HAYATI Journal of Biosciences December 2010
Vol. 17 No. 4, p 179-182
EISSN: 2086-4094

Available online at:
http://journal.ipb.ac.id/index.php/hayati
DOI: 10.4308/hjb.17.4.179

Quinoline Alkaloids in Suspension Cultures of *Cinchona ledgeriana* Treated with Various Substances

DIAH RATNADEWI[1]*, SUMARYONO[2]

[1]*Department of Biology, Faculty of Mathematics and Natural Sciences, Bogor Agricultural University, Darmaga Campus, Bogor 16680, Indonesia*
[2]*Indonesian Biotechnology Research Institute for Estate Crops, Jalan Taman Kencana No. 1, Bogor 16151, Indonesia*

Received April 30, 2010/Accepted December 27, 2010

Cinchona alkaloids are in extensive uses, not only for drugs but also for soft drink industries. They are harvested from the bark of trees *Cinchona* spp. after certain ages and therefore are available over a limited time. Cell culture is an alternative way to continuously produce such secondary metabolites in a much shorter time. Various substances were added in the normal growth media to promote quinoline alkaloids production by cell cultures of *Cinchona ledgeriana*. At the sixth week of culture, quinine and cinchonine contents were suppressed by paclobutrazol (PBZ), abscisic acid (ABA), or even by precursor tryptophan, while cinchonidine content was enhanced by 0.2 mg/l tryptophan to 43 fold of that produced by untreated cells (2.8% dry weight). At the seventh week of culture, the production of quinine and quinidine started to grow whereas the production of cinchonine and cinchonidine tended to decrease. An addition of 5 mg/l PBZ to culture media yielded the highest level of total quinine/quinidine after seven weeks, e.g. quinine 11 times more abundant and quinidine 23 fold higher compared to the untreated cells. Particularly the level of quinine which is the most demanded for medical and industrial purposes still need to be improved to approach to or even higher than that of extracted from the conventional source.

Key words: alkaloids, paclobutrazol, abscisic acid, tryptophan, cell suspension culture, *Cinchona ledgeriana*

INTRODUCTION

Cinchona bark contains quinoline alkaloids. Quinine, quinidine, cinchonine, and cinchonidine are the major substances among over thirty others (McCalley 2002). The demand for quinine is increasing due to their extensive uses as antimalaria and also as ingredient in the preparation for treatments of colds, cough, influenza, and various fevers. In addition to its drug values, quinine is substantially used in the manufacture of tonic drinks. The salts of quinine are also added to hair oils, sunburn lotions, moth repellents and insecticides. Quinidine is known as a remedy against cardiac ailments. Cinchonidine, having weaker action than quinine, is useful as an antispasmodic in whooping cough (Peter *et al*. 2007). The presence of cinchonine in a mixture of quinine and quinidine was proven to be more effective against quinine-resistant strains of *Plasmodium falciparum* (Druilhe *et al*. 1988).

Production of cinchona alkaloids in cell suspension cultures and its enhancement by using stress, precursors, elicitors, and the use of hairy roots system have been reported (Wijnsma *et al*. 1986; Hamill *et al*. 1989; Toruan-Mathius *et al*. 2006). By treating leaf, shoot, and organ cultures of *C. ledgeriana* with benzyladenin in Murashige-Skoog (MS) media, the content of alkaloids augmented with the increase in cultures age. Thirty two-week-old tissue cultures contained the same amount of alkaloids as one-year-old plant. Feeding various precursors to eight-week-old leaf shoot cultures increased the total alkaloids content by 66% with tryptophan, 42% with secologanin, and 5% with strictosidine-type alkaloid intermediates (Peter *et al*. 2007).

The aim of this research work was to improve the content of quinoline alkaloids, particularly quinine, in cell suspension cultures. In addition to that, the influence of various substances incorporated in the media to the cells' alkaloids production was also investigated.

MATERIALS AND METHODS

Plant Material. Fast growing callus initiated from leaves of axenic seedlings of *C. ledgeriana* was used as material source for suspension cultures. Cells were harvested after two weeks of homogenization in the baffle flasks (Sumaryono & Riyadi 2005). Approximately one gram of cells filtered through a mesh of 500 µm were transferred into 20 ml of basic WP medium (Lloyd & McCown 1981) containing 30 g/l sucrose, 1 µM phloroglucinol, 15 µM picloram, and 0.5 µM benzyladenin (BA), added with certain substances as treatments.

Treatments of Cell Cultures. Treatments with growth retardants paclobutrazol (PBZ: 1, 3, and 5 mg/l, annotated as PBZ 1, 3, and 5) and a precursor for quinoline synthesis, tryptophan, at 0.2 and 2 mg/l (Tryp 02 and Tryp 2) were applied to enhance the production of quinoline, from the beginning of the culture. This constituted the starting

*Corresponding author. Phone/Fax: +62-251-8622833, E-mail: dratnadewi@yahoo.com

point of treatments on the cell cultures. Some other cell cultures were instead exposed to 1, 3, and 5 mg/l of PBZ or abscisic acid (ABA) 1 and 3 mg/l at the fifth week (annotated as PBZ 1-5, 3-5, 5-5, and ABA 1-5, 3-5). Ten flasks represented each treatment.

The treated cell suspension cultures and controls were agitated on an orbital shaker at 100 rpm, under the light intensity of 10 µmol/m^2/sec. and temperature of 25 °C. The cell growth rate was measured by CVS (cell volume after sedimentation) method every week up to the harvesting day.

