Somatic embryogenesis of sago palm (*Metroxylon sagu* Rottb.) from different origins in Indonesia

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Abstract. Sago palm (*Metroxylon sagu* Rottb.) is considered as one of the prospective carbohydrate-producing crops in Indonesia. The development of commercial sago plantations has been hampered by the shortage of superior planting materials in a large quantity. Rapid and large-scale propagation of superior varieties could be accomplished by tissue culture. The Indonesian Research Institute for Biotechnology and Bioindustry has developed tissue culture of sago through somatic embryogenesis. The research objective was to determine the response of sago origins to previously developed somatic embryogenesis procedure. Explants of young suckers were taken from six different sago areas in Indonesia: Riau, West Java, South Kalimantan, Moluccas, Papua, and Southeast Sulawesi. Callus initiation was conducted on a solid modified-MS medium. The frequency of callus initiation was low and highly varied between 0.7% (Southeast Sulawesi and Papua) and 12.5% (West Java). The first callus was formed at the fourth culture (20 weeks) on explants from West Java and South Kalimantan, and at the sixth culture (29 weeks) on explants from Southeast Sulawesi. Callus multiplication rate was generally very high, 2-3.4 folds in 4 weeks. Calli produced from all explant origins could be successfully developed for somatic embryos and plantlets.

1. Introduction

Sago palm (*Metroxylon sagu* Rottb.) is one of the most potential carbohydrate-producing crops in the tropical area. Sago palm is monocarpic in which at 10-15 years of age produces a large branched terminal inflorescence above the leaves. The starch is stored in the trunk and harvested just before flowering. Sago palm yields 15-25 ton dry starch/ha/year [1]. Sago starch has long been a staple food for people in many parts of Indonesia such as Mentawai islands, Moluccas, and Papua. In addition to being a raw material for food, sago starch is also used in the production of white bread, noodles, high-fructose syrup, biodegradable filler in plastics, adhesive, bioethanol, animal feed, and many other derivative products [1].

The palm is distributed in tropical Southeast Asia mostly in Indonesia and Papua New Guinea, also in Malaysia, Thailand, Philippines, and the Melanesian islands of the Pacific Ocean. Sago in Indonesia is spread over several sago areas, such as in Java, Riau, Moluccas, Papua, Kalimantan, Sulawesi and Mentawai islands. Sago is able to grow in areas where most other crops cannot grow well such as in the riverbanks, lowland swampy areas, peat soils, flooded lands, and coastal lowland areas.

Sago palm can be propagated using suckers and seeds. Seed production is rare because the trees are harvested prior to the onset of flowering; therefore, suckers commonly propagate this sago palm.
However, to establish large-scale sago plantations, the availability of uniform suckers in a large number is a major constraint [2]. Tissue culture is a promising alternative means for rapid and mass clonal propagation of superior sago palms. In addition, tissue culture can be used as a method of in vitro conservation of sago palm. Tissue culture of sago palm through somatic embryogenesis has been successfully established by the Indonesian Research Institute for Biotechnology and Bioindustry (IRIBB) [3, 4]. Apical meristematic tissues of young suckers of selected sago palms were used as explants. The research objective was to determine the effect of plant origins on somatic embryogenesis of sago palm.

2. Materials and Methods

2.1. Explants and culture conditions
The research was conducted at the Laboratory of Plant Cell Culture and Micropropagation, IRIBB, Bogor from 2001 to 2010. Apical shoot tissues of young suckers of the field grown sago palms in six sago-producing areas: Bogor (West Java), Banjarmasin (South Kalimantan), Selat Panjang (Riau, Sumatra), West Seram (Moluccas), Merauke (Papua), and Kendari (Southeast Sulawesi) were used as explant materials (Figure 2a). All cultures except for callus initiation were incubated in the light culture room under white fluorescent lamps providing 20 μmol photon/m²/s over a 12-h photoperiod at a constant temperature of 26 °C. Culture medium was sterilized in an autoclave at 121°C for 20 min before being used.

2.2. Callus culture
Apical shoot tissues of young suckers were cultured on a modified MS (MMS) medium [3]. The cultures were placed in a dark culture room and were sub cultured every 6-8 weeks. Callus formed was sub cultured on the same medium with a lower concentration of 2,4-D for callus proliferation under light conditions.

The primary calli formed were sub cultured on solid MMS media in jar bottles added with 10-20 mg/L 2,4-D and 0.1 mg/L kinetin for callus proliferation. These cultures were placed in a dark room for 6 to 8 weeks. Calli formed were used as a material source for somatic embryo induction or for further callus proliferation.

2.3. Somatic embryo induction on a solid medium
The embryogenic callus was cultured on MMS solid media supplemented with 0.1 mg/L kinetin and 5 mg/L 2,4-D. The cultures were placed in a light culture room for 4 weeks when the frequency of somatic embryo formation was determined.

