Clonal Propagation of Sago Palm (*Metroxylon sagu* Rottb.) through Tissue Culture

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Abstract
Sago palm (Metroxylon sagu Rottb.) is one of the most productive carbohydrate-producing crops but still underutilized. Sago starch has been known for a long time as a staple food for people in eastern parts of Indonesia and is becoming an important source of industrial and many other derivative products. The development of commercial sago plantations is hampered by the availability of superior planting materials in a large quantity. The supply of superior planting materials by tissue culture is a promising alternative. Tissue culture of sago through somatic embryogenesis has been developed by the Indonesian Biotechnology Research Institute for Estate Crops. Somatic embryogenesis of sago was started with the initiation of callus from shoot apical tissue of young suckers, proliferation of callus, induction of somatic embryos, maturation and germination of somatic embryos, and development of plantlets. All the stages are conducted on solid media. Problems found in tissue culture of sago are asynchronous development of somatic embryos, weak plantlets, and low survival rate of plantlets in the acclimatization stage. These problems make tissue culture process of sago inefficient. Liquid cultures using temporary immersion system has been developed to improve somatic embryogenesis production of sago. The availability of superior planting materials of sago will support the development of sago plantations and the rehabilitation of sago areas.
***Keywords** : clonal propagation, Metroxylon sagu Rottb, sago, superior genotype, tissue culture*

INTRODUCTION

Sago palm (*Metroxylon sagu* Rottb.) is one of the most potential carbohydrate-producing crops in supporting Indonesian food security program (Tarigans, 2001). Total area of sago palm in the world is predicted around 2.47 millions ha where 1.4 millions ha in Indonesia, 1.02 millions ha in Papua New Guinea, and the rest is in Malaysia, Thailand, Philippines and other countries (Flach, 1997).

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Sago has long been a staple food for people in eastern parts of Indonesia especially in Papua and Moluccas. Apart from being a raw material for food, sago starch is also used in the production of noodles, white bread, high-fructose syrup, biodegradable filler in plastics, animal feed, adhesive, bioethanol and many other derivative products (Flach, 1997).

Sago palm has many advantages over other carbohydrate-producing crops especially for its higher yield (15-25 ton dry starch/ha/year) (Flach, 1997). This palm can grow along riverbanks and in swampy areas where are not suitable for other crops, consequently the development of sago palm does not compete with the use of land for food crops. In addition, sago is a perennial crop which means once planted it can produce for many years and harvest can be done regularly by managing the suckers (Rostiwati *et al*., 1998).

Sago palm propagates vegetatively by suckers and generatively by seeds. Seed production is rare because the palms are commonly harvested by cutting the trees just before flowering. 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 from 2 to 5 kg (Rostiwati *et al*., 1998) make them more difficult in the transportation.

Tissue culture is a promising alternative means to propagate superior sago palm clonally. The criteria 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 from 150 to 700 kg with the average of 300 kg fresh starch per trunk (Haryanto & Pangloli, 1992). 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: Tissue culture of sago has been established through zygotic embryogenesis (Hisajima *et al*., 1991) and somatic embryogenesis (Tahardi *et al*., 2002). Somatic embryogenesis is one the most important of vegetative plant propagation methods in a large-scale (van Arnold *et al*., 2002). The development of somatic embryos

resembles to zygotic embryos is composed of globular, heart-shape, torpedo, cotyledon and germinant stages.

Tahardi *et al*. (2002) used apical meristematic tissues of young suckers as explants. The explants were cultured on a modified MS (Murashige & Skoog, 1962) medium containing 2,4-D and kinetin to initiate callus. Callus was usually formed after three consecutive cultures on the same medium in a dark culture room. In the callus proliferation media, the concentration of 2,4-D has been decreased gradually. Somatic embryos were then induced from the nodular callus after several subcultures (Riyadi *et al*., 2005). In the development of somatic embryos of sago, Kasi & Sumaryono (2006) reported that there were morphological variations in size, shape and color of the embryos. Somatic embryos have been able to grow and develop into normal plantlets. The next step was to induce roots on plantlets grown on a solidified medium with the addition of plant growth regulators especially from auxin group. Vigorous plantlets with good rooting system are then transferred and acclimatized in a greenhouse. Procedure of tissue culture of sago palm is illustrated in the next diagram (Figs. 1 and 2).

