

Effect of Carbohydrate Source on Growth and Performance of *In Vitro* Sago Palm (*Metroxylon sagu* Rottb.) Plantlets

SUMARYONO^{1*}, WIRDHATUL MUSLIHATIN², DIAH RATNADEWI³

¹Indonesian Biotechnology Research Institute for Estate Crops, Jalan Taman Kencana No. 1, Bogor 16151, Indonesia

²Faculty of Teacher Training and Education, Muhammadiyah University Surabaya,
Jalan Sutorejo 59, Surabaya 60113, Indonesia

³Department of Biology, Faculty of Mathematics and Natural Sciences, Bogor Agricultural University,
Kampus Dramaga, Bogor 16680, Indonesia

Received January 4, 2012/Accepted June 4, 2012

Sago palm (*Metroxylon sagu* Rottb.), grown mostly in the tropics, is one of the most productive carbohydrate-producing crops. However, it is still underutilized. Tissue culture of sago through somatic embryogenesis has been developed. The plantlets derived from somatic embryos, however, are usually weak with few leaves and roots and have low survival rates during acclimatization. Carbohydrate is commonly added into culture medium as an energy source and an osmotic agent. Research was conducted to determine a suitable carbohydrate for plantlets growth in order to produce vigorous plantlets of sago. The basal medium used was a modified MS medium with a half-strength of salts. Different types of carbohydrate (sucrose, maltose, glucose, and fructose) at various concentrations (30, 45, and 60 g/l) were added into the medium. A single 2 cm plantlet derived from somatic embryo was cultured on a culture tube. Each treatment consisted of 15 plantlets. The cultures were incubated in a culture room with light intensity at 20 $\mu\text{mol}/\text{m}^2/\text{s}$ and temperature at 26 °C. The results show that different types and concentrations of carbohydrate influenced the growth of sago plantlets significantly, but there was no interaction between the two factors. Sucrose was better than other types of carbohydrate, and the concentration of 30 g/l was better than concentrations of 45 or 60 g/l for the growth and vigor of sago plantlets. Medium with a sucrose level at 30 g/l gave the best performance of sago plantlets based on plantlet height, leaf number, biomass fresh weight, stem diameter, and rooting percentage.

Key words: carbohydrate, *in vitro* culture, *Metroxylon sagu*, plantlet growth, sago palm

INTRODUCTION

Sago palm (*Metroxylon sagu* Rottb.), native to Papua, Indonesia, is one of the most productive carbohydrate-producing crops, but it has not been grown commercially in a large area. Sago has long been a staple food for people in eastern parts of Indonesia especially in Papua and Moluccas. Apart from being raw material for food, sago starch is also used for noodles, white bread, high-fructose syrup, biodegradable filler in plastics, animal feed, adhesive, bioethanol and many other derivative products (Flach 1997).

Sago palm can be propagated by seeds and suckers. Seed production is rare because the palms are commonly harvested by cutting the trees just before flowering. Sago seed germination rate is also very low. Therefore, the most common propagation method of sago is by suckers. To establish large-scale plantations, the availability of uniform suckers is a major constraint (Jong 1995). In addition, the weights of good-sized suckers which are 2 to 5 kg (Rostiwati *et al.* 1998) make them more difficult to transport to other places.

Tissue culture is a promising alternative means to propagate superior sago palm clonally. The criterions of superior sago palm are high-yield starch production, quick bole maturation, high pith starch density, thin bark, and white starch (Flach 1997). There is a high variability in starch yield of sago in many areas in Indonesia which range from 150 to 700 kg, with the average yield of 300 kg fresh starch per trunk. If these high-yielding genotypes can be clonally propagated through tissue culture, the productivity of sago palm will increase significantly.

Tissue culture of sago palm has been established through zygotic embryogenesis (Hisajima *et al.* 1991) and somatic embryogenesis (Tahardi *et al.* 2002). Tahardi *et al.* (2002) used apical meristematic tissues from young suckers of sago. Riyadi *et al.* (2005) found medium compositions for somatic embryo induction, embryo maturation, and plantlet formation. Morphological variations in form, size and color of somatic embryos were found during its maturation (Kasi & Sumaryono 2007). High morphological variation of somatic embryos may inhibit *in vitro* plant mass propagation of sago (Riyadi *et al.* 2005).

