
January 2022

THE SECOND INTERNATIONAL CONFERENCE ON GENETIC RESOURCES AND BIOTECHNOLOGY: Harnessing Technology for Conservation and Sustainable Use of Genetic Resources for Food and Agriculture

# Committees: The Second International Conference on Genetic Resources and Biotechnology

Cite as: AIP Conference Proceedings **2462**, 010002 (2022); https://doi.org/10.1063/12.0008934
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# Preface: The Second International Conference on Genetic Resources and Biotechnology

The Second International Conference on Genetic Resources and Biotechnology, which is the continuation of the first event held in 2018, focuses on topics related to advances in biotechnology to create more opportunities for effective conservation and sustainable utilization of genetic resources for food and agriculture. This year conference's theme is Harnessing Technology for Conservation and Sustainable Use of Genetic Resources for Food and Agriculture. The conference was organized by Indonesian Agency for Agricultural Research and Development (IAARD), Ministry of Agriculture, Indonesia, in collaboration with Indonesian Biotechnology Consortium and held on 24$^{th}$-25$^{th}$ of May 2021virtually due to the pandemic of COVID-19.

The conference aims to share and exchange current scientific information and technological developments on biotechnology and their applications for conservation and sustainable use of genetic, to encourage and promote quality, efficiency, and modernization of management and utilization of genetic resources, and to facilitate national and international collaboration among participants. There are five scopes discussed in this conference. They are effective management of conservation and sustainable use of genetic resources for food and agriculture, application of genomics and molecular markers for genetic resource conservation and crop adaptation to climate change, application of innovative crop improvement techniques for conservation and sustainable use of plant genetic resources for food and agriculture, plant cell and tissue culture for conservation and effective utilization of genetic resources, and the use of microbial genetic resources as biological control agents of agricultural pests and diseases, and for soil bioremediation.

Five speakers from the United States of America, Japan, India and Indonesia were invited to discuss about their expertise and knowledge on relevant subjects in the plenary sessions. This conference was attended by more than 100 participants including 75 presenters and 44 listeners worldwide. They came from diverse governmental, private, or academic institutions and also scientific communities. The presented materials have undergone peer review processes and only qualified papers were selected. Furthermore, all papers were subjected to double blind peer-review and expected to meet the scientific criteria of significance and academic excellence to be published in a conference proceedings indexed in a well-known, reputable service.

We would like to express our sincere gratitude to our speakers, presenters and all participants for their contributions in this conference. We would also like to express our appreciation for the generosity of our sponsors that support this conference: PT CropLife, PT ITS Science Indonesia, PT Fajar Mas Murni and PT Prima Instrument Analitika. Lastly, special thanks to all committee members for their exceptional work and contributions in the conference and publication.

Chair of Organizing Committee

Dr. Toto Hadiarto

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# The application of gamma ray irradiation to increase triterpenoid compounds in embryogenic calli of *Centella asiatica* L. Urban

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**Ika Roostika, Suci Rahayu and Nurliani Bermawie**

# The Application of Gamma Ray Irradiation to Increase Triterpenoid Compounds in Embryogenic Calli of *Centella asiatica* L. Urban

Ika Roostika[1, a)], Suci Rahayu[1] and Nurliani Bermawie[2]

[1]*Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development, IAARD, Jln. Tentara Pelajar No. 3A, Bogor 16111, West Java, Indonesia*
[2]*Indonesian Spice and Medicinal Crops Research Institute, IAARD, Jln. Tentara Pelajar No. 3, Bogor 16111, West Java, Indonesia*

[a)]Corresponding author: roostikatambunan@gmail.com

**Abstract.** *Centella asiatica* L. Urban has bioactive compounds from triterpenoid group. Raw materials that are not standardized are one of the obstacles in supplying the needs of the pharmaceutical industry. The *in vitro* culture technology may solve this problem. The purpose of this study was to determine the effect of gamma ray irradiation on growth and triterpenoid content of embryogenic calli of *C. asiatica*. The three accessions tested were CASI 016, CASI 002, and released varieties Castina 1. Gamma ray irradiation was carried out at doses of 0, 1, 2, 3, 4, and 5 Krad. The results showed that the *in vitro* response of each accession was genotype dependent. Castina 1 contained higher triterpenoid compounds than the other accessions. However, the gamma ray irradiation treatment up to 5 Krad has not been able to increase the content of these compounds. The treatment could only increase the content of asiaticoside and asiatic acid of accession CASI 016 at the dose of 5 Krad. There was an increase of asiaticoside and madecasoside content of accession CASI 002. It is expected that the increase of gamma ray irradiation dose will increase the triterpenoid compounds content in embryogenic calli of *C. asiatica*.

