Efficiency for Shoot Multiplication of 'Srivijaya' New Hybrid Pineapple Variety Using Three Tissue Culture Systems

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Abstract

A new hybrid pineapple variety, 'Srivijaya, has superior fruit quality and is resistant to disease. The planting materials of the 'Srivijaya' pineapple variety are still limited for farmers. The tissue culture technique provides for rapid mass shoot production. This research, 'Srivijaya' pineapple tissue culture was invested. Suckers were *sterilized* using 70% ethanol for 5 minutes and were moved to new bottle containing 40 ml of NaOCl (6 % w/w), 60 ml of distill water and 3 drops of tween-20 for 35 minutes. The sucker was cut and cultured to induce shoots. Shoot multiplication of 'Srivijaya' pineapple was studied on three different *in vitro* culture systems; solid, liquid, and bioreactor. A completely randomized design was used with four treatments; 1/2 MS+ 0.5 mg/L BA, 1/2 MS+1 mg/L BA, 1/2 MS+ 2 mg/L BA and MS+ 2 mg/L BA. The data were analyzed by ANOVA and DMRT for mean different analyses. The results showed that MS+ 2 mg/L BA medium gave the highest total number of shoots in all culture systems. For solid culture, 17.75, 21.50, 25 and 30.25 shoots were produced from 1/2 MS + 0.5, 1, 2 and MS+ 2 mg/L BA media respectively. Similar to liquid culture, 21.75, 23.50, 22.50 and 34.75 shoots were produced from four media respectively. The bioreactor was the most efficient in shoot multiplication of 'Srivijaya' for all treatments giving 51.5, 76, 95.50 and 124.5 shoots from 1/2 MS + 0.5, 1, 2 and MS+ 2 mg/L BA media respectively.

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1. Introduction

Pineapple is a tropical fruit crop commodity with economic value. It contains many nutrients. Every 100 g of pineapple contains 50 kcal of energy, 10 types of minerals as much as 144,547 mg, 17 kinds of amino acids as much as 761 mg, 0.54 g of protein, 0.12 g of fat, 0.22 g of ash, 13.12 g of carbohydrates, 1.4 g of fiber, 9.85 g of total sugar, and various types of vitamins . These nutrients are for the general pineapple cultivar. The high nutrition content of pineapple is a source of ingredients beneficial to health by supporting the body's metabolic system.

Pineapple plants have adapted well to drought due to morphological, anatomical and physiological characteristics and are widely cultivated by more than 90 countries worldwide. There are wide pineapple varieties in the world. There are both clones and hybrids. One of the superior hybrid pineapples with high quality and disease resistance had been bred by Sripaoraya and this variety was approved as a new pineapple variety in 2021 by Department of Agriculture named ‘Srivijaya’ . It has fruit weight 1,120 gm, depth of cup 0.98 cm, sweetness 17.80 brix, and a crunchy and aromatic texture. More fruit qualities were reported, such as antioxidant activity of 88.52%, protein of 4.84%, fiber of 3.86%, and bromelain of 6,332.85 mg/ml. Since "Srivijaya is a new pineapple variety, the availability of planting material is still limited. If new pineapple cultivation uses conventional propagation techniques, it takes longer, and the amount of planting material produced is less. Therefore, alternative technology is needed to provide superior planting material for pineapples.

Propagation through plant tissue culture techniques is an alternative to producing pineapple planting material on a mass scale in a relatively short time . There are several culture systems used in plant tissue culture, such as solid culture, liquid shake culture, and bioreactors. Each culture system has advantages and disadvantages for the multiplication of pineapple shoots. Solid media was developed many years ago and is still a favorite today. Liquid culture conducted by Karyanti *et al*. showed that liquid culture was able to increase the percentage of shoot formation better than solid culture. Bioreactors are applied to the culture of ornamental plants and fruit crops in the health sector .