Alkaloid Extraction and Analysis. The cells were collected from five flasks through filtration, at the sixth and the seventh week for quinoline analysis. The levels of quinoline, i.e. quinine, quinidine, cinchonine, and cinchonidine were determined.

For extraction and purification, 0.5 g oven-dried cells powder was boiled in 95 ml aquadest. Five milliliters aliquot were taken from the decanted solution, filtered through Millipore 0.45 µm, and 5 µl of it was injected into the HPLC column (Pursuit XRs 3 µ C_{18}, column length 150 cm X 4.6 mm id), and performed at 30 °C. Quinoline standards were used for determination. The eluent was the mixture of water:acetonitrile:glacial acetic acid = 81:18:1. The flow rate was adjusted to 0.6 ml/min. and the attenuation was 6. UV-Vis 250 nm was employed as detector (Klink 1979). Data from each treatment and age of culture were average of duplo determinations.

RESULTS

The Growth of Treated Suspension Cell Cultures. The cell growth started to enhance remarkably in all cultures at the third week, and it continued to increase until the sixth week. The application of PBZ 1 at the beginning of the treatment did not reduce the growth capacity but higher levels of PBZ (PBZ 3 and 5) lowered their growth (Figure 1a), with PBZ 5 affected more significantly. Contrary to that, the application of the same growth retardant to the cells after letting them grow in basic media for five weeks (PBZ 1-5, 3-5, 5-5) gave no negative effects to the cells (Figure 1b); there was even a tendency to promote. The use of ABA in the cultures five weeks later (ABA 1-5 and 3-5) also demonstrated cell growth enhancement.

Tryptophan is a precursor in the synthesis pathway of quinoline alkaloid. Tryptophan feeding into the culture media maintained the growth rate of cinchona cells although it was lower than that of the untreated ones (Figure 1c).

The Alkaloids Content. Quinoline is alkaloids of *Cinchona*, secondary metabolites which have been proven being synthesized by cultured cells *in vitro* (Payne *et al*. 1987; Robins *et al*. 1987). Table 1 demonstrated that the untreated cells also produced quinoline after six weeks of culture. Supply of growth retardants PBZ and ABA as well as precursor of alkaloids L-tryptophan reduced the synthesis of quinine and mostly of cinchonine. Quinidine was not detected in all treatments at the sixth week of

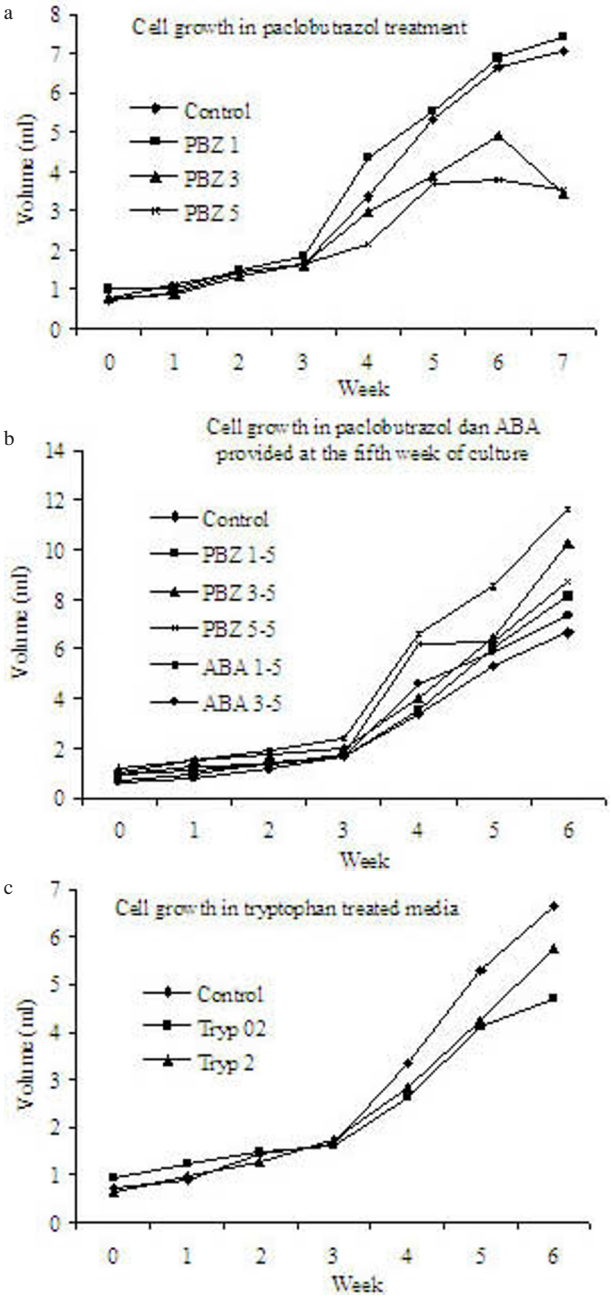

Figure 1. The growth curves of cells in media treated with various substances: a: PBZ; b: PBZ; and ABA supplied after five weeks of culture; c: Tryptophan. Means of ten flasks of cell cultures per treatment.

culture. In contrast to those results, the production of cinchonidine was favored by Tryp 02 and Tryp 2 as well as by PBZ 1 incorporated one week before analysis (PBZ 1-5); the other treatments resulted in lower content of cinchonidine.

At the seventh week of culture, production of quinine and particularly quinidine remarkably increased from the sixth week state. However, cinchonidine drastically dropped compared to one week before. Cinchonine appeared only at the seventh week from the treatments PBZ 1, PBZ 3, both levels of tryptophan, and PBZ 1-5 while none of PBZ 5, PBZ 3-5, and ABA 3-5 gave