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EFFECT OF GAMMA IRRADIATION ON THE GROWTH AND DEVELOPMENT OF SAGO PALM (*Metroxylon sagu* Rottb.) CALLI

*Pengaruh Iradiasi Sinar Gamma terhadap Pertumbuhan dan Perkembangan Kalus Tanaman Sagu (*Metroxylon sagu *Rottb.)*

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ABSTRACT

The application of gamma irradiation on plant materials may increase the genetic variation of the offspring with useful traits. The experiment was conducted to determine the effect of irradiation dosage of gamma ray on growth and development of sago palm (*Metroxylon sagu*) calli. Friable calli of sago palm derived from suspension culture were used as a material source. The primary calli were initiated from apical meristematic tissues of sago palm suckers of Alitir variety from Merauke, Papua. The treatments used were dosage of gamma ray irradiation at 0, 5, 10, 15, 20 and 25 Gy. The treated calli were then subcultured on modified Murashige and Skoog (MMS) solid medium containing 3% sucrose and 0.1% activated charcoal and added with 1 mg l^{-1} 2,4-D and 0.1 mg l^{-1} kinetin. The results showed that at all irradiation dosages, calli biomass increased significantly. The highest proliferation of calli biomass of 5.33 folds from the initial culture after 4 weeks was achieved at gamma irradiation of 25 Gy, whereas the lowest proliferation of calli biomass of 3.4 folds was achieved at control. The best development of embryogenic calli was obtained at 10 Gy that produced 100% somatic embryos, whereas the lowest somatic embryo formation at 0% was obtained at 0 and 25 Gy after one subculture. High response of somatic embryo induction to gamma irradiation at 10 Gy may increase production of somatic embryos. These results can be used in *in vitro* breeding of sago palm via mutagenesis to create new elite varieties.

[***Keywords***: *Metroxylon sagu*, gamma irradiation, embryogenic calli, somatic embryo]

ABSTRAK

*Aplikasi iradiasi sinar gamma pada bahan tanaman dapat meningkatkan keragaman genetik pada keturunan baru dengan sifat-sifat unggul yang bermanfaat. Percobaan ini bertujuan untuk menentukan pengaruh dosis iradiasi sinar gamma pada pertumbuhan dan perkembangan kalus sagu (*Metroxyon sagu*). Kalus remah sagu yang berasal dari kultur suspensi digunakan sebagai sumber bahan penelitian. Kalus primer tersebut berasal dan hasil induksi jaringan meristem pucuk dari anakan sagu varietas Alitir yang berasal dari Merauke, Papua. Perlakuan yang diuji adalah dosis iradiasi sinar gamma yang terdiri atas 0, 5, 10, 15, 20, dan 25 Gy. Kalus yang telah diberi perlakuan iradiasi sinar gamma kemudian disubkultur pada media padat Murashige dan Skoog yang dimodifikasi (MMS) mengandung 3% sukrosa dan 0,1% arang aktif, serta zat pengatur tumbuh 2,4-D 1 mg l^{-1} dan kinetin 0,1 mg l^{-1}. Hasil penelitian menunjukkan bahwa semua perlakuan dosis iradiasi sinar gamma dapat meningkatkan biomassa kalus secara nyata. Penggandaan biomassa kalus tertinggi sebesar 5,33 kali lipat dari awal kultur setelah empat minggu dicapai pada perlakuan dosis sinar gamma 25 Gy, sedangkan penggandaan biomassa kalus terendah sebesar 3,4 kali lipat diperoleh pada kontrol. Perkembangan kalus embriogenik terbaik dicapai pada perlakuan 10 Gy yang mampu menghasilkan embrio somatik 100%, sedangkan pembentukan embrio somatik terendah diperoleh pada kontrol dan 25 Gy setelah disubkultur satu kali. Respons yang tinggi dari induksi embrio somatik terhadap sinar gamma pada dosis 10 Gy meningkatkan produksi embrio somatik. Hasil yang diperoleh dapat digunakan dalam pemuliaan* in vitro *tanaman sagu melalui mutagenesis untuk menghasilkan varietas unggul baru.*

[***Kata kunci***: *Metroxylon sagu,* iradiasi sinar gamma, kalus embriogenik, embrio somatik]

INTRODUCTION

Sago palm (*Metroxylon sagu* Rottb.) is an important crop for supporting Indonesian food security (Tarigans 2001; Ehara 2009) and its distribution is abundant particularly in eastern parts of Indonesia such as Maluku and Papua (Bintoro *et al*. 2007). The area of sago palm was estimated 2.47 million ha world wide and more than half was grown in Indonesia. Sago starch has been used for many years as a staple food especially in eastern Indonesia and as material sources for various foods such as cakes and noodles (Flach 1997).

