Effect of Chemical Compounds on Quinine Content of *Cinchona ledgeriana* in Cell Suspension Culture[1].

Dedi Satriawan[1)], Diah Ratnadewi[1)], Sumaryono[2)]

[1]Department of Biology, Faculty of Mathematics and Natural Sciences, Bogor Agricultural University, Darmaga Campus, Bogor 16680, Indonesia
e-mail: dedi_nendra@yahoo.com
[2]Indonesian Biotechnology Research Institute for Estate Crops, Jalan Taman Kencana No. 1, Bogor 16151, Indonesia

ABSTRACT

Quinine has been used as a tonic, appetite enhancer, antipyretic, antimalarial agent, cosmetic ingredients and tanning. Quinine can be obtained from the bark of seven or more years old *Cinchona* tree. Cell suspension culture is an alternative means to produce secondary metabolites rapidly and continuously. Quinine, one of alkaloid compounds, is expected to increase with the addition of abscisic acid (ABA) and paclobutrazol (PBZ) as growth inhibitors, tryptophan as a precursor and mannitol as an osmotic stress inducer. ABA was used at 1 and 3 ppm, PBZ at 5 and 7 ppm combined with 5.3 g/l mannitol, tryptophan at 0.2 and 2 ppm combined with 5.3 g/l mannitol. ABA at 1 and 3 ppm increased the cell suspension growth but reduced the content of quinine. PBZ at 7 ppm combined with 5.3 g/l mannitol and 20 g/l sucrose significantly reduced the cell suspension growth but remarkably increased quinine content, even higher than in the bark of *Cinchona* tree in the field. Combination of mannitol and paclobutrazol was effective to inhibit the cell growth but enhance the production of quinine.

Keywords: abscisic acid, cell suspension culture, *Cinchona*, mannitol, paclobutrazol, quinine, tryptophan

Introduction

- Quinine has many uses especially as an antimalarial drug and as a bittering agent of soft drinks.
- Quinine is conventionally harvested from the bark of *Cinchona* trees after 7 to 10 years of planting.
- Cell culture can be used to produce secondary metabolites, including alkaloids, in very short and continuous cycles.
- The objective was to determine the effect of different chemical substances on the growth and level of quinine in a cell suspension culture of *Cinchona*.

Materials and Methods

- Culture conditions: under lamps at light intensity 20 µmol photon/m^2/sec for 12 h photoperiod and temperature 26 ^{0}C.
- Explant: young leaves of *C. ledgeriana* clone QRC 315.
- Callus initiation: leaf cuts were cultured on WP medium with 3.5 g/l gelrite, 30 g/l sucrose, 15 µM picloram, 2 µM BA, 1 µM phloroglucinol (Sumaryono & Riyadi 2005).
- Callus proliferation: on media similar to the initiation of callus.
- Cell culture: WP liquid medium with 30 g/l sucrose, 15 µM picloram, 0.5 µM BA and 1 µM ploroglucinol in baffle flasks. The flasks were placed on a shaker at 90 rpm
- Treatment: The cells were cultured into WP liquid medium with 15 µM picloram, 2 µM BA, 1 µM phloroglucinol and the treatments were: 1) 30 g/l sucrose (control), 2) 30 g/l sucrose and 1 ppm ABA (A_1), 3) 30 g/l sucrose and 3 ppm ABA (A_3), 4) 20 g/l sucrose, 5.3 g/l mannitol and 0.2 ppm tryptophan ($T_{0.2}$), 5) 20 g/l sucrose, 5.3 g/l mannitol and 2 ppm tryptophan (T_2), 6) 20 g/l sucrose, mannitol g/l 5.3 and 5 ppm paclobutrazol (P_5), 7) 20 g/l sucrose, 5.3 g/l mannitol and 7 ppm paclobutrazol (P_7).

Results and Discussion

- Callus Culture

Callus was formed in the second week after planting. It produced a white callus slightly browned crumbs mixed with brown compact callus (Figure 1).

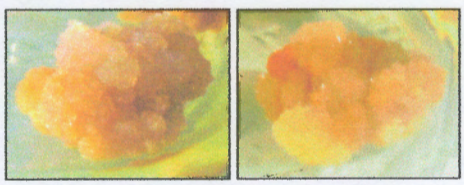

Figure 1. Callus of *Cinchona ledgeriana*

- Cell Culture

Suspension cultures were able to produce enough new cells in just one week on the homogenization phase.

- Cell Growth

The best cell growth was obtained in A_3 treatment (up to 15 times from the initial cell volume) and lowest was in P_7 treatment (only 5 times the initial cell volume). Cell growth in A_3 and A_1 treatments were higher than the control treatment while in $T_{0.2}$, T_2, P_5 and P_7 treatments were lower. Generally, the peak growth was reached at the 42nd day, except in the treatment of T_2 which was still growing (Figure 2). The cell growth of *Cinchona* observed by CVS method can be seen in Figure 2.

