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Pineapple Rooting in Vitro from Sipahutar, North Sumatra, Indonesia, with Addition of Sucrose and IBA

Fauziyah Harahap[1*], Nusyirwan Nusyirwan[1], Arisah Hasanah[2], Syahmi Edi[1], Suci Rahayu[3], Rifa Fadhillah Munifah Hasibuan[4]

[1] Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Negeri Medan, Jl. Willem Iskandar Psr V Medan Estate, 20221 North Sumatra, Indonesia

[2] Postgraduate Program, Department of Biology Education, Universitas Negeri Medan, Jl. Willem Iskandar Psr V Medan Estate, 20221, Indonesia

[3] Department of Biology, Faculty of Mathematics and Natural Sciences, University of North Sumatera, Jln. Bioteknologi No.1, Kampus USU Medan, 20155, Indonesia

[4] International Environmental Agricultural Science, Tokyo University of Agriculture and Technology, Tokyo-Fuchu-Harumicho, 3-Chrome 8-1, Tokyo 183-0001, Japan

Abstract: The advantages of Sipahutar Pineapple are that it tastes sweeter, has a low content of water, softer texture, yellowish color, and is suitable with people preference, but up until now, the production is very limited. In vitro rooting was carried out to determine the effect of sucrose addition and Plant Growth Regulator (PGR) Indole Butyric Acid (IBA) on base media MS + 1 ppm BAP of Sipahutar pineapple (*Ananas comosus* L.) plantlet roots with in vitro. This research was conducted using a completely randomized factorial design. Factor 1: Sucrose in a row, 0, 10, 20, 30 gram L^{-1}. Factor 2: PGR IBA with the dose is 0, 1.5, and 3 ppm. The interaction of sucrose and IBA on MS + 1 ppm BAP basic medium significantly affected the number of roots, the number of leaves, leaf length, leaf width and had no significant effect on other parameters.

Keywords: rooting, indole butyric acid, sucrose, *Ananas comosus* L., Sipahutar pineapple.

来自印度尼西亚北苏门答腊西帕胡塔的菠萝在体外生根，并添加了蔗糖和国际律师协会

摘要：西帕胡塔菠萝的优点是味道更甜，水分含量低，质地更柔软，颜色偏黄，适合人们的喜好，但到目前为止，产量非常有限。 进行体外生根以确定添加蔗糖和植物生长调节剂 (遗传资源) 吲哚丁酸 (国际律师协会) 对西帕胡塔菠萝 (凤梨升.) 小植物根的基础培养基多发性硬化症+ 1 百万分之一 BAP 的影响。 这项研究是使用完全随机的因子设计进行的。 因子 1 ：连续蔗糖，0、10、20、30 克 L-1。 因素 2：遗传资源国际律师协会的剂量为 0、1.5 和 3 百万分之一。蔗糖和国际律师协会在多发性硬化症+1 百万分之一 BAP 基础培养基上的相互作用显着影响根数、叶数、叶长、叶宽，对其他参数无显着影响。

关键词：生根，吲哚丁酸，蔗糖，凤梨，西帕胡塔菠萝。

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About the authors: Fauziyah Harahap, Nusyirwan Nusyirwan, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Negeri Medan, Indonesia; Arisah Hasanah, Postgraduate Program, Department of Biology Education, Universitas Negeri Medan, Indonesia; Syahmi Edi, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Negeri Medan, Indonesia; Suci Rahayu, Department of Biology, Faculty of Mathematics and Natural Sciences, University of North Sumatera, Medan, Indonesia; Rifa Fadhillah Munifah Hasibuan, International Environmental Agricultural Science, Tokyo University of Agriculture and Technology, Tokyo, Japan

Corresponding author Fauziyah Harahap, fauziyahharahap@unimed.ac.id

1. Introduction

Sipahutar Pineapple is a horticultural commodity that has long been cultivated. It has prospects and potential to continue to be developed in Sipahutar District. Pineapple has the widest harvest of 1,819 ha, with a production of 32,532.82 tons [1]. Pineapple is a vegetatively propagated plant; now, this commodity is very important in tropical countries [2]. Pineapple contains sugar, acids and is rich in vitamins [3]. Pineapple from Sipahutar is a source of income for the people of North Tapanuli [4].

Generally, farmers plant pineapple from Sipahutar by using seedlings derived from tillers stem and crown [5]. By utilizing seedlings derived from crowns and tillers stem, which are relatively limited in number to plant huge land. The obstacle faced by pineapple farmers in Sipahutar is the difficulty in getting the pineapple seeds [6]. One of the alternatives that can be used to solve the needs of many seedlings is using in vitro culture techniques. In vitro culture has several advantages, including preparing large-scale seedlings in rare, endemic, and endangered plants [7]. The seedlings produced are more tolerant of pests and disease (aseptic propagation) and have a good uniformity of plant material.

The success of in vitro culture is strongly affected by growth regulators (PGR) [8], [9], [10]. MS media is often used in plant tissue culture applications both in embryogenic and organogenesis [11]. According to [12], one of the media components that determines the success of tissue culture is the type and concentration of PGR used. The type and concentration of PGR depend on the purpose and stage of the culture [13]. Auxins are phytohormones that are known to play an important role in plant growth and root development. However, the effect of auxin can vary in various plant species [14]. Indole Butyric Acid (IBA) is a growth regulator which is more often used than other types of auxin because IBA is a synthetic auxin, which is superior and effective in stimulating rooting activities; also, IBA has more stable chemical content and is higher to stimulate root formation [15].

In addition to growth regulators, the carbon source is also an important factor in determining the success of tissue culture. The carbon source serves as a source of energy that is needed by cells to be able to grow. This research will show the further influence of sucrose as an energy source. As a result of sucrose hydrolysis, glucose and fructose can stimulate the growth of some plants [16]. Based on research that has been recorded, the sucrose and IBA test for plant growth on a pineapple is still very limited, while the combination of sucrose + IBA has never been done on Sipahutar pineapple. Therefore, this research is important and has a high degree of novelty for farmers and science in the future.

2. Materials and Methods

This research was carried out in the YAHDI tissue culture laboratory, Perum Pelabuhan Jl. Lambung No. 18 Tanah 600 Medan Marelan Indonesia in March – September 2018. The tools used are Laminar Air Flow Cabinet (LAFC), autoclaves, analytical balance, refrigerators, and standard tissue culture tools. The material used was Pineapple Explants from Sipahutar, which was developed at the Yahdi Tissue Culture Laboratory in Medan.

This research used a factorial Completely Randomized Factorial Design experimental method, with 12 treatments. With the basic media Murashige and Skoog (MS) media + Plant Growth Regulator (PGR) of 1 ppm Benzyl Amino Purine (BAP), the factors in this study are: Factor I: Sucrose, consists of 4 levels of treatment: S_0 = 0 ppm (control), S_{10} = 10 gram L^{-1}, S_{20} = 20 gram L^{-1}, S_{30} = 30 gram L^{-1}. Factor II: IBA (PGR) consisted of 3 levels of treatment: I_0 = 0 ppm (control), $I_{1.5}$ = 1.5 ppm, I_3 = 3 ppm. Each treatment was repeated three times, resulting in 36 bottles in this research.

All tools used in this study were sterilized in two stages. The first stage was washed thoroughly and followed up by using an autoclave at 121°C with a pressure of 17.5 psi for 1 hour. All ingredients are used according to a predetermined dose. MS + Sucrose media (0; 10; 20; 30) grams L^{-1} combined with IBA (0; 1.5; 3 ppm) were dissolved in 1 liter of sterile aquades, then measured media pH (5.8) and added to (7 gram L^{-1}).

Explant planting used is in vitro Sipahutar Pineapple shoots. Planting is carried out in a LAFC with a sterile planting tool. The in vitro shoots were planted into the rooting medium according to treatment. After all the explants were planted, Sipahutar pineapple explants were transferred to the culture room on the plant rack.

Maintenance was done by placing an explant-filled bottle on a research rack at a temperature range of 22ºC. For light treatment, the application was made with a 3000-3200 lux fluorescent lamp in a 16-hour photoperiod.

After planting, the next step was observing plant growth, starting from one week old until 12 Weeks After Planting (WAP). The parameters observed in this research were:

(1) Time of root emergence is characterized by swelling of the bulb buds. White bumps appear, followed by the release of fine roots. Observations are carried out every week to see when the roots emerged;

(2) The number of roots. Counting of the number of roots starts from week 1 to 12 weeks of observation, by counting the number of roots each week;

(3) Time of emergence of leaves. The time of the emergence of the leaves is calculated since the new leaves are formed. Observations are carried out every week to see when exactly the leaves appear;

(4) The number of leaves. Total leaf counted every week, from the first week to 12 weeks after treatment;

(5) The number of tillers is calculated every week by counting the total tillers every week, from the first week to 12 weeks after treatment;

(6) Time of emergence of tillers. Observations were every week to see how many tillers appear in a specific week;

Other observation parameters:

(7) Root length. This parameter was measured from the root base to the tip of the root;

(8) Leaf width. This parameter was measured by measuring the width of the leaf in the center of the leaf;

(9) Leaf length. This parameter was measured from the leaf base to the tip of the leaf;

(10) Height of tillers. This parameter was observed by measuring the height of new shoots formed;

(11) Plant height. This parameter was measured from the base of the stem to the top (leaf tip);

(12) Overall plant height. This parameter measured from leaf tips to root tips.

Parameters 7–12 were observed on 12 Weeks after Planting (12 WAP). After all the data was obtained, the data is processed by analysis techniques using a factorial completely randomized design model as follows:

$Yijk = \mu + \alpha i + \beta j + (\beta j)\ ij + \varepsilon ijk$

where: Yijk - the results of observations on the replication of the k-test which received the treatment of myo-inositol on the i-level and IBA treatment on the j-level; μ - middle value; αi - concentration effect of myo-inositol on the i-level; βj - concentration effect of IBA on the j level; (βj) ij - interaction effect of myo-inositol on the i-level with IBA on the j-level; εijk - error effect of myo-inositol on the i-level with IBA on the j-level at the k-test.