## INTRODUCTION

Traditionally, *Centella asiatica* L. Urban, commonly known as *pegagan*, is used as medicine in various countries, such as China, India, Nepal, Malaysia, Bangladesh, Fiji Islands, Madagascar, and Brazil [1] as well as Indonesia. *Pegagan* has bioactive compounds from the triterpenoid group. Pharmacologically and clinically, *pegagan* products (both in the form of extracts and bioactive compounds) have been proven to treat various diseases, including wounds, stomach ulcers, tumors, disorders of the nerves, heart, and liver, be able to protect the body from irradiation, used as antistress, antidepressant, anti-inflammatory, and sedative, strengthens the vascular system, improves mental retardation, strengthens memory, and for the treatment of Alzheimer's disease [2–4].

Because of its many uses, it become a valuable tropical medicinal plant [5, 6]. Currently, various kinds of its products have been circulating in the world market. The increase in its demand is not balance by the availability of raw materials because conventional propagation methods are not able to meet the demand of pharmaceutical industry [6, 7]. In Indonesia, the plant materials are one of the main ingredients of herbal medicine besides javanese turmeric, *Kaempferia galanga*, ginger, and green chiretta. The demand for herbal medicines or herbal medicine from Indonesia is very large. The herbal ingredients have been exported to China in the form of extracts or simplicia1. Raw materials that are not standardized are one of the obstacles in supplying the needs of the pharmaceutical industry.

To solve this problem, it is advisable to use *in vitro* culture technology instead of using the whole plant for the extraction of the desired secondary metabolites [8]. It was reported that medicinal crop *purwoceng* (*Pimpinella pruatjan*) callus culture could increase the content of stigmasterol and sitosterol compounds by 10–100 times than

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those contained in the roots of 9 months old plants [9]. Induced mutation is an effective strategy for increasing secondary metabolites [10]. Because *C. asiatica* is clonally propagated, somaclonal variation technique is a suitable method to be applied. Somaclonal variation is an accumulative result of genetic changes from explants under *in vitro* conditions. This diversity can be increased through induced mutation with gamma ray irradiation [11]. The purpose of this study was to determine the effect of gamma ray irradiation on callus growth from three accessions of *C. asiatica* and the content triterpenoid compounds.

## MATERIALS AND METHODS

This research was conducted at Tissue Culture Laboratory in Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development (ICABIOGRAD), Bogor from March until November 2015. The plant material used was three accessions of *C. asiatica*, namely CASI 016, CASI 002, and Castina 1 which were collected at the Manoko Experimental Station, Lembang, West Bandung belong to the Indonesian Spice and Medicinal Plants Research Institute. The explants sources were 2 months seedlings from cuttings that were maintained in a greenhouse belong to ICABIOGRAD, Bogor. Tendrils were sterilized using fungicides and bactericides for 1 h, then sterilized using 70% alcohol for 7 min, 15.75% NaOCl for 5 min, and 10.5% NaOCl for 10 min. The explants were planted on MS medium. Leaves from *in vitro* shoots were isolated and planted on callus induction media consisted of MS medium with the addition of 4 mg/l dicamba. The calli induced from leaves were then used as the explants in this experiment. The callus was maintained on MS medium supplemented with 4 mg/l dicamba. After 4 weeks incubation period (WIP), the calli were irradiated with gamma rays at doses of 0, 1, 2, 3, 4, and 5 Krad. The irradiation was carried out at the National Atomic Energy Agency, Jakarta. The treatments were repeated 8 times. Post irradiation treatment, the calli were transferred onto the same medium for recovery. They were incubated in the dark condition at 25±20°C. The variables observed were the number of brown calli, the number of proliferated calli, the growth score of calli, and the texture of calli.

