plant utilized anti-diabetic and anti-oxidant property. This anti-oxidant property interferes with free radicals, chelating, catalytic metals and also by acting as oxygen scavengers. It also contains enzyme α-amylase, which has an immense industrial application. The plant is in its wild form has a very much chance of exposure to chromosomal cross over which ultimately can lead to loss of potency of the medicinal property of this wild form after mutation. *Plant tissue culture of this wild plant can ultimately provide* a continuous wild and original source of natural products so that a intact genome can be identified to preserve it in the form of a genome. Callus generation was done from the various parts of the plant namely stem, leaves and matured fruit parts. The presence of α-amylase was detected, in comparison to normal plant, in the callus by observing the zone of clearance due to starch breakdown in a MS media containing 1% starch in. Stem callus gave maximum enzyme activity and zone of clearance on weight basis. For anti-oxidant property fruits showed the highest activity and callus showed the moderate activity. The current study depicts industrial application of vulnerable species without being imperiled.

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3.2.39

Effects of phytohormones and macro- and microelements on growth and alkaloid accumulation in *Lycopodiella inundata* (L.) holub plant cell cultures

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Neurologically degenerative disorders including Alzheimer's disease (AD) are growing in impact on human health. Memory deficits associated with AD may be partly due to impairment of cholinergic neurotransmission. Drugs acting as acetylcholinesterase inhibitors are the focus of symptomatic therapeutic strategies. *Huperziaceae* and *Lycopodiaceae* species have been used in traditional Chinese medicine for the treatment of memory deficiencies and alkaloids of these species have been shown to produce positive effects on learning and memory. Among these bioactive compounds Huperzine A is one of the most promising drug for the treatment of symptoms of AD.

These alkaloids often possess unusual skeletons and are challenging but difficult targets for total synthesis. Natural plants are up to now the main source of these compounds and may soon become extinct if harvest practices are not curtailed. Alternative production methods of Lycopodium alkaloids by plant cell cultures are therefore of growing interest.

Previously established *in vitro* cultures of *Lycopodiella inundata* have been shown to produce alkaloids but the growth was very slow. Our efforts were focused on improving biomass production and alkaloid accumulation by optimizing cell culture media using factorial experimental design. The alkaloid production was evaluated by an optimized ultrasonic selective extraction followed by HPLC and NMR analysis. The effects of phytohormones and of macro- and microelements were investigated. Our results analyzed by using response surface methodology showed that all these parameters have a strong influence on the plant cell cultures: the growth could be increased by a factor of ten and the analytical results showed that the alkaloid accumulation (from 0.9 to 3.1 mg/g) is closely correlated with slight modifications of culture media. The conditions leading to optimized growth and alkaloid accumulation have been applied on a larger scale culture. Structural separation and identification of the main alkaloids produced *by Lycopodiella inundata plant cell cultures are in progress.*

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Modification of an amaranth seed protein and its effect in the expression into *E. Coli*

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Amarantin is the most predominant storage protein in *Amaranthus hypochondriacus* seeds; it belongs to the 11S globulin class, and shows a high content of essential aminoacids, making this protein important from a nutritional view point. This protein has two disulfