Petcharut Chuntaratin 2006: Production of Plumbagin by Hairy Root, Callus and Cell Suspension Cultures of *Plumbago indica* L.. Doctor of Philosophy (Agricultural Biotechnology), Major Field: Agricultural Biotechnology, Interdisciplinary Graduate Program. Thesis Advisor: Assistant Professor Sermsiri Chanprame, Ph.D. 133 pages.
ISBN 974-16-2187-6

Chettamuun Phloeng Daeng (*Plumbago indica* L.) is an important medicinal plant of Thailand which root is the main source of plumbagin, a naphthoquinone derivative of commercial interest for pharmacological properties. Toward the main objective of *in vitro* production of plumbagin, the specific objectives of this research were i) to develop the immunolocalization technique for plumbagin detection in *P. indica* L., ii) to investigate the plumbagin production from *P. indica* L. hairy root, callus and cell suspension cultures and iii) to enhance plumbagin production using biotic and abiotic elicitors. To understand the pattern of plumbagin accumulation in plant tissue, the immunolocalization method was developed. The plumbagin-BSA and plumbagin-OVA conjugates were successfully prepared using glutaraldehyde reaction and were confirmed by SDS-PAGE and MALDI-TOF MS. The 1 mg aliquots of this conjugate mixed with Freund's adjuvant was immunized into a rabbit for 4 times at weekly interval. Inspite of the low titers of approximately 625 in rabbit, the antiserum was specifically recognized with plumbagin. The immunolocalization study of plumbagin in *P. indica* L. plant using fluorescein-labelled goat anti rabbit IgG specific for plumbagin demonstrated that plumbagin was localized mainly in cell membrane and intercellular space.

For the *in vitro* production of plumbagin, the best medium for hairy root growth was 1/2 MS liquid medium with 20 g/l sucrose and the optimal culture medium for callus and cell suspension culture was MS salts with B5 vitamins supplemented with 0.2 mg/l NAA, 0.2 mg/l 2,4-D and 0.5 mg/l kinetin. For plumbagin production, the hairy root culture yielded the highest plumbagin content follows by cell suspension and callus cultures, respectively. However, cell suspension cultures grew in the dark condition yielded higher plumbagin content than in the light condition. The age of culture which yielded the maximum plumbagin content was 12 days after subcultured which was at the mid-exponential phase of cell growth.

Various elicitors were added to hairy root and cell suspension cultures to enhance the plumbagin accumulation. The addition of chitin or chitosan in hairy root and cell cultures strongly promoted the release of plumbagin into culture media whereas fungal and yeast elicitor showed little effect. In cell suspension cultures, chitin could increase the plumbagin production about 20 folds. Chitin and chitosan elicitation resulted in the increased plumbagin accumulation of 3-4 folds in hairy root culture.

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29 / May / 2006