Another factor affecting plantlet production is plant growth regulators. The most commonly used growth regulator for shoot multiplication *in vitro* is 6-benzyladenine (BA). BA has the advantages of being relatively stable, cheaper, effective, and strong compared to kinetin . Previous studies have shown success for pineapple multiplication *in vitro* such as Zuraida *et al*. using BA up to 5 ppm. An average of 2 ppm BA induces an average of 6–9 plantlets per explant . The multiplication of pineapple shoots with a BA concentration of 2.5 mg/L into MS medium also resulted in a large number of calluses according to Ibrahim *et al*.

The application of BA in MS medium has generally been widely studied for the multiplication of pineapple shoots, among others . While the study by Nikumbhe *et al*. using BA produced an average number of shoots of 2.40 shoots after 3–4 weeks in 1/2 MS + BA 0.5 ppm, Reducing the concentration of MS content and the use of BA in various concentrations has the opportunity to reduce production costs. Thus, it is important to determine the effect of media composition and tissue systems on the multiplication of new 'Srivijaya' pineapple variety shoots.

2. Methods

2.1 Explant materials and explants sterilization

The explant used in this study was the Srivijaya' pineapple cultivar. Explant materials are sourced from suckers of Srivijaya pineapple in the field. The mature leaves were taken from the sucker. The suckers were cleaned up with tap water. The suckers were then soaked and *sterilized* using 70% ethanol for 5 minutes. The materials were moved to new glass bottles containing 40 ml of 6% w/w sodium hypochlorite (NaOCl), 60 ml of autoclaved distill water, and 3 drops of tween-20 for 35 minutes.

The next step was done in a laminar air flow cabinet. The sucker was cleaned three times using sterile distilled water. Every single time, the cleaning process took 5 minutes. The sample was trimmed to four pieces on the plate. The explants were transferred to MS + 2 mg/L BA solid media for shoot induction.

2.2 BA growth regulator and medium preparation

Fifty milliliters of BA stock solution were prepared to contain 0.05 g of 6-benzylaminopurine. The stock solution was stored at 4 °C. The different concentrations of BA (0.5, 1, 1.5, and 2 mg/L were piped from stock solutions depending on treatments.

The medium used for this research was MS and 1/2 MS medium stock. The solution was adjusted to pH 5.8. Equipment and materials were autoclaved for 20 minutes at a temperature of 121^0 C and a pressure of 15 pounds/in^2.

2.3 Experimental design

A completely randomized design (CRD) was used with four treatments combining two factors: BA and MS concentrations following as 1/2 MS+ 0.5 mg/L BA, 1/2 MS+ 1 mg/L BA, 1/2 MS+ 2 mg/L BA, and MS+ 2 mg/L BA as a control treatment. Solid culture and liquid shake culture were replicated four times. Total of 16 experimental units and 5 young shoots of 'Srivijaya' pineapple explants per replication were set. Two replications were used for bioreactor culture. Each experimental unit used 10 young shoots of 'Srivijaya' pineapple explants, with a totally of 8 experimental units.

2.4 Culture

The conditions for the culture room were maintained at 22 $\pm$ 20 °C, with lighting 12 hours per day. In solid culture, explants were planted with five young shoots per bottle. For liquid culture, five explants were immersed in the media in an Erlenmeyer glass. Each liquid culture treatment was put on a shaker with a speed of 130 RPM, which worked automatically. Stirring each treatment simultaneously starts at 08.10 and ends at 16.00. In a culture of the bioreactor, 10 young shoots were planted per culture glass using tweezers. The bioreactor used has a temporary immersion system. Shoots were automatically fed 8 times daily for 5 minutes by the time controller . All cultures were incubated at 22 $\pm$ 20 °C under a 12-hour photoperiod of excellent daylight illumination per day with a humidity of 70–80%.

2.5 Measurements and data analysis

The total number of shoots, height, and a classified number of shoots based on the height categories (<1.0 cm, 1.0–1.5 cm, >1.5 cm) were collected after culture for 30 days. All data were tested by analysis of variance at the 5% level. The analysis of variance test results that were significantly different was analyzed using the Duncan Multiple Range Test through the Costat program.