To analysis the content of triterpenoid compounds, the fresh calli were weighed with analytical balance and then they were dried in the laminair air flow cabinet overnight. The calli were then heated in the oven at 40°C overnight and their dry weight were weighed. Each dry material from the three accessions was ground, then ±0.25 g was taken and put into a 25 ml volumetric flask. Technical ethanol was added in ±1/2 volume of the flask. Shaking was done using a shaker for 2 h. The mixture was left for 24 h, then filtered until the filtrate was obtained. For the preparation of standard solutions, each of ±0.001 g of standard powder (asiaticoside, madecasoside, and asiatic acid) was put into a 10 ml volumetric flask and absolute ethanol was added to the mark. The standard solution was ready to be measured. A total of 5 µl of the filtrate and 5 µl of the standard solution were placed on the Aluminum Silica gel 60 F254 plate with a distance of 2 cm between the spots. The plate was then inserted into a chamber containing eluent which consisted of $CHCl_3$ p.a. and ethanol absolute + ethyl acetate p.a. with the ratio of 49:1. Elution was carried out for ±45 min then the plate was moved and dried in the open air. After drying, the plates were measured using a TLC scanner at a wavelength of 276 nm. The level of triterpenoid compounds was calculated by measuring the concentration of the sample (ppm) multiplied by the volume of the filtrate and the dilution factor, then divided by the dry weight of the sample and multiplied by 100%.

## RESULTS AND DISCUSSIONS

The result showed that the proliferation rate of Castina 1 callus was not inhibited even it had been irradiated with gamma rays up to 5 Krad (Table 1). The fresh weight of calli was increased with increasing irradiation dose followed by the increase of browning rate. The irradiation treatment caused their texture to be friable (Table 1, Fig. 1). It is suspected that gamma ray irradiation treatment stimulates cells development of *C. asiatica* to form embryogenic calli. Such calli were potentially to be regenerated into somatic embryos. The similar observation was reported in previous research that the calli of *purwoceng* and *Citrus remiculata* cv. Limau Madu could differentiate more quickly to form mature somatic embryos after gamma ray irradiation treatment [9, 12].

**TABLE 1.** The effect of gamma ray irradiation dose on callus growth of *Centella asiatica* Castina 1 variety at 6 WIP.

| Irradiation dose (Krad) | Fresh weight of callus (g) | Proliferated callus (%) | Score of callus growth | Score of browning callus | Callus texture |
|---|---|---|---|---|---|
| 0 | 0.96 | 100 | +++ | 1.90 | Compact, dry |
| 1 | 1.71 | 100 | +++ | 1.52 | Friable, dry |
| 2 | 1.31 | 100 | ++ | 2.72 | Friable, dry |
| 3 | 1.07 | 100 | +++ | 2.52 | Friable, dry |
| 4 | 1.04 | 100 | ++ | 2.67 | Friable, watery |
| 5 | 1.00 | 100 | +++ | 2.62 | Friable, watery |

+ = low, ++ = medium, +++ = high.

