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MORPHOLOGICAL CHANGES DURING THE DEVELOPMENT OF SOMATIC EMBRYOS OF SAGO (*Metroxylon sagu* Rottb.)

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ABSTRACT

Development of somatic embryos of sago (*Metroxylon sagu* Rottb.) on agar-solidified medium are highly varied producing heterogeneous seedlings. Understanding of this phenomenon may help in improving the cultural procedures and conditions of sago somatic embryogenesis to obtain uniform seedlings in a large scale. This experiment was conducted at the laboratory for plant cell culture and micropropagation, Indonesian Biotechnology Research Institute for Estate Crops from January to March 2006 to examine morphological changes i.e. color and development stages of sago during their somatic embryo development on an agar-solidified medium. Twenty single globular somatic embryos of sago with specific color (yellowish, greenish, and reddish) were cultured in a Petri dish supplemented with a solid medium. The medium was a micronutrients-modified MS (MMS) with half strength of macronutrients containing 0.01 mg l^{-1} ABA, 2 mg l^{-1} kinetin, 20 g l^{-1} sucrose, 0.5 g l^{-1} activated charcoal, and 2 g l^{-1} gelrite. Parameter observed was the percentage of embryo's number based on color and developmental stage. The result showed that at the end of 6-week culture passage, most originally greenish (80.8%) and reddish (95.8%) embryos remained unchanged in their colors, whereas almost half of the originally yellowish embryos turned to greenish and only 30% remained yellowish. At the same time, single globular embryos have changed gradually into the next developmental stages, although not all of the embryos were germinated. The initial color of embryo affected the rate of the developmental stage changes. Yellowish and greenish globular embryos developed more rapidly into cotyledon or germinant stages at 58% and 55% respectively, in 6 weeks than the reddish ones (41%). Therefore, the yellowish and greenish embryos are the best sources of material for *in vitro* mass propagation and synthetic seed production of sago.

[***Keywords***: *Metroxylon sagu*, somatic embryogenesis, embryo development, embryo color]

INTRODUCTION

The sago palm (*Metroxylon sagu* Rottb.) is a monocotyledonous tree grown mostly in the swamplands of hot humid tropics of South East Asia and Oceania. Sago palm can grow along the riverbanks and in swampy areas which are not suitable for other crops. Sago palm is one of the most efficient starch-producing crops. The carbohydrate reserve stored in the trunk can reach 700 kg of fresh starch per tree, giving a yield of 15-25 ton dry starch per ha per year (Flach 1997).

In Indonesia, sago starch is extracted from sago trunk following the cutting down of the trees from their natural habitat. Sago palms are propagated from seeds and suckers. The later is traditionally practiced for planting material because fertile seeds are relatively scarce. This traditional way to produce planting material is slow and time consuming, therefore an improved procedure to mass propagate planting materials such as using tissue culture technique is essential. To establish large scale sago plantation, the availability of uniform suckers of superior trees is a major constraint (Jong 1995).

Tissue culture of sago palm has been conducted for more than 20 years, however only a few studies have been published. Hisajima *et al.* (1991) reported the induction of a few multiple shoots or mini-suckers from excised sago zygotic embryos. Tahardi *et al.* (2002) have achieved embryogenic callus by culturing shoot apical tissues as explants. Somatic embryogenesis of sago is initiated by embryogenic callus formation followed by induction of somatic embryos. Somatic embryogenesis is favored over other micropropagation means due to its potential to scale up the propagation process (van Arnold *et al.* 2002). Somatic embryo development of sago is similar to zygotic embryo development that consists of globular, heart-shape, torpedo, cotyledon, and germinant stages.

Medium composition for induction of somatic embryo, embryo maturation, and plantlets conversion has been reported by Riyadi *et al.* (2005). *In vitro* culture of sago on a maturation agar-solidified medium produced somatic embryos of different sizes, colors, and developmental stages during one culture period indicating that the sago cultures were highly varied. Kasi and Sumaryono (2006) revealed that the average size of sago somatic embryos did not change significantly over the culture period, however, the embryo size was already highly varied at the beginning and increased gradually as the embryo developed. By the end of culture, the composition of embryo colors has changed. Globular embryos were

yellowish and greenish, while cotyledon and germinant embryos were mostly reddish. The same phenomenon was observed by Riyadi *et al.* (2005) where reddish embryos were found at the later developmental stages of sago somatic embryos. Red color of sago plantlets is unique, it is not commonly found in any other perennial plants.