Problems in tissue culture of sago: The first problem encountered in tissue culture of sago is high variability of somatic embryos. At present somatic embryogenesis of sago has been conducted mostly on solid media. Somatic embryos obtained were heterogeneous; in the culture of sago can be found somatic embryos of different sizes, colors and developmental stages (Kasi & Sumaryono, 2006). Therefore, the procedure for somatic embryogenesis of sago should be refined in order to generate more uniform somatic embryos with a higher multiplication rate. The use of liquid culture with a periodic immersion for rapid and uniform production of somatic embryos of oil palm was reported by Tahardi (1998) possibly can be used to resolve this problem.

The second problem is that *in vitro* plantlets are not vigorous. The leaves are narrow and elongated, and the roots are small and without root hairs. These weak plantlets would have an effect on low survival rate of *vitro* plants during acclimatization in *ex vitro* conditions. The use of plant growth regulators, different sources of nitrogen or carbohydrate, pH medium, and *in vitro* environments such light intensity and temperature are now being tested in order to improve the development of plantlets.

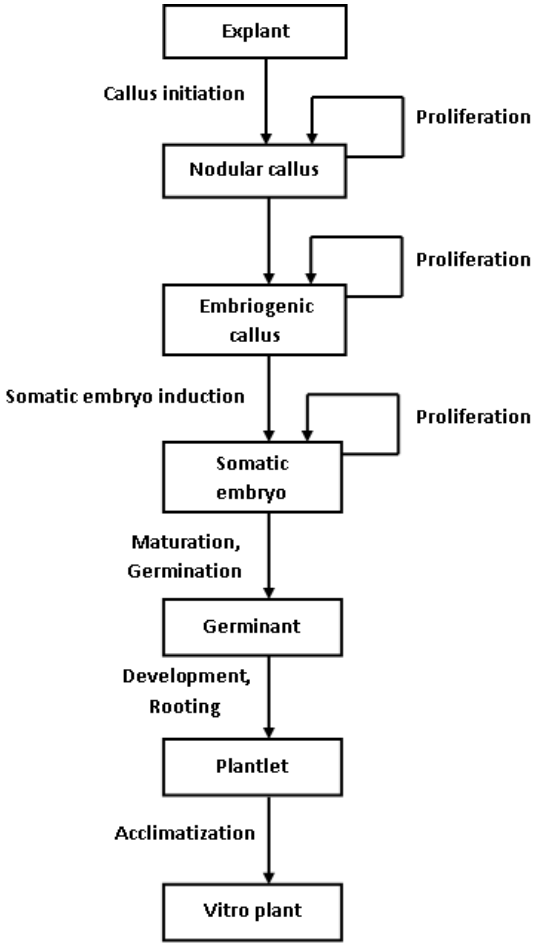

Fig.1. A diagram of tissue culture procedure of sago

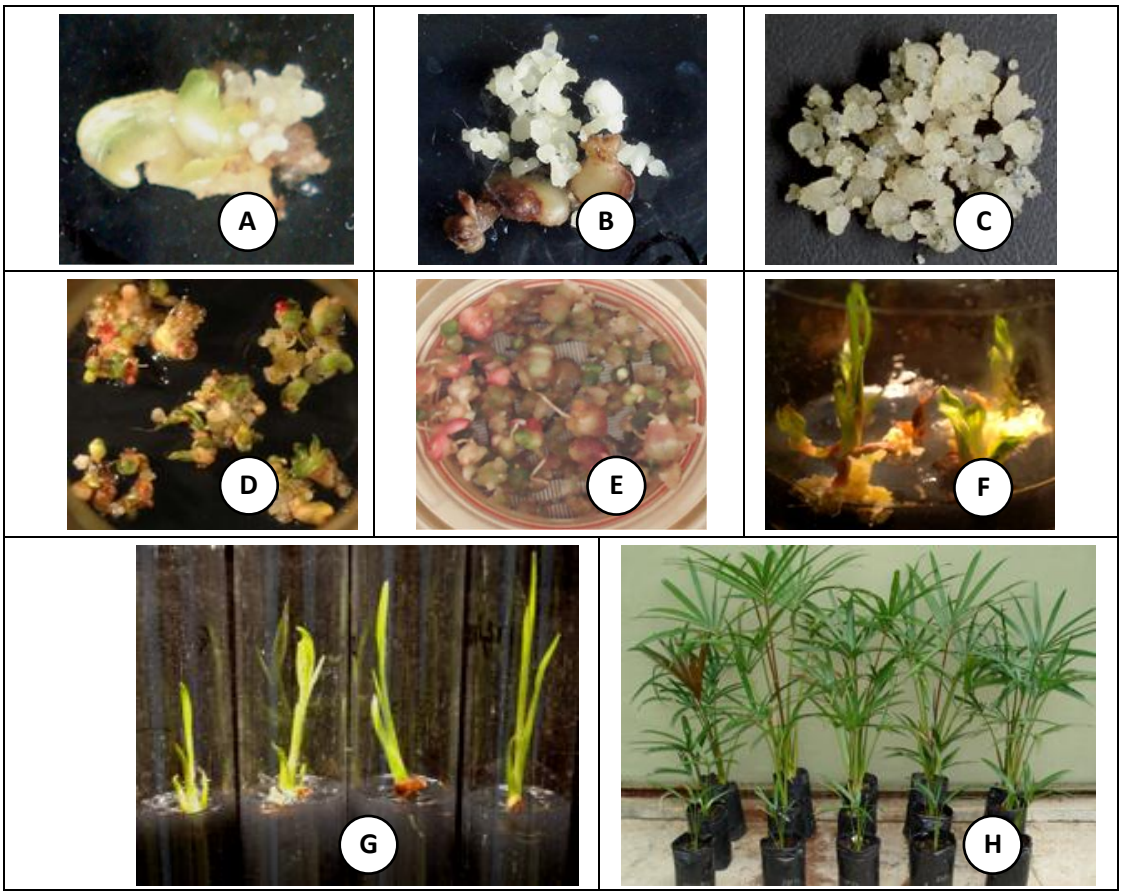

Fig. 2. Stages of clonal propagation of sago palm through tissue culture :

A. Initiation of callus on a solid medium.
B. Fast growing friable callus.
C. Nodular callus.
D. Maturation of somatic embryos on a solid medium.
E. Maturation of somatic embryos in temporary immersion system.
F. Development of germinants to plantlets on a solid medium.
G. Growth of plantlets on a solid medium.
H. Young vitro plants of sago in the nursery.