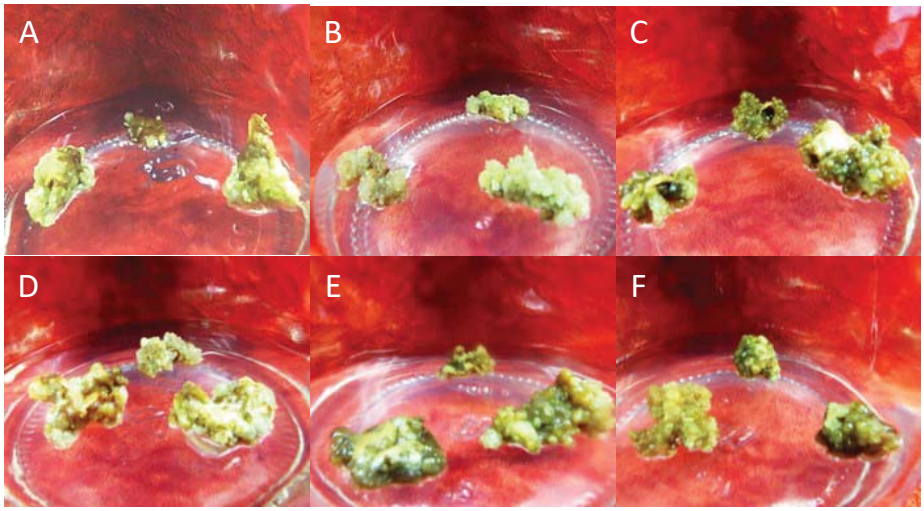


**FIGURE 1.** Performance of *Centella asiatica* calli Castina 1 variety post gamma ray irradiation: control (a), 1 Krad (b), 2 Krad (c), 3 Krad (d), 4 Krad (e), and 5 Krad (f).

In contrast to Castina 1, calli from accession CASI 016 had an increased fresh weight after gamma ray irradiation at doses of 1 and 2 Krad. The browning rate increased and the growth decreased with increasing irradiation dose (Table 2, Fig. 2). The results were similir with gamma ray irradiation effect on shoot growth and multiplication of patchouli *in vitro* [13]. The proliferation rate of CASI 016 callus was also not inhibited up to 5 Krad of gamma rays. The irradiation treatment caused their texture to be friable and dry. A different response was shown by accession CASI 002. The fresh weight of the calli decreased with increasing irradiation doses, high doses (4 and 5 Krad) caused the calli to be compact or not friable (Table 3, Fig. 3).

These results indicated that the response of *A. asiatica* after gamma ray treatment was very dependent by genotype. Similar response was also reported by Pangestuti *et al*. [14] when apply gamma ray irradiation treatment to garlic (*Allium sativum*) and *lulo* (*Solanum quitoense* Lam.) genotypes [15].

**TABLE 2.** The effect of gamma ray irradiation dose on callus growth of *Centella asiatica* accession CASI 016 at 6 WIP.

| Irradiation dose (Krad) | Fresh weight of callus (g) | Proliferated callus (%) | Score of callus growth | Score of browning callus | Callus texture |
|---|---|---|---|---|---|
| 0 | 2.37 | 100 | +++ | 1.10 | Friable, dry |
| 1 | 4.42 | 100 | +++ | 1.00 | Friable, dry |
| 2 | 2.76 | 100 | ++ | 1.10 | Friable, dry |
| 3 | 2.15 | 100 | ++ | 1.14 | Compact, dry |
| 4 | 1.77 | 100 | ++ | 1.24 | Compact, dry |
| 5 | 1.27 | 100 | ++ | 2.48 | Friable, dry |

+ = low, ++ = medium, +++ = high.

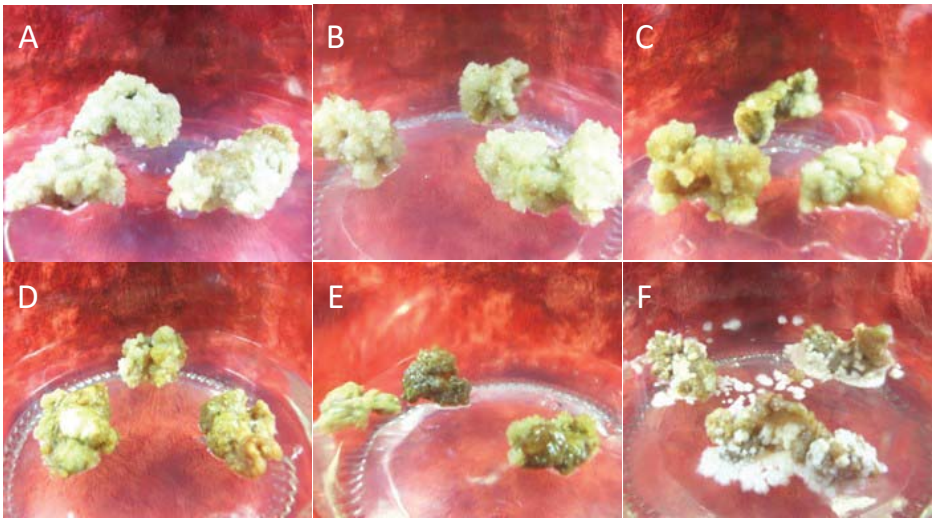


**FIGURE 2.** Performance of *Centella asiatica* calli accession CASI 016 post gamma ray irradiation: control (a), 1 Krad (b), 2 Krad (c), 3 Krad (d), 4 Krad (e), and 5 Krad (f).

