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BIOMASS PRODUCTION OF ROOT AND SHOOT OF *Talinum Paniculatum* Gaertn. BY LIQUID AND SOLID MS MEDIUM WITH PLANT GROWTH HORMONE IBA

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ABSTRACT

Talinum paniculatum Gaertn. is one of traditional medicinal plant in Indonesia which has benefits such as for vitality and maintain blood circulation. The aim of this research is to obtain biomass production of root and shoot of *T. paniculatum* Gaertn. by liquid and solid MS medium with IBA. This research conducted to provide biomass as raw material for secondary metabolites test. Stems as explant were induced with four treatments (liquid MS, solid MS, liquid MS + 2 ppm IBA and solid MS + 2 ppm IBA) with five replicates. Observation has done for 28 days. The parameters are the percentage of explants which formed the root and shoot, morphology, fresh and dry biomass. Result shows that percentage of root and shoot have 100% in liquid and solid MS + 2 ppm IBA. Fresh biomass of root and shoot in solid MS + 2 ppm IBA were 5.929 g and 5.351 g. Dry biomass of root and shoot in solid MS + 2 ppm IBA were 0.623 g and 0.562 g. This research found callus in liquid and solid MS + 2 ppm IBA. Morphology of root in liquid MS + 2 ppm IBA has thin, long and friable, but thick in solid MS. Shoot in solid and liquid MS has thin, short and sturdy.

1. Introduction

Prakash (1993) classified *in-vitro* culture medium into two groups such as solid and liquid medium. Solid medium was prepared from adding agent of solidifying into the liquid medium. Several studies showed that many brands and grades of agar such as agarose, phytigel and gerlite, which are used as solidifying agents (Debergh, 1983; Prakash *et al.*, 2000). Mohamed *et al.* (2009) described adding those agents into liquid medium can increase viscosity and reinforce that explants in medium.

Plant growth hormones like auxins are an important class in all aspects of growth and development. Davies (1995) and Hobbie (1998) described that physiological and genetic studies have shown auxins to be involved enlargement and division of cell, lateral branching of roots and shoots, vascular differentiation and early embryonic development. Indole-3-butyric acid (IBA) is widely used in agriculture because it induces rooting. Early studies that examined the effects of exogenous auxin application found IBA to be more effective than IAA in promoting the formation of adventitious root (Ludwig-Muller, 2000). Nordstrom *et al.* (1991) has demonstrated that internal IBA levels increase and stay elevated during IBA-induced root formation. Kim *et al.* (2003) reported that IBA able to increase root accumulation of *Panax ginseng*. Lulu *et al.* (2015) explained that treatment with IBA made the roots to be more thick and long, in contrast with NAA made the roots to be more thin and short. Hartmann *et al.* (1997) explained that IBA had become the preferred auxin to induce root formation on cuttings and in tissue culture.

T. paniculatum Gaertn. (Portulacaceae family) has a bulging shape of the roots such as root of *Panax*

ginseng and used as traditional medicinal plant in Indonesia namely Java ginseng or Java som. It has many functions such as for vitality and maintain blood circulation. Beside, these functions can increase testosterone levels, number and motility of sperm at the low testosterone condition. One of the chemical compound of the roots is saponins which were used as aphrodisiac (vitality). Root of java ginseng grew very slowly in their natural habitat. Further, it has known that saponins level of java ginseng roots of three months was lower than the java ginseng roots growing *in vitro* for 28 days (Manuhara *et al.*, 2015). Beside, shoots or leaves of *T. paniculatum* Gaertn. also used to similar function. So, aim of this research is to obtain biomass production of root and shoot of *T. paniculatum* Gaertn. by liquid and solid MS medium with IBA. This research conducted to provide biomass as raw material for secondary metabolites test.