The survival rate of *vitro* plants of sago in the greenhouse during acclimatization up to now is very low. The survival frequency so far is only 40%. Besides the weak plantlets when be transferred from *in vitro* conditions, micro environments (temperature, light intensity, relative humidity) and medium compositions (soil, sand, peat, cocopeat, zeolite and compost) may have significant effects on survival rate and growth of the *vitro* plants. Water immersion in the media may influence the survival and growth of young *vitro* plants as the natural environment of sago is swampy areas. Association of sago roots with mycorrhizae or other microbes is predicted to have a good influence on the young *vitro* plants.

Planting materials for the development of sago plantations: Indonesian production of sago starch in the present time is about 200.000 tons dry starch per year or only 5% of its potential yield. The low national yield is also contributed by still simple and traditional processing technology used by most smallholders. In Indonesia the exploitation of sago depends greatly on wild stands and semi-cultivated stands. The natural growth of sago palm may not keep up with the rate of over-harvesting (Tarigans, 2001). Therefore, the development of large-scale commercial sago plantations is necessary.

In large-scale plantations the planting distance is arranged 8x8 m or 9x9 m depending on cultivation regime and sago variety. Single sago tree will produce suckers from the lowest part of the trunk, forming a cluster in various stages of development. The number and age of suckers in one cluster are managed to be harvested regularly one tree every one and half or two years. On average one tree produces 175 kg dry starch (Flach, 1997), therefore, sago plantations produce from 15 to 25 tons dry starch/ ha/ year. This yield level is one of the highest yields of carbohydrate-producing crops.