The composition of the culture medium is one of the most important factors determining the growth of plants *in vitro*. Mineral salts and carbohydrates as carbon

*Corresponding author. Phone: +62-251-8327449,
Fax: +62-251-8328516, E-mail: sumaryonobogor@yahoo.com

sources are the major components of *in vitro* culture medium. Carbohydrate is an important component for plant growth and development *in vitro* where the conditions are not suitable for photosynthesis; thus, carbohydrate is supplemented as a carbon source to maintain carbon supply as well as to maintain osmotic potential of cells. Types of carbohydrate commonly used are sucrose, fructose, and glucose. Sucrose is widely used in plant tissue culture due to its most favorable effect on growth and relatively low cost. Novero *et al.* (2010) used sucrose and sorbitol in *in vitro* culture of sago palm and found that the best medium for explant growth was MS supplied with 22.5 g/l sucrose and 7.5 g/l sorbitol. They suggested that sago plantlet may withstand an environment with high solute concentration.

Based on the previous research, plantlets of sago palm derived from tissue culture were generally weak with only few leaves and roots. Consequently, the survival rate of plantlets during acclimatization to *ex vitro* conditions was very low. The objective of this research was to determine the type and concentration of carbohydrate suitable for producing vigorous plantlets of sago palm *in vitro*.

MATERIALS AND METHODS

Plant Materials and Medium. Somatic embryos of sago (Figure 1) were derived from apical meristematic tissues of young suckers of sago palm grown in South Kalimantan. Plant materials used for the experiment were plantlets without roots with the height of 1-2 cm derived from somatic embryos. Culture medium for plantlet growth was a solid modified MS medium (Tahardi *et al.* 2002) supplemented with 0.5 mg/l GA₃, 2 mg/l IBA, 3 mg/l NAA, 30 g/l sucrose, 1 g/l activated charcoal, and 2.8 g/l gelrite. The pH of the medium was adjusted to 5.7 before being autoclaved at 121 °C and 1 kg/cm² for 20 minutes. All cultures were incubated in the culture room at 25 °C under cool-white fluorescent lamps providing light intensity of 20 μmol/m²/s over a 12-h photoperiod.

Treatments and Observations. Treatments used were different types and concentrations of carbohydrate. Type of carbohydrate used was sucrose, maltose, fructose, and glucose at concentration of 30, 45, and 60 g/l. Each treatment was replicated 15 times; one replicate consisted of one culture tube containing one plantlet.

Observations were conducted on plantlet height, leaf number, plantlet diameter, plantlet fresh weight, and rooting frequency. Plantlet height and leaf number were determined every 2 weeks. Relative growth rate (RGR) for plantlet height and leaf number was also determined. The RGR was calculated from the additional growth (size) divided by the original size.

Experimental Design and Statistical Analysis. The experimental design used was a completely randomized design. Treatment of carbohydrate consisted of two factors (type and concentration). The data was analyzed with the general linear model (GLM) and subjected to analysis of variance (F test). If there was any significant difference, the differences among treatment means would be determined by Duncan's multiple range test at P = 0.05 using SAS version 9.1.

RESULTS

Plantlet Height RGR. All plantlets of sago in different types and concentrations of carbohydrate grew steadily during 12 weeks of culture (Figure 1c). Statistical analysis revealed no interactive effect between the types and the concentrations of carbohydrate to the RGR of the plantlet height. Plantlets cultured on a medium containing sucrose was higher than those of other carbohydrates (Figure 2). Carbohydrate at 30 g/l added to the medium gave the highest plantlets on average 5.1 cm, followed by carbohydrate at 60 and 45 g/l. The growth of plantlet on a medium with 30 g/l carbohydrate was 2.4 times its initial height whereas the addition of 45 g/l carbohydrate only increased 1.9 times from its initial height.

The results show that type and concentration of carbohydrate significantly affected RGR of sago plantlet height. Compared with other types and concentrations of carbohydrate, the RGR of plantlet heights (0.12-0.14) were higher on media containing glucose at 30 g/l and sucrose at 30 and 60 g/l. The medium containing sucrose produced the best RGR and the plantlets were also more vigorous.