2. Material and method

2.1. Plant Material and Optimization Culture Conditions

The study was carried out in the plant physiology laboratory at Departement of Biology, Faculty of Science and Technology, Airlangga University between March-June 2016. Root and shoot were induced from stem segments (2 cm in lengths) of *T. paniculatum* Gaertn. on solid MS (Murashige and Skoog's, 1962) gelled (0.7% agar, w/v) medium and liquid MS supplemented with 30 gL⁻¹ sucrose; 2 ppm indole-3-butyric acid (IBA) (Manuhara *et al.*, 2014; Manuhara *et al.*, 2015). Different types of cultures namely solid and liquid MS medium supplemented with 2 ppm IBA to verify the suitable culture method for accumulation of biomass. Stems as explant were induced with four treatments (liquid MS, solid MS, liquid MS + 2 ppm IBA and solid MS + 2 ppm IBA) with five repetitions. Set of experiments were established solid and liquid MS medium supplemented 30 mgL⁻¹ sucrose without IBA. In another set of experiment, solid and liquid MS medium supplemented 30 mgL⁻¹ sucrose and 2 ppm IBA. The cultures were established in 300 ml flasks containing 50 ml MS medium. The solid MS medium was modified with spon (thick of 1,5 cm) (Fig. 1D-1F). The liquid MS medium cultures were kept under continuous agitation at 100 rpm on the shaker. All the cultures were maintained for 28 days at 25 ± 2°C, with a 16 hour light (40 μmol m⁻² s⁻¹)/8 hour dark photoperiod cycle provided by 40-W white fluorescent tubes. The growth parameters on fresh and dry biomass were assessed at 4 weeks after cultivation.

2.2. Determination of Root and Shoot Biomass and Morphology

After 4 weeks of culture, the adventitious root and shoot were separated from the medium through a stainless steel sieve and washed with distilled water and dried at 60°C for 48 hour. Each 7 days of culture, the root and shoot were observed from nodal stem. All of root and shoot were recorded until 28 days.

2.3. Measurement of Conductivity, Total Sugar and Hydrogen Ion Concentration in The Medium

Measurement was conducted using liquid MS medium. The electrical conductivity (EC) was measured using a conductivity meter (Ezdo, Cond 5021, Taiwan), total sugar was measured using a refractometer (Atago, Japan) and the hydrogen ion concentration (pH) of the culture medium was measured using a pH meter (Boeco, BT-600, Germany) at the beginning and the end of culture.

2.4. Statistical Analysis

All experiments were set up in a completely randomized design and the data were collected from five replicates and mean values are subjected to Duncan's multiple range test using SPSS software (version 17.0).

3. Result and discussion

3.1. Effect of Culture Method on Production of Root and Shoot Biomass and Morphology

The present study showed that the growth of roots and shoots of *T. paniculatum* Gaertn. in solid and liquid MS medium. roots and shoots revealed that its proliferation and biomass accumulation were more efficient in solid MS medium supplemented 2 ppm IBA (Table 1) (Fig. 1). To verify the suitability of culture methods, solid and liquid MS medium were used in the present study. All (100%) explants that grown in solid MS medium supplemented with 2 ppm IBA produced roots and shoots, on the other hand using liquid MS medium there was no shoot formed from explant. In solid MS medium with 2 ppm IBA, the fresh and dry biomass accumulation of root were 5.929 g and 0.623 g. Besides, the fresh and dry biomass accumulation of shoot were 5.351 g and 0.562 g, respectively than liquid medium. (Table 1).

Table 1. Biomass and percentage of root and shoot of *T. paniculatum* Gaertn. after 28 days of culture

Treatments	Fresh Biomass (g)		Dry Biomass (g)		Percentage (%)	
	Root	Shoot	Root	Shoot	Root	Shoot
Liquid MS	2.224	1.346	0.178	0.114	60%	40%
Solid MS	1.789	2.886	0.268	0.476	60%	60%
Liquid MS + 2 ppm IBA	4.862	2.301	0.438	0.198	100%	60%
Solid MS + 2 ppm IBA	5.929	5.351	0.623	0.562	100%	100%

Data represents mean values (n=5), Mean separation within column by Duncan's multiple range test at $P \leq 0.05$

In this study, the stem segments of *T. paniculatum* Gaertn. whose were induced with IBA showed that solid medium produced greater numbers of roots and shoots per explants (Table 1) (Fig 3). This result was contradicted with the plant tissue in liquid medium culture are continuously submerged permitting efficient nutrient and hormones uptake which improved the growth of the plantlets (Yan *et al.*, 2010) and liquid culture provides better homogenization. The highest of roots and shoot biomass in solid medium compared with liquid culture (Table 1).