Establishment of a large-scale plantation of sago requires planting materials in a large quantity. Vegetative propagation using suckers is by far the most

common and effective method of sago propagation by smallholders. To provide good suckers in a large quantity is a major problem in the development of large-scale plantations of sago (Jong, 1995). Therefore, tissue culture is a promising alternative for mass production of good quality planting materials that will solve sucker shortage problems.

Tissue culture of sago has been developed successfully through somatic embryogenesis, however, some problems are encountered for a mass production. The problems hopefully can be solved in the next several years consequently commercial-scale production of superior genotypes of sago can be accomplished. *Vitro* plants must be field planted in several locations and assessed for growth, yield and defects (abnormality).

In conclusion, tissue culture of sago palm for mass propagation has been developed through somatic embryogenesis, although there are still some problems to be dealt with. It is predicted that an established procedure of sago palm tissue culture will be accomplished in the next few years. However, the *vitro* plants must be first field-tested in order to determine its performance, productivity and possibly abnormality. The availability of superior planting materials of sago will support the development of large scale sago plantations and the rehabilitation of sago areas. The higher productivity of tissue culture derived plants may convince farmers or entrepreneurs to cultivate sago palm as a commercial crop considering the important of sago for industry, food and energy.

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REFERENCES

Flach, M. 1997. Sago palm *Metroxylon sagu* Rottb. Promoting the Conservation and Use of Underutilized and Neglected Crops. 13. International Plant Genetic Resources Institute, Rome-Italy. 76.

Haryanto, B. & P. Pangloli. 1992. Potensi dan Pemanfaatan Sagu. Penerbit Kanisius. 140p.

Hisajima S., F.S. Jong, Y. Arai & E.S. Sim. 1991. Propagation and Breeding of Sago Palm (*Metroxylon sagu* Rottb.) Plant *In Vitro*: 1. Embryo Culture and Induction of Multiple Shoots from Sago Embryos *In Vitro*. *Jap. J. Trop. Agric*. 35 (4): 259-267.

Jong, F.S. 1995. Research for the Development of Sago Palm (*Metroxylon sagu* Rottb.) Cultivation in Sarawak, Malaysia. Sadong Press. Sdn, Bhd. 139.

Kasi, P.D. & Sumaryono. 2006. Keragaman Morfologi selama Perkembangan Embrio Somatik Sagu (*Metroxylon sagu* Rottb.). *Menara Perkebunan*. 74 (1): 44-52.

Murashige, T. & F. Skoog. 1962. A Revised Medium for Rapid Growth and Biassays with Tobacco Tissue Culture. *Physiol. Plant*. 15: 473-497.

Riyadi, I., J.S. Tahardi & Sumaryono. 2005. The Development of Somatic Embryos of Sago Palm (*Metroxylon sagu* Rottb.) on Solid Media. *Menara Perkebunan*. 69 (2): 46-57.

Rostiwati, T., F.S. Jong & M. Natadiwirya. 1998. Penanaman Sagu (*Metroxylon sagu* Rottb.) Berskala Besar. Badan Penelitian dan Pengembangan Kehutanan dan Perkebunan. Jakarta. 60.

Tahardi, J.S., N.F. Sianipar & I. Riyadi. 2002. Somatic Embryogenesis in Sago Palm (*Metroxylon sagu* Rottb.). *In* Kaimuna, K. *et al*. (Eds.). New Frontiers of Sago Palm Studies. Universal Academic Press, Inc. Tokyo-Japan. 75-81.

Tahardi, J.S. 1998. Improvement of Oil Palm Somatic Embryogenesis by Periodic Immersion in Liquid Medium. *In* Jatmika, A. *et al*. (Eds.). *Proc. Int. Oil Palm Conf. Bali. Indonesia*. 595-601.

Tarigans, D.D. 2001. Sagu Memantapkan Swasembada Pangan. *Warta Penelitian dan Pengembangan Pertanian*. 23 (5): 1-3.

van Arnold, S., I. Sabala, P. Bozhkov, J. Dyachok & L. Filonova. 2002. Development Pathways of Somatic Embryogenesis. *Plant Cell. Tiss. Org. Cult*. 69: 233-249.