Leaf Number RGR. The type and concentration of carbohydrate and its interaction significantly affected the leaf number RGR of sago plantlets. Media with sucrose produced higher RGR than other types of carbohydrate. The highest leaf number RGR was obtained from medium supplemented with 30 g/l sucrose (0.14) (Figure 3).

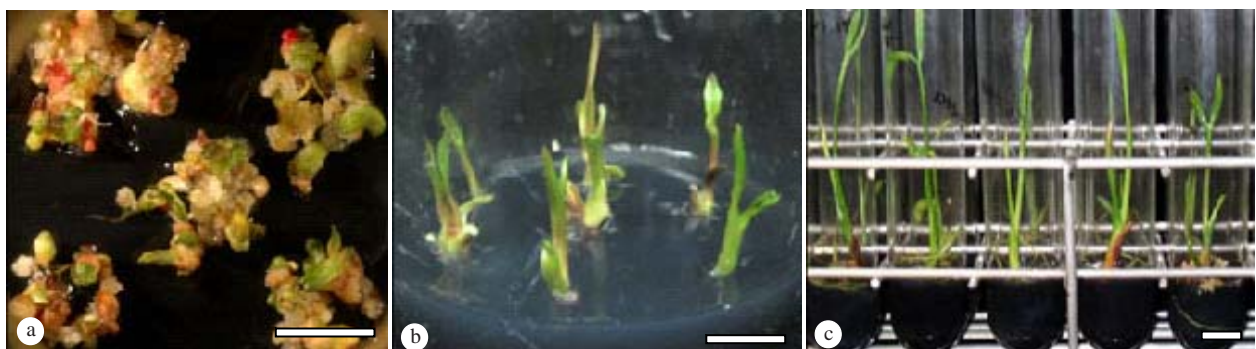


Figure 1. a. Somatic embryos of sago, b. Initial plantlets of sago derived from somatic embryos used for the experiment, c. The sago plantlets after 12 weeks of culture. Bar = 1 cm.

Increasing the concentration of carbohydrate tended to decrease the leaf number RGR, except for maltose.

Fresh Weight of Plantlet. The type and concentration of carbohydrate significantly affected plantlet fresh weight, but there was no interaction between the two factors. Media supplemented with sucrose at 30 g/l produced higher fresh weight than other types or concentrations of carbohydrate (Table 1). Sucrose was better than other carbohydrate types on plantlet fresh weight. The fresh weight of plantlet on media with carbohydrate at 30 g/l was significantly higher than those at 45 and 60 g/l.

Plantlet Diameter. The type and concentration of carbohydrate significantly influenced the diameter of sago plantlets, but there was no interaction between the two factors. The largest diameter of plantlets was obtained from the media supplemented with sucrose (6.5 mm). The concentrations of carbohydrate at 30 and 60 g/l produced relatively the same diameter of plantlets, whereas 45 g/l produced smaller diameter. Sucrose at 30 g/l produced the largest diameter of plantlet (7.3 mm) (Table 2).

Rooting Frequency. The highest percentage of plantlet root formation was found on the medium added with 30 g/l sucrose (40%). There was no root formation in plantlet of sago grown on the medium with 45 g/l fructose (0%) (Table 3). The lowest rooting percentage was obtained in plantlets of sago grown on medium supplemented with fructose.

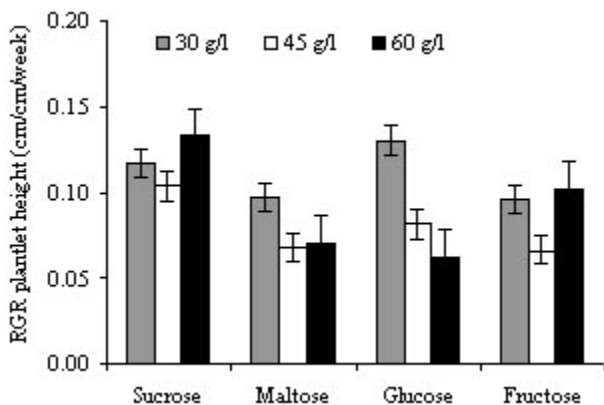


Figure 2. Effect of type and concentration of carbohydrate on RGR of sago plantlet height.