3. Results

3.1. Number of Roots

It appears that as a single factor, sucrose or PGR IBA do not affect increasing the number of roots (Table 1), but the interaction of both has a significant and very significant effect on increasing the number of roots, especially starting from Week 3 until the end of observation (Week 12). The highest number of roots (2 ± 0.49) strands resulted from the treatment of medium base MS + 1 ppm BAP with the addition of 20 grams of sucrose L^{-1} and without the addition of IBA (S_{20} I_0), followed by 20 grams of sucrose L^{-1} treatment and addition of 3 ppm IBA ($S_{20}I_3$) produced 1.96 ± 0.67 roots. The lowest root number was 0.71 ± 0.00, resulting from the treatment without sucrose and 1.5 ppm IBA ($S_0I_{1.5}$).

3.2. Number of Leaves

Sucrose, PGR IBA, and their interactions had not affected the initial stage of the plant. In subsequent observations, sucrose has a significant and very significant effect, especially starting at Week 5 until the end of the observation (Table 1). PGR IBA from the beginning to the end of the observation did not have a significant effect. The interaction between the two (sucrose and PGR IBA) formed a significant effect from week 8 to the 12^{th} week of observation. The highest number of leaves was produced from MS + 1 ppm BAP base medium with the addition of 20 grams of sucrose L^{-1} and 3 ppm IBA (S_{20} I_3) with an average number of leaves 31.33 ± 15.89. Without the addition of sucrose but given 3 ppm IBA (S_0I_3) produced the lowest number of leaves (2.67 ± 0.58) (Table 1).

3.3. Number of Tillers Buds

Sucrose generally has a significant and very significant effect, especially starting in Week 5 until the end of the observation. In contrast, PGR IBA and the interaction of both have no significant effect on the number of tiller buds (Table 1). During Weeks 3-12, the highest number of shoots resulted from 20 grams L^{-1} sucrose treatment and 3 ppm PGR IBA ($S_{20}I_3$), with an average number of shoots of 2.67. Treatment of 0 gram L^{-1} sucrose and 3 ppm IBA (S_0I_3), 10-gram L^{-1} sucrose and 3 ppm IBA ($S_{10}IB_3$), and 30-gram L^{-1} sucrose and 1.5 ppm IBA ($S_{30}I_{1.5}$) did not produce shoots shown in Fig. 1.

3.4. Leaf Length

Table 2 shows that the rooting induction treatment with MS + 1 ppm BAP base medium with 12 combinations of treatments above gives different responses for the observed parameters, namely number of roots, number of leaves, leaf length, and leaf width. The highest leaf length was produced from the treatment without sucrose and 3 ppm IBA (S_0I_3) with an average leaf length of 2.35 ± 0.04, then decreased with a leaf length of 2.28 ± 0.10, resulting from 20 grams L^{-1} sucrose treatment of and PGR 3 ppm IBA (S_{20} I_3). The shortest leaves averaged 1.15 ± 0.13, resulting from the treatment of 20 grams sucrose L^{-1} and 1.5 ppm IBA (S_{20} $I_{1.5}$). Table 2 shows that sucrose has no effect, PGR IBA has a significant effect, and the interaction between sucrose and PGR IBA has a very significant effect on leaf length.

3.5. Leaf Width

In general, leaf width is near evenly distributed. The highest leaf width resulted from 10 grams sucrose L^{-1} treatment and 1.5 ppm IBA (S_{10} $I_{1.5}$) with leaf width of 1.12 ± 0.11 (Table 2), while the lowest leaf width resulted from administering the same dose of 10 grams sucrose L^{-1} and 3 ppm IBA ($S_{10}I_3$) with leaf width 0.97 ± 0.03 (Fig. 2). The widest leaf is not followed by the longest leaf length and vice versa, while the narrowest leaf does not have the shortest leaf length. Data from

Fig. 2 shows that sucrose, PGR IBA have no effect, but the interaction between the two has a very significant effect on leaf width.

3.6. Root Length

Table 3 shows that sucrose, PGR IBA, and their interactions do not affect the root length, buds height, and plant height. The highest root length was obtained from the treatment of base media MS +1 ppm BAP with the addition of 20-gram L^{-1} sucrose and 0 ppm PGR IBA ($S_{20}I_0$), with an average root length of 2 cm, with the same amount of sucrose concentration and 3 ppm PGR IBA producing root length an average of 1.96 cm. The lowest root length resulted from treatment of 0-gram sucrose L^{-1} and 1.5 ppm IBA (Fig. 3).

Table 1 Recapitulation of variance of various variables observation of pineapple rooting from Sipahutar

Week	Observation 1 - 12 WAP								
	Number of root			**Number of leaves**			**Number of tillers buds**		
	S	**I**	**S&I**	**S**	**I**	**S&I**	**S**	**I**	**S&I**
1	nd	nd	nd	0.21^{ns}	2.91^{ns}	0.60^{ns}	1^{ns}	1^{ns}	1^{ns}
2	1.11^{ns}	1.54^{ns}	2.46^{ns}	1.84^{ns}	2.77^{ns}	0.35^{ns}	2.2^{ns}	0.55^{ns}	0.55^{ns}
3	1.52^{ns}	0.19^{ns}	2.6^{*}	2.04^{ns}	0.08^{ns}	2.78^{*}	3.94^{*}	2.05^{ns}	1.21^{ns}
4	1.88^{ns}	0.34^{ns}	5.14^{**}	2.75^{ns}	0.13^{ns}	0.99^{ns}	4.92^{**}	0.46^{ns}	0.91^{ns}
5	1.5^{ns}	0.35^{ns}	5.59^{**}	4.33^{*}	0.08^{ns}	1.84^{ns}	6.1^{**}	0.76^{ns}	1.7^{ns}
6	0.6^{ns}	1.08^{ns}	4.9^{**}	4.53^{*}	0.19^{ns}	2.16^{ns}	6.58^{**}	0.31^{ns}	1.33^{ns}
7	0.07^{ns}	1.85^{ns}	3.64^{*}	4.08^{*}	0.10^{ns}	2.27^{ns}	6^{**}	0.18^{ns}	1.43^{ns}
8	1.6^{ns}	1.21^{ns}	2.13^{ns}	5.62^{**}	0.36^{ns}	3.15^{*}	5.45^{**}	0.26^{ns}	1.58^{ns}
9	1.96^{ns}	0.89^{ns}	3.22^{*}	4.94^{**}	0.27^{ns}	3.37^{*}	5.47^{**}	0.46^{ns}	1.75^{ns}
10	2.24^{ns}	1.31^{ns}	2.91^{*}	5.70^{**}	0.79^{ns}	5.14^{*}	4.9^{**}	0.27^{ns}	1.46^{ns}
11	2.24^{ns}	1.31^{ns}	2.91^{*}	5.91^{**}	0.37^{ns}	3.62^{*}	5.47^{**}	0.36^{ns}	1.59^{ns}
12	2.24^{ns}	1.31^{ns}	2.91^{*}	6.13^{**}	0.29^{ns}	3.11^{*}	5.58^{**}	0.4^{ns}	1.55^{ns}

Notes: ** - highly significant; * - significant; nd - no data; ns - not significant; S - sucrose; I - IBA; S&I - sucrose and IBA interaction

Table 2 Average number of roots, number of leaves, leaf length, and leaf width on the observation of root formation of Sipahutar pineapple in 12 WAP

Treatment	Number of root	Number of leave	Leaf length	Leaf width
$S_0\ I_0$	1.34 ± 0.21^{abc}	9.00 ± 6.24^{ab}	1.80 ± 0.25^{bcde}	0.98 ± 0.08^{ab}
$S_0\ I_{1,5}$	0.71 ± 0.00^{a}	10.00 ± 6.08^{ab}	1.45 ± 0.26^{ab}	1.00 ± 0.00^{abcd}
$S_0\ I_3$	1.76 ± 0.31^{bc}	2.67 ± 0.58^{a}	2.35 ± 0.04^{e}	1.11 ± 0.05^{cd}
$S_{10}\ I_0$	1.65 ± 0.38^{bc}	12.33 ± 3.06^{a}	2.10 ± 0.32^{cde}	1.08 ± 0.09^{bcd}
$S_{10}\ I_{1,5}$	1.81 ± 0.56^{bc}	13.33 ± 6.43^{ab}	1.97 ± 0.33^{bcde}	1.12 ± 0.11^{d}
$S_{10}\ I_3$	1.46 ± 0.21^{bc}	3.00 ± 1.73^{a}	1.51 ± 0.44^{ab}	0.97 ± 0.03^{a}
$S_{20}\ I_0$	2.00 ± 0.49^{c}	12.00 ± 12.12^{a}	1.78 ± 0.49^{bcde}	0.98 ± 0.08^{abc}
$S_{20}\ I_{1,5}$	1.25 ± 0.94^{ab}	16.00 ± 12.12^{b}	1.15 ± 0.13^{a}	0.91 ± 0.08^{a}
$S_{20}\ I_3$	1.96 ± 0.67^{bc}	31.33 ± 15.89^{c}	2.28 ± 0.10^{de}	1.09 ± 0.12^{bcd}
$S_{30}\ I_0$	1.56 ± 0.33^{bc}	6.33 ± 5.13^{ab}	1.80 ± 0.58^{bcde}	1.00 ± 0.08^{abcd}
$S_{30}\ I_{1,5}$	1.83 ± 0.44^{bc}	6.33 ± 1.15^{ab}	1.71 ± 0.39^{bcd}	1.05 ± 0.08^{bcd}
$S_{30}\ I_3$	1.34 ± 0.21^{abc}	11.33 ± 6.03^{ab}	1.64 ± 0.40^{abc}	1.06 ± 0.10^{bcd}

Note: Numbers followed by the same lowercase letter in the same column or row show no significant difference at the Duncan Multiple Range Test (DMRT) 5%

Table 3 Recapitulation of variance of root length, leaf length, leaf width, buds' height, overall plant height, plant height

Week	Observation 12 WAP								
	Root Length			**Leaf Length**			**Leaf Width**		
	S	**I**	**S&I**	**S**	**I**	**S&I**	**S**	**I**	**S&I**
12	1.30^{ns}	0.73^{ns}	1.09^{ns}	0.61^{ns}	4.95^{*}	4.95^{**}	1.32^{ns}	1.74^{ns}	3.98^{**}
	Buds height			Plant height					
12	2.79^{ns}	0.13^{ns}	2.24^{ns}	2.11^{ns}	0.81^{ns}	1.66^{ns}			

Notes: ** - highly significant; * - significant; nd - no data; ns - not significant; S - sucrose; I - IBA; S&I - sucrose and IBA interaction

3.7. Tiller Buds Height

Data from observations of the average height of shoots of pineapple shoots from Sipahutar showed the results of 20 grams sucrose L^{-1} treatment and 3 ppm PGR IBA ($S_{20}I_3$) higher than all treatments with an average of 1.72 ± 0.56 and the lowest in the treatment of 30 grams sucrose L^{-1} and 0 ppm PGR IBA ($S_{30}I_0$) with an average of 0.81 ± 0.17, while in the 0 grams sucrose L^{-1} treatment and 3 ppm IBA (S_0I_3), 10 grams sucrose L^{-1} and 3 ppm IBA ($S_{10}IB_3$), and 30-gram L^{-1} sucrose and 1.5 ppm IBA ($S_{30}IB_{1.5}$) did not produce new shoots (Fig. 4).