3. Results and Discussion

3.1 Shoot multiplication of 'Srivijaya' hybrid pineapple on solid culture

Treatments significantly affected all the pineapple shoot multiplication variables (Table 1). The coefficient of variation was lower than 20% except for the total number of shoots based on height (1 cm–1.5 cm). Solid cultures had no shoots based on height greater than 1.5 cm except the explants. Multiplication of 'Srivijaya' pineapple shoots in solid culture showed that MS + 2 mg/L BA gave the best results for almost all variables after further testing with DMRT. The variables that showed these results were the total number of shoots and the number of shoots with a height of less than 1 cm.

Table 1 Analysis of variance of solid medium culture for four variables

Variables	Computed F treatment	Probability	Coefficient of variation
Total number of shoots	13.50**	0.0004	12.25
Shoot height	3.92*	0.0365	14.67
Total number of shoots based on height (<1cm)	4.64*	0.0224	19.71
Total number of shoots based on height (1 cm -1.5 cm)	3.54*	0.0482	72.36

*significantly different, **very significantly different on 5 % level of significance

Table 2 Total number of shoot, shoot height and number of shoot based on height (<1 cm) of 'Srivijaya' hybrid pineapple in different media on solid culture

Media	Total number of shoots*	Shoot height (cm)*	Total number of shoots based on height <1 cm*
1/2 MS+ 0.5 mg/L BA	17.75 c	1.65 a	11.75 b
1/2 MS+ 1 mg/L BA	21.50 bc	1.36 ab	14.50 ab
1/2 MS+ 2 mg/L BA	25.00 b	1.24 b	18.75 a
MS+ 2 mg/L BA	30.25 a	1.22 b	18.50 a

*numbers followed by the same letters in the same column were not significantly different at 5% level.

The excellent result performance with MS + 2 mg/L BA was due to the media using MS with full concentration while the other treatments used half strength MS. This report was in line with research from Atawia *et al*. who reported that full MS nutrition was the best culture medium for pineapple culture after comparison. The ratio used was MS medium with full strength MS, 3/4, 1/2, and 1/4 added with 30 mg/L sucrose and 0.7% agar. The next effect is to have an impact on the overall appearance of the pineapple shoots.

Half strength MS + 2 mg/L BA medium gave good results but was significantly different from MS + 2 mg/L BA. 1/2 MS + 2 mg/L BA that was able to induce shoots as much as 25 or had a difference of 4.75 lower in MS medium with full strength MS concentration (Table 2). The number of obtained shoots indicated that the full strength MS medium showed the best result, which was consistent with the research of Atawia *et al*., who stated that the full-strength MS medium was the best concentration for producing 1.77 new shoots using crown explants. The treatment with the lowest BA concentration of 1/2 MS + 0.5 mg/L BA gave the least total number of shoots. This effect of BA concentration was consistent with the study conducted by Zuraida *et al*. This research reported that 5 mg/L BA concentration was the best treatment for producing an average of 7 plantlets per explant compared to BA (0, 0.5, 1.0, and 2.0 mg/L).

For shoot height, the new hybrid pineapple shoot height averaged no more than 2 cm in solid medium after culture 30 days. The best average shoot height was 1.65 cm in the 1/2 MS + 0.5 mg/L BA treatment. The results of this study indicated that the higher the BA concentration, the smaller the average value of shoot height. These results were in contrast with Feryati *et al*., who reported that the higher the BA concentration, the more shoots were formed.

The higher mean of 'Srivijaya' pineapple shoot height was thought to be the result of higher BA concentrations. The higher the concentration, the faster the growth of explant is focusing on the multiplication rather than the elongation of new shoots. These results agreed with the study of Atawia *et al*., who reported that full strength MS medium with 0.5, 1 and 2 mg/L BA induced shoot heights of 4.6 cm, 4.03 cm, and 3.4 cm, respectively. However, the results of this study were not in line with the finding of Harahap *et al*., who explained that a higher BA concentration was directly proportional to shoot height.