2.4. Somatic embryo maturation and germination in TIS
Somatic embryos formed were cultured on a liquid MMS medium with a half-strength of macro salts, 0.01 mg/L ABA, 1 mg/L kinetin and 0.1 mg/L GA₃. Flask of TIS consists of an upper containment for the somatic embryos and a lower containment for the media (Figure 2d). The volume for liquid culture was 75-100 ml per flask. To avoid contamination, 15 mg/l rifampicin and 15 mg/l tetracycline were added into the medium. When the lower containment was pressured, liquid medium was pumped into the upper containment immersing the plant culture for a specific period and interval of immersion adjusted automatically. The somatic embryos were immersed for 3 min every 6 h.

Somatic embryos at advanced developmental stages of scutellar and coleoptilar were subcultured in liquid MMS media supplemented with 0.5 mg/L GA₃ and 0.5-1.0 mg/L kinetin. The cultures were placed in a light culture room for 4-6 weeks. TIS was adjusted with 3 min immersion duration and immersion interval every 6 or 12 h [5].
2.5. Plantlet development
The resulting germinant were sub cultured on an MMS solid medium or in a liquid medium in the test tubes added with 0.5-1.0 mg/L GA3 and 1.0 mg/L kinetin. The cultures were placed in a light room for 6 - 8 weeks [6]. Healthy vigorous plantlets with height more than 10 cm, had more than 3 leaves and good root systems were ready for acclimatization in ex vitro conditions.

3. Results and Discussion

3.1. Origin and description of sago palm
Sago palms collected from six regions in Indonesia had different local names (Table 1, Figure 1a-f). The sago palm trees had spines or without spines (spineless). The spines of sago palm were usually not more than 4 cm in length. This spininess characteristic is important for sago cultivation because sago with the spine is more difficult in managing the suckers than that of spineless one. In addition, explants collection from spined sago was more difficult than unspined sago palms.

In this research, unspined sago palms were collected from West Java, South Kalimantan and Papua, while spined sago palms were from Riau and Southeast Sulawesi. Both types of sago palms were obtained from West Seram, the Moluccas with different local names, Tuni for spined sago and Molat for unspined sago (Table 1).

| Explant Origin | District       | Date of Collection | Local Name | Spines |
|----------------|----------------|--------------------|------------|--------|
| West Java      | Bogor          | May 2001           | Kirai      | No     |
| South Kalimantan| Banjarmasin    | August 2004        | Sagu       | No     |
| Riau           | Selat Panjang  | August 2007        | Duri       | Yes    |
| Moluccas       | West Seram     | July 2008          | Tuni       | Yes    |
| Moluccas       | West Seram     | July 2008          | Molat      | No     |
| Papua          | Merauke        | November 2008      | Alitir     | No     |
| Southeast Sulawesi | Kendari    | October 2009       | Runggamano | Yes    |

Figure 1. Sago palm from different origins: (a). West Java, (b). South Kalimantan, (c). Riau, (d). Moluccas, (e). Papua, and (f). Southeast Sulawesi.
3.2. Callus culture

Explants from different origins responded differently to the media used. The first callus was formed at the fourth culture (20 weeks), the fastest response on explants from West Java and South Kalimantan, and at the sixth culture (29 weeks) the slowest response on explants from Southeast Sulawesi (Table 2). The frequency of callus initiation was low ranging from 0.7% (Papua and Southeast Sulawesi) to 12.5% (West Java). White friable callus emerged from the edge of the explants (Figure 2b). There was no correlation between spined or unspined sago on callus formation.

The results show that callus formation on explants from eastern region (West Java, South Kalimantan and Riau) was slower and lower in frequency than those of explants from the western region (Moluccas, Papua and Southeast Sulawesi). All calli formed were friable and white in color (Figure 2b) with proliferation rate between 2 to 3.4 folds (Table 2). Sago from Riau had relatively low callus formation (2%) but the calli proliferation rate was high.

Table 2. Callus culture of different sago origins.

| Explant origin   | First callus emerge (week) | Callus formation (%) | Callus type       | Nodular callus formation (%) | Nodular callus proliferation (times) |
|------------------|---------------------------|----------------------|-------------------|-----------------------------|-------------------------------------|
| West Java        | 20                        | 12.5                 | Friable, white    | 100                         | 3.0                                 |
| South Kalimantan | 20                        | 1.4                  | Friable, white    | 100                         | 3.0                                 |
| Riau             | 24                        | 2.0                  | Friable, white    | 100                         | 3.4                                 |
| Moluccas Tuni    | 27                        | 1.4                  | Friable, white    | 100                         | 3.0                                 |
| Moluccas Molat   | 27                        | 0.9                  | Friable, white    | 100                         | 2.0                                 |
| Papua            | 26                        | 0.7                  | Friable, white    | 100                         | 2.0                                 |
| Southeast Sulawesi | 29                     | 0.7                  | Friable, white    | 100                         | 2.0                                 |

3.3. Callus proliferation

Calli began to multiply at 4 weeks after culture. Callus biomass increased 3-5 times of the initial biomass. The biomass increase in TIS culture was much higher than that of on solid media culture. In TIS cultures, biomass increase at 6 weeks of age (at harvest time) reached up to 6.5 times of the initial biomass [5]. The calli from this proliferation stage were used as material sources for somatic embryo induction culture both on solid media and in TIS.