3.8. Plant Height

Data from observations of the highest average plant height is from 10 grams L^{-1} sucrose and 0 ppm IBA treatments, with an average plant height of 5.67 cm, not significantly different from 10 grams sucrose L^{-1} treatment with 1.5 ppm IBA or 0-gram sucrose L^{-1} and 3 ppm IBA and 20-gram L^{-1} sucrose and 3 ppm IBA (Fig. 5).

3.9. Time of Root Emergence

The emergence of roots produced from a base medium of MS +1 ppm BAP with the addition of 20-gram sucrose L^{-1} with 3 ppm IBA (S_{20} I_3) began in the second week. The rest of the roots generally begin at week 4 (Fig. 6). In this study, media without the addition of sucrose combined with 1.5 ppm IBA ($S_0I_{1.5}$) did not show the appearance of new roots (Table 4).

3.10. Time of Leaves' Increase

The number of leaves has begun to increase since the first and second week after planting. Exception for media without the addition of sucrose and PGR IBA (S_0I_0), and media with the addition of 10 grams sucrose L^{-1} and 3 ppm PGR IBA ($S_{10}I_3$), new leaves begin to appear in the third week.

3.11. Time of Tillers' Bud Emergence

The emergence time of tillers bud varies greatly from the first to the eighth. With moderate treatment, the addition of 20 grams sucrose L^{-1} and 1.5 ppm PGR IBA (S_{20} $I_{1.5}$) produced the fastest tillers bud that is one week after planting (Table 4). Certain treatments did not produce new tillers bud (Fig. 7).

Table 4 Time of root emergence, time of number of leaves increases, time of tillers bud emergence

Treatment	Time of root emergence	Time of number of leaves increases	Time of tillers bud emergence
S_0 I_0	Week 5	Week 3	Week 6
S_0 $I_{1,5}$	No root appeared	Week 1	Week 5
S_0 I_3	Week 4	Week 2	No tillers appeared
S_{10} I_0	Week 5	Week 1	Week 8
S_{10} $I_{1,5}$	Week 4	Week 1	Week 4
S_{10} I_3	Week 4	Week 3	No tillers appeared
S_{20} I_0	Week 4	Week 1	Week 4
S_{20} $I_{1,5}$	Week 8	Week 1	Week 1
S_{20} I_3	Week 2	Week 1	Week 2
S_{30} I_0	Week 4	Week 1	Week 7
S_{30} $I_{1,5}$	Week 4	Week 1	No tillers appeared
S_{30} I_3	Week 3	Week 2	Week 3

Abbreviations

S_0I_0	= Sucrose 0gram L^{-1} + IBA 0 gram/l	S_{20} I_0	= Sucrose 20 gram L^{-1} + IBA0 gram L^{-1}
$S_0I_{1,5}$	= Sucrose 0gram L^{-1} + IBA1.5 gram/l	S_{20} $I_{1,5}$	= Sucrose 20 gram L^{-1} + IBA1.5 gram L^{-1}
S_0I_3	= Sucrose 0gram L^{-1} + IBA3 gram/l	S_{20} I_3	= Sucrose 20 gram L^{-1} + IBA3 gram L^{-1}
S_{10} I_0	= Sucrose 10 gram L^{-1} + IBA0 gram L^{-1}	$S_{30}I_0$	= Sucrose 30 gram L^{-1} + IBA0 gram L^{-1}
S_{10} $I_{1,5}$	= Sucrose 10 gram L^{-1} + IBA1.5 gram L^{-1}	S_{30} $I_{1,5}$	= Sucrose 30 gram L^{-1} + IBA1.5 gram L^{-1}
S_{10} I_3	= Sucrose 10 gram L^{-1} + IBA3 gram L^{-1}	S_{30} I_3	= Sucrose 30 gram L^{-1} + IBA3 gram L^{-1}

4. Discussion

This study showed information that sucrose was needed for root formation. This can be clarified by the research results showing that the highest number of roots is produced from sucrose-treated treatments, without ignoring the role of PGR IBA. It was seen in all treatments with PGR IBA. Also, the acceleration of root emergence is faster than without PGR IBA. The data above showed that media without the addition of sucrose combined with 1.5 ppm IBA did not produce roots, meaning that although IBA is present but without an energy source, namely sucrose, the roots will not appear. Research from [17] found that sugar has provided the tissue culture plant with carbon in an organic form that is not required for those grown from seeds. This process indicates that the PGR IBA serves to accelerate the emergence of roots.

Early emergence of roots is a success factor in pineapple rooting from Sipahutar with in vitro technique. The interaction between sucrose and IBA gives a better effect than a single factor. Data from Table 4 indicate that the addition of IBA to the base media of MS without adding the sucrose is not enough to induce the emergence of roots. It is shown from the treatment of sucrose 0 gram L^{-1} with 1.5 ppm IBA ($S_0I_{1.5}$). Hence, to induce the emergence of new roots, it takes a source of energy to increase metabolic processes, in this study is sucrose.

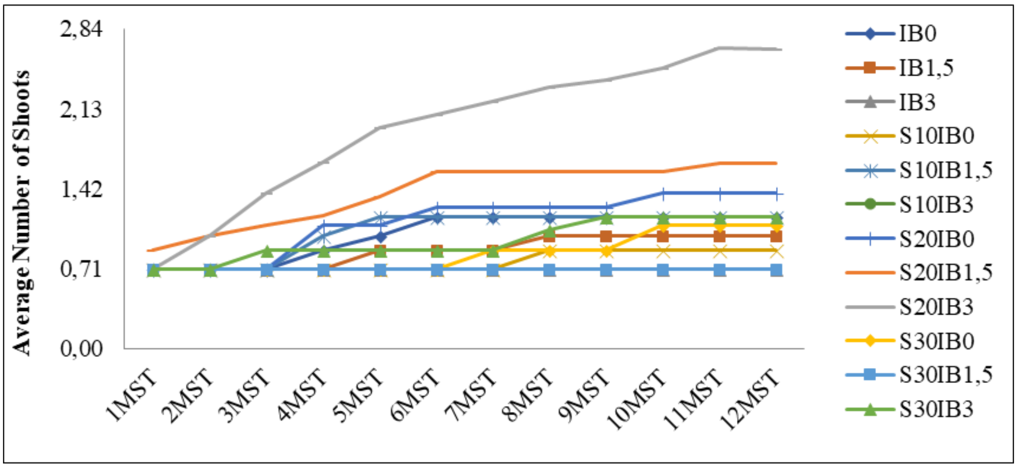

Fig. 1 Number of shoots from Sipahutar pineapple for 12 weeks

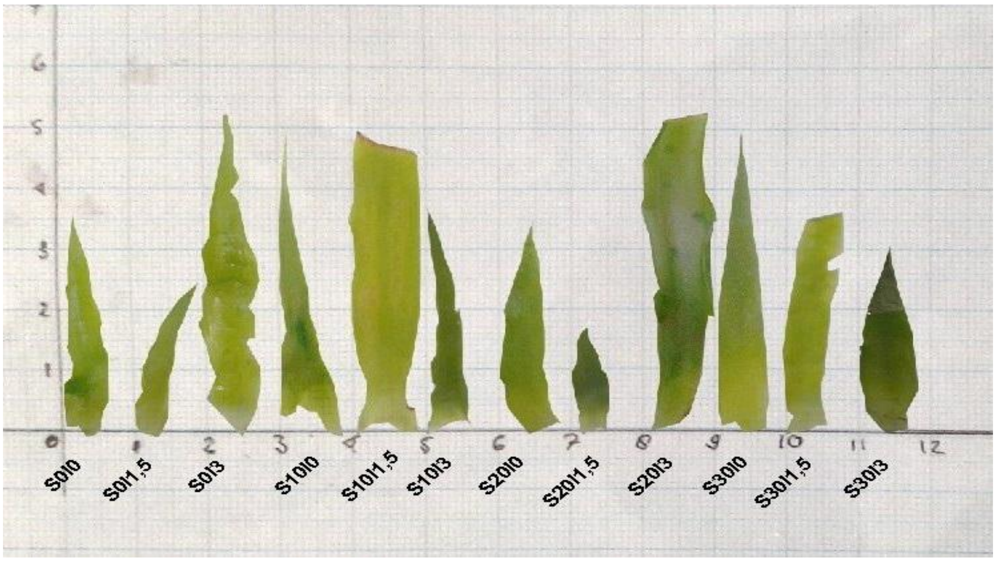

Fig. 2 Documentation of leaf length and leaf width on various concentrations of sucrose and IBA 12 WAP, with treatment S_0I_0, $S_0I_{1.5}$, S_0I_3, $S_{10}I_0$, $S_{10}I_{1.5}$, $S_{10}I_3$, $S_{20}I_0$, $S_{20}I_{1.5}$, $S_{20}I_3$, $S_{30}I_0$, $S_{30}I_{1.5}$, $S_{30}I_3$

