Biosciences, Biotechnology Research Asia Vol. **3**(2), 341-345 (2005)

INFLUENCE OF SUCROSE CONCENTRATION ON GROWTH AND AJMALICINE PRODUCTION FROM *Catharanthus roseus* SUSPENSION CULTURES

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(Received: September 25, 2005; Accepted: November 21, 2005)

ABSTRACT

Sucrose is a major ingredient of culture medium. Present study was performed to investigate the influence of sucrose concentration on growth and ajmalicine production. Maximum packed cell volume (72%), fresh weight (65g 100 ml^{-1}), and dry weight (6.2148g 100 ml^{-1}) was recorded in medium containing 4% sucrose. About 9-fold increase in dry weight was recorded in medium with 4% sucrose. All growth parameters increased upto 25th day of incubation after which a gradual decline was observed. Ajmalicine accumulation started from 15th day of incubation and reached to maximum (60 µg g^{-1} dry weight) in 25th day of incubation in medium with 4% sucrose, which however gradually reduced on further incubation.

Key words: *Catharanthus roseus*, sucrose, ajmalicine, suspension culture.

INTRODUCTION

High value and low yield of bioactive compounds *viz*. vinblastine, vincristine and ajmalicine from naturally grown *Catharanthus roseus* attracted the attention world over to search a viable alternative process through plant biotechnology. Several strategies have been adopted to enhance the accumulation of these compounds in cell cultures of *C. roseus*, including organ culture[1], optimization of nutrient media[2], growth hormones[3], chemical treatment[4], large-scale cultivation in bioreactor[5] and elicitation[6].

Sucrose, the major constituent of medium, is used in the synthesis of cell constituents and provides the energy required for growth and maintenance of plant cells[3]. It also plays an important role in growth and ajmalicine production in cell suspension cultures of *C. roseus*. Present study was carried out to optimize the sucrose concentration for better growh and higher ajmalicine accumulation in cultures of *C. roseus*. Suspension culture was established by dispensing friable callus in continuously agitated liquid medium.

Considerable efforts like, medium composition, growth hormones, pH, temperature, light, aeration, agitation, stress factors and precursor feeding have been done to optimize growth and production of ajmalicine in cell suspension cultures of *C. roseus*[7,8].

MATERIALS AND METHODS

Establishment of callus and suspension culture

Leaf explants of *C. roseus* were washed in running tap water (30 min), 10 % Dettol (5 min) followed by distilled water. Explants were then treated with 70 % ethyl alcohol (2 min) and freshly prepared mercuric chloride (0.1 % w/v) in sterilized distilled water (2 min). Finally, the explants were thoroughly rinsed with sterilized distilled water. Surface sterilized explants (5-6 mm) were transferred aseptically to MS medium[9] supplemented with 2,4 D (4.52 µM) + Kn (4.65 µM).

Culture tubes were incubated at 25 ± 2ºC under white light (H" 1600 lux), maintaining 16 h photoperiod for callus initiation. Three-week-old friable callus was inoculated into 500 ml Erlenmeyer flasks containing 150 ml of MS medium supplemented with same growth hormones and incubated on gyratory shaker (100 rpm) at 25 ± 2°C. Packed cell volume (PCV), fresh weight (FW), dry weight (DW), medium pH, residual sugar and ajmalicine accumulation were recorded after regular interval of 5 days.

Extraction of Ajmalicine

The harvested cells were separated from the medium by filtration, homogenized in indigenously designed homogenizer and extracted with cold methanol for 12 h (three times). The pooled methanol extract was filtered and evaporated. The residue was extracted with 2N HCl. The acidic layer was basified with NaOH to pH 10 and extracted three times with chloroform. The chloroform extract was evaporated to dryness, which gave crude basic fraction. The basic fraction was first analyzed by thin layer chromatography (TLC) using ethyl acetate: methanol (98:2) as solvent system and visualized by spraying with cerric ammonium sulfate solution followed by heating at 100°C.

High Performance Liquid Chromatography

HPLC was carried out on Shimadzu HPLC system, Japan, (LC-10AT/SPD-10A, CR6A. The HPLC column used was Intersil ODS-3 C_{18}, reverse phase silica gel column (GL Science) with a size of 4.6 X 250 mm. Solvent system used was methano l/ 0.1 µM diammonium hydrogen phosphate solution in 70:30 proportion with a flow rate of 1 ml/min and peak detection was done at 254 nm. Quantitative estimation of ajmalicine was performed by using a standard curve obtained from authentic sample.