The use of sago palm is very diverse not only for foods but also for various industrial products including bioethanol (Kanro *et al*. 2003). In the food industry, sago starch is a material source for noodles, cakes and other foods (Bintoro *et al*. 2007). In the

beverage industry, sago is used for producing syrup. Sago palm is also used for other industrial materials such as adhesive gels, pharmaceuticals, cosmetics, bioplastics and papers (Flach 1997; Rostiwati *et al*. 2008; Singhal *et al*. 2008).

Tissue culture of sago palm has been established through somatic embryogenesis (Tahardi *et al*. 2002; Sumaryono *et al*. 2009), where early development of somatic embryos has been achieved by culturing shoot apical tissues as explants on a modified MS medium containing high concentrations of 2,4-D and 1 mg l^{-1} kinetin (Tahardi *et al*. 2002). Somatic embryogenesis has been chosen over bud multiplication and organogenesis because of its higher multiplication rate in producing an abundant supply of uniform superior planting materials (Muruganantham *et al*. 2010).

In vitro mutagenesis has been successfully done for developing elite clones of commercial crops (Barakat and El-Sammak 2011). Two types of mutagens are chemical mutagens such as DMSO, EMS, MMS, dNS; and physical mutagens such as cosmic ray, ion beam, and irradiation using alpha, beta and gamma rays. Irradiation technique is more popular than chemical and other physical mutagenesis (IAEA 2012) because irradiation is rapid, convenient and more extensive and the ionizing energy penetrates rapidly through the polysaccharide granule (Bao *et al*. 2005). The most popular irradiation is gamma ray irradiation from Co^{60} which is widely used in agriculture (Piri *et al*. 2011) as a mutagenesis agent for large commercial crops like ornamental plants (Barakat and El-Sammak 2011), food crops (IAEA 2012), pharmaceutical plants (Sung *et al*. 2013) and oil palm (Rohani *et al*. 2012).

The objective of this study was to determine the optimum dosage of gamma ray irradiation from Co^{60} for the growth and development of sago palm calli particularly callus proliferation, somatic embryo induction, and regeneration into plantlets.

MATERIALS AND METHODS

Plant Materials and Culture Conditions

The research was conducted at the Laboratory of Plant Cell Culture and Micropropagation, the Indonesian Research Institute for Biotechnology and Bioindustry, Bogor, West Java. Friable calli were used as materials (Fig. 1a). The calli were initiated from apical meristematic tissues of young suckers of sago palm of Alitir variety from Merauke, Papua. The calli have been cultured several times before being used as plant materials.

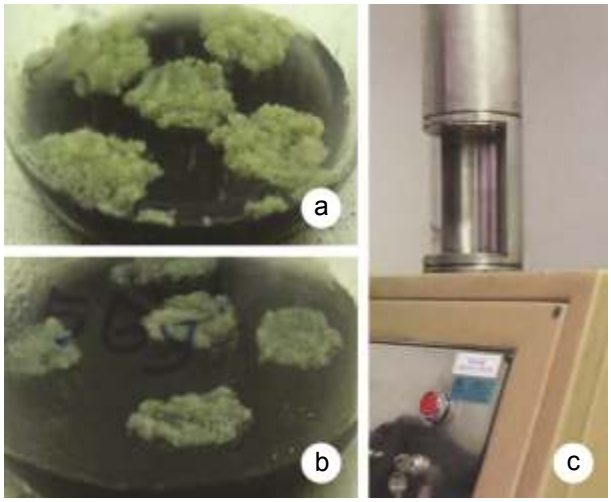

Fig. 1. Irradiation on sago palm calli: (a) clumps of friable calli before treated, (b) calli culture after irradiated (one week after culture) and (c) apparatus of gamma irradiator.

The irradiated calli were cultured on a solid modified MS (MMS) in a culture bottle (jar) with 5 callus clumps per bottle. The initial callus weight was 1 g per bottle. Each treatment was replicated five times. The media were supplemented with 3% sucrose, 1 g l^{-1} activated charcoal, 1 mg l^{-1} 2,4-D and 0.1 mg l^{-1} kinetin (Riyadi *et al*. 2005). The pH of the medium was adjusted to 5.8 before autoclaved at 121° C and 1 kg cm^{-2} pressure for 20 minutes.

