discussed [11].

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Direct organogenesis of JengkaPine

Direct organogenesis was investigated in three stages: initiation (Figure 2b), multiplication (Figure 2c), and rooting (Figure 2d). The effects of different concentrations of PGRs such as BAP, NAA, kinetin, myo-inositol, and IAA were investigated according to the study's objectives. After 8 weeks, it was observed that 20% of explants cultured on MS medium supplemented with 2.0–3.0 mg/L BAP responded to organogenesis. The optimum total number of clonal shoot initiations at 1.0 ± 0.12 and average shoot length of 0.16 ± 0.16 and 0.14 ± 0.14 were recorded, respectively. No results were obtained from other treatments, including control (MS0). The rate of explants responding to direct organogenesis improved significantly after 12 weeks of culture (60%) on optimum medium, MS + 3.0 mg/L BAP, with a total number of 6.0 ± 0.70 clonal shoots and mean length of 0.45 ± 0.24 (Table 1). The addition of myo-inositol did not enhance direct organogenesis in JengkaPine. Some explants that did not respond were found to turn brownish and eventually die. In contrast, optimum results of direct organogenesis on pineapple cv. MD2 published by [12] reported optimum organogenesis response by adding 0.1 g/L of myo-inositol but with reduced sucrose concentration (30.0 g/L).

The transfer of explants onto multiplication media of similar composition increased the total number of clonal shoots' development. MS media containing 3.0 – 6.0 mg/L BAP promotes the highest total number of 12 and 18 clonal shoots after 8 and 12 weeks, respectively (Table 2). MS media containing a high concentration of kinetin (6.0 mg/L) also performs well for JengkaPine clonal proliferation. The best mean length of clonal shoots established from the study ranges from 2.20 ±0.05 to 2.40 ±0.02. However, based on the established clonal shoot morphology and length, the final BAP concentration of 3.0 mg/L is considered optimal. BAP is a form of synthetic cytokinin and is a common component of plant tissue culture media such as Murashige and Skoog (MS). Researchers frequently employ this plant growth regulator for the *in vitro* multiplication of clonal shoots in a variety of plant species [13], [14] in addition to the numerous varieties of pineapple [15], [16]. This synthetic cytokinin interacts with auxins, which are naturally present in the plant, to encourage cell proliferation. It works very effectively when used with kinetin.

Table 1: The establishment of direct organogenesis initiation on sucker explants of *Ananas comosus* cv. MD2 (JengkaPine) after 8 and 12 weeks of culture.

Treatment(s)	Composition (mg/L)		Rate of organogenesis (%)		Total number of clonal shoots		Mean length (cm ± SE)	
	BAP	NAA	8w	12w	8w	12w	8w	12w
T0 (Control)	-	-	-	-	-	-	-	-
T1	1.0	-	-	20	-	1	-	-
T2	2.0	-	20	40	1	5	0.14 ± 0.14[a]	0.08 ± 0.08[a]
T3	3.0	-	20	60	1	6	0.16 ± 0.16[a]	0.48 ± 0.30[a]
T4	4.0	-	-	20	-	1	-	0.45 ± 0.24[a]
T5	1.0	0.1	-	40	-	-	-	-
T6	2.0	0.1	-	40	-	2	-	0.12 ± 0.10[a]
T7	3.0	0.1	-	20	-	1	-	0.10 ± 0.10[a]
T8	4.0	0.1	-	-	-	-	-	-

[1] Values represent means ± standard error (SE). N=5. 2Mean values followed by the same letter within a column are not significantly different by the Tukey test at P ≤ 0.05. W = weeks

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Table 2: The establishment of direct organogenesis proliferation on sucker explants of *Ananas comosus* cv. MD2 (Jengkapine) after 8 and 12 weeks of culture.