RESULTS AND DISCUSSION

Effect on packed cell volume and fresh weight

Fig. - 1 represents the effect of sucrose concentration on PCV and FW of *C. roseus* cells grown in MS medium containing 2,4 D (4.52 mM) + Kn (4.65 µM). The PCV increased upto 25th day of incubation and declined on further incubation. Maximum packed cell volume (72%) was recorded in medium containing 4% sucrose followed by 65% and 59% in medium containing 3% and 5% sucrose respectively. At all concentrations of sucrose, fresh weight persistently increased upto 25th day after which a gradual decline in fresh weight was

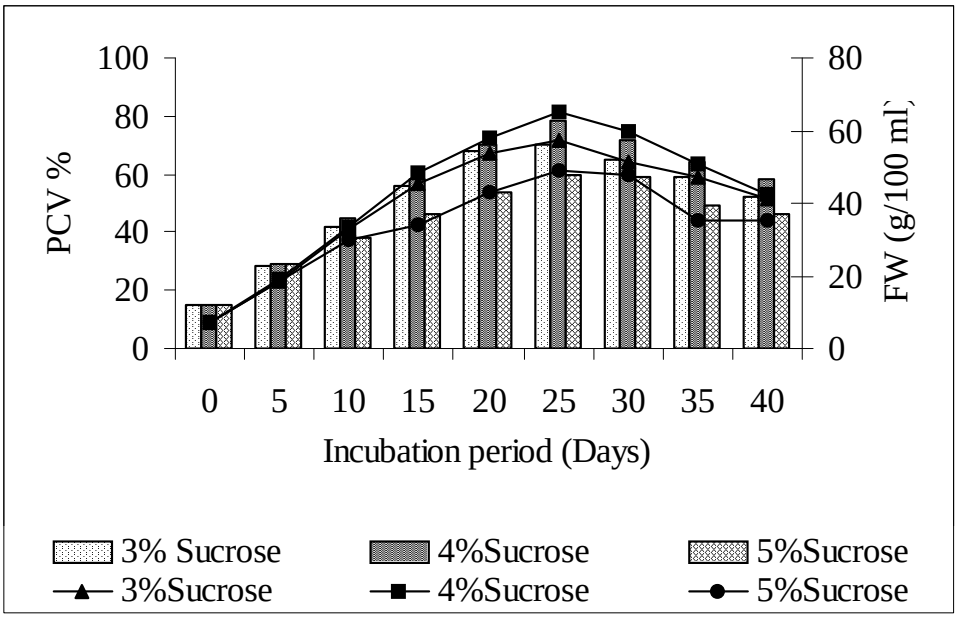

Fig. - 1: Effect of sucrose concentration on packed cell volume and fresh weight in suspension cultures of *C. roseus*

observed. Maximum fresh weight biomass (65g 100 ml^{-1}) was observed in medium containing 4% sucrose followed by 57 and 49 g 100 ml^{-1} biomass in 3% and 5% sucrose respectively.

Effect on dry weight and pH

Effect of sucrose concentration on dry weight and pH was illustrated in Fig. - 2. As compared to initial dry weight, about 9-fold increase in dry weight was recorded in medium supplemented with 4% sucrose, whereas medium containing 3% and 5% sucrose yielded 8-fold and 7-fold increase respectively. The pH of the medium with 3 % sucrose remained unchanged till 20th day and dropped from 5.6 to 5.4 on 25th day and remained almost stable till the end of incubation period. In medium containing 4% sucrose, pH dropped from 5.5 to 5.3 on 15th day and then gradually increased upto 5.6. With slight drop on 5th day, pH of medium containing 5% sucrose increased slowly to 5.7 in 35 days.

Residual sugar and ajmalicine production

Fig. - 3 shows the effect of sucrose concentration on sucrose consumption (residual sugar) and ajmalicine production by cells of *C. roseus*. Sucrose consumption was almost similar in media containing different concentrations of sucrose. Ajmalicine accumulation started from 15th day of incubation in medium containing 4% sucrose,