Morphological variation of somatic embryos was also found in *in vitro* culture of tea (Akula and Dodd 1998; Sumaryono *et al.* 2001). The morphological variation of somatic embryos restrains the scalling up of *in vitro* mass propagation (Tautorus and Dunstan 1995), because somatic embryos at different developmental stages usually need different culture conditions. It is known that synthetic seed technology requires large numbers of uniform high-quality somatic embryos especially at cotyledonary stage (Attree and Fowke 1993; Onishi *et al.* 1994).

This experiment was conducted to determine morphological changes during sago somatic embryo development on agar-solidified medium with respect to color and developmental stage. An understanding of this phenomenon may help in improving the cultural procedures and conditions of sago somatic embryogenesis to obtain uniform seedlings of sago in a large scale.

MATERIALS AND METHODS

Plant Material

This experiment was conducted at the laboratory for plant cell culture and micropropagation, Indonesian Biotechnology Research Institute for Estate Crops from January to March 2006. Globular somatic embryos of sago were initiated from shoot apical tissues cultured on a micronutrients-modified MS (MMS) agar-solidified medium based on Riyadi *et al.* (2005). The shoot apical tissue was taken from young suckers of field-grown sago palm in Parung district, West Java. The cultures were subcultured regularly every 4-5 weeks on a germination medium.

Medium and Culture Conditions

Single globular embryos were collected and separated according to their color (yellowish, greenish, and reddish) and cultured on a solid germination medium. The germination medium was MMS containing half-strength of macrosalts, 0.01 mg l^{-1} ABA, 2 mg l^{-1} kinetin, 20 g l^{-1} sucrose, 0.5 g l^{-1} activated charcoal, and 2 g l^{-1} gelrite. As much as 35 ml of the medium was placed in a 10-cm-diameter Petri dish. Culture media were adjusted to pH 5.7, and autoclaved at 121°C and 1.0 kg cm^{-2} for 20 minutes. All cultures were incubated in the culture room at 25°C under cool-white fluorescent lamps providing approximately 30 µmol photon m^{-2} s^{-1} over a 14-hour photoperiod. Twenty single globular somatic embryos with the same color were placed on the medium and replicated six times. The percentage of embryo's number was counted weekly for 6 weeks on the basis of color and developmental stage.

Statistical Analysis

Data were subjected to analysis of variance test (F test). Differences among treatment means were determined by Duncan's multiple range test at P = 0.05.

RESULTS AND DISCUSSION

Color Changes

After completing 6-week culture period, most (80.8%) of the single globular originally greenish embryos remained unchanged in their color, but the rest became yellowish and reddish. Similar results were found in the originally reddish embryos, where most of the embryos remained reddish. Only few of them turned to greenish and yellowish. In contrast, almost half of the originally yellowish embryos turned to greenish, and the rest were yellowish and reddish (Table 1 and Fig. 1). By the end of the culture, the total numbers of yellowish embryos (13.3%) were less than those of the greenish and reddish embryos (43.6% and 43.0%).

The results showed that the color of somatic embryos was changed during the culture on agar-solidified medium, especially in the originally yellowish embryos. These changes might be associated with light conditions during culture. Most of the newly formed somatic embryos, particularly through secondary embryogenesis were yellowish which then turned greenish or reddish.

Table 1. The percentage of color distribution of sago somatic embryos after 6 weeks of culture on a solid MMS medium.