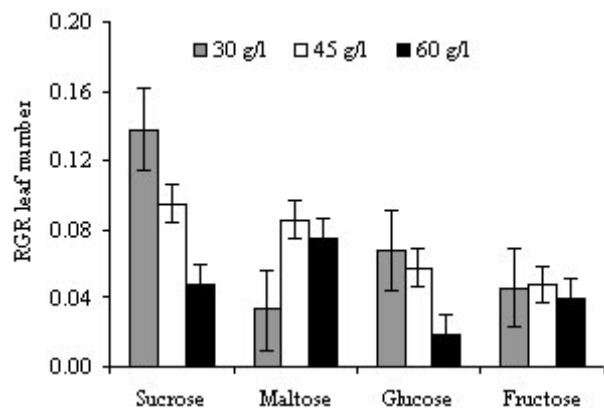


Figure 3. Effect of type and concentration of carbohydrate on RGR of leaf number of sago plantlet.

Table 1. Effect of type and concentration of carbohydrate on the fresh weight of sago plantlet

Carbohydrate type	Carbohydrate concentration (g/l)			Average
	30	45	60	
-----Plantlet fresh weight (g)-----				
Sucrose	0.40	0.33	0.35	0.36a
Maltose	0.27	0.23	0.22	0.24b
Glucose	0.29	0.21	0.19	0.23b
Fructose	0.32	0.24	0.22	0.26b
Average	0.32a	0.25b	0.25b	

Means followed by the same letters are not significantly different according to Duncan's multiple range test at P =5 %.

Table 2. Effect of type and concentration of carbohydrate on plantlet diameter of sago

Carbohydrate type	Carbohydrate concentration (g/l)			Average
	30	45	60	
-----Plantlet diameter (mm)-----				
Sucrose	7.3	5.1	7.2	6.5a
Maltose	5.6	5.9	5.8	5.7ab
Glucose	5.7	3.7	5.3	4.9b
Fructose	6.1	5.0	6.3	5.8ab
Average	6.2a	4.9b	6.1a	

Means followed by the same letters are not significantly different according to Duncan's multiple range test at P =5 %.

Table 3. Effect of type and concentration of carbohydrate on rooting percentage of sago plantlets

Carbohydrate type	Carbohydrate concentration (g/l)			Average
	30	45	60	
-----Rooting percentage (%)-----				
Sucrose	40.0	13.3	6.7	20.0
Maltose	20.0	33.3	26.7	26.7
Glucose	26.7	26.7	6.7	20.0
Fructose	20.0	0.0	13.3	11.1
Average	26.7	18.3	13.3	

DISCUSSION

Sucrose produced the best plantlet height RGR, and they looked more vigorous than those from the other types of carbohydrate. The same results were reported by Gubis *et al.* (2005) who stated that medium supplemented with 30 g/l sucrose produced healthier and more vigorous plantlets of tomato (*Lycopersicon esculentum*) than those of other types and concentrations of carbohydrate. Other research revealed, however, that plantlet height RGR was better achieved on medium supplemented with 30 g/l glucose in culture of *Vaccinium vitis-idaea* (Debnath 2005) and *Eclipta alba* (Baskaran & Jayabalan 2005).

Sucrose at 30 g/l demonstrated the highest leaf number RGR in sago. Similarly, in banana (*Musa spp. cv Shima*), Buah *et al.* (2000) proved that plantlets on media containing sucrose produced higher numbers of leaves than on media with glucose and fructose. They reported that fructose at high temperatures released a poisonous substance known as *5-hydroxymethyl-2-furaldehyde* that enhanced hyperhydricity and decreased leaf water potential. That would hamper the enlargement of leaves of the plantlets.

The fresh weight of plantlet biomass is affected, since sucrose is used as an energy source to enhance cell

division. This, in turn, increases the volume and weight of plantlets. In addition, Jain *et al.* (1997) mentioned that in rice plant cultures, sucrose is a disaccharide that produces optimum plant fresh weight. Novero *et al.* (2010) stated that the highest mean weight increase of sago palm explants was obtained in medium containing 22.5 g/l sucrose and 7.5 g/l sorbitol. Again, this research revealed that sucrose has a better effect on the fresh weight of sago plantlets (the best concentration was 30 g/l). The use of sucrose and sorbitol at lower concentrations reduced the weight of sago plantlets. However, increasing concentration of sucrose produced a negative effect on the biomass of wheat plantlets (Javed & Ikram 2008) and *Alocasia amazonica* (Jo *et al.* 2009). Decreasing biomass due to higher concentration of sucrose might be caused by the decrease of the osmotic potential of the medium that restricts the ability of the cultures to absorb nutrition.

