Propagation of Sukun (*Artocarpus altilis* (Parkinson) Fosberg) through *In Vitro* Shoot Proliferation

Maria Imelda*, Aida Wulansari and Laela Sari
Research Centre for Biotechnology, Indonesian Institute of Sciences (LIPI), Cibinong16911
** maria_tahardi@yahoo.com*

ABSTRACT

Sukun (*Artocarpus altilis* (Parkinson) Fosberg) of the Moraceaae is a big tree which can grow to 15-20 m in height and native to the Asia-Pasific region. Beside its delicious fruit, sukun is also known as a traditional herbal medicinal plant in the region, including Indonesia. Nearly all parts of the plant, such as roots, stems and leaves are believed by local communities to be capable of curing liver disease, hypertension, cardiac arrest, toothache, renal problem and even skin itchiness. The collaborative research between LIPI and PR China, on developing herbal medicines indicated that sukun has a great potential for treating cardiovascular disease. However, the availability of raw materials still poses a big constraint for the industry of herbal medicines. Generally, sukun is propagated by root or stem cuttings, since in Indonesia sukun does not produce any seeds. However such method only produces limited planting materials. In general tissue culture propagated plants have many advantages, namely being clonal, free from pest and diseases, more uniform, and allowing a high rate of plant multiplication. Therefore, the technique for sukun propagation has been developed by the LIPI Research Centre for Biotechnology. In this research the effects of 1-5 mg/l benzyl amino purine (BAP) and 20-40 mg/l adenine sulphate (AS) on shoot bud proliferation were investigated using lateral shoot buds on a modified Murashige and Skoog (MS) medium with addition of 150 ml/l coconut water(CW). Shoots were rooted on MS medium without plant growth regulators (PGRs). The results showed that the best medium for *in vitro* shoot proliferation was a modified MS medium containing 2 mg/l BAP, 40 mg/l AS and 150 ml/l CW. The best medium for rooting is MS medium containing 1 mg indole butyric acid (IBA), producing roots within 3 weeks.

Keywords : Sukun (*Artocarpus altilis*), *in vitro* shoot proliferation, Benzyl amino purine (BAP), Adenine sulphate (AS), coconut water (CW), Indole butyric acid (IBA)

INTRODUCTION

Sukun (*Artocarpus altilis* (Parkinson) Fosberg) of the Moraceaae is a big tree which is 15-20 m in height and native to Asia-Pasific region. Beside its delicious fruit, sukun is also used as a traditional herbal medicine in the region, including Indonesia. Nearly all parts of the plant, such as roots, stems and leaves are believed to be capable of curing liver disease, hypertension, cardiac arrest, toothache, renal problems and even skin itchiness by local communities (Wang *et al.*, 2007 and Amarasinghe *et al.*, 2008).

It has been reported that the plant contains active compounds of the flavonoid group such as cycloaltilisin, artomunoxanthotrione epoxide, cyclocommunol, cyclomulberrin, cyclocommunin, artocarpanone (Wang *et al.*, 2007) and artonins B, cyclochampedol, artoindonesianin L, P, X, Y, U and V (Jennie *et a*l, 2009). The collaborative research between LIPI and PR China, in developing herbal medicines has shown the potential of flavonoid and phytosterol of sukun leaves for treating cardiovascular disease similar to aspirin as antithrombosis and *Ginko biloba*, which has been marketed as a cardiovascular medicine (Jennie *et al*, 2009). At present, the availability of raw

materials (leaves) from sukun for the herbal industry is very limited. An extensive sukun plantation has to be developed to ensure a continuous supply of leaf materials. For that purpose, a large number of superior planting materials are needed.

In Indonesia, sukun is generally triploid (3n + 84) and seedless although it has a diploid variety (2n = 56). Conventionally, sukun is propagated by root or stem cuttings. However, such method can only produce limited and low quality (not uniform) planting materials.