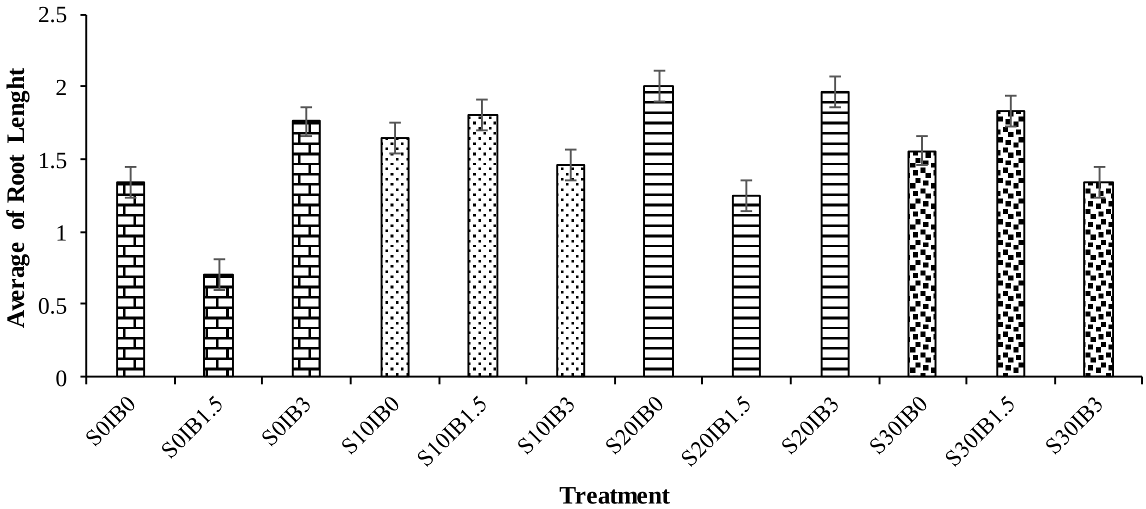

Fig. 3 Average length of pineapple roots from Sipahutar age 12 WAP

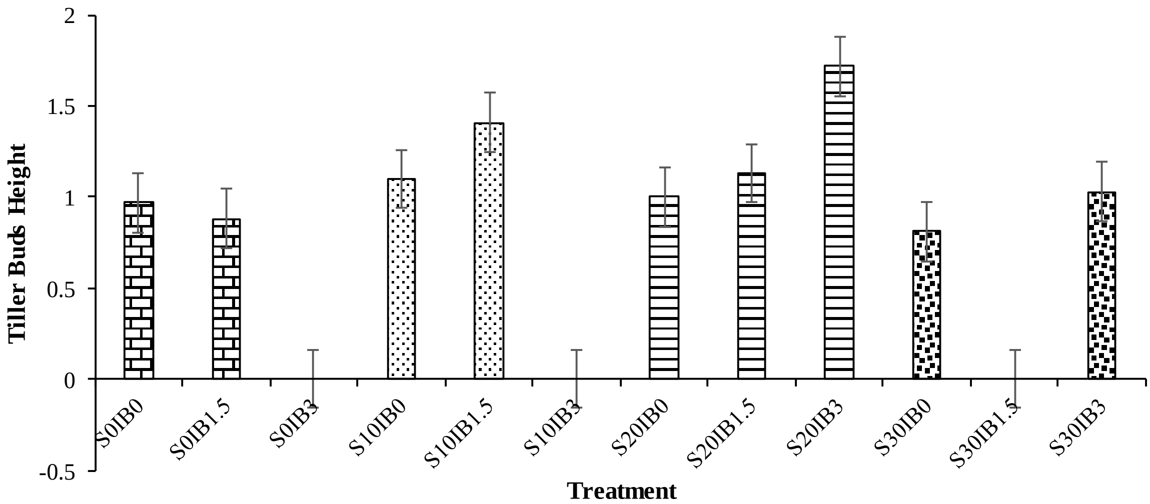

Fig. 4 The average tiller buds height of Sipahutar pineapple

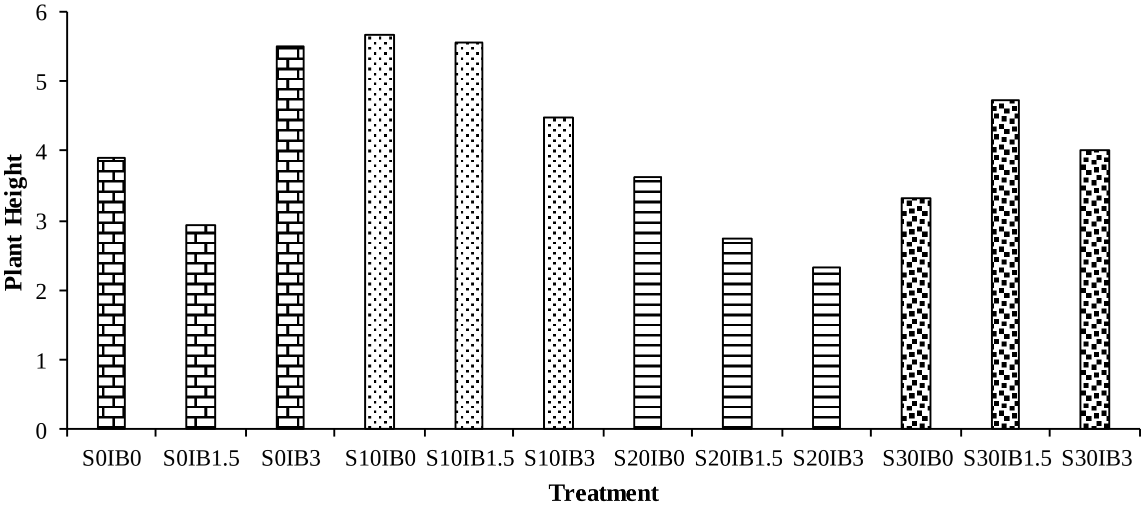

Fig. 5 Plant height of pineapple from Sipahutar age 12 WAP

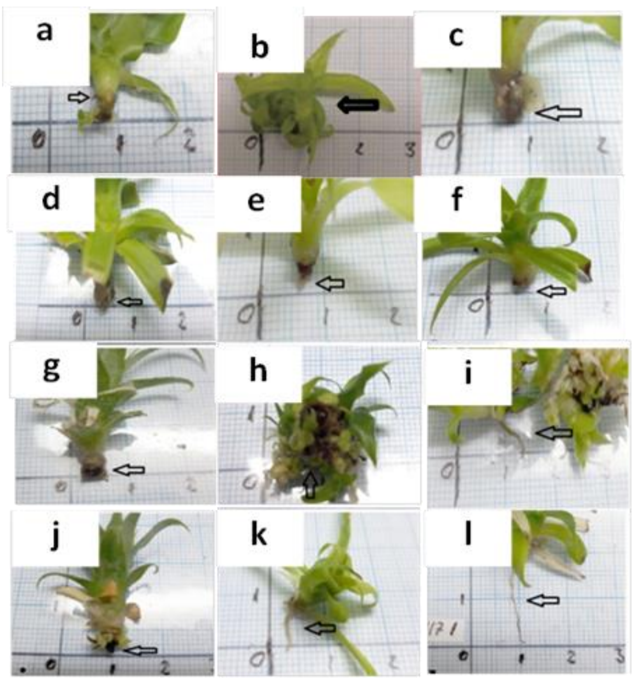

Fig. 6 Documentation of root lengths of various concentrations of sucrose and IBA 12 WAP, on the treatment

a. A S_0I_0 = Sucrose 0 gram L^{-1} + IBA 0 gram L^{-1}
b. B $S_0I_{1,5}$ = Sucrose 0 gram L^{-1} + IBA1.5 gram L^{-1}
c. C S_0I_3 = Sucrose 0 gram L^{-1} + IBA3 gram L^{-1}
d. D S_{10} I_0 = Sucrose 10 gram L^{-1} + IBA0 gram L^{-1}
e. E S_{10} $I_{1,5}$ = Sucrose 10 gram L^{-1} + IBA1.5 gram L^{-1}
f. F S_{10} I_3 = Sucrose 10 gram L^{-1} + IBA3 gram L^{-1}
g. G S_{20} I_0 = Sucrose 20 gram L^{-1} + IBA0 gram L^{-1}
h. H S_{20} $I_{1,5}$ = Sucrose 20 gram L^{-1} + IBA1.5 gram L^{-1}
i. I S_{20} I_3 = Sucrose 20 gram L^{-1} + IBA3 gram L^{-1}
j. J $S_{30}I_0$ = Sucrose 30 gram L^{-1} + IBA0 gram L^{-1}
k. K S_{30} $I_{1,5}$ = Sucrose 30 gram L^{-1} + IBA1.5 gram L^{-1}
l. L S_{30} I_3 = Sucrose 30 gram L^{-1} + IBA3 gram L^{-1}

According to [18], IBA is a type of auxin that is most often used in inducing roots compared to other types of auxin because of its high ability to initiate roots. Likewise, according to [19], the concentration of 20 grams of sucrose L^{-1} is the best in influencing pineapple plant growth. The addition of 20 grams of sucrose L^{-1} and 1.5 ppm IBA (S_{20} $I_{1.5}$) resulted in the acceleration of the emergence of new tiller buds.