Morphology of root in liquid MS medium supplemented with 2 ppm IBA was thin and friable. Shoot has founded in solid, liquid and solid MS medium supplemented with 2 ppm IBA. Further, shoots in liquid and solid MS were thin, short and sturdy, meanwhile in solid MS supplemented with 2 ppm IBA was thick, long and sturdy (Table 2). Roots growth in solid medium have grown on bottom, meanwhile roots on liquid medium were in upper (Fig 2B and 2E). The shoots have grown in nodal stem (Fig. 2A – 2C).

Table 2. Morphology of root, shoot and callus in four treatments after 28 days of culture

Treatments	Morphology		
	Root	Shoot	Callus
Liquid MS	-	Thin, short, sturdy	Friable, greenish
Solid MS	-	Thin, short, sturdy	-
Liquid MS + 2 ppm IBA	Thin, long, friable	-	Friable, green-brownish
Solid MS + 2 ppm IBA	Thick, long, friable	Thick, long, sturdy	Friable, green-brownish

Biomass production was influenced by concentration of MS medium (Cui *et al.*, 2014; Baque *et al.*, 2014), culture method (Cui *et al.*, 2014), sucrose (Mariateresa *et al.*, 2014) and plant growth hormone (Lulu *et al.*, 2015). Study of Kuria *et al.* (2008), demonstrated that the liquid medium with supporting material such as luffa (*Luffa acutangula*), filter paper and double phase have given more shoots and more leaves per plantlet than that in solid medium. The authors reported biomass accumulation in liquid medium higher than in solid medium. In several studies, it had been reported that the use of liquid media frequently enhanced the growth of shoot and root (Sandal *et al.*, 2001; Preece 2011). However, in this study showed contradiction in liquid and solid medium for *T. paniculatum*. The stem segments of *T. paniculatum* as explants resulted biomass production of adventitious root in solid medium with IBA is higher than liquid medium with or without IBA.

In Addition, IBA has potent for induction of

root formation rather than IAA or synthetic auxins (Ludwig-Muller, 2000). Nordstrom *et al.* (1991) has demonstrated that internal IBA levels increase and stay elevated during IBA-induced root formation. Yang and Davies (1999) showed further evidence of IBA for basipetal transport. IBA can stimulate elongation of subtending nodes, suggesting IBA is transported basipetally in intact pea plants and it has been examined the radiolabeled distribution after application of a rooting solution to the explants base. Zolman *et al.* (2000) has examined the IBA and IAA sensitivity at root elongation and lateral root formation of *Arabidopsis* developmental processes. Rashotte *et al.* (2003) have examined the hypocotyl elongation sensitivity to IBA and IAA using hypocotyls grown in dark, low, or high light. IBA is potent to stimulate hypocotyl elongation in high-light conditions at concentrations ranging from 1 to 10 μm , with 50% stimulation at a concentration of 3 μm IBA. In similar, Poupart and Waddell (2000) demonstrated that specificity of IBA transport tissue

can support the possibility of endogenous auxin in growth and development of some *Arabidopsis* tissues. IBA can inhibit root elongation and induces lateral root formation. Furthermore, IBA affects stem elongation in seedlings of pea, but its effect has not been examined on *Arabidopsis* hypocotyl elongation previously. The authors examined the growth sensitivity of *Arabidopsis* hypocotyls to IAA and IBA. In high-light conditions at low concentration of IBA, hypocotyls were sensitive to growth stimulation, but insensitive to growth stimulation if dark or low light (Rashotte *et al.*, 2003).

Adelberg *et al.* (2010) had shown that with gelling agents the nutrients dissolved in the liquid phase are not completely available as water is bound to the gel by a matrix force. That study compared the spent gelled medium and spent the liquid medium and revealed that nutrients remained in agar, and therefore nutrients were somehow unavailable to be uptaken to the explant plant. It also has been shown that in presence of agar, the resistance of sucrose transfer from medium to the plant is 300×

greater than that of sucrose transfer from a shaken liquid to the plant per unit of surface area (Adelberg and Fári, 2010). Liquid culture is often associated with hyperhydricity such as a physiological disorder characterized by high water retention capacity due to adverse culture conditions and was detected in the study of Shaik *et al.* (2010).

The results showed that roots and shoots in the liquid medium present hyperhydricity symptoms. The fresh biomass of roots and shoots were differ between treatments, however the number of roots and shoots per explants were higher in solid medium culture supplemented with IBA (Table 1) (Fig. 1).