In line with the global trend of "*back to nature*", the use of medicinal plants as herbal materials is significantly increasing. Industries which use herbal materials for medicines, cosmetics or healthy food and beverages have developed rapidly. In Indonesia, herbal medicines have long been used since centuries ago for curing several diseases.

In this reseach, tissue culture technique, which has many advantages, such as being clonal, free from pest and diseases, providing a high rate of multiplication, will be developed to produce high quality planting materials of sukun. The objective of this research is to develop an efficient and effective method for the propagation of sukun through *in vitro* shoot proliferation.

Fig 1 : (a) Mature plant of sukun bearing young fruits; (b) Young shoot as a source of explant

MATERIALS AND METHODS

a. Preparation and inoculation of explants

Shoots about 10 cm in length from branch cuttings of sukun grown in the glass house, were cut into 1.5-cm segments containing a lateral shoot bud. The segments were washed in running tap water, immersed in 70% ethanol for 1 minute, then rinsed several times with distilled water.

Under a laminar air flow cabinet, the branch segments were further sterilized in 0.075 % $HgCl_2$ with a drop of soap solution (Tween 20) for 15 minutes, then rinsed three times with sterile distilled water. The shoot-buds were disected and inoculated on a modified MS agar-solidified medium (Murashige and Skoog, 1962).

All cultures were incubated in a growth room at 25ºC and under a 16-h photoperiod with a light intensity of 30 µmol/m2/sec provided by cool white influorescent tubes. Subculture to the same medium was done every 4 weeks.

b. Preparation of medium

A basal MS medium containing 3% sucrose and 2.3 g/l Gelrite was used in the experiment. The pH of the medium was adjusted to 5.8 by the addition of 1 N KOH or 0.1 N HCl before autoclaving at 121° C for 15 min at 1 atm.

c. Treatments

Shoot proliferation was induced by the addition of 1-5 mg/l Benzyl Amino Purine (BAP) in combination with 20-40 mg/l adenine sulphate (AS) and 150 ml/l coconut water (CW) into the medium. Totally there were 16 treatments with 3 replicates. Rooting of shoots was induced in MS medium with the addition of 0.5 -1 mg/l indole butyric acid (IBA).

d. Acclimatization

Rooted plantlets were removed from culture, rinsed in running tap water to remove the agar medium and planted in plastic pots containing soil and cocopeat (1:1). The potted plants were watered to saturation and covered with transparent plastic bags for about 2 weeks and placed in the greenhouse.

e. Observations

Observations were done at intervals of 4 weeks on the number of shoots produced by each treatments. The data were statistically analyzed using the Least Significant Difference (LSD) test

RESULTS AND DISCUSSION

Establishment of explant in culture began with the swelling of a shoot-bud followed by the appearance of a new shoot about 7-10 days after culture (Fig.1 a). In the medium containing BAP, multiple shoots were developed (Fig.1 b).

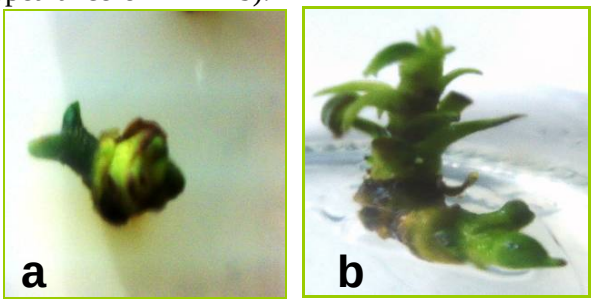

Figure 1 : Establishment of explants on modified MS medium

Woody plant (WP) medium was reported to be the optimal medium in terms of survival, root production and shoot growth of sukun. The addition of 2.5 mg/l BAP to the WP medium could enhance shoot multiplication (Tuia *et al*, 2000). In line with this report, Rouse-Miller & Duncan (2000), cultured shoot tip explants from mature tree of sukun on Murashige & Skoog (MS) basic medium with the addition of sucrose (30 g/L), benzyladenine (0.99 mg/L) and agar (8 g/L). This research also proved that MS medium (Murashige & Skoog, 1962) could also be a good medium for sukun micropropagation.