The cultures were incubated in the culture room at 26° C under dark conditions in callus proliferation (first culture) and under cool-white fluorescent lamps providing approximately 20 µmol photon m^{-2} s^{-1} of light intensity over a 12-h photoperiod in somatic embryo induction or formation (second culture) and also in somatic embryo regeneration phase. The duration of each culture was 4 weeks.

The somatic embryos formed after 8 weeks were subsequently regenerated into shoots on solid media in culture bottles. The development and rooting of plantlets were conducted in liquid media in culture tubes for 6–8 weeks, then subcultured again into the same media. Two plantlets were placed in one culture tube.

Application of Gamma Ray Irradiation

The application of irradiation was conducted at the National Nuclear Energy Agency of Indonesia,

Jakarta. The selected friable calli of all irradiation dosage treatments were brought to the Agency to be treated with gamma ray irradiation using an irradiator apparatus (Fig. 1c).

The dosage of gamma ray irradiation was 0 (control treatment), 5, 10, 15, 20 and 25 Gy. After irradiation, all calli were brought back immediately to the Laboratory of Plant Cell Culture and Micropropagation to be subcultured on fresh media (Fig. 1b).

Statistical Analysis

The experiment was arranged using a completely randomized design. Data were subjected to one-way analysis of variance test (F test) using IBM SPSS Statistics 19.0 for Windows. Differences among treatment means were determined by Duncan's multiple range test at $\alpha < 0.05$.

RESULTS AND DISCUSSION

Callus Proliferation

Calli of sago palm survived from the treatments of gamma ray irradiation up to 25 Gy. All calli grew well with a very high biomass proliferation rate. The highest calli biomass proliferation of 5.3 folds from the initial culture in the first 4 weeks (first culture) and 4.8 folds in the next 4 weeks (second culture) was achieved in 25 Gy irradiation dosage. The lowest biomass yield of 3.4 folds from the initial culture in the first 4 weeks (first culture) and 3.2 folds in the next 4 weeks (second culture) was obtained in untreated control (Table 1). Similar results were obtained in embryogenic calli of oil palm derived from zygotic embryo explants (Rohani *et al*. 2012) and in embryogenic calli of citrus derived from nucellus explants (Agisimanto *et al*. 2016) treated with gamma irradiation where callus growth decreased at 40 Gy and above. In embryogenic callus of sugarcane, however, gamma irradiation at 20 Gy already decreased callus proliferation (Yasmin *et al*. 2011).

The biomass calli proliferation rate in the first culture was higher than that in the second culture for all treatments tested. In early second culture, proembryos and somatic embryos started to emerge particularly in gamma irradiation dosage of 10 Gy. The first embryogenic calli or proembryos emerged at 3 weeks after culture (Fig. 2). For untreated control and 25 Gy dosage of gamma irradiation, the cultures remained as friable calli.

Biomass increase of calli is an important aspect in order to yield a higher multiplication rate of the calli, however it is not necessarily in the best culture for *in*

Table 1. Fresh weight of sago palm calli after treated with different dosages of gamma ray at four weeks (first culture) and eight weeks (second culture).

Gamma ray irradiation (Gy)	Fresh weight (g)	
	4 weeks	8 weeks
0	3.4d	11.0e
5	4.2c	15.4d
10	5.0b	24.2b
15	5.0b	22.5c
20	3.6d	11.4e
25	5.3a	25.7a

Numbers in the same column followed by the same letters are not significantly different according to Duncan's multiple range test at $\alpha < 0.05$.

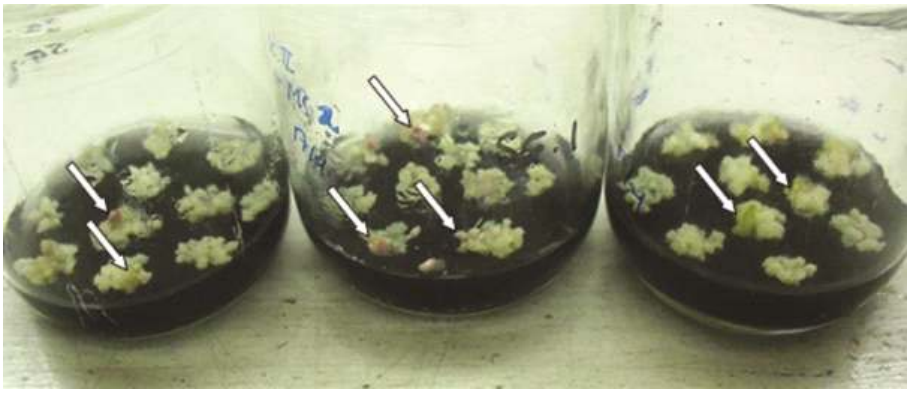

Fig. 2. Growth and development of irradiated sago palm calli after eight weeks (second culture): somatic embryos started to emerge. Arrows indicate the somatic embryos.