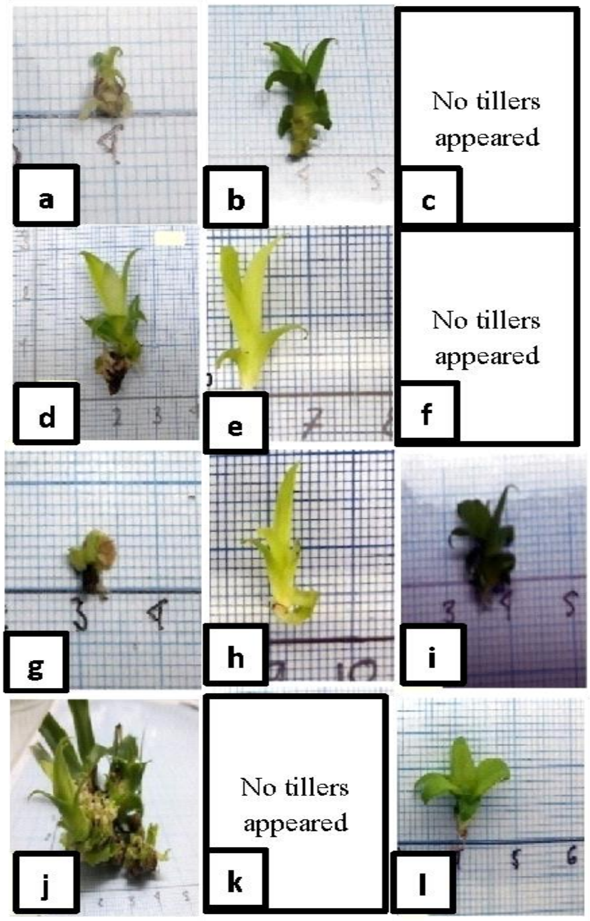

Fig. 7 Documentation of tillers' bud of various concentrations of sucrose and IBA 12 WAP, on the treatment

a.	A	S_0I_0	= Sucrose 0 gram L^{-1} + IBA 0 gram L^{-1}
b.	B	$S_0I_{1,5}$	= Sucrose 0 gram L^{-1} + IBA1.5 gram L^{-1}
c.	C	S_0I_3	= Sucrose 0 gram L^{-1} + IBA3 gram L^{-1}
d.	D	S_{10} I_0	= Sucrose 10 gram L^{-1} + IBA0 gram L^{-1}
e.	E	S_{10} $I_{1,5}$	= Sucrose 10 gram L^{-1} + IBA1.5 gram L^{-1}
f.	F	S_{10} I_3	= Sucrose 10 gram L^{-1} + IBA3 gram L^{-1}
g.	G	S_{20} I_0	= Sucrose 20 gram L^{-1} + IBA0 gram L^{-1}
h.	H	S_{20} $I_{1,5}$	= Sucrose 20 gram L^{-1} + IBA1.5 gram L^{-1}
i.	I	S_{20} I_3	= Sucrose 20 gram L^{-1} + IBA3 gram L^{-1}
j.	J	$S_{30}I_0$	= Sucrose 30 gram L^{-1} + IBA0 gram L^{-1}
k.	K	S_{30} $I_{1,5}$	= Sucrose 30 gram L^{-1} + IBA1.5 gram L^{-1}
l.	L	S_{30} I_3	= Sucrose 30 gram L^{-1} + IBA3 gram L^{-1}

Sucrose, which at first prioritized to stimulate root growth, is less effective in giving effect to the rooting of pineapple from Sipahutar. It is suspected that the level of treatment factor and concentration are not suitable, making the Sipahutar pineapple plantlet less optimal in rooting. The use of optimal concentration is a factor that can determine the emergence of root explants planted by [18]. Sucrose has a significant effect on the number of leaves and the number of tillers bud.

This study showed that the interaction between sucrose and IBA has a significant effect on the parameters of root number. In $S_{20}I_3$ treatment with an average of 1.96, the concentration of 20 grams of sucrose L^{-1} with the addition of 3 ppm IBA significantly affected the number of pineapple roots from Sipahutar. The study of [20] also stated that the interaction of sucrose with IBA in medium concentrations was more effective in increasing the number of roots. In contrast, high concentrations resulted in a decreasing number of roots. Likewise, according to [21], the interaction of sucrose and IBA can also increase root growth, but a single factor of sucrose is more to stimulate the formation of the buds. According to [22], sucrose will provide energy for the growth of pineapple explants from Sipahutar and, as a building substance, produce larger molecules needed for growth. Therefore, giving IBA as rooting stimulation interacting with sucrose can affect the growth of the number of pineapple explant roots from Sipahutar.

According to [23], sucrose, as a carbohydrate synthesized and transported naturally in plants, helps the number of roots grow. According to [24], IBA has better properties and effects of accelerating root growth, increasing the number of roots, and improving root quality. The effect of auxin on plant roots depends on concentration, method of application, time of addition, part of the plant, and age of plant tissue.

Sucrose is also needed to increase the number of leaves; this can be seen from the treatment without sucrose, which is given with a high dose of 3 ppm PGR IBA, which will produce a low number of leaves. This pictures sucrose contributes greatly to the supply of energy for the process of metabolism in tissue culture. [25] stated that carbohydrate is supplemented as a carbon source to maintain the carbon supply and osmotic potential of cells. Sucrose is widely used in plant tissue culture due to its most favorable effect on growth. This is a basic description that a very basic physiological process happened, that the treatment which produced the fewest number of leaves will have the longest leaves (Table 2).

The delayed emergence of new leaves causes of there is no addition of sucrose and IBA. However, high concentrations (3 ppm) of IBA also cause new leaves to be delayed. It can be obtained that to maximize growth (producing new leaves), it is needed an additional source of energy and growth regulators in certain doses. In tissue culture, certain optimum concentrations are needed for growth. This study illustrates that there is an optimum dose to increase the acceleration of Sipahutar pineapple rooting. This is indicated by adding 20 grams of sucrose L^{-1} and 1.5 ppm IBA (S_{20} $I_{1.5}$), resulting in the acceleration of the emergence of new tiller buds.

The average time to increase the number of leaves in all treatments is 1 WAP. This showed that both single and combined factors could increase the number of leaves quickly due to the balance between the composition of growth regulators in the media. According to [26], the balance of growth regulators in the media with the addition of the right dose of sucrose can provide optimal nutrients in plants to accelerate the growth of the number of pineapple leaves from Sipahutar. Likewise, according to [27], in their role as determining the direction of tissue development, the addition of auxins and cytokines must consider its concentration and comparison in the media.

The 20-gram sucrose L^{-1} treatment gave a good stimulation for the growth of the number of pineapple leaves from Sipahutar. During plant tissue culture, sucrose acts as a fuel source to maintain photomixotrophic metabolism and a carbon precursor or metabolite signal to ensure optimal development [28]. The leaves are one of the important organs in plants, the higher the leaves, the better the growth of a plant. According to [29], leave is one of the most important plant organs, especially for photosynthesis, to produce photosynthate experience optimum growth. According to [30], the number of leaves is one of the indicators of plant growth and can be used as supporting data to explain the growth process; sucrose as a carbohydrate substance for plant growth and development affects the increase of pineapple leaves from Sipahutar.

$S_{20}I_3$ treatment with an average of 31.33 showed that the addition of 20-gram sucrose L^{-1} and 3 ppm IBA gave a good stimulation for the growth of the number of pineapple leaves from Sipahutar. Sucrose is the most common source of carbohydrates and carbon used in plant tissue culture media. This is because carbohydrates supply energy to plants, especially when it is not ready to photosynthesize during the early stages of tissue culture [31]. Increased addition of sucrose and auxin is proven can increase the number of leaves. [32] stated in her research that MS media added with sucrose can optimally stimulate leaf length. According to [30], the number of leaves is one of the indicators of plant growth that can be used as supporting data to explain the growth process. The addition of sucrose as a carbohydrate substance for plants for growth and development affects the increase of pineapple leaves from Sipahutar.

Observation showed that the earliest for the emergence of tillers bud was in the sucrose treatment of 20 gram L^{-1} by giving 1.5 ppm IBA ($S_{20}I_{1.5}$). This shows sucrose as a carbon source, and the energy available in the media can be absorbed by the Sipahutar pineapple plantlet. According to [33], the growth and propagation of shoots in vitro are influenced by many factors, one of which is the type of carbon source added to the media. Carbon sources function as energy and osmotic agents to support plant tissue growth. Likewise, according to [34], the addition of sucrose in the media will be a source of energy and a source of carbon for explant cells to grow. Increasing the concentration of sucrose will cause the explant to obtain more energy and carbon sources to accelerate the growth of explants. The higher energy sources resulted in faster cell division so that the growth of tillers bud will be faster.

Explants treated with sucrose have a large number of tillers which first appear like white lumps, then these lumps form young tillers with large numbers. According to [44], sucrose with high concentrations and added with high BAP will stimulate cell division multiple times and produce higher tillers. [35] stated that high BAP concentrations would inhibit the work of auxin so that it will accelerate the growth of tillers in explants.

According to [19], the concentration of sucrose 20 grams L^{-1} can affect the growth of the number of roots, but in this study, sucrose has a significant effect on the growth of the number of leaves and the number of Sipahutar pineapple tillers. This might be caused by adding 1 ppm BAP to base media whose main function is to promote tillers' growth. According to [36], adding sucrose will produce lots of tillers because sucrose and BAP can stimulate cell division in plants, sucrose as a source of carbohydrates to meet the needs of plants in cell division and plant tillers growth. Formed tillers are indicated as a success of the regeneration of explants that were inoculated in tissue culture media [37]. The faster the tillers appear, the faster the plant propagation material is produced. Exogenous applications of cytokinins and auxins have been known to be important for the induction of buds and elongation in many plant species in vitro [38].

The results of the research analysis showed that IBA has a significant effect on leaf length and has no significant effect on the following parameters: the number of leaves, leaf width, length of tillers bud, number of tillers, root length, and number of roots. IBA has a significant effect on the leaf length of Pineapple from Sipahutar at IBA 3 ppm. The addition of auxin, which is often used for root growth, can also affect the growth of leaf length showed in this study; therefore, the basal medium used in this research is MS medium + 1 ppm BAP. [39] stated that the number and length of roots influence the height and number of leaves in plants which with a positive correlation between the number and lengths of roots with the nutrients absorbed are relatively larger, so plants have the opportunity to form more leaves and vice versa, if a little amount of the roots are produced and short, the nutrients absorbed are relatively small and the leaves are formed smaller. Indol-3-Butyric-Acid (IBA) is better than other auxins for rooting stimulation [40]. The main function of IBA as a root growth stimulation hormone does not provide a significant effect on the growth of pineapple roots from Sipahutar. It is suspected because BAP given to the base media, which was already high enough in concentration, made IBA stop stimulating the growth of pineapple roots from Sipahutar. According to [39], IBA has activity as a rooting hormone, so that IBA activity can affect the number of roots. If the addition of IBA is too high, it can inhibit root growth. It is suspected that the use of a high concentration of IBA following the addition of 1 ppm of BAP in base media is too high to stimulate the growth of pineapple roots. When combined with high concentrations, auxin and cytokinin can inhibit the growth of pineapple roots from Sipahutar. However, in the study of [41], the interaction of IBA and BAP can stimulate long and dense root growth compared to the single factor.