The role of cytokinin such as BAP in plant tissue culture has long been known in inducing cell division, release of lateral bud dormancy, inducing elongation of lateral buds and adventive shoot formation (Gaspar *et al*, 1996, Gaspar *et al*, 2003). BAP is the most effective cytokinin in inducing *in vitro* shoot formation and proliferation in many plant species, such as *Phaseolus vulgaris* (Malik & Saxena, 1992), buah merah (*Pandanus conoideus*) (Imelda *et al*, 2008), *Sida cordifolia* (Sivanesan & Jeong, 2007).

In this experiment, the addition of BAP to the MS medium affected shoot formation significantly. Without BAP, only a single shoot was produced within 1 month.. However, in the medium containing BAP, the number of shoots increased up to 2 mg/l BAP, and then decreased thereafter (Table 1). The

highest shoot number was obtained in the medium containing 2 mg/l BAP, 20 mg/l AS and 150 ml/l coconut water, producing 4,33 shoots after 1 month and 11,44 shoots after 2 months of culture. From 18 shoots, Rouse-Miller & Duncan (2000) produced 162 shoots every 6 weeks.

Table 1 . The effects of BAP, AS and coconut water on the number of *in vitro* shoots of sukun

No	Treatment	*In vitro* shoots number	
		1 month	2 months
1	Control	0,00 [a]	1,56 [abc]
2	B1K	0,44 [ab]	1,89 [abcd]
3	B2K	1,00 [abcd]	3,00 [abcd]
4	B3K	1,33 [abcdef]	3,56 [abcd]
5	B4K	2,22 [bcdef]	2,67 [abcd]
6	B5K	1,56 [abcdef]	2,67 [abcd]
7	B1A2K	2,67 [cdefg]	5,78 [def]
8	B2A2K	4,33 [g]	11,44 [f]
9	B3A2K	3,33 [fg]	7,22 [ef]
10	B4A2K	3,22 [efg]	5,22 [bcdef]
11	B5A2K	3,00 [defg]	5,44 [cdef]
12	B1A4K	1,22 [abcde]	3,00 [abcd]
13	B2A4K	0,89 [abc]	1,89 [abcd]
14	B3A4K	0,44 [ab]	1,44 [ab]
15	B4A4K	0,33 [ab]	0,89 [a]
16	B5A4K	0,22 [ab]	1,22 [a]

Note : Means within column followed by the same letters are not significantly different according to LSD test at 5% level
B = BAP; B1 = 1 mg/l; B2 = 2 mg/l; B3 = 3 mg/l; B4 = 4 mg/l; B5 = 5 mg/l;
A = Adenine Sulphate, A2 = 20 mg/l; A4 = 40 mg/l

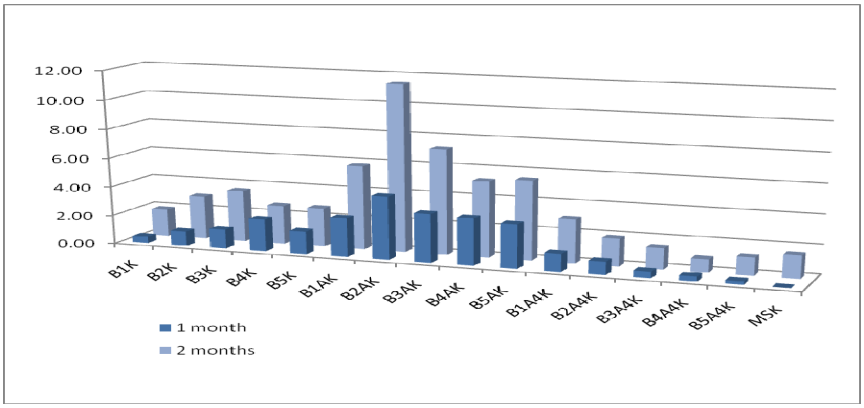

Figure 2 **:** The effects of BAP, AS and coconut water on the umber of *in vitro* shoots of sukun