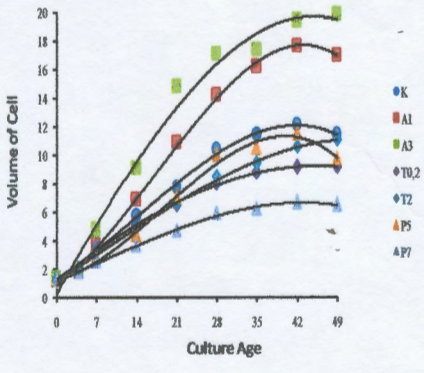

Figure 2. Cell growth curve of *C. ledgeriana*

ABA is known to enhance as well as to inhibit the growth (Finkelstein and Rock 2002). In this study ABA increased cell growth of *Cinchona* in A_1 and A_3 treatments.

ABA can cause imperfection in the process of cell wall formation during cell division due to impaired synthesis of cellulose. Imperfection in the cell wall increases the rate of required nutrients to move in and out of cells make the cells to multiply more quickly. ABA induces the accumulation of protein reserves in somatic embryos of white spruce. The availability of protein reserves increases the process of multiplication and division of plant cells.

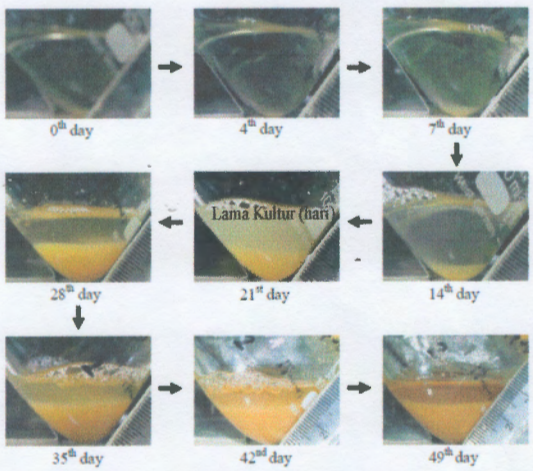

Figure 3. The growth of *C. ledgeriana* cell suspension observed by CVS method.

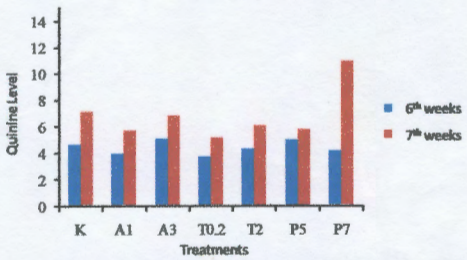

Figure 4. Quinine level in *C. ledgeriana* cell suspension

- Quinine Level

Quinine levels of all treatments at 7 weeks of culture were higher than at 6 weeks. The highest quinine level at 10.9% was in P_7 treatment at 7 weeks of culture and the lowest was in treatment $T_{0.2}$ at 6 weeks of culture (Figure 4).

Quinine level in all treatments (on average at 5.6%) was the higher than the yields obtained by Robins *et al.* (1986) at 0.01%, Rhodes *et al*: (1986) at 0.003%, and Ratnadewi & Sumaryono (2010) at 0.12%.

The highest level of total quinoline (quinine, quinidine, cinchonine and cinchonidine) was obtained in P_7 treatment at 6 weeks of culture followed by P_7 treatment at 7 weeks of culture and the lowest was in T_2 treatment at 6 weeks of culture.

Conclusions

- WP liquid medium added with 3 ppm ABA and 30 g/l sucrose was the best medium for the growth of cell suspension of *C. ledgeriana*.
- Paclobutrazol at 7 ppm, 5.3 g/l mannitol and 20 g/l sucrose at 7 weeks of culture was the best for quinine production in *Chincona* cell culture.

References

Ratnadewi D, Sumaryono. 2010. Quinoline alkaloids in suspension cultures of *Cinchona ledgeriana* treated with various substances. *HAYATI J. Biosci.* 17(4): 179-182.

Rhodes MJC, Payne J, Robins RJ. 1986. Cell suspension cultures of *Cinchona ledgeriana*; II. the effect of a range of auxins and cytokinin on the production of quinoline alkaloids. *Planta Medica*: 52: 226-229.

Robins RJ, Payne J, Rhodes MJC. 1986. Cell suspension cultures of *Cinchona ledgeriana*; I. growth and quinoline alkaloid production. *Planta Medica*: 52: 220-225.

Sumaryono, I. Riyadi 2005. Pertumbuhan kultur kalus dan suspensi sel kina (*Cinchona ledgeriana* Moens). *Menara Perkebunan* 73(1): 1-11.

Presented at the IGN TTRC International Conference, 17-18 July 2012, Bogor, Indonesia