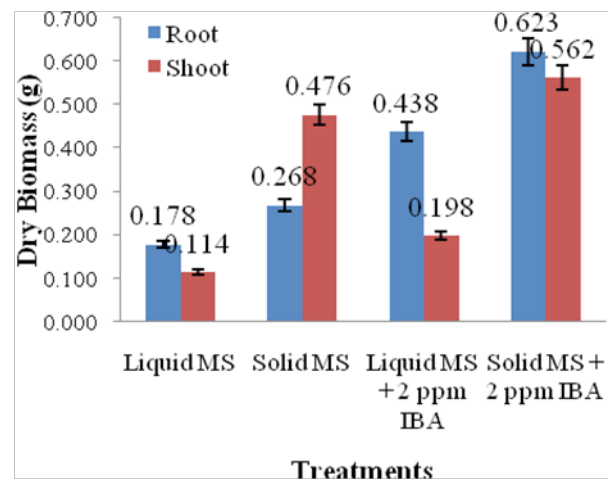
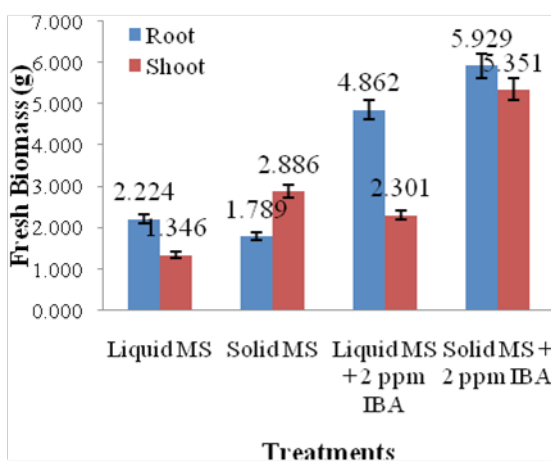


Figure 1. Fresh and dry biomass of roots and shoots of *T. paniculatum* Gaertn. after 28 days on four treatments. Data are presented as the means \pm standard error, n=5