vitro breeding process of sago palm. Other aspects must be considered such as embryogenic and regeneration capacity, somatic embryo or plant abnormality, starch yield, starch quality and harvesting time. In term of callus proliferation rate, the best treatment was gamma irradiation at 25 Gy (Table 1), while for somatic embryogenesis, the best treatment was gamma irradiation at 10 Gy (Table 2).

Somatic Embryo Induction

The first somatic embryos emerged at the end of the second culture (the second 4 weeks of culture) mostly at 10 Gy dosage of gamma ray. Somatic embryos were not formed at untreated control. The highest somatic embryo formation at 100% in the second culture was achieved at 10 Gy dosage of gamma ray irradiation (Table 2). Somatic embryos were in the form of aggregates or clusters. The surface of somatic embryos were reddish-white or yellowish-green and opaque (Fig. 2).

Proembryos and somatic embryos of sago palm were obtained only at 5 Gy until 20 Gy of gamma irradiation dosage at 8 weeks after culture. The best gamma irradiation dosage was 10 Gy then somatic embryo induction decreased as the dosage of gamma ray increased (Table 2). It indicates that treatments of gamma irradiation up to 20 Gy increased somatic embryo formation in sago palm. Gamma ray irradiation has been successfully applied in many crops for *in vitro* mutagenesis (Barakat and El-Sammak 2011; IAEA 2012; Sung *et al*. 2013). The somatic embryos of sago palm were able to regenerate into small shoots in the following 4 to 6 weeks of culture.

Table 2. Effect of gamma ray irradiation on somatic embryo and germinated somatic embryo induction of sago palm after eight weeks of culture.

Gamma ray irradiation (Gy)	Proembryo induction (%)	Number of germinated somatic embryos (%)
0	0.0d	4.6d
5	14.3c	5.4c
10	100.0a	9.8a
15	35.0b	7.4b
20	16.7c	5.8c
25	0.0d	1.2e

Numbers in the same column followed by the same letters are not significantly different according to Duncan's multiple range test at $\alpha < 0.05$.

Somatic Embryo Regeneration

Gamma irradiation affected significantly the number of germinated somatic embryos (Table 2). Untreated control culture produced 4.6 embryos per jar. Increasing the gamma ray dosages increased the number of somatic embryos up to 10 Gy, then decreased gradually. Culture with gamma ray at 10 Gy produced the highest number of embryos on average 9.8 somatic embryos per culture jar, while at 25 Gy it produced only 1.2 embryos per jar.

Somatic embryos germinated into small shoots within 8 weeks of culture (Fig. 3a). All the somatic embryos were able to develop into normal shoots.

Fig. 3. Development of somatic embryos of sago palm: (a) small shoots on solid media and (b) growth and rooting of plantlets in liquid media.

The shoots were then transferred into test tubes containing liquid media for plantlet growth and rooting for 6–8 weeks, and subcultured into the same media for another 6–8 weeks. At this stage the plantlets had 2–4 leaves and good root systems (Fig. 3b). These plantlets were ready to be acclimatized to *ex vitro* conditions.

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^{-1} sucrose and 7.5 g l^{-1} sorbitol. They suggested that *in vitro* culture of sago palm is more favorable in an environment with high solute concentration or low osmotic potential. However, results reported by Sumaryono *et al*. (2012) revealed that increasing the concentration of sucrose or other carbohydrates did not favor the growth and vigor of sago plantlets. Rooting of the plantlets was conducted in liquid medium as commonly performed in oil palm plantlets (Konan *et al.* 2007). Good vigor and root system of plantlets increased the survival rate of plantlets during acclimatization.

CONCLUSION

Application of gamma ray irradiation affected callus proliferation and somatic embryo induction of sago palm. The highest calli biomass proliferation of sago palm was obtained at 25 Gy gamma irradiation, whereas the highest somatic embryo induction was obtained at 10 Gy gamma irradiation. The somatic embryos of sago palm was successfully regenerated into vigorous plantlets. This result may support *in vitro* mutagenesis of sago palm breeding to generate new elite varieties.

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