In Vitro Propagation of *Curcuma Caesia* Roxb

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Abstract

Curcuma caesia Roxb. is a species belong to genus *Curcuma*, under family Zingiberaceae. It is called 'Kunyit Hitam' (Malay), Kala haldi (Bengal), Manupasupu (Telugu) and Lampuyangdorac (Visayan). It is a non – native plant in Malaysia. Kunyit Hitam offers several advantages included contain antioxidant activity and the pharmacological effects for future clinical used. As its vegetatively propagated underground rhizome, it is not quickly reproduced. Therefore, an alternative method of reproduction for this species is by plant tissue culture method. The study was determined the effect of varies concentration (1, 2, 3, 4 and 5 mg/L) of 2,4-Dichlorophenoxyacetic acid (2,4-D) in combination with 0.5 mg/L of 6-benzylaminopurine (BAP) and 0,2.5, 5.0, 7.5 and 10 mg/L of picloram alone on callus induction. Leaves, roots and stems of *C.caesia* were used as explants and were cultured on half strength solid MS medium. The highest mean number of callus formed was 3.44 ± 0.98 in 2.5 mg/L of picloram. Then, in the effect of 2,4 D in combination with BAP, the highest mean number of callus formed was 1.99 ± 0.67 in 1.0 mg/L 2,4 D with 0.5 mg/L BAP and 3.0 mg/L 2,4 – D with 0.5 mg/L BAP.

Keywords: *Curcuma caesia,* callus induction, Murashige and Skoog (MS)

1.0 Introduction

Zingiberaceae is one of the largest families of plant kingdom and consist of 52 genera and 1500 species (Sirirugsa, 1999). As stated by Sirirugsa (1999), these family are highest distributed in Malesian regions like Indonesia, Malaysia, Singapore, Brunei, Philippines and Papua New Guinea. One of the genera listed under this family is the genus *Curcuma* that consist of more than 80 species of rhizomatous herbs. It was first introduced by Linnaeus in 1753 (Velayudhan *et al.*, 1994). The *Curcuma caesia* Roxb. is a species that is classified under this genus. This species is native to North – East and Central India.

Generally, the species in family Zingiberaceae is very important as natural resources that provide many useful uses like dyes, medicinal properties, spices in the culinary sector, perfume, colouring materials in pharmacy and food industries. The part of these plants that make they are so valuable is their rhizome part. Commonly, the value of each species in this genus is considered based on their rhizome.

Most of *Curcuma* species contain an antioxidant activity and the pharmacological effects for future clinical used (Miquel, Bernd, Sempere,

Diaz-Alperi & Ramirez, 2002). Besides, antioxidant can prevent the body from the reaction of excess free radical that can destroy the body cells and tissues (Noor Zainah & Muhammad Khairuddin, 2008). It is present naturally in the food like vegetables and fruits. Therefore, the human body really needs the antioxidant substances to keep healthy.

As *C. caesia* Roxb. and other *Curcuma* species are vegetatively propagated by underground rhizome, they are do not reproduce rapidly like the other monocotelydon plants. Besides, most of the *Curcuma* species is limited availability at high yielding genotypes, expensive field maintenance of planting material and high possibility exposure to rhizome rot diseases. Therefore, the only alternative method of reproduction for the species that have a low multiplication rate is the plant tissue culture technique.

Plant tissue culture technique for *Curcuma* species either through rhizomes buds or leaves has been reported widely nowadays especially the *Curcuma longa* or turmeric that is very important in several sectors like food industries, colouring industries and pharmacy industries. According to Ashmore (1997), the *in vitro* techniques is a relatively safe method for conservation and international exchange with limited quarantine of various vegetatively propagated corp germplasm.

Since there is limited work has been done for *C. caesia* Roxb., this study would be carried out to determine rapid clonal multiplication, conservation of important plant species, making the stock disease free and improve their genotype. The main objective of this study determined the effect of 2,4 D and BAP on callus induction and development and to determine the effect of picloram on callus formation.

2.0 Literature Review

The *C. caesia* Roxb. that belongs to genus *Curcuma* and under family Zingiberacea have several local names. For instance, this species is commonly known as Kala haldi, Kalo halud and Nilkantha (Bengal), Kali haldi (Hindi), Nar kacchur (Marathi), Manupasupu (Telugu) and Lampuyangdorac (Visayan) (Velayudhan *et al.*, 1994). Besides that, this plant species also known as Kunyit hitam or Temu hitam in Malay language.

Each of *Curcuma* species has their own chemical contents. By doing extraction and analysis of target species, the chemical content can be identified. Curcumin is a yellow bioactive pigment which is one of the major component in most of *Curcuma* species. Pratap Singh and Jain (2011) claims that curcumin hasa wide spectrum of biological activities like antifungal, antioxidant, anticancer, antibacterial, anti-HIV and anti-tumor. In addition, anti -oxidants can be classified into two types of compound which are phenolic and β – ketone. The antioxidant in curcumin is eight times more powerful than in Vitamin E (Reddy & Lokesh, 1992).