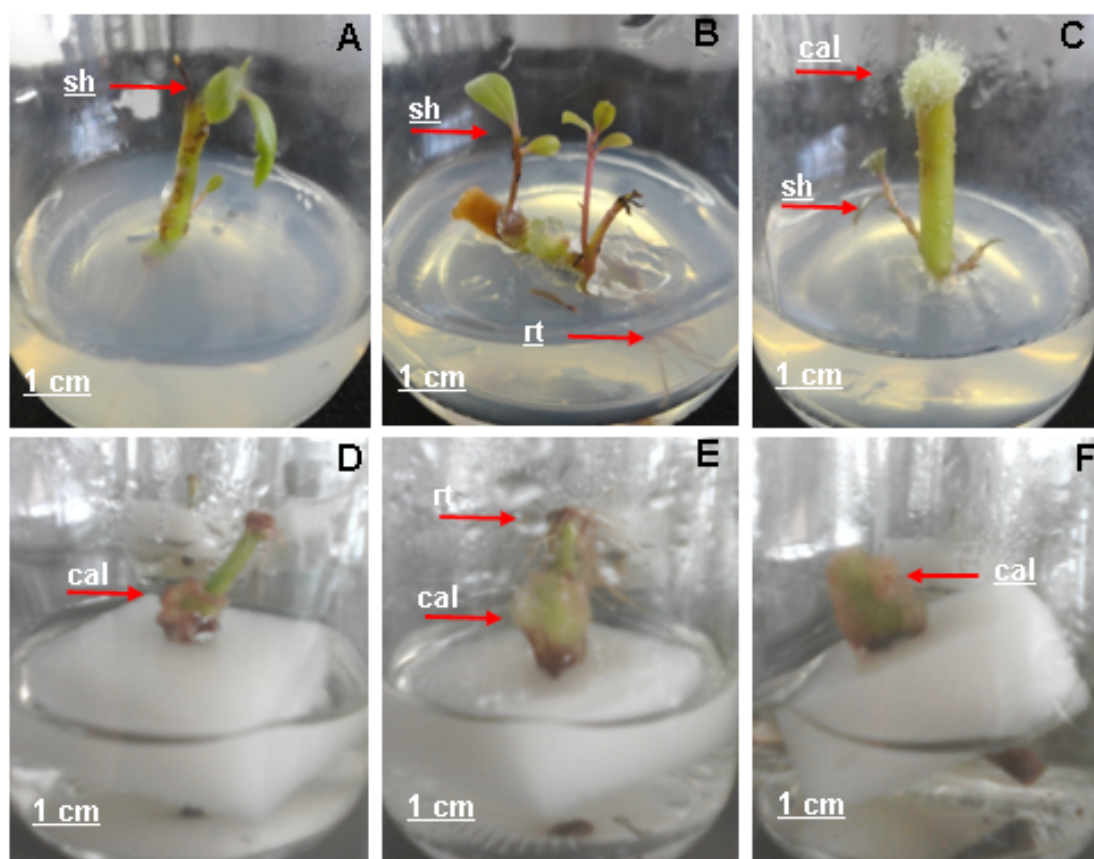


Figure 2. The growth of stem segment explants of *T. paniculatum* Gaertn. after 14 days *in vitro* culture: (A) Shoot formed from stem segment on solid MS medium with 30 gL⁻¹ sucrose, (B-C) shoot and callus formed from stem segment supplemented with 30 gL⁻¹ sucrose and 2 ppm IBA, (D) Callus formed on liquid MS with 30 gL⁻¹ sucrose, (E-F) root and callus formed on liquid MS medium with 30 gL⁻¹ sucrose and 2 ppm IBA. rt ; root, sh ; shoot, cal ; callus

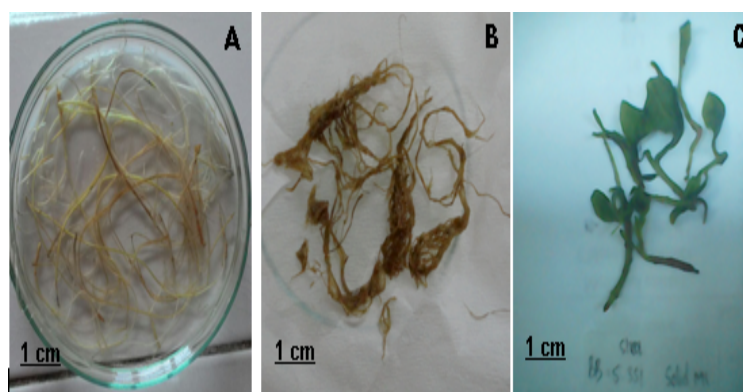


Figure 3. Accumulation of biomass production in shoot culture of *T. paniculatum* Gaertn. at 28 days after cultivated on solid MS supplemented with 30 gL⁻¹ sucrose and 2 ppm IBA : (A-B) fresh biomass of roots

The electrical conductivity of liquid MS in this study is not shown. Several studies described that liquid medium was examined by electrical conductivity, pH and total sugar. The high conductivity of medium showed that the adventitious root unavailable to absorb inorganic compounds. Thanh *et al.*, (2006) explained that electrical conductivity was used as indirect method to estimated of biomass in the cell culture, because it was reflected of uptake inorganic compounds by the cell during cultivation. Decreasing organic compounds showed the activity of cell has been working and increased biomass of cell. Further, this condition were supported by the decreasing pH of medium at the end of cultivation. Decreasing pH can cause absorption ability of cell is low to absorb inorganic compound from the medium (Manuhara *et al.*, 2015). That decreasing pH during adventitious root culture was caused by MS medium contain ammonium. Ammonium nitrate as source of nitrogen and

that necessary as a buffer. The cell can release H⁺ when its need nitrogen from ammonium. Releasing of H⁺ continuously into medium caused acid condition. Source of carbon in both MS medium (liquid and solid) is sucrose. It had been hydrolyzed to glucose and fructose in the beginning of culture. During cultivation, cells have consumed monosaccharides, so measurement of total sugar were reflected by the growth of cell growth (Gorret *et al.*, 2004).

5. Conclusion

The present study has successfully established root and shoot biomass of *T. paniculatum* in solid and liquid MS medium. The best treatment was in solid MS with 2 ppm IBA. The fresh and dry biomass accumulation of root were 5.929 g and 0.623 g higher than the others. This research found callus in liquid MS + 2 ppm IBA. Root morphology in liquid MS was thin and friable, but in contrast it was thick in solid MS. Shoots in solid and liquid MS were thin, short and sturdy.

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