It has been reported that adenine has a cytokinin activity in that it improves the action of cytokinin in the medium (Gatica Arias, 2010). Adenine sulphate (AS) has

been used for *in vitro* shoot multiplication of pawpaw (Carica papaya) (Saha *et al*, 2004), banana (Musa balbisiana x paradisiaca) (Imelda, 1991), and common bean (*Phaseolus vulgaris*) (Gatica Arias *et al*, 2010). The benefit of AS was often only noticed when it was associated with cytokinins like BAP or TDZ (van Staden *et al*, 2008). In the present study, the addition of AS at 2 concentrations (20 and 40 mg/l) in the presence of BAP did not produce a relevant increase in shoot number of sukun. Increasing the concentration of AS to 40 mg/l even decreased the number of shoots (Table 1, Fig. 3).

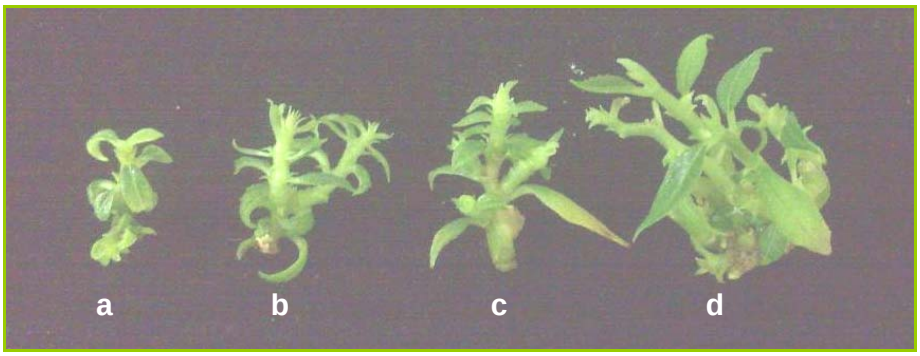

Figure 3 : *In vitro* shoots of sukun in modified MS medium after 2 months in culture,
a. Control; b. B2K; c. B2A4K; d. B2A2K

Coconut water is traditionally added to the medium as a growth supplement in plant tissue culture (Yong *et al*, 2009). Coconut water or coconut liquid endosperm at the level of 15 % was first successfully used in shoot tips culture of *Phalaenopsis* (Intuwong & Sagawa, 1974). Coconut water contains a large spectrum of biochemicals that act as growth factors individually or synergistically such as diphenylurea, zeatin riboside and zeatin which shows a cytokinin-like activity (Arditti & Ernst, 1993). The presence of biotin and folic acid in coconut water might be the other reason for its promotion of shoot proliferation and elongation (Kalimuthu *et al*, 2006).