Treatment(s)	Composition (mg/L)			Rate of organogenesis (%)		Number of clonal shoots		Mean length (cm ± SE)	
	BAP	NAA	Kin	8w	12w	8w	12w	8w	12w
P0 (Control)	-	-	-	60	60	5	5	0.44 ±0.18^{b}	1.00 ±0.16^{b}
P1	2.0	1.0	-	60	60	6	7	0.60 ±0.26^{b}	1.20 ±0.21^{ab}
P2	2.0	2.0	-	80	100	6	7	1.14 ±0.10^{ab}	1.20 ±0.21^{ab}
P3	3.0	-	-	80	100	12	14	1.48 ±0.04^{ab}	2.40 ±0.10^{a}
P4	6.0	-	-	100	100	12	18	1.12 ±0.04^{ab}	2.40 ±0.02^{a}
P5	7.0	-	-	100	100	11	11	0.88 ±0.06^{b}	2.20 ±0.05^{a}
P6	-	-	2.0	100	100	5	6	0.720 ±0.19^{b}	1.00 ±0.12^{b}
P7	-	-	4.0	80	80	7	11	0.92 ±0.23^{b}	1.40 ±0.20^{ab}
P8	-	-	6.0	100	100	12	13	1.1 ±0.05^{ab}	1.80 ±0.04^{ab}

[1] Values represent means ± standard error (SE). N=5. 2Mean values followed by the same letter within a column are not significantly different by the Tukey test at P ≤ 0.05. W = weeks

In vitro rooting of JengkaPine

Clonal roots of JengkaPine were initiated on an MS medium containing different concentrations of plant growth regulators such as BAP, NAA, and IAA (Table 3). MS medium devoid of plant growth regulators served as a control treatment. *In vitro* roots were first observed to develop after 4 weeks, and data was recorded and analyzed at 8 and 12 weeks of culture. With a mean number of 3.6±0.40 and length of 0.44±0.05 after 8 weeks, it was found that MS media with 1.0 mg/L IAA had the best root establishment out of all the treatments studied. By week 12, the data readings had increased to a mean number and length of 5.6±0.40 and 1.0±0.10, respectively. Similar findings were made by [17], who found that pineapple shootlets cultured on MS media containing a higher concentration of IAA at 3.0 mg/L produced the tallest length of roots. The highest numbers of *in vitro* pineapple roots were produced on MS media supplemented with 1.0 mg/L IAA with 10.67 roots per shootlet. Aside from IAA, *in vitro*, rooting of pineapple has been successfully initiated using additional plant growth regulators such as IBA (indole-3-butyric acid) [18] and BAP alone [19]. Some researchers reported using plant growth regulators in combinations at different concentrations to obtain optimum rooting [20]. The control treatment produced no results.

Table 3: The initiation of *in vitro* rooting of *Ananas comosus* cv. MD2 (JengkaPine) clonal shootlets after 8 and 12 weeks.

Week (s)	Treatment (s)	Media Composition(s)	Number of roots per explants	Root Length (cm ± SE)
8	T0	MSO	-	-
	T1	MSO + 1.0 mg/L IAA	3.6 ± 0.40^{a}	0.44 ± 0.05^{b}
	T2	MS + MS + 1.0 g/L NAA	0.4 ± 0.40^{b}	0.1 ± 0.10^{b}
12	T0	MSO	-	-
	T1	MSO + 1.0 mg/L IAA	5.6 ± 0.40^{a}	1.0 ± 0.10^{ab}
	T2	MS + MS + 1.0 mg/L NAA	2.2 ± 0.58^{a}	0.38 ± 0.11^{b}

[1] Values represent means ± standard error (SE). N=5. 2Mean values followed by the same letter within a column are not significantly different by the Tukey test at P ≤ 0.05. W = weeks