Generally, each of the *Curcuma* species has their own importance value in various aspects. This plant species are found very useful in medical aspect. It had been proved by several studies. Curcumin that comes from the extraction of *C. longa* is believed to have an antiviral activity being an HIV -1 integrase inhibitor. This may be developed as anti – AIDS drugs (Vijay, Pramond, Nitin, Rupesh & Jonish, 2011).

Futhermore, there are several uses of *Curcuma* species like they act as the colouring agent or dye. Next, the rhizome of *C.longa* can be used as insect repellent of houseflies (Asolkar, Kakkar & Chakre, 1992). The juice from the tubers of *C. longa* is good for children. In term of cosmetics, these species also play an important role. It has been proved by Nadkarni (1998) that the *C. zedoaria* is used as the ingredient in the cosmetics to cure the chronic skin diseases.

3.0 Materials and Method

3.1 Plant materials

Three parts of the plantlets were used in this experiment; leaves, stems or pseudostems and roots. Then, they were cut into five pieces from each part with the size 1 cm × 1 cm by using sterilized scarpel and cutter. Lastly, the smaller pieces of plants were transferred into the prepared culture medium, sealed and incubated in the dark condition at the culture room.

3.2 Callus Induction

There were two different combinations of hormone in callus induction. First treatment were combination of 2,4-Dichlorophenoxyacetic (1,2,3,4,5 mg/L) (2,4-D) with 0.5mg/L 6- benzyl amino purine (BAP). The second treatment was picloram in varies concentration (0, 2.5,5.0,7.5 and 10 mg/L). Each treatment had three replicates and each replicates consisted of 5 explants for leaves, roots and stems. The development of callus was observed every week. The explants were cultured for 8 weeks and kept in dark places for callus induction purposes.

3.3 Parameter Measured

Parameters recorded were the number of callus formation from the two treatments. The experimental design was arranged by Completely Randomized Design (CRD). The data were analysed by using ANOVA.

4.0 Result and discussion

4.1 Callus Induction by using picloram

The highest mean number of callus formed was 3.44 ± 0.98 in 2.5 mg/L of picloram as shown in Figure 4.1. Calli were recorded during the fifth week of the culture. The calli obtained were white and yellowish in colour. Mostly, all of the axenic explants were able to produce fully developed calli. After two months of observation, the calli formation became slow down. It was noticed that the size of calli were not increased. Then, the selected calli were being used for the next experiment; shoot induction and multiplication. In this experiment, the medium without any plant growth regulators acts as control treatment. No callus formation was observed.

After 2 months of culture, the calli formed from these three part of explants were soon become dry, brown then died. As stated by Collin and Edwards (1998), the calli become brown and slow in growth due to unsuitable substitute medium. Hence, some precaution should be applied in order to overcome this problem. For example, the friable and light – colored callus should be choosen and inoculate into a new medium with new concentration of picloram.

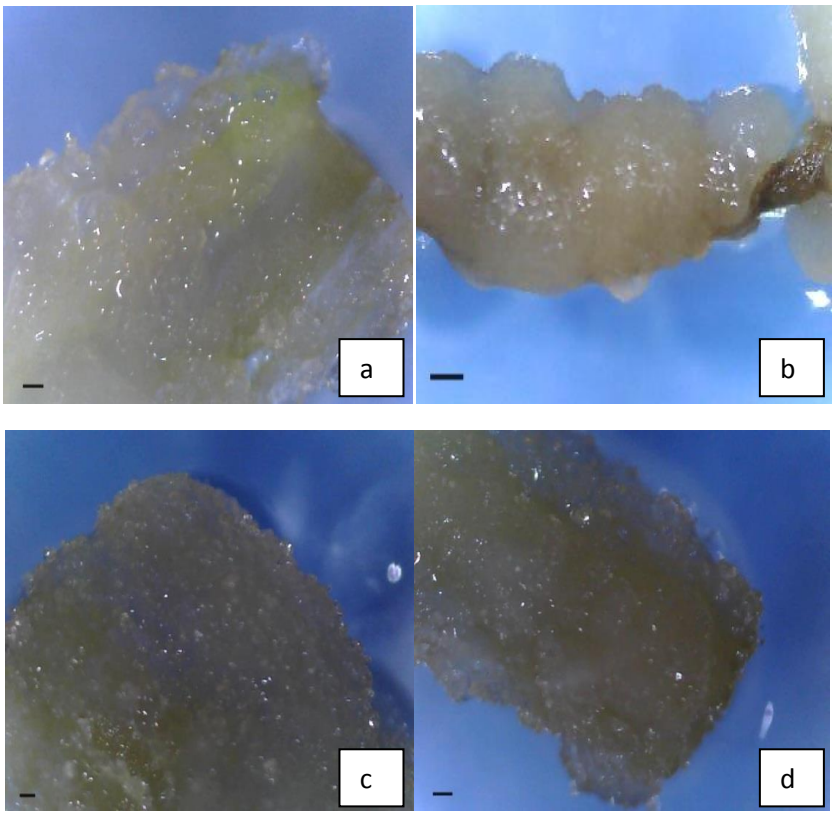

Figure 4.1: Appearance of callus from leaf, root and stem of *C. caesia.* Bar = 0.5 cm