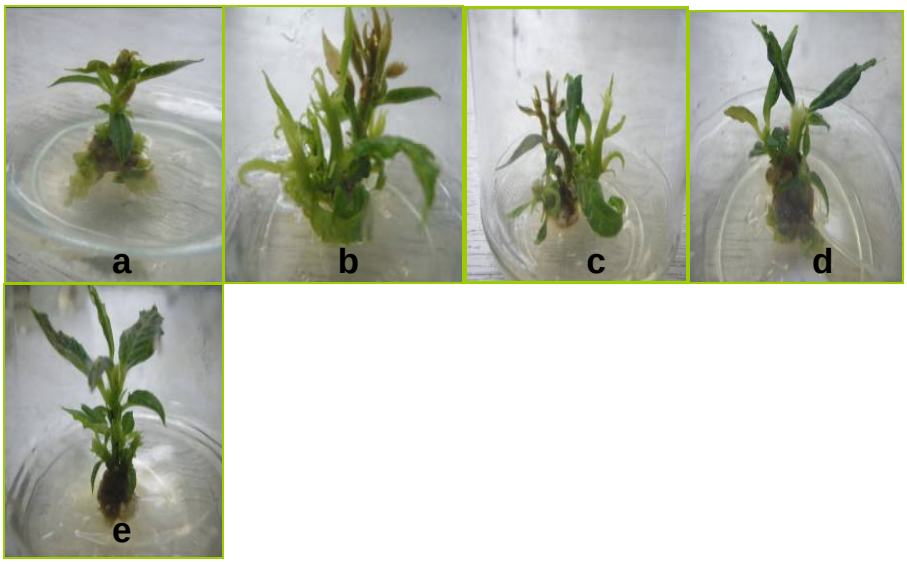

Figure 4 : *In vitro* shoots of sukun in modified MS medium after 2 months in culture, a. B1A2K b. B2A2K; c. B3A2K; d. B4A2K; e. B5A2K

AS may act as a synergist of cytokinins, where the presence of BAP (1-5 mg/l) in combination with AS (20 mg/l) and CC (150 ml/l) showed a synergic effect in enhancing growth of *in vitro* shoots of sukun (Fig. 4).

Indole butyric acid (IBA) was used to promote the formation of roots in plantlets and to generate new roots in cuttings. Rooting of *in vitro* shoots of sukun was successfully done on a medium containing indole butyric acid (IBA) at the concentration of 0.5 (Fig. 5.a1) and 1,0 mg/l (Fig. 5.a2). However, the best roots were produced at IBA concentration of 1.0 mg/l. More roots were produced after 4 weeks (Fig. 5 b). Rouse-Miller & Duncan (2000), got a success rate of 60 % root formation using a hormone-free MS medium with sucrose reduced to 20 g/l, while Mariska *et al* (2004), using WPM medium with 3 mg/l IBA for root production in sukun.

Acclimatization is an adaptation process for plantlets from *in vitro* condition where both temperature and humidity was under control, to the *ex vitro* green house condition which is more fluctuative. All regenerated plantlets from tissue culture need to be acclimatized since they are very sensitive to green house condition and easily attacked by diseases (Ziv, 1994). In this research , acclimatization of sukun plantlet was achieved on soil and coco peat medium (Fig. 5 c).

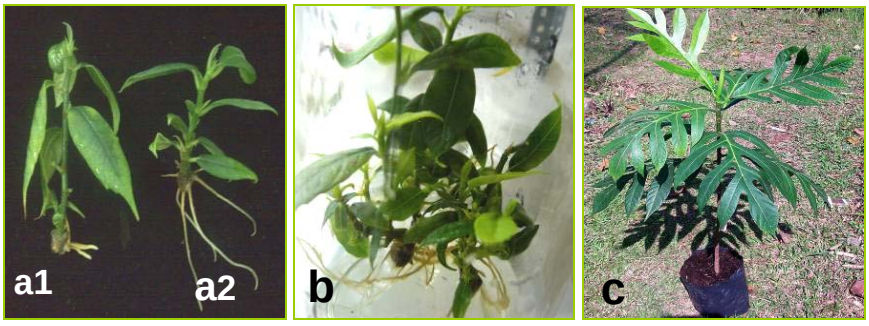

Figure 5 : a1. Rooted plantlet from MS + 0.5 mg/l IBA; a2. Rooted plant from MS + 1.0 mg/l IBA; b. Rooted plantlets after 4 weeks; c. Planting material before transplanted to the field

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REFERENCES

Amarasinghe NR, L Jayasinghe, N Hara & R Rujimoto. 2008. Chemical constituents of the fruits of *Artocarpus altilis*. Biochemical Systematics and Ecology 36 : 323-325

Arditti J & R Ernst. 1993. Micropropagaion of orchids. John Willey & Sons, Inc.. New York. 682 pp.

Gatica Arias AM, JM Valverde, PR Fonseca & MV Melara. 2010. *In vitro* plant regeneration system for common bean (*Phaseolus vulgaris*) effect of N6-benzylaminopurine and adenine sulphate. Electronic Journal of Biotechnology 13 (1) : 1-8.