Table 3 Analysis of variance of liquid culture for five variables

Variables	Computed F treatment	Probability	Coefficient of variation
Total number of shoots9	7.58 **	0.0420	17.36
Shoot height	0.39 ns	0.7626	13.09
Total number of shoots based on height (< 1.0 cm)	11.81 **	0.0007	99.38
Total number of shoots based on height (1.0-1.5 cm)	0.45 ns	0.7227	32.90
Total number of shoots based on height (> 1.5 cm)	1.02 ns	0.4187	32.49

**very significantly different, ns not significant on 5 % level of significance

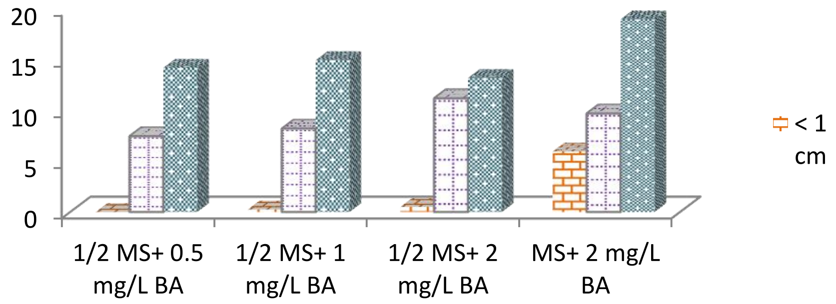

Figure 1 Effect of BA concentrations on the total number of shoots based on shoot height in liquid culture

Table 4 Total number of the shoots after liquid culture in different media of 'Srivijaya' hybrid pineapple variety

Media	Total number of shoot
1/2 MS+ 0.5 mg/L BA	21.75 b
1/2 MS+ 1 mg/L BA	23.50 b
1/2 MS+ 2 mg/L BA	22.50 b
MS+ 2 mg/L BA	34.75 a

*numbers followed by the same letters in the same column were not significantly different at 5% level.

3.2 Shoot multiplication of 'Srivijaya' hybrid pineapple in liquid shake culture

According to the 5 variables observed, there were 2 variables: total number of shoots and total number of shoots based on height (1.0 cm), which were very significantly impacted by treatment and media composition (Table 3). The total number of 'Srivijaya' pineapple shoots in liquid shake culture was significant. The highest total number of shoots was 34.75 shoots cultured in MS + 2 mg/L BA. These results were significantly different from other treatments, namely 1/2 MS + BA 2 mg/L, 1/2 MS + 1 mg/L BA, and 1/2 MS + 0.5 mg/L BA, giving 22.5 shoots, 23.5 shoots, and 21.75 shoots, respectively (Figure 1 and Table 4). The total number of pineapple shoots in liquid culture was directly proportional to the increase in BA and MS media concentrations. This result agreed with the study conducted by Ibrahim *et al*. The number of shoots increased along with the provision of more BA. MS medium concentrations also affected shoot growth. The effect of MS medium is that it provides of both macronutrients and micronutrients needed for explant growth.

The number of ‘Srivijaya’ pineapple shoots based on three categories of shoot height, namely <1.0 cm, 1-1.5 cm, and > 1.5 cm, indicated that shoots were dominated by shoots with a height of more than 1.5 cm, followed by shoots with a height of 1.0–1.5 cm. Overall, the liquid culture triggered the shoots to grow faster and taller. Most of the ‘Srivijaya’ pineapple shoots were dominated by large shoots with a height of more than 1.5 cm. Hvoslef-Eide and Preil also reported that the liquid culture system had a good effect on shoot morphology.

The liquid culture leads to the production of more height growth due to the continuous shaking and provides enhanced nutrient uptake and oxygen with uniform distribution. In addition, growing faster in liquid shake culture is caused by these movements, which make all tissues connect with the nutrient solution. This method has advantages that produce higher shoots that acclimatize faster and survive more than small shoots. Liquid medium disperses phenolic exudates from the explants, consequently resulting in a faster growth rate .

Table 5 Total number of the shoots after culture in different media for 4 weeks with bioreactor

Media	Total number of shoot
1/2 MS+ 0.5 mg/L BA	51.50 c
1/2 MS+ 1 mg/L BA	76.00 bc
1/2 MS+ 2 mg/L BA	95.50 ab
MS+ 2 mg/L BA	124.50 a

*numbers followed by the same letters in the same column were not significantly different at 5% level.