Interaction of sucrose with IBA significantly affected the length of pineapple leaves from Sipahutar, with an average of 2.35 in the $S_{20}I_3$ treatment. The addition of 20 grams of sucrose L^{-1} with a concentration of 3 ppm IBA and 1 ppm BAP on MS base media can affect the length of pineapple plantlets from Sipahutar. The study of [40] stated that MS media with BAP could affect the length of the leaves in the tillers bud that emerges. According to [42], the addition of sucrose and BAP to MS media can initiate the growth of plant parts because sucrose, which provides carbohydrates for plants, acts optimally in the growth of plant parts.

The interaction between sucrose and IBA significantly affected leaf width. Observation results showed that the highest average value in leaf width was in the $S_{10}I_{1.5}$ treatment with an average of 1.12, interactions of 10 grams sucrose L^{-1} with 1.5 ppm IBA giving a significant effect on the width of pineapple leaves from Sipahutar. According to [14], adding auxin and cytokinins in accordance with plants can affect the development of plant parts; adding organic substances such as sucrose with the appropriate concentration can provide energy substances for plants. Sucrose is easily available to cells and directly participates in the glycolytic and pentose phosphate pathways for cell growth and is also found to act as osmotic [43].

5. Conclusion

The addition of sucrose significantly affected the number of leaves and the number of tillers. While IBA (Indole Bricty Acid) as PGR significantly affects the length of the pineapple leaves from Sipahutar. This showed that IBA stimulates root growth and can stimulate leaf length with the appropriate concentration. The interaction between sucrose and IBA significantly affects the number of roots, leaf length, and leaf width of pineapple plants from Sipahutar. IBA, which interacted with sucrose, can stimulate the number of roots and growth of pineapple from Sipahutar. The limitation of this study is that the test was only conducted in vitro and not yet in vivo. In addition, the best results from each treatment need to be further tested in vivo.

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References

[1] SILABAN S. S. E., & GINTING R. Analisis efisiensi produksi nanas (*Ananas comusus* L (Merr) (studi kasus: Desa Siabal-Abal 2, Kec. Sipahutar, Kab. Tapanuli Utara). *Journal on Social Economic of Agriculture and Agribusiness*, 2017, 8: 1-13. http://repositori.usu.ac.id/handle/123456789/10734

[2] BE L.V., & DEBERGH P. C. Potential low-cost micropropagation of pineapple (*Ananas comosus*). *South African Journal of Botany*, 2006, 72: 191–194. https://doi.org/10.1016/j.sajb.2005.07.002

[3] GAMA P. B. S., & IBRAHIM T. Low cost growth enhancement of in vitro produced pineapples (*Ananas comosus* L. Merr) propagules under nursery conditions. *International Journal of Agricultural Research and Review*, 2015, 3: 425-431. http://springjournals.net/full-articles/springjournals.netijarrarticlesindex=8gamaandibrahim.pdf?view=inline

[4] HASANAH A., HARAHAP F., and SILABAN R. The effects of MYO-inositol and Indole Butyric Acid (IBA) on the formation of pineapples root (*Ananas comosus* L.) from Sipahutar North Sumatera in vitro. *International Journal of Biological Research*, 2018, 6: 23-28. http://dx.doi.org/10.14419/ijbr.v6i2.13699

[5] HARAHAP F., POERWANTO R., SOBIR S., HASRUDDIN H., SURIANI C., SIALLAGAN J., and ROHYANA R. Sterilization of pineapple explant from Sipahutar, North Sumatera, Indonesia (*Ananas comosus* L.) and in vitro growth induction. *Asian Journal of Microbiology, Biotechnology & Environmental Sciences*, 2015, 17: 469-478. http://digilib.unimed.ac.id/id/eprint/1426

[6] INSANI H., HARAHAP F., and DININGRAT D.S. The effect of coconut water and Benzyl Amino Purine (BAP) addition to the growth of pineapple from Sipahutar North Sumatera in vitro condition. *International Journal of Biological Research*, 2018, 6: 29-33. http://dx.doi.org/10.14419/ijbr.v6i2.13697

[7] MAHIPAL S., SHEKHAWAT N., KANNAN M., MANOKARI C. P., and RAVINDRAN R. In vitro regeneration of shoots and ex vitro rooting of an important medicinal plant *Passiflora foetida* L. through nodal segment cultures. *Journal of Genetic Engineering and Biotechnology*, 2015, 13: 209-214. https://doi.org/10.1016/j.jgeb.2015.08.002

[8] HARAHAP F., POERWANTO R., SUHARSONO S., SURIANI C., and RAHAYU S. In vitro growth and rooting of mangosteen (*Garnicia mangostana* L.) on medium with different concentration of plant growth regulator. *HAYATI Journal of Bioscience*, 2014, 21: 151-158. https://doi.org/10.4308/hjb.21.4.151

[9] HARAHAP F., DININGRAT D. S., POERWANTO R., NASUTION N. E. A., and HASIBUAN R. F. M. In vitro callus induction of Sipahutar pineapple (*Ananas comosus* L.) from North Sumatra Indonesia. *Pakistan Journal of Biological Sciences*, 2019, 22: 518-526. https://doi.org/10.3923/pjbs.2019.518.526

[10] HARAHAP F., DJULIA E., PURNAMA D., NUSYIRWAN N., ALTIO V., RAHAYU S., ROSMAYATI R., POERWANTO R., and HASIBUAN R. F. M. Pineapple callus induction from Sipahutar North Sumatera Indonesia (*Ananas comosus* L.) with bud as a source explant. *IOP Conference Series: Journal of Physics*, 2020, 1462: 012008. https://iopscience.iop.org/article/10.1088/1742-6596/1462/1/012008/meta

[11] JAIN M. S. In vitro mutagenesis for improving date palm (*Phoenix dactylifera* L.). *Emirates Journal of Food and*

Agriculture, 2012, 24: 400-407. https://www.ejfa.me/index.php/journal/article/view/927

[12] NATARAJAN V., & DHANAVEL D. In vitro production of *Stevia rebaudiana* Bertoni. *Emirates Journal of Food and Agriculture*, 2010, 22: 216-222. https://www.ejfa.me/index.php/journal/article/view/275

[13] ANNIASARI E. D., PUTRI R. B. A., and MULIAWATI E. S. Penggunaan BA dan NAA untuk merangsang pembentukan tunas lengkeng dataran rendah (*Dimocarpus longan*) secara in vitro. *Bioteknologi*, 2016, 13: 43-53. https://digilib.uns.ac.id/dokumen/detail/12313

[14] HARAHAP F. *Kultur jaringan tanaman.* Unimed Press, Medan, 2011. http://digilib.unimed.ac.id/1640/4/Bab%20IV.pdf

[15] SHOFIANA A., RAHAYU Y., LUKAS S., and BUDIPRAMANA B. Pengaruh pemberian berbagai konsentrasi hormon IBA (Indole Butyric Acid) terhadap pertumbuhan akar pada stek batang tanaman buah naga (*Hylocereus undatus*). *LenteraBio: Berkala Ilmiah Biologi*, 2013, 2: 101–105. https://jurnalmahasiswa.unesa.ac.id/index.php/lenterabio/article/view/1424

[16] SHOFIYANI A., & PURNAWANTO A. M. Pertumbuhan kalus kencur (*Kaemferia galanga* L) pada komposisi media dengan perlakuan sukrosa dan zat pengatur tumbuh (2, 4 D dan Benzil Aminopurin). *Agritech: Jurnal Fakultas Pertanian Universitas Muhammadiyah Purwokerto*, 2017, 19: 55-64. http://dx.doi.org/10.30595/agritech.v19i1.2098

[17] NILANTHI D., & YANG Y. S. Effects of sucrose and other additives on in vitro growth and development of purple coneflower (*Echinacea purpurea* L.). *Advances in Biology*, 2014, 2014: 1-4. https://www.hindawi.com/archive/2014/402309/abs/

[18] ARLIANTI T., SYAHID S., KRISTINA N., and ROS O. Effect of auxin IBA and NAA on in vitro rooting of stevia (*Stevia rebaudiana*). *Buletin Penelitian Tanaman Rempah dan Obat*, 2017, 24: 57-62.

[19] MOHAJER R., TAHA M., and ADEL M. In vitro environmental factors controlling root morphological traits of pineapple (*Ananas comosus* L. Merr.). *International Journal of Biotechnology and Bioengineering*, 2015, 9: 1155–115. http://eprints.um.edu.my/id/eprint/15052

[20] QAMAR M., QURESHI T. S., KHAN A. I., NIZAMANI S. G., and BURIRO A. Effect of sucrose on optimal rooting of *Musa* spp. plantlets in-vitro condition. *Pakistan Journal of Biotechnology*, 2015, 12: 169-172. http://pjbt.org/index.php/pjbt/article/download/160/156

[21] PÉREZ P. L., OLMEDO G. J., RODRÍGUEZ R., and NORMAN O. Effects of different culture conditions (photoautotrophic, photomixotrophic) and the auxin indole-butyric acid on the in vitro acclimatization of papaya (*Carica papaya* L. var. Red. Maradol) plants using zeolite as support. *African Journal of Biotechnology*, 2015, 14: 2622-2635. https://doi.org/10.5897/AJB2015.14814

[22] INAYAH T. Pengaruh konsentrasi sukrosa pada induksi embrio somatik dua kultivar kacang tanah (*Arachis hypogaea* L.) secara in vitro**.** *Jurnal Agribisnis*, 2015, 9: 61–70. https://doi.org/10.15408/aj.v9i1.5086

[23] FATRIYATUN N. Pengaruh pemberian berbagai kombinasi konsentrasi sukrosa dan kinetin terhadap induksi umbi mikro kentang (*Solanum tuberosum* L.) kultivar granola kembang secara in-vitro. *LenteraBio: Berkala Ilmiah Biologi*, 2012, 1: 41–48. https://jurnal.unesa.ac.id/index.php/lenterabio/article/view/193/127

[24] RUGAYAH R., ANGGALIA I., and GINTING C. Y. Pengaruh konsentrasi dan cara aplikasi IBA (Indole Butiric Acid) terhadap pertumbuhan bibit nanas (*Ananas comosus* [L.] Merr.) asal anakan mahkota. *Jurnal Agrotropika*, 2013, 17: 35-38. http://dx.doi.org/10.23960/ja.v17i1.4279

[25] SUMARYONO S., MUSLIHATIN W., and RATNADEWI D. Effect of carbohydrate source on growth and performance of *in vitro* sago palm (*Metroxylon sagu* Rottb.) plantlets. *HAYATI Journal of Biosciences*, 2012, 19: 88-92. https://doi.org/10.4308/hjb.19.2.88

[26] HARAHAP F., HASANAH A., INSANI H., HARAHAP N. K., PINEM M. D., EDI S., SIPAHUTAR H., and SILABAN R. *Kultur jaringan nanas.* Media Sahabat Cendekia, Surabaya, 2019.