Gaspar TH., C Kevers, C Penel, H Greppin, DM Reid & TA Thorpe. 1996. Plant hormones and plant growth regulators in plant tissue culture. *In vitro* Cellular and Developmental Biology Plant 32 (4) : 272-289.

Gaspar TH, C Kevers, O Faivre-Rampant, M Crevecoeur, C Penel, H Greppin & J Dommes.2003.Changing concepts inplant hormoneaction. *In vitro* Cellular and Developmental Biology Plant 39 (2) : 85-106.

Imelda, M. 1991. Penerapan teknologi *in vitro* dalam penyediaan bibit pisang. Prosiding Seminar Bioteknologi Perkebunan dan Lokakarya Biopolimer untuk Industri. PAU Bioteknologi IPB, Bogor : 71-79.

Imelda M, A Wulansari & Sumarnie. 2008. In vitro propagation of buah merah (*Pandanus conoideus* Lam). Annales Bogorienses n.s. 12 (1) : 39-43.

Intuwong O & Y Sagawa. 1974. Clonal propagation of *Phalaenopsis* by shoot tip culture. Am. Orchid Soc. Bull 43 :893-895.

Jenie U, LB Kardono, T.Mozef.2008. Potensi ekstrak daun sukun (*Artocarpus altilis*) sebagai obat herbal untuk kardiovaskular. Usulan Paten

Kalimuthu K, R Senthilkumar & N Murugalatha. 2006. Regeneration and mass multiplication of *Vanilla planifolia* Andr., a tropical orchid. Current Science 91 (10) : 1401-1403.

Malik KA & PK Saxena. 1992. Regeneration in *Phaseolus vulgaris* L : High-frequency induction of direct shoot formation in intact seedlings by N^6-benzylaminopurine and thidiazuron. Planta 186 (3): 384-389.

Mariska I, Y Supriati & S Hutami. 2004. Mikropropagasi sukun (*Artocarpus communis* Forrst.), Tanaman Sumber Karbohidat. Makalah disajikan pada Seminar Hasil Penelitian BB Biogen, Bogor, 188 hal.

Murashige T & F Skoog. 1962. A revised medium for rapid growth and bio-assays with tobacco tissue cultures. Physiol. Plant. 15: 473-497.

Rouse-Miller J & JE Duncan. 2000. *In vitro* propagation of *Artocarpus altilis* (Park.) Foosberg (Breadfruit) from mature plant material. *In Vitro Cellular and Developmental Biology. Plant* 36(2) : 115-117

Santalla M, J Power & MR Bavey. 1998. Efficient *in vitro* shoot regeneration responses of *Phaseolus vulgaris* and *P.coccineus*. Euphytica 102 (2) : 195-202.

Tuia VS, MB Taylor & D Ragone. 2000. Studies on *in vitro* culture of breadfruit cultivars in the Pacific. ISHS Acta Horticulturae 757

Van Staden J, E Zazimalova & EF George. 2008. Plant growth regulators II : Cytokinins, their analougues and antagonist.In : George, EF, M Hall and GJ De Kleck eds. Plant Propagation by Tissue Culture Vol I, The Background. Springer, The Netherlands : 205-226.

Wang Y, K Xu, L Lin, Y Pan & X Cheng. 2007. Geranyl flavonoids from the leaves of *Artocarpus altilis*. Phytochemistry 68 : 1300-1306.

Yong Jean W.H., LY Ge, YF Ng & SN Tan. 2009. The chemical composistion and biological properties of coconut (*Cocos nucifera* L.) water. Molecules 14 (12) : 5144-5164

Ziv M. 1994. *In vitro* acclimatization. In: J.Aitken-Christie, T.Kozai & M.A.L. Smith (Eds.). *Automation and Environmental control in Plant Tissue Culture*. Kluwer Academic Publisher, Dordrecht, 493-516.