3.3 Shoot multiplication of ‘Srivijaya’ hybrid pineapple in bioreactor

The total number of ‘Srivijaya’ pineapple shoots was 95.50 in 1/2 MS + 2 mg/L BA and was not significantly different with MS + 2 mg/L BA (Table 5). The 1/2 MS + 2 mg/L BA had the potential to save on the use of MS medium with half concentration but it gave the total number of shoots lower than MS+ 2 mg/L BA medium (124.5). However, shoot height showed contrast with the number of shoots as shown in Figure 2. A higher number of shoots gave less shoot height. The highest total number of hybrid ‘Srivijaya’ shoots reached 124.5 shoots. There were 10 explants per experimental unit so one explant obtained 12.45 of new shoots from MS + 2 mg/L BA after culture for 4 weeks. This result is similar to the report of Ayenew *et al*., which produced 13.17 shoots per explant on MS + 2 mg/L BA using a bioreactor. However, greater shoot multiplication by Ayenew *et al*. is due to a longer culture period of 6 weeks. The total number of pineapples plantlets directly proportional to the length of the cultural period.

The ability of the bioreactor agrees with Reis *et al*., who state that gas exchange is able to increase the production of shoots and the rate of photosynthesis of ornamental pineapples *in vitro* culture. Previous research by Sripaoraya *et al*. reported that MS + 2 mg/L BA was effective in producing 151 pineapple shoots in the bioreactor system. The total number of shoots was higher than in this study. The factors causing the difference in the total number of shoots can be influenced by internal hormones in the explants.

3.4 Efficiency of shoot multiplication of 'Srivijaya' hybrid pineapple in different culture system

Shoot multiplication of explants will grow well if diffusion and absorption of nutrition elements are efficiently performed by phloem and xylem tissues. Nevertheless, the type of culture system had a different impact on it due to the different processes. The relationship between nutrient absorption in culture media and its efficiency can be seen from its efficiency. Efficiency can be determined by the number of explants per milliliter of medium.

Table 6 Efficiency of shoot multiplication of 'Srivijaya' hybrid cultured in solid media for 4 weeks after culture

Media	First		Last		Efficiency
	∑shoot	∑shoot/ml	∑shoot	∑shoot/ml	
1/2 MS+ 0.5 mg/L BA	5	0.200	17.8	0.70	3.50
1/2 MS+ 1 mg/L BA	5	0.200	21.5	0.86	4.30
1/2 MS+ 2 mg/L BA	5	0.200	25.0	1.000	5.00
MS+ 2 mg/L BA	5	0.200	30.3	1.210	6.05
				Average	4.71

3.4.1 Shoot multiplication efficiency of 'Srivijaya' hybrid pineapple in solid culture

The average efficiency value of 'Srivijaya' pineapple shoot multiplication in solid culture reached 4.71 (Table 6). These results indicated that the addition of BA was relevant to the increase in multiplication of 'Srivijaya' pineapple shoot. The multiplication efficiency of 'Srivijaya' pineapple shoots on MS + 2 mg/L BA was 6.05. The efficiency of MS medium with full strength concentration in solid culture was greater than that of 1/2 MS. The solid medium system used agar binds water and adsorbs compounds from the media, it was thought that it may limit the uptake of growth regulators due to the hard texture of media. Hence, it impacts to the shoot multiplication efficiency.

Table 7 Efficiency of shoot multiplication of 'Srivijaya' hybrid pineapple in liquid culture for 4 weeks after culture

Media	First		Last		Efficiency
	∑shoot	∑shoot/ml	∑shoot	∑shoot/ml	
1/2 MS+ 0.5 mg/L BA	5	0.05	21.75	0.21	4.20
1/2 MS+ 1 mg/L BA	5	0.05	23.50	0.23	4.60
1/2 MS+ 2 mg/L BA	5	0.05	22.50	0.22	4.40
MS+ 2 mg/L BA	5	0.05	34.75	0.34	6.80
				Average	5.13

3.4.2 Shoot multiplication efficiency of 'Srivijaya' hybrid pineapple in liquid culture

Media with a half strength MS concentration of 0.5 mg/L, 1 mg/L, and 2 mg/L BA showed that the difference in efficiency values obtained did not reach 1 (Table 7). MS + BA 2 mg/L treatment gave higher efficiency value of 2.4 compared to 1/2 MS + 2 mg/L BA treatment. The presence of BA was essential for inducing shoots *in vitro*. The results showed that MS + 2 mg/L BA gave the highest efficiency value (6.80). This efficiency value indicated that MS with half strength concentration could not match the efficiency of MS with full strength concentration in liquid culture.