Stem diameter, referring to the lateral growth of plants, indicates the size enlargement. Carbohydrates control morphogenesis which is responsible for enlargement, hardness, and composition of the cell wall (Baskaran & Jayabalan 2005). Sucrose is important for cell enlargement and maintaining osmotic pressure of cells, therefore increasing the diameter of sago plantlets.

Al-Khateeb (2001) found that, in *Phoenix dactylifera*, the longest and highest numbers of roots were produced on media with 30 g/l sucrose. This result is similar to that demonstrated by sago in this research.

Sucrose is the most common carbohydrate used as a carbon and energy source in plant *in vitro* culture. This is because sucrose is a disaccharide with a role as a molecule transporter that has high solubility in water and easily goes through plasma membrane (Baskaran & Jayabalan 2005; Javed & Ikram 2008).

The addition of carbohydrate into the medium of *in vitro* culture enhances plantlet growth. Photosynthesis activity of plantlets *in vitro* is considered low due to low light intensity, high air relative humidity, and lack of gas exchange; therefore, the plantlets need energy source from carbohydrate (Kozai *et al.* 1997). This research shows that the best growth of sago plantlet and its rooting were obtained from media added with 30 g/l of sucrose. Similar results were obtained by Al-Khateeb (2001) who reported that 30 g/l of sucrose gave the best growth of plantlets of date palm (*Phoenix dactylifera*) cv. Khanezi and by Jo *et al.* (2009), in plantlets of *Alocasia amazonica*. Plantlets of *A. amazonica* also survived better during acclimatization when previously planted *in vitro* on medium added with 30 g/l of sucrose (Jo *et al.* 2009).

Sucrose influences cell enlargement by maintaining osmotic pressure in the cell that increases the growth or size of plantlets. Maltose is hydrolyzed 20 times slower than that of sucrose, therefore the absorption and metabolism of maltose take a longer time than sucrose (Blanc *et al.* 2002). Fructose and glucose belong to monosaccharides that are easier to be decompose than sucrose. However, fructose is an intermediate product of glucose catabolism that affect the growth of *in vitro* plants slowly. Fructose is a hexose monosaccharide that does not stimulate plant tissue differentiation but it promotes

sucrose activity. Glucose enhances the growth of both shoots and roots (Tiexeira da Silva 2004).

Carbohydrate, as a source of carbon, at high concentrations, can be toxic and can inhibit the growth and development of plantlets (Tiexeira da Silva 2004). Increasing the concentration of carbohydrate in the medium reduced the growth of sago plantlets in terms of plantlet height and diameter, leaf number, biomass weight, and root formation because of it lowers the osmotic potential. A similar result was reported by Novero *et al.* (2010) who used low concentrations of sucrose added with sorbitol, with the best treatment for the growth of sago plantlet being 22.5 g/l sucrose + 7.5 g/l sorbitol. Lower concentrations of sucrose and sorbitol slowed the weight increase in sago plantlets. They suggested that *in vitro* culture of sago palm is more favorable in an environment with high solute concentration or low osmotic potential. However, our results revealed that increasing the concentration of sucrose or other carbohydrates did not favor the growth and vigor of sago plantlets. Over-accumulation of sugars due to higher carbohydrate supply might have induced osmotic stress, thus altered the metabolic processes in the cells, which subsequently decreased the growth rate of the plantlets (Jo *et al.* 2009).

ACKNOWLEDGEMENT

This study was a part of sago tissue culture project funded by the Indonesian State Ministry of Research and Technology through an Applied Research Incentive Program with contract number: 091/RT/D.PSIPTN/Insentif/PPK/I/2009.