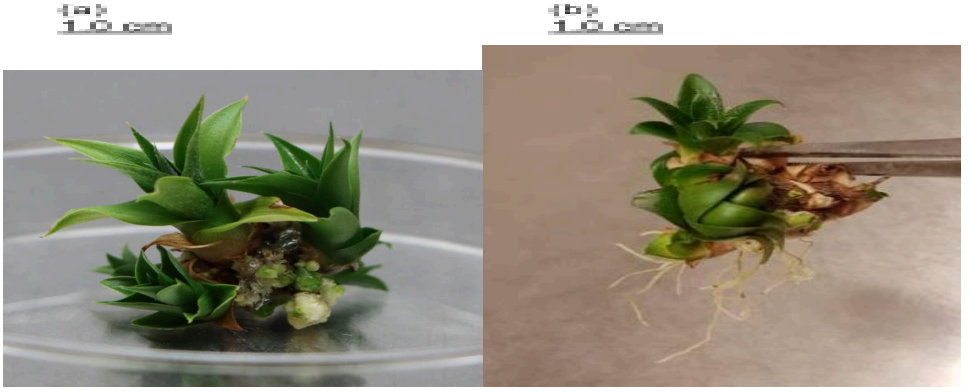

Figure 2. Direct organogenesis of *Ananas Comosus* Cv. MD2 (JengkaPine) from (a) aseptic sucker explants. Adventitious shoots formation after (b) 4 weeks, (c) 12 weeks on MS + 3.0 mg/L BAP. (d) The *in vitro* rooting initiation of clonal shootlets of JengkaPine on optimum culture medium after 12 weeks

CONCLUSION

Organogenesis of *Ananas comosus* cv. MD2 (JengkaPine) has been successfully obtained. The optimal clonal shoot initiation was observed on MS media containing 2.0 mg/L–3.0 mg/L BAP, while optimal shoot proliferation was observed on MS + 3.0 mg/L BAP. Cultures successfully initiated clonal rooting on MS + 1.0 mg/L IAA. Improvement on *Phytophtora*-reduced contamination during surface sterilization was minimally reduced by applying 3% hydrogen peroxide. Plantlets produced will be subjected to acclimatization and proceeded with future greenhouse and field planting investigations.

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CONFLICT OF INTEREST STATEMENT

The authors agree that this research was conducted without any self-benefits or commercial or financial conflicts and declare the absence of conflicting interests with the funders.

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AUTHORS' CONTRIBUTIONS

Nazatul Asikin, Nur Atiqah, Noorshilawati, and Danial were responsible for the project's conception, research, and writing. Neni Kartini and Norsharina contributed input on the theoretical and conceptual frameworks. Additionally, Khairi and Nurulatika were responsible for revising the article and supervising the research development.

REFERENCES

[1] Chen, H. et al. (2019). Differential expression analysis of reference genes in pineapple (*Ananas comosus* L.) during reproductive development and response to abiotic stress, hormonal stimuli. *Tropical Plant Biology*, *12(2),* 67–77.

[2] Halim, N.A. (2016). Policy Intervention for the Development of the Pineapple Industry in Malaysia. Malaysia: FFTC Agricultural Policy Article.

[3] Fazidah, R. & Shamsuddin, I. (2023). Jengkapine: Nanas MD2 UiTM Cawangan Pahang. *UiTM News Hub*. Retrieved from https://news.uitm.edu.my/jengkapine-nanas-md2-uitm-cawangan-pahang/.

[4] Sapak, Z., Mohd Faisol Mahadeven, A.N., Nurul Farhana, M.H., Norsahira, S. & Mohd Zafri, A. W. (2021). A review of common diseases of pineapple: The causal pathogens, disease symptoms, and available control measures. *Food Res*, *5(4),* 1-14.

[5] Nor, A.A.M., Zainol, R., Abdullah, R., Jaffar, N.S., Rasid, M.Z.A., Laboh, R., Shafawi, N.A. & Aziz, N.B.A. (2023). Dissemination pattern of bacterial heart rot (BHR) disease and screening of the disease resistance among commercial pineapple varieties in Malaysia. *Malaysian Journal of Microbiology*, *15(4),* 346–350.

[6] Shamsuddin, I. (personal communication, January 1, 2023).