[27] WIDIASTOETY W. Effect of auxin and cytokinin on the growth of mokara orchid plantlets. *Journal of Horticultura*, 2014, 24: 230-238. https://media.neliti.com/media/publications/98313-ID-pengaruh-auksin-dan-sitokinin-terhadap-p.pdf

[28] GAGO J., NUNEZ M., LANDIN L., FLEXAS M., PEDRO J. P., and GALLEGO G. Modeling the effects of light and sucrose on in vitro propagated plants: a multiscale system analysis using artificial intelligence technology. *PLoS ONE*, 2014, 9: 1-11. https://doi.org/10.1371/journal.pone.0085989

[29] ARIMARSETIOWATI, R., & ARDIYANI F. Pengaruh penambahan auxin terhadap peranakanan dan perakaran kopi arabika perbanyakan somatik embriogenesis. *Pelita Perkebunan*, 2012, 28: 82-90. https://doi.org/10.22302/iccri.jur.pelitaperkebunan.v28i2.201

[30] HARTATI S. Pengaruh macam ekstrak bahan organik dan ZPT terhadap pertumbuhan planlet anggrek hasil persilangan pada media kultur. *Caraka Tani: Journal of Sustainable Agriculture*, 2010, 25: 101-105. https://doi.org/10.20961/carakatani.v25i1.15752

[31] KOZAI T. Photoautotrophic mic-ropropagation. *In Vitro Cellular & Developmental Biology - Plant*, 1991, 27: 47–51. https://doi.org/10.1007/BF02632127

[32] ZAHARA M., DATTA A., BOONKORKAEW P., and MISHRA A. The effects of different media, sucrose concentrations and natural additives on plantlet growth of *Phalaenopsis* hybrid 'Pink'. *Brazilian Archives of Biology and Technology*, 2017, 6: 1-15. https://doi.org/10.1590/1678-4324-2017160149

[33] MORFEINE E. A. Effect of sucrose and glucose concentrations on micropropagation of *Musa* sp.cv. Grand Naine. *Journal of Applied and Industrial Sciences*, 2014, 2: 58-62.

[34] SITORUS N., HASTUTI E., and SETIARI N. Induksi kalus binahong (*Basella rubra* L.) secara in vitro pada media Murashige & Skoog dengan konsentrasi sukrosa yang berbeda. *Bioma*, 2011, 13: 1-7. https://doi.org/10.14710/bioma.13.1.1-7

[35] PAMUNGKAS M. S., RAHAYUNINGSIH E., and KUSUMASTUTI Y. Effect of glucose, sucrose, and lactose solution on the stability of betacyanin pigment from red dragon fruit (*Hylocereus polyrhizus*) peels. *IOP Conference Series: Earth and Environmental Science*, 2020, 572: 012014. https://iopscience.iop.org/article/10.1088/1755-1315/572/1/012014/meta

[36] SANTOSO R. D., & SOBIR S. Pertumbuhan planlet nenas (*Ananas comosus* L. Merr.) varietas Smooth Cayenne

hasil kultur in vitro pada beberapa konsentrasi BAP dan umur plantlet. *Buletin Agrohorti*, 2013, 1: 54–61. https://doi.org/10.29244/agrob.1.1.54-61
[37] ELIMASNI E. *Perbanyakan bibit kemenyan Sumatrana (Styrax benzoin Dryander) secara kultur jaringan.* Universitas Sumatera Utara Repository, Medan, 2006.
[38] ISLAM S. M. S., ISLAM T., BHATTACHARJEE B., MONDAL K. T., and SUBRAMANIAM S. In vitro pseudobulb based micropropagation for mass development of *Cymbidium finlaysonianum* Lindl. *Emirates Journal of Food and Agriculture*, 2015, 27: 469-474. https://doi.org/10.9755/ejfa.2015.04.017
[39] FEBRIYANTI E., SUWIRMEN S., and DRIS M. Induksi perakaran anakan *Tetrastigma rafflesiae* Miq. pada media Murashige-Skoog dengan penambahan beberapa konsentrasi Indole-3-Butyric Acid (IBA) secara in-vitro. *Jurnal Biologi Universitas Andalas*, 2013, 2: 188-193. https://doi.org/10.25077/jbioua.2.3.%25p.2013
[40] AHMED S., SHARMA A., SINGH A. K., WALI V. K., and KUMARI P. In vitro multiplication of banana (*Musa* sp.) cv. Grand Nain. *African Journal of Biotechnology*, 2014, 13: 2696-2703. https://doi.org/10.5897/AJB2014.13750
[41] SAFDARI Y., & KAZEMITABAR S. K. Plant tissue culture study on two different races of purslane (*Portulaca oleracea* L.). *African Journal of Biotechnology*, 2009, 8: 5906-5912. https://doi.org/10.5897/AJB09.816
[42] KAFINDRA L., KHUMAIDA N., and ARDIE S. W. Induksi rimpang mikro *Kaempferia parviflora* secara in vitro dengan penambahan BAP dan Sukrosa. *Jurnal Hortikultural Indonesia*, 2015, 6: 54–63. https://doi.org/10.29244/jhi.6.1.54-63
[43] JULKIFLEE L. A., UDDAIN J., and SUBRAMANIAM S. Efficient micropropagation of *Dendrobium sonia*-28 for rapid PLBs proliferation. *Emirates Journal of Food and Agriculture*, 2014, 26: 545-551. https://doi.org/10.9755/ejfa.v26i6.18020
[44] ZAKARIA D. *Pengaruh konsentrasi sukrosa dan BAP (Benzil Amino Purine) dalam media Murashige Skoog (MS) terhadap pertumbuhan dan kandungan reserpin kalus pule pandak (Rauvolfia verticillate Lour.).* Universitas Sebelas Maret, Surakarta, 2010. https://digilib.uns.ac.id/dokumen/detail/17651

参考文:

[1] SILABAN S. S. E., & GINTING R. 菠萝生产效率分析（菠萝 comusus 升(梅尔)）(案例研究：西亚巴尔-阿巴尔 2 村, 西帕胡塔区, 北塔帕努里区）. 农业和农业社会经济杂志，2017, 8: 1-13. http://repositori.usu.ac.id/handle/123456789/10734
[2] BE L.V., & DEBERGH P.C. 菠萝 (凤梨) 的潜在低成本微繁殖。南非植物学杂志，2006，72：191-194。https://doi.org/10.1016/j.sajb.2005.07.002
[3] GAMA P. B. S., & IBRAHIM T. 在苗圃条件下体外生产的菠萝 (凤梨升·梅尔) 繁殖体的低成本生长促进。国际农业研究与评论杂志，2015，3：425-431。http://springjournals.net/full-articles/springjournals.netijarrarticlesindex=8gamaandibrahim.pdf?view=inline
[4] HASANAH A.、HARAHAP F. 和 SILABAN R. 米奥-肌醇和吲哚丁酸 (国际律师协会) 对来自西帕胡塔北苏门答腊的菠萝根 (凤梨升.) 体外形成的影响。国际生物研究杂志，2018，6：23-28。http://dx.doi.org/10.14419/ijbr.v6i2.13699
[5] HARAHAP F.、POERWANTO R.、SOBIR S.、HASRUDDIN H.、SURIANI C.、SIALLAGAN J. 和 ROHYANA R. 来自印度尼西亚北苏门答腊西帕胡塔（凤梨升.）和体外生长诱导。亚洲微生物学、生物技术与环境科学杂志，2015，17：469-478。http://digilib.unimed.ac.id/id/eprint/1426
[6] INSANI H.、HARAHAP F. 和 DININGRAT D. S. 添加椰子水和苄基氨基嘌呤 (BAP) 对西帕胡塔北苏门答腊菠萝生长的影响。国际生物研究杂志，2018，6：29-33。http://dx.doi.org/10.14419/ijbr.v6i2.13697
[7] MAHIPAL S.、SHEKHAWAT N.、KANNAN M.、MANOKARI C. P. 和 RAVINDRAN R. 重要药用植物西番莲升.芽的体外再生和体外生根，通过节段培养。基因工程与生物技术杂志，2015，13：209-214。https://doi.org/10.1016/j.jgeb.2015.08.002
[8] HARAHAP F.、POERWANTO R.、SUHARSONO S.、SURIANI C. 和 RAHAYU S. 山竹 (山竹升.) 在不同浓度植物生长调节剂的培养基上的体外生长和生根。哈亚蒂生物科学杂志，2014，21：151-158。https://doi.org/10.4308/hjb.21.4.151
[9] HARAHAP F.、DININGRAT D. S.、POERWANTO R.、NASUTION N. E. A. 和 HASIBUAN R. F. M. 来自印度尼西亚北苏门答腊的西帕胡塔菠萝 (凤梨升.) 的体外愈伤组织诱导。巴基斯坦生物科学杂志，2019，22：518-526。https://doi.org/10.3923/pjbs.2019.518.526
[10] HARAHAP F.、DJULIA E.、PURNAMA D.、NUSYIRWAN N.、ALTIO V.、RAHAYU S.、ROSMAYATI R.、POERWANTO R. 和 HASIBUAN R. F. M. 来自印度尼西亚西帕胡塔北苏门答腊（凤梨升）的菠萝愈伤组织诱导.) 以芽作为来源外植体。眼压会议系列：物理学杂志，2020，1462：012008。https://iopscience.iop.org/article/10.1088/1742-6596/1462/1/012008/meta
[11] JAIN M. S. 用于改良枣椰树（凤仙花升.）的体外诱变。阿联酋食品和农业杂志，2012，24：400-407。https://www.ejfa.me/index.php/journal/article/view/927
[12] NATARAJAN V., & DHANAVEL D. 甜叶菊的体外生产。阿联酋食品和农业杂志，2010，22：216-222。https://www.ejfa.me/index.php/journal/article/view/275
[13] ANNIASARI E. D.、PUTRI R. B. A. 和 MULIAWATI E. S. 使用文学士和美国国家航空航天局刺激低地龙眼（龙眼）的枝条形成体外。生物技术，2016，13：43-53。https://digilib.uns.ac.id/dokumen/detail/12313
[14] HARAHAP F. 植物组织培养。联合医学出版社，棉兰，2011。http://digilib.unimed.ac.id/1640/4/Bab%20IV.pdf
[15] SHOFIANA A.、RAHAYU Y.、LUKAS S. 和 BUDIPRAMANA B. 给予不同浓度的效果激素国际律师协会（吲哚丁酸）关于火龙果 (火龙果) 茎插条的根生长。兰黛生物：生物科学期刊, 2013, 2: 101–105。https://jurnalmahasiswa.unesa.ac.id/index.php/lenterabio/article/view/1424
[16] SHOFIYANI A., & PURNAWANTO A. M. 愈伤组织 (山茱萸) 在用蔗糖和生长调节剂（2、4 D 和苄基氨基嘌呤）处理的培养基组合物上的生长。农业科技：穆罕默