REFERENCES

- Al-Khateeb AA. 2001. Influence of different carbon source on *in vitro* root formation of date palm (*Phoenix dactylifera* L.) cv. Khanezi. *J Agric Res* 28:597-608.
- Baskaran P, Jayabalan N. 2005. Role of basal medium, carbon source and growth regulator in micropropagation of *Eclipta alba* - a valuable medicinal herb. *KMITL Sci J* 5:469-682.
- Blanc G, Lardet L, Martin A, Jacob JL, Carron MP. 2002. Differential carbohydrate metabolism conducts morphogenesis in embryogenic callus of *Hevea brasiliensis* Mull. *Arg J Exp Bot* 53:1453-1462. <http://dx.doi.org/10.1093/jexbot/53.373.1453>
- Buah JN, Kawamitsu Y, Yonemori S, Hayashi M. 2000. Effect of various carbon sources and their combination on *in vitro* growth and photosynthesis of banana plantlets. *Plant Prod Sci* 3:392-397. <http://dx.doi.org/10.1626/pp.s.3.392>
- Debnath SC. 2005. Effects of carbon source and concentration on development of lingonberry (*Vaccinium vitis-idaea* L.) shoots cultivation *in vitro* from nodal explants. *In Vitro Cell Dev Biol* 41:145-150.
- Flach M. 1997. Sago Palm (*Metroxylon sago* Rottb.). Promoting and Conservation and Use of Underutilized and Neglected Crops 13. Rome: Institute of Plant Genetics and Crop Plant Research, International Plant Genetic Resources Institute.
- Gubis J, Lajchova Z, Klčova L. 2005. The effect of carbon source on plant regeneration in tomato. *Hort Sci* 32:6-8.
- Hisajima S, Jong FS, Arai Y, Sim ES. 1991. Propagation and breeding of sago palm (*Metroxylon sago* Rottb.) plant *in vitro*: 1. Embryo culture and induction of multiple shoots from sago embryos *in vitro*. *Japanese J Trop Agric* 35:259-267.

- Jain K, Davey MR, Cocking EC, Wu R. 1997. Carbohydrate and osmotic requirement for high frequency plant regeneration from protoplast-derived colonies of indica and japonica rice varieties. *J Exp Bot* 48:751-758. <http://dx.doi.org/10.1093/jxb/48.3.751>
- Javed F, Ikram S. 2008. Effect of sucrose induced osmotic stress on callus growth and biochemical aspects of two wheat genotypes. *Pakistan J Bot* 40:1487-1495.
- Jo EA, Tewari RK, Hahn EJ, Paek KY. 2009. *In vitro* sucrose concentration affects growth and acclimatization of *Alocasia amazonica* plantlets. *Plant Cell Tiss Organ Cult* 96:307-315. <http://dx.doi.org/10.1007/s11240-008-9488-4>
- Jong FS. 1995. Research for the Development of Sago Palm (*Metroxylon sagu* Rottb.) Cultivation in Sarawak, Malaysia. Sadong Pr.
- Kasi PD, Sumaryono. 2007. Morphological changes during the development of somatic embryos of sago (*Metroxylon sagu* Rottb.). *Indonesian J Agric Sci* 8:43-47.
- Kozai T, Kubota C, Jeong BR. 1997. Environmental control for the large scale production of plants through *in vitro* techniques. *Plant Cell Tiss Organ Cult* 51:49-56. <http://dx.doi.org/10.1023/A:1005809518371>
- Novero A, Delima AG, Acaso J, Baltores LM. 2010. The influence of osmotic concentration of media on the growth of Sago Palm (*Metroxylon sagu* Rottb.) *in vitro*. *Australian J Crop Sci* 4:453-456.
- Riyadi I, Tahardi JS, Sumaryono. 2005. The development of somatic embryos of sago palm (*Metroxylon sagu* Rottb.) on solid media. *Menara Perkebunan* 73:33-40.
- Rostiwati T, Jong FS, Natadiwirya M. 1998. Planting of Sago (*Metroxylon sagu* Rottb.) in Large Scale. Agency for Forestry and Estate Crops Research and Development, Indonesian Dept of Forestry. Jakarta. p 60.
- Tahardi JS, Sianipar NS, Riyadi I. 2002. Somatic embryogenesis in sago palm (*Metroxylon sagu* Rottb.). In: Kainuma K *et al.* (eds). *New Frontiers of Sago Palm Studies*. Tokyo: Cecil Universal Acad Pr. p 75-81.
- Teixeira da Silva JAT. 2004. The effect of carbon source on *in vitro* organogenesis of chrysanthemum thin cell layers. *Bragantia Campinas* 63:165-177. <http://dx.doi.org/10.1590/S0006-87052004000200002>