**TABLE 3.** The effect of gamma ray irradiation dose on callus growth of *Centella asiatica* accession CASI 002 at 6 WIP.

| Irradiation dose (Krad) | Fresh weight of callus (g) | Proliferated callus (%) | Score of callus growth | Score of browning callus | Callus texture |
|---|---|---|---|---|---|
| 0 | 2.65 | 100 | +++ | 1.29 | Friable, dry |
| 1 | 2.29 | 100 | +++ | 1.11 | Friable, dry |
| 2 | 1.77 | 100 | +++ | 1.05 | Friable, dry |
| 3 | 1.85 | 100 | +++ | 1.22 | Friable, dry |
| 4 | 1.60 | 100 | ++ | 1.38 | Compact, dry |
| 5 | 1.66 | 100 | ++ | 1.55 | Compact, dry |

+ = low, ++ = medium, +++ = high.

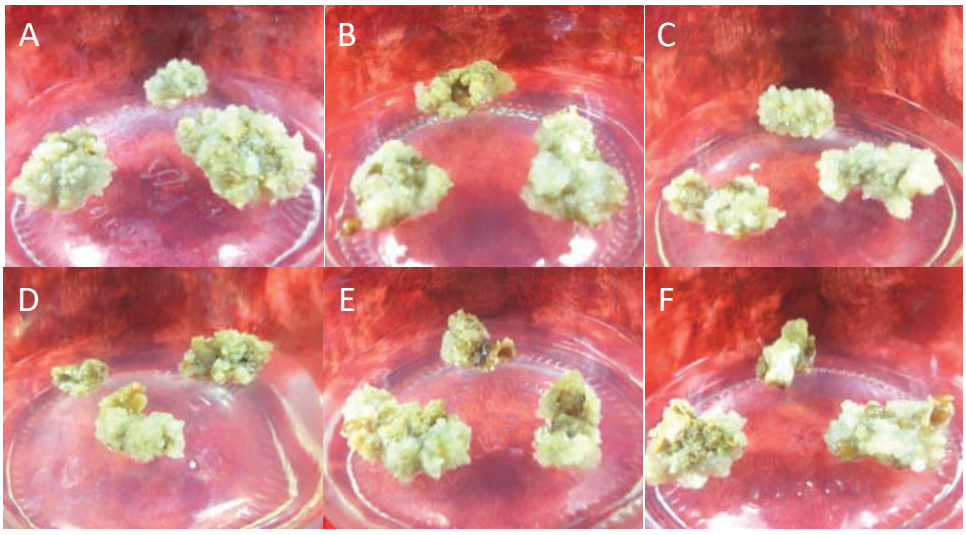


**FIGURE 3.** Performance of *Centella asiatica* calli accession CASI 002 post gamma ray irradiation: control (a), 1 Krad (b), 2 Krad (c), 3 Krad (d), 4 Krad (e), and 5 Krad (f).

Based on fresh and dry weight measurements, the water content of the calli of two accessions of *C. asiatica* (Castina 1 and CASI 016) tended to decrease after gamma ray irradiation (Tables 4–6). This indicates an increase in biomass after irradiation applications. The biomass increase of *C. asiatica* was also reported by Tan *et al.* (2010). The TLC scanner analysis showed that all calli from the three accessions of *C. asiatica* had a higher asiaticoside content than the minimum standard for its herb at 0.9% (Tables 4–6). The calli of Castina 1 had a higher triterpenoid content than the other two accessions, but irradiation treatment of up to 5 Krad was not able to increase the content of those compounds (Table 4). For accession CASI 016, irradiation treatment could only increase the asiaticoside and asiatic acid content of callus treated with 5 Krad irradiation (Table 5). For accession CASI 002, there was an increase in the asiaticoside and madecasoside content of the calli treated with gamma ray irradiation. Conversely, irradiation treatment caused a decrease in the asiatic acid content (Table 6).