德·普沃克托大学农学院学报，2017，19：55-64。http://dx.doi.org/10.30595/agritech.v19i1.2098
[17] NILANTHI D., & YANG Y. S. 蔗糖和其他添加剂对紫锥花 (紫锥菊升.) 体外生长和发育的影响。生物学进展, 2014, 2014: 1-4 。https://www.hindawi.com/archive/2014/402309/abs/
[18] ARLIANTI T.、SYAHID S.、KRISTINA N. 和 ROSO. 生长素国际律师协会和美国国家航空航天局对甜菊（甜叶菊）体外生根的影响。班尼利蒂安·塔纳曼·伦帕·丹·奥巴特公报, 2017, 24: 57-62。
[19] MOHAJER R.、TAHA M. 和 ADEL M. 控制菠萝根部形态特征的体外环境因素（凤梨升. 梅尔。）。国际生物技术与生物工程杂志，2015，9：1155-115。http://eprints.um.edu.my/id/eprint/15052
[20] QAMAR M.、QURESHI T. S.、KHAN A. I.、NIZAMANI S. G. 和 BURIRO A. 蔗糖对芭蕉科最佳生根的影响。幼苗体外条件。巴基斯坦生物技术杂志，2015，12：169-172。http://pjbt.org/index.php/pjbt/article/download/160/156
[21] PÉREZ P. L.、OLMEDO G. J.、RODRÍGUEZ R. 和 NORMAN O. 不同培养条件（光合自养、光合营养）和生长素吲哚-丁酸对木瓜（番木瓜升. 变种 红色的。）体外驯化的影响。马拉多尔) 使用沸石作为载体的植物。非洲生物技术杂志，2015，14：2622-2635。https://doi.org/10.5897/AJB2015.14814
[22] INAYAH T. 蔗糖浓度对两个花生品种（花生升.）体细胞胚诱导的影响 体外塞卡拉。期刊阿格里比尼斯, 2015, 9: 61–70。 https://doi.org/10.15408/aj.v9i1.5086
[23] FATRIYATUN N. 不同浓度的蔗糖和激动素组合对马铃薯微型块茎（马铃薯升.）格兰诺拉麦片花栽培品种体外灯笼生物的影响：生物学科学时期, 2012, 1: 41–48。https://jurnal.unesa.ac.id/index.php/lenterabio/article/view/193/127
[24] RUGAYAH R.、ANGGALIA I. 和 GINTING C. Y. 专注力的影响以及如何应用国际律师协会（吲哚丁酸）菠萝苗的生长（凤梨[升.] 梅尔。）冠树苗的起源。农业杂志，2013，17：35-38。http://dx.doi.org/10.23960/ja.v17i1.4279
[25] SUMARYONO S.、MUSLIHATIN W. 和 RATNADEWI D. 碳水化合物源对体外西米棕榈 (甲氧龙萨古·罗特布.) 植株生长和性能的影响。哈亚提生物科学杂志，2012，19：88-92。https://doi.org/10.4308/hjb.19.2.88
[26] HARAHAP F.、HASANAH A.、INSANI H.、HARAHAP N. K.、PINEM M. D.、EDI S.、SIPAHUTAR H. 和 SILABAN R. 菠萝组织培养。媒体学者朋友，泗水，2019。
[27] WIDIASTOETY W. 生长素和细胞分裂素对莫卡拉兰花植株生长的影响。园艺杂志，2014，24：230-238。https://media.neliti.com/media/publications/98313-ID-pengaruh-auksin-dan-sitokinin-terhadap-p.pdf
[28] GAGO J.、NUNEZ M.、LANDIN L.、FLEXAS M.、PEDRO J. P. 和 GALLEGO G. 模拟光和蔗糖对体外繁殖植物的影响：使用人工智能技术的多尺度系统分析。公共图书馆一号, 2014, 9: 1-11 。https://doi.org/10.1371/journal.pone.0085989
[29] ARIMARSETIOWATI, R., & ARDIYANI F. 添加生长素对阿拉比卡咖啡繁殖及根系体细胞繁殖的影响胚胎发生。佩丽塔·佩克布南, 2012, 28: 82-90 。https://doi.org/10.22302/iccri.jur.pelitaperkebunan.v28i2.201
[30] HARTATI S. 各种有机物提取物和 PGR 对生长的影响行星来自文化媒体上十字架的兰花。卡拉卡塔尼：可持续农业杂志，2010，25：101-105。https://doi.org/10.20961/carakatani.v25i1.15752
[31] KOZAI T. 光合自养微繁殖。体外细胞与发育生物学 - 植物，1991，27：47-51。https://doi.org/10.1007/BF02632127
[32] ZAHARA M.、DATTA A.、BOONKORKAEW P. 和 MISHRA A. 不同培养基、蔗糖浓度和天然添加剂对蝴蝶兰杂交“粉红色”幼苗生长的影响。巴西生物技术档案，2017，6：1-15。https://doi.org/10.1590/1678-4324-2017160149
[33] MORFEINE E. A. 蔗糖和葡萄糖浓度对穆萨品种微繁殖的影响。大奈奈。应用与工业科学杂志，2014，2：58-62。
[34] SITORUS N.、HASTUTI E. 和 SETIARI N. 比纳红愈伤组织诱导（红底芽孢杆菌升.）体外在具有不同蔗糖浓度的村重和斯库格培养基上。生物，2011，13：1-7。https://doi.org/10.14710/bioma.13.1.1-7
[35] PAMUNGKAS M. S.、RAHAYUNINGSIH E. 和 KUSUMASTUTI Y. 葡萄糖、蔗糖和乳糖溶液对红火龙果 (火龙果) 果皮中甜菜青素色素稳定性的影响。眼压会议系列：地球与环境科学，2020，572：012014。https://iopscience.iop.org/article/10.1088/1755-1315/572/1/012014/meta
[36] SANTOSO R. D. 和 SOBIR S. 佩通布汉植株菠萝 (凤梨升. 梅尔。) 来自体外培养的光滑卡宴品种，不同浓度的 BAP 和苗龄农家乐，2013，1：54-61。https://doi.org/10.29244/agrob.1.1.54-61
[37] ELIMASNI E. 通过组织培养繁殖苏门答腊乳香（安息香安息香）幼苗。北苏门答腊大学知识库，棉兰，2006。
[38] ISLAM S. M. S.、ISLAM T.、BHATTACHARJEE B.、MONDAL K. T. 和 SUBRAMANIAM S. 基于体外假球茎的微繁殖用于大花蕙兰的大规模发育。阿联酋食品和农业杂志，2015，27：469-474。https://doi.org/10.9755/ejfa.2015.04.017
[39] FEBRIYANTI E.、SUWIRMEN S. 和 DRIS M. 在体外添加几种浓度的吲哚-3-丁酸 (国际律师协会) 在村重-斯科格培养基中诱导莱佛士四柱花。。期刊生物学安达拉斯大学，2013，2：188-193。https://doi.org/10.25077/jbioua.2.3.%25p.2013
[40] AHMED S.、SHARMA A.、SINGH A. K.、WALI V. K. 和 KUMARI P. 香蕉 (芭蕉科.) 简历的体外增殖。大纳因。非洲生物技术杂志，2014，13：2696-2703。https://doi.org/10.5897/AJB2014.13750
[41] SAFDARI Y., & KAZEMITABAR S. K. 两种不同马齿苋（马齿苋升.）的植物组织培养研究。非洲生物技术杂志，2009，8：5906-5912。https://doi.org/10.5897/AJB09.816
[42] KAFINDRA L.、KHUMAIDA N. 和 ARDIE S. W. 添加 BAP 和蔗糖对小花山奈微根茎的体外诱导。印度尼西亚园艺杂志，2015，6：54-63。https://doi.org/10.29244/jhi.6.1.54-63
[43] JULKIFLEE L. A.、UDDAIN J. 和 SUBRAMANIAM S. 石斛-28 的高效微繁殖用于快速公共小巴增殖。阿联

酋食品和农业杂志，2014，26：545-551。https://doi.org/10.9755/ejfa.v26i6.18020
[44] ZAKARIA D. 村重史库格(多发性硬化症) 培养基中蔗糖和 BAP（苄基氨基嘌呤）浓度对愈伤组织 (萝芙木轮叶.) 生长和利血平含量的影响。塞贝拉斯马雷特大学，苏腊卡尔塔，2010。https://digilib.uns.ac.id/dokumen/detail/17651