Origin color of embryos	Embryo color (%)		
	Yellowish	Greenish	Reddish
Yellowish	30.0	47.5	22.5
Greenish	8.3	80.8	10.8
Reddish	1.7	2.5	95.8
Average	13.3	43.6	43.0

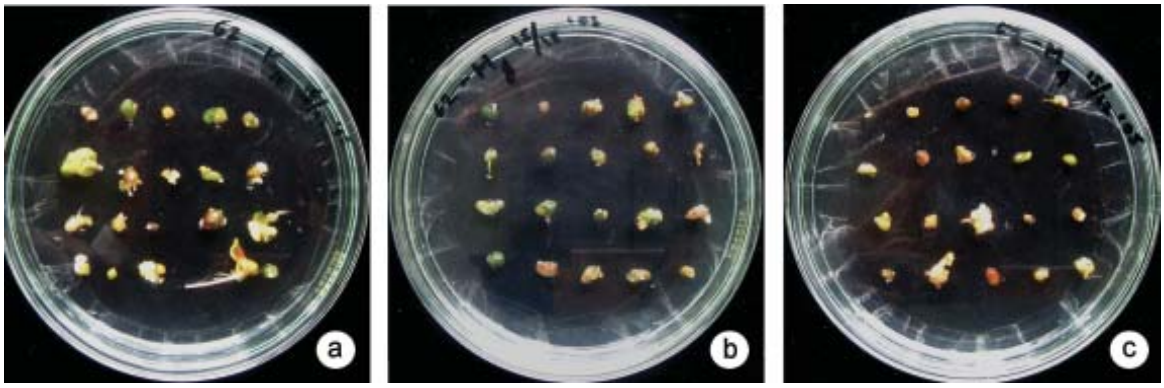

Fig. 1. Somatic embryo cultures of sago that originally from single globular embryo with the same color after 6 weeks of culture on solid MMS medium; a = originally yellowish embryos, b = greenish embryos, and c = reddish embryos. Bar = 1 cm.

Developmental Stage of Embryo

Globular embryos developed gradually into more advanced developmental stages as the cultures progressed, those are heart-shape, torpedo, cotyledon, and germinant. One example of the pattern of developmental stage changes was taken from originally yellowish embryos (Fig. 2). The figure showed developmental changes of the embryos indicated by the occurrence of non-globular stages of embryos during the culture period. At the first week, the torpedo stage was already found, and the number increased until the third week. The germinant stage was initially found at the third week and the number increased at the fourth week and then stayed the same up to the end of culture.

The results indicated that single somatic embryo was changed gradually into the later developmental stages but not at the same time for each embryo. For example, only 30% of somatic embryos were at germinant stage by the end of culture. Asynchronous development of these somatic embryos may be due to the differences in embryo sources and culture conditions. Single globular somatic embryos used might not be exactly the same in terms of size and developmental stage. In addition, only a part of embryo surface was contacted to the surface of solid medium. Asynchronous development of somatic embryos was also found in other woody species (Tautorus and Dustan 1995; Akula and Dodd 1998; Sumaryono *et al.* 2001). The high variability of sago somatic embryos restraints the development of mass clonal production and the production of synthetic seeds. Therefore, attempt to synchronize somatic embryo development of sago could be done by using a suspension culture that has been conducted in tea (Tahardi *et al.* 2000) and oil palm (Tahardi 1999).

Somatic Embryo Distribution

After 6-week observation, most of the originally yellowish embryos were in torpedo, cotyledon and germinant stages (Figs. 2 and 3). Some of the embryos (9%) were in globular and heart-shape stages. The same percentage was also found in the originally greenish and reddish embryos. These results demonstrated that not all of globular embryos were converted into the later developmental stages. The initial color of embryos affected the rate of stage changes of embryos on solid medium. At the sixth week, 58% originally yellowish embryos and 55 % for originally greenish embryos were at cotyledon and germinant stages, whereas only about 41% originally reddish embryos were at those stages (Fig. 3). Origi-nally yellowish and greenish embryos developed more rapidly into cotyledon and germinant stages than originally reddish embryos.