Based on those data, it is assumed that the calli of *C. asiatica* may still survive and proliferate when the irradiation dose is increased more than 5 Krad. Increasing the irradiation dose may caused the increase of somaclonal variations as well as the content of triterpenoid compounds. An increase in antioxidant compounds and carotenoids from *C. asiatica* has been reported by Norhayati *et al*. [16] through callus culture and an increase in eights bioactive flavonoid content. Moghaddan *et al*. [17] and Jeon *et al*. [18] stated that irradiation with light spectrum was closely related to the enhancement of growth and bioactive compounds of dropwort (*Oenanthe stolonifera*).

**TABLE 4.** The effect of gamma ray irradiation dose on triterpenoid contents in calli of *Centella asiatica* Castina 1 variety at 6 WIP.

| Irradiation dose (Krad) | Water content (%) | Asiaticoside (%) | Asiatic acid (%) | Madecasoside (%) |
|---|---|---|---|---|
| 0 | 96.57 | 2.87 | 2.18 | 2.49 |
| 1 | 93.47 | 1.91 | 1.20 | 1.46 |
| 2 | 92.02 | 2.56 | 1.44 | 2.00 |
| 3 | 90.58 | 1.93 | 1.00 | 1.61 |
| 4 | 88.75 | 2.43 | 1.23 | 2.07 |
| 5 | 90.31 | 1.91 | 0.93 | 1.76 |

**TABLE 5.** The effect of gamma ray irradiation dose on triterpenoid contents in calli of *Centella asiatica* accession CASI 016 at 6 WIP.

| Irradiation dose (Krad) | Water content (%) | Asiaticoside (%) | Asiatic acid (%) | Madecasoside (%) |
|---|---|---|---|---|
| 0 | 96.76 | 1.37 | 1.61 | 1.94 |
| 1 | 95.14 | 1.41 | 1.58 | 1.66 |
| 2 | 94.95 | 1.41 | 1.52 | 1.61 |
| 3 | 94.39 | 1.42 | 1.52 | 1.54 |
| 4 | 94.35 | 1.49 | 1.57 | 1.51 |
| 5 | 93.44 | 1.70 | 1.83 | 1.53 |

**TABLE 6.** The effect of gamma ray irradiation dose on triterpenoid contents in calli of *Centella asiatica* accession CASI 002 at 6 WIP.

| Irradiation dose (Krad) | Water content (%) | Asiaticoside (%) | Asiatic acid (%) | Madecasoside (%) |
|---|---|---|---|---|
| 0 | 96.37 | 1.49 | 1.35 | 1.30 |
| 1 | 91.97 | 1.50 | 1.13 | 1.30 |
| 2 | 94.22 | 1.45 | 0.96 | 1.33 |
| 3 | 93.12 | 1.60 | 0.88 | 1.44 |
| 4 | 91.93 | 1.60 | 0.79 | 1.45 |
| 5 | 92.06 | 1.69 | 0.73 | 1.57 |

## CONCLUSION

The *in vitro* response of post-irradiated calli of *C. asiatica* was genotype dependent. The asiaticoside content of the calli from three accessions was higher (1.37−2.87%) than the minimum standard for herb medicine (0.9%). The calli of Castina 1 had a higher triterpenoid content than the other two accessions, but irradiation treatment of up to 5 Krad was unable to increase the content of these compounds. Irradiation treatment could increase the content of asiaticoside and asiatic acid from accession CASI 016, which irradiated at dose of 5 Krad. There was an increase in the asiaticoside and madecasoside content of the calli from accession CASI 002 after gamma ray irradiation. Conversely, irradiation treatment caused a decrease in their asiatic acid content. It is assumed that the calli of *C. asiatica* may still survive and proliferate when the irradiation dose is increased more than 5 Krad. Increasing the irradiation dose may caused the increase of somaclonal variations as well as the content of triterpenoid compounds.

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