The rate of callus proliferation varied with sago origins. In general, the proliferation rate of sago callus originating from the western region was faster and higher than that of from the eastern region. The highest proliferation rate was obtained from sago plants originated from Riau at 3.4 times in 6 weeks (Table 2). The low frequency of callus initiation of sago is not a limitation in sago propagation using somatic embryogenesis technique. This condition can be reinstated with high sago callus proliferation rate, and the ability of calli to form somatic embryos that reached 100%.

3.4. Somatic embryo induction and regeneration

All nodular calli of sago can be induced into somatic embryos (Figure 1c). The difference between the sago types and origins was on emergent time and proliferation rate of somatic embryos. Sago origin of Riau and Kalimantan showed the fastest and highest response on induction of somatic embryos. On the other hand, sago origin of Southeast Sulawesi and Papua showed the slowest response on somatic embryo induction and proliferation.

Maturation and germination of somatic embryos of sago were successfully conducted both on solid and in TIS media (Figure 2). TIS allows explants to be exposed to the culture media periodically in a very short time with a good air circulation. The efficiency of maturation and germination of somatic embryos was almost the same for all sago origins from both western and eastern Indonesia. TIS has been used successfully for SE of oil palm [7]. In case of flowering abnormality such as mantled fruits...
in oil palm due to the long-term duration of the culture, is not a problem in sago plants because sago palms are harvested before flowering.

Secondary somatic embryos (SSE) appeared during somatic embryo maturation stage [4]. The formation of SSE has been occurred subsequently at the germination stage of somatic embryos, especially on sago originated from Riau, Kalimantan, Moluccas (Tuni), and Papua (Alitir) [6]. Plantlet development and rooting were conducted on solid or in liquid media before acclimatization. Selection of plantlets to be acclimatised was done by determining the criteria of plantlets that had more than 3 leaves, the height was more than 7 cm, and had a good root system.

Plantlets of sago were successfully acclimatized in the nursery. The survival rate of plantlets on average was 40-60% in all sago origins. For the commercial purposes, this survival rate frequency is considered low, therefore more efforts should be done to increase sago plantlets survival rate. The most critical period in the acclimatization stage was in the first stage of acclimatization where the plantlets were placed in a closed plastic tunnel for 12 weeks. When this period was successfully accomplished, the plantlets had a high chance to survive afterwards. In the second stage, the plantlets were transferred to larger polybags (30 cm x 25 cm) and placed below the 50-60% shading plastic net for 6-8 weeks and then without shading net for 6-8 weeks. After this stage, sago plants were ready to be transplanted to the field.

Figure 2. Sago palm tissue culture: (a). Young suckers as explant sources, (b). Callus emerged from shoot apical explants, (c). Somatic embryos, (d). Embryo maturation on TIS, (e). Plantlets, and (f). Tissue culture derived sago plants in the nursery.

4. Conclusions

Callus initiation of sago was varied according to the plant origins. Sago explants from the western region of Indonesia formed callus earlier and in higher frequency than those of eastern regions. Sago explants from West Java had the best response for callus initiation. The proliferation rate of nodular callus was different depending on explant origins where explants from Riau was the fastest in callus proliferation. Calli from all explant origins could be converted to somatic embryos and plantlets.

5. Acknowledgments

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6. References

[1] 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. 76pp

[2] Jong F S 1995 Research for the development of sago palm (Metroxylon sagu Rottb.) cultivation in Sarawak, Malaysia Sadong Press Sdn. Bhd. 139pp

[3] Tahardi J S, N F Sianipar and I Riyadi 2002 Somatic embryogenesis in sago palm (Metroxylon sagu Rottb.), p.75-81 In K Kaimuna, M Okazaki, Y Toyoda and J E Cecil (Eds.). New Frontiers of Sago Palm Studies. Universal Academy Press, Tokyo, Japan

[4] Riyadi I, J S Tahardi and Sumaryono 2005 Menara Perkebunan 69 46

[5] Kasi P D and Sumaryono 2008 Menara Perkebunan 76 1

[6] Riyadi I, Efendi D, Purwoko B S and Santoso D 2016 Somatic embryogenesis of sago palm (Metroxylon sagu Rottb) using liquid culture method for technological development of elite clone seedling propagation [Dissertation] Bogor: Agricultural University 97pp

[7] Sumaryono, Riyadi I, Kasi P D and Ginting G 2008 Indon. J. Agric. 1 109