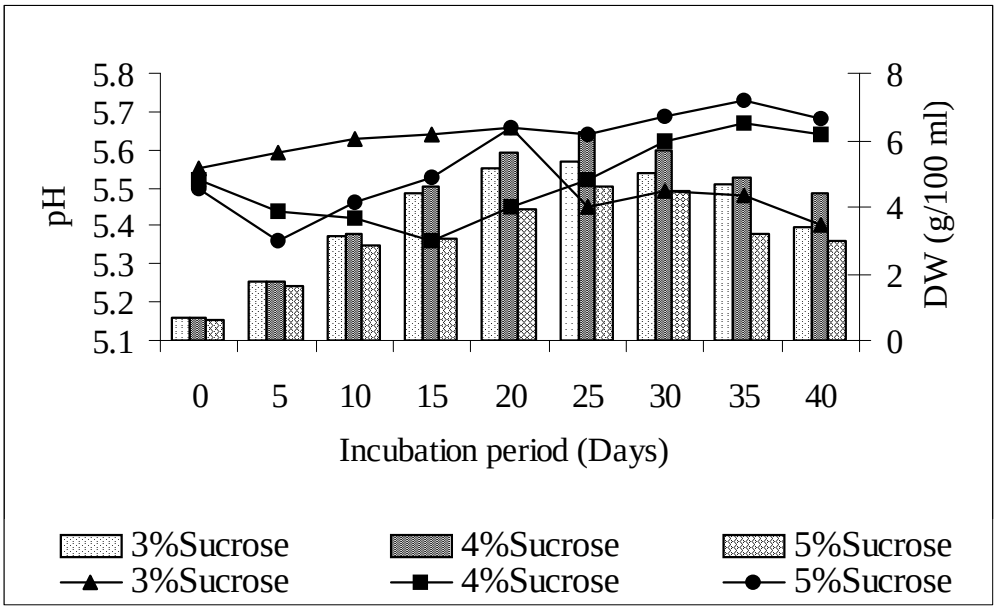

Fig. - 2: Effect of sucrose concentration on pH and dry weight biomass in suspension cultures of *C. roseus*

whereas it was from 20th day in medium containing 3% and 5% sucrose. In medium containing 4% sucrose, a maximum of 60 µg g^{-1} dry weight accumulation was recorded on 25th day of incubation, which gradually reduced on further incubation.

In the present investigation, *C. roseus* cells showed a uniform growth measured as packed cell volume, fresh weight and dry weight in all concentrations of sucrose. Maximum biomass production and ajmalicine accumulation was achieved on 25th day of incubation in medium

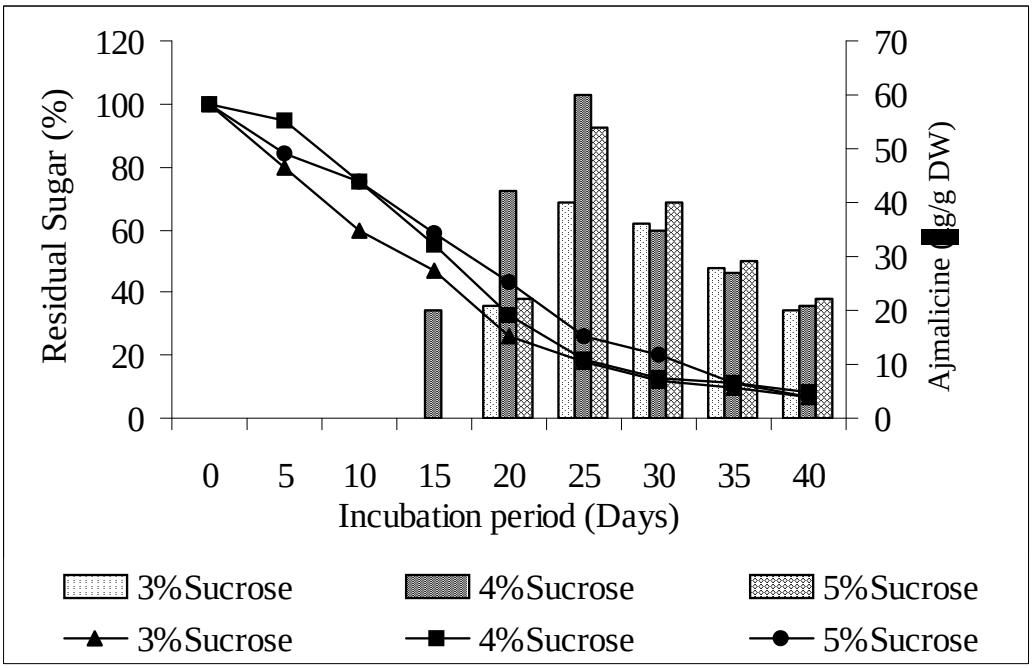

Fig. - 3: Effect of sucrose concentration on residual sugar and ajmalicine accumulation in suspension cultures of *C. roseus*

supplemented with 4% sucrose. However, higher concentration of 5% sucrose adversely affected the growth and ajmalicine production. Earlier studies reported that sucrose might also influence secondary metabolism in cell cultures of *C. roseus*[10]. Increase in sucrose concentration in the culture medium has been reported to increase alkaloid synthesis in *C. roseus* cells[11,12].