(a) A whitish callus of *C. caesia* leaf after two months cultured in ½ MS medium supplemented with 2.5 mg/L of picloram.
(b) A yellowish callus of *C. caesia* root after two months cultured in ½ MS medium supplemented with 2.5 mg/L of picloram.
(c) A white yellowish and fully developed of *C. caesia* leaf callus after two months cultured in ½ MS medium supplemented with 2.5 mg/L of picloram.
(d) A white yellowish callus of *C. caesia* stem after two months of cultured in ½ MS medium supplemented with 2.5 mg/L of picloram.

4.2 Callus induction by using 2,4-D and BAP

According to this treatment the highest mean number of callus formed was 1.99 ± 0.67 in 1.0 mg/L of 2,4 – D with 0.5 mg/L of BAP and 3.0 mg/L of 2,4 – D with 0.5 mg/L of BAP. While, in Figure 2 showed the callus formation in 1.0 mg/L of 2,4 – D with 0.5 mg/L of BAP and 3.0 mg/L of 2,4 – D with 0.5 mg/L of BAP for leaf, root and stem explants.

During the week eight, the calli were recorded. The calli obtained were whitish and yellowish brown in colour (Figure 4.2). In this experiment, 1.0 mg/L of 2,4 – D with 0.5 mg/L of BAP and 3.0 mg/L of 2,4 – D with 0.5 mg/L of BAP were recorded to induce the highest number of callus for all three different part of explants. The lower concentration of 2,4 – D used with combination of BAP gave a good result. The MS medium supplemented with 0.5 mg/L of BAP and 0.5 mg/L of 2,4 – D could induce callus formation from shoot buds and rhizome explants of *Zingiber officinalis* (Ilahi & Jabeen, 1987). The callus formation also appeared in the other combinations of 2,4 – D concentration with 0.5 mg/L of BAP. However, the number of callus formed in these medium were less compared to medium supplemented with 1.0 mg/L of 2,4 – D with 0.5 mg/L of BAP and 3.0 mg/L of 2,4 – D with 0.5 mg/L of BAP.

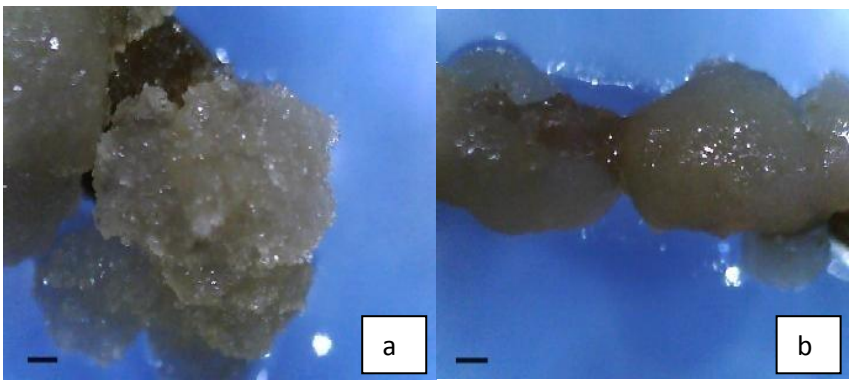

Figure 4.2: Appearance of callus from leaf and root and of *C. caesia*. Bar = 0.5 cm

a) A whitish and partially developed callus of *C. caesia* leaf after two months of cultured in ½ MS medium supplemented with 1.0 mg/L of 2,4 – D and 0.5 mg/L of BAP
b) A yellowish and fully developed callus of *C. caesia* root after two months of cultured in ½ MS medium supplemented with 1.0 mg/L of 2,4 – D and 0.5 mg/L of BAP

5.0 Conclusion

As a conclusion, two of the effects; picloram, and 2, 4 – D with BAP that were tested for callus induction was being compared. Based on the result obtained, picloram which was the first effect gave the highest mean number of callus formed. This was followed by the effect of 2, 4 – D in combination with BAP. Based on the observation, the root explants was one of the fast part producing callus. This was followed by stem and leaf. However, the leaf was the worse part for callus induction in this experiment. This was due to their low ability to produce callus. Besides, this explant can only produce slight amounts of callus. Hence, the root part was recommended to be used for callus induction of *C. caesia.*

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