Table 8 Efficiency of shoot multiplication of 'Srivijaya' hybrid pineapple in bioreactor for 4 weeks after culture

Media	First		Last		Efficiency
	∑shoot	∑shoot/ml	∑shoot	∑shoot/ml	
1/2 MS+ 0.5 mg/L BA	10	0.06	51.5	0.34	5.66
1/2 MS+ 1 mg/L BA	10	0.06	76.0	0.50	8.33
1/2 MS+ 2 mg/L BA	10	0.06	95.5	0.63	10.50
MS+ 2 mg/L BA	10	0.06	124.5	0.83	13.83
				Average	9.58

3.4.3 Shoot multiplication efficiency of 'Srivijaya' hybrid pineapple in bioreactor

MS + 2 mg/L BA had the highest efficiency with a value of 13.83. It was followed by the treatment of 1/2 MS + 2 mg/L BA with an efficiency value of 10.50 (Table 8). The two efficiency values of the two treatments indicate that the same BA concentration at different MS concentrations affects the resulting efficiency values.

The shoot multiplication efficiency of 'Srivijaya' hybrid pineapple showed different values in solid, liquid and bioreactor culture systems. The results showed that the culture systems with the greatest efficiency were the bioreactor, with an average efficiency value of 9.58. The MS + 2 mg/L BA gave the highest efficiency value of all treatments in different *in vitro* culture systems (Figure 2 and 3).

Figure 2 Plantlets after 30 days cultured in different systems: (A) solid culture; (B) liquid culture and (C) bioreactor

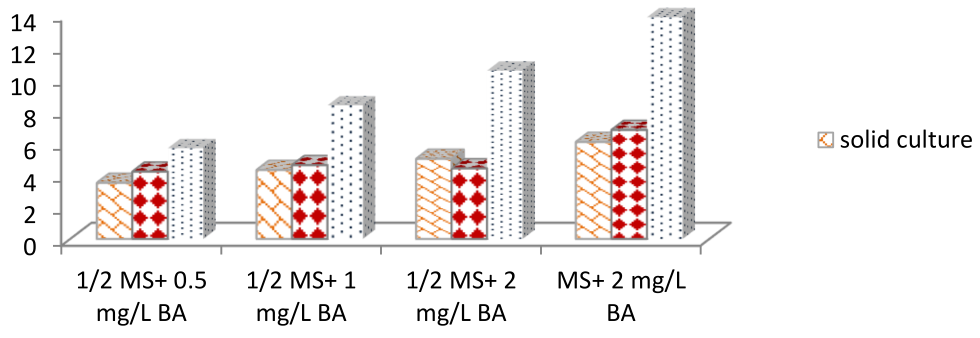

Figure 3 Efficiency of shoot multiplication of 'Srivijaya' pineapple variety in 3 *in vitro* cultures: solid; liquid and bioreactor

MS + 2 mg/L BA in the bioreactor made it possible to produce 958 shoots per 1 liter of medium for 30 days after culture. Other treatments have not been able to provide more performance than the bioreactor system. This was agreed to the study by Reis *et al*, who revealed that BA at the concentration of 2 mg/L in bioreactor was an efficient treatment for the multiplication and growth of ornamental pineapple plants. This study found that the bioreactor increased the multiplication rate 2.03 times better than the solid culture system.

4. Conclusion

4.1 Nutrient reduction to half strength MS concentration and BA reduction could not increase the total number of shoots. MS + 2 mg/L BA medium gave the highest total number of shoots being 30.25, 34.75 and 124.5 shoots in solid culture, liquid culture, and bioreactor, respectively.

4.2 Bioreactor system was obtained the highest efficiency value *in vitro* propagation of 'Srivijaya' hybrid pineapple shoots.

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