Suspension culture is a homogenous system and allows easy environmental control of cells for large-scale cultivation. Sucrose has been most commonly used carbon source in plant cell suspension media as it provides the energy required for growth and maintenance of plant cell constituents[3]. Concentration of sucrose influenced the growth and accumulation of bioactive compounds in cell suspension cultures of *C. roseus*[11,12]. Present investigation revealed that MS medium containing 4% sucrose yielded maximum biomass growth and ajmalicine accumulation by *C. roseus* cells. Concentrations, lower or higher than 4% of sucrose, however, adversely affected the growth and ajmalicine accumulation. Carew and Krueger[13] also observed the adverse effect of increased sucrose concentration on alkaloid production. Similarly, ajmalicine and serpentine accumulation increased by 9 and 4 fold on increasing the sucrose concentration from 2% to 6% respectively[14].

Conclusion

Sucrose has been most commonly used carbon source in plant cell suspension media as it provides the energy required for growth and maintenance of plant cell constituents. Concentration of sucrose influenced the growth and accumulation of bioactive compounds in cell suspension cultures of *C. roseus.* Present investigation revealed that MS medium containing 4% sucrose yielded maximum biomass growth and ajmalicine accumulation by *C. roseus* cells. Concentrations, lower or higher than 4% of sucrose, however, adversely affected the growth and ajmalicine accumulation.

REFERENCES

1. Hirata, K., Yawanaka, A., Kurano, N., Miyamoto, K. and Miura, Y.: Production of indole alkaloids in multiple shoot cultures of *Catharanthus roseus* (L.) G. Don. *Agri. Biol. Chem.* **51,** 1311-1317 (1987)
2. Van der Heijden, R., Verpoorte, R. and Ten Hoopen, H.J.G.: Cell and tissue cultures of *Catharanthus roseus* (L.) G. Don: a literature Survey. *Plant Cell Tiss. Org.Cult.* **18**, 231-280 (1989)
3. Ganapathi, G. and Kargi, F.: Recent advances in indole alkaloid production by *Catharanthus roseus* (Periwinkle). *J. Exptl. Bot.* **41**, **259**-267 (1990)
4. Zhao, J., Zhu, W.H., Hu, Q., He, X.W.: Improved alkaloid production in *Catharanthus roseus* suspension cultures by various chemicals. *Biotechnol Lett.* **22,** 1221-1226 (2000)
5. Fulzele, D.P. and Heble, M.R.: Large-scale cultivation of *Catharanthus roseus* cells: Production of ajmalicine in a 20 l airlift bioreactor. *J. Biotechnol.* **35(1**), 1-7 (1994)
6. Namdeo, A., Patil, S., Fulzele D.P.: Influence of fungal elicitors on production of ajmalicine by cell cultures of *Catharanthus roseus*. *Biotechnol. Prog.* **18**, 159-162 (2002)
7. Lounasmaa, M. and Galambos, J.: Indole alkaloid production in *Catharanthus roseus* cell suspension cultures. In *Progress in the Chemistry of Organic Natural Products*. **Vol. 55**. Herz, W., Grisebach, H., Kirby, G.W. and Tamm, C. (eds.), Springer-Verlag, Wien-New York. 89-115 (1989)
8. Moreno, P.R.H., Van der Heijden, R., Verpoorte, R. Cell and tissue cultures of *Catharanthus roseus:* A literature survey: II. Updating from 1988 to 1993. *Plant Cell Tiss. Org. Cult.* **2 (1)**, 1-25 (1995)
9. Murashige, T. and Skoog, F. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant.* **15**, 473-497 (1962)
10. Zenk, M.H., El Shagi, H., Arens, H., Stockigst, J., Weiler, E.W. and Deus, D. Formation of indole alkaloids serpentine and ajmalicine in cell suspension cultures of *Catharanthus roseus*. *In Plant Tissue culture and its Biotechnological Application*. Barz, W., Reinhard, E. and Zenk, M.H. (eds.), Springer-Verlag, Berlin. 27-44 (1977)
11. Knobloch, K.H. and Berlin, J.: Influence of medium composition on the formation of secondary compounds in cell suspension cultures of *Catharanthus roseus* (L.) G. Don. *Z. Naturforsch.* **35 c**, 551-556, (1980)
12. Balague, C. and Wilson, G.: Growth and alkaloid biosynthesis by cell suspensions of *Catharanthus roseus* in a chemostat under sucrose and phosphate limiting conditions. *Physiologie Vegetale*. **20**, 515-22, (1982)
13. Carew, D.P. and Krueger, R.J. *Catharanthus roseus* tissue culture: The effects of medium modifications on growth and alkaloid production. *Lloydia*. **40 (4)**, 326-36 (1977)
14. Merillon, J. M., Rideau, M., and Chenieux, J. C.: Influence of sucrose on levels of ajmalicine, serpentine and tryptamine in *Catharanthus roseus* cells *in vitro*. *Planta Med.* **50**, 497-501, (1984)