Different colors of sago somatic embryos presumably have different levels of storage reserves such as

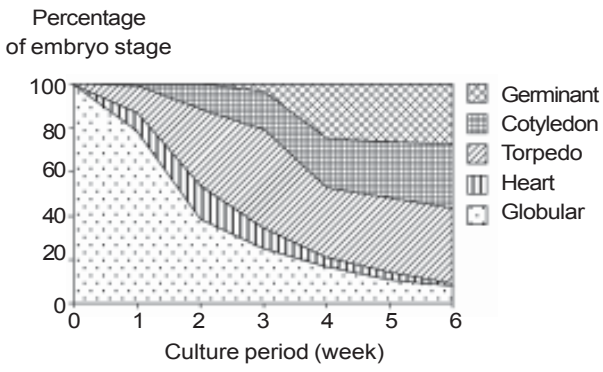

Fig. 2. Developmental stage changes of originally yellowish globular embryos of sago over 6 weeks of culture on solid MMS medium.

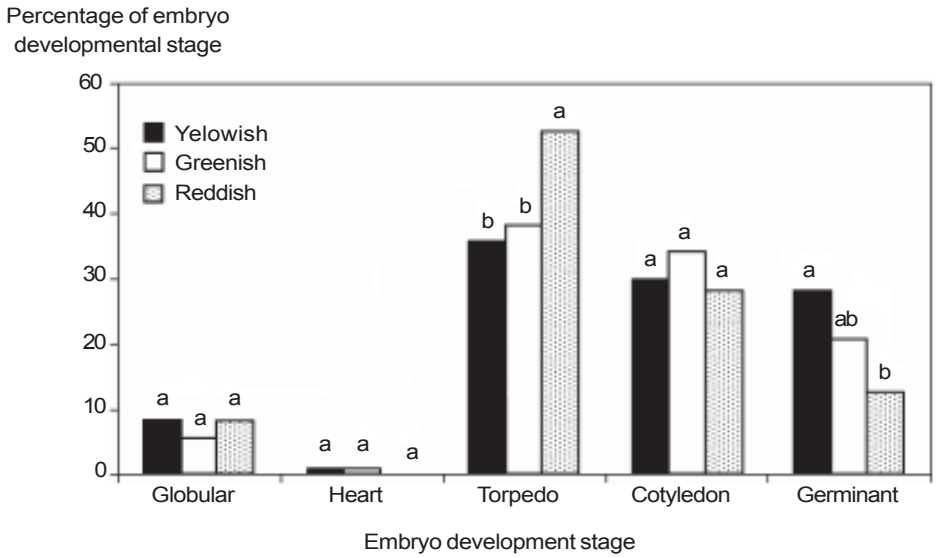

Fig. 3. The distribution of sago somatic embryo with respect to the developmental stage after 6 weeks of culture on a solid MMS medium. (Means in the same embryo stage followed by the same letters are not significantly different according to Duncan's multiple range test at P = 0.05).

starch and triacyl-glycerol required for germination of somatic embryos into plantlets as shown in *Hevea* rubber (Cailloux *et al.* 1996). The color of embryo is correlated with the development of somatic embryo. On rubber, for example, white embryos would develop into plantlets, while the green embryos would develop into callus and produce embryogenic callus by secondary embryogenesis (Veisseire *et al*. 1994).

Somatic embryo color has been used for quality evaluation of synthetic seeds of carrot (Sakamoto *et al*. 1992) and for assessing somatic embryo conversion potential of sweet potato (Padmanabhan *et al.* 1998) and walnut (Deng and Cornu 1992). In developing synthetic seed production, the availability of somatic embryos at the developmentally mature stages, especially cotyledon and germinant, is important as material sources. These results suggested it is important to use yellowish and greenish embryos as the sources of material for production of synthetic seeds of sago.

CONCLUSION

The color and developmental stage of sago embryos were changed during the course of the culture. At the end of 6-week culture passage, the total numbers of yellowish embryos (13.3%) were less than those of the greenish (43.6%) and reddish (43.0%) embryos.

Every single globular somatic embryo has changed into later developmental stages gradually at different rates, although not all of the embryos were germinated. The initial color of embryos affected the rate of developmental stage changes. Yellowish and greenish embryos developed more rapidly into cotyledon and germinant stages (58% and 55%, respectively) than the reddish embryos (41%). Therefore, the yellowish and greenish embryos are the best sources of material for *in vitro* mass propagation and the production of synthetic seeds of sago.

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