[7] Halim, N. A. A., Tan, B. C., Midin, M. R., Madon, M., Khalid, N. & Yaacob, J. S. (2018). Abscisic acid and salinity stress induced somaclonal variation and increased histone deacetylase (HDAC) activity in *Ananas comosus* var. MD2. *Plant Cell, Tissue and Organ Culture*, *133*, 123-135.

[8] Bhojwani, S. S. & Dantu, P. K. (2013). Micropropagation. Plant tissue culture: An introductory text. India: Springer, pp. 45-274.

[9] Singh, A. (2023). Organogenesis in Plants. *Plant Cell Technology*. Retrieved from https://www.plantcelltechnology.com/blog/organogenesis-in-plants/

[10] Hesami, M., Naderi, R. & Tohidfar, M. (2019). Modeling and optimizing in vitro sterilization of chrysanthemum via multilayer perceptron-non-dominated sorting genetic algorithm-II (MLP-NSGAII). *Front. Plant Sci*., *10*, 282.

[11] Ali, G.A., Metwaly, A., Salama, G. M. Y. & Ali, G. A. (2018). Using hydrogen peroxide for reducing bacterial contamination in date palm tissue culture. *International Journal of Advances in Agricultural Science and Technology*, *5(4),* 25–33.

[12] Hamid, N. S., Bukhori, N. & Jalil, M. (2013). Direct and indirect plant regenerations of pineapple var. MD2. *Malaysian Applied Biology*, *42(1),* 61-66. [13] Rustikawati, R., Herison, C., Inoriah, E. & Dwisari, V. (2021). Effect of BAP (6-benzyl aminopurine) on *in vitro* shoot growth of curcumas. *AGRITROPICA*, *4(1),* 82–92.

[14] Ramayana, S., Supriyanto, B., Sunaryo, W., Susylowati, S. & Adiastie, S. (2022). Benzyl amino purine (BAP) growth regulator application and shoot origin stem lai (*Durio kutejensis*) against growth durian (*Durio zibethinus* Murr) grafting seedlings in *Advances in Biological Sciences Research*.

[15] Awal, A., Nor Fazilah, N., Azvin, M. P., Najwa, M., Shamsiah, A. & Norrizah, J. S. (2011). Micropropagation of Pineapple (*Ananas Comosus* L. Merr. 'Josapine'). *Acta Horticulturae*, 923, 163–168.

https://doi.org/10.24191/sl.v18i3.27826

[16] Sani, L. A., Usman, I. S., Nasir, A. U. & Abdulmalik, M. M. (2020). Micropropagation of pineapple (*Ananas comosus* L. Var. Smooth cayenne) in temporary immersion bioreactor system (TIPS). *Bayero Journal of Pure and Applied Sciences*, *12(2),* 207-209.

[17] Atawia, A., Fathy El-Gioushy, S., Saied, S., Mohammed, O., El, A. & Kotb, S. (2016). Studies on Micropropagation of Pineapple (*Ananas comosus* L.)". *Middle East Journal of Agriculture Research*, *5(2),* 224–232.

[18] Lakho, M. A., Jatoi, N., Solangi Abul-Soad, A.A., Qazi, M. A. & Abdi, G. (2023). Optimizing *in vitro* nutrient and *ex vitro* soil mediums-driven responses for multiplication, rooting, and acclimatization of pineapple. *Scientific Reports*, *13 (1),* 1275.

[19] Al-Saif, A.M. (2011). Effects of benzylaminopurine and naphthalene acetic acid on proliferation and shoot growth of pineapple (*Ananas comosus* L. Merr) *in vitro*. *African Journal of Biotechnology*, *10(27),* 5591–5595.

[20] Ab Rahman, N.A., Abdul Latif, N., Udin, E.Z., Awal, A. & Shamsiah, A. (2020). *In vitro* regeneration and acclimatization of pineapple (*Ananas comosus* L. Merr. var. MD2). *Food Research*, *4(5),* 164 – 172.

https://doi.org/10.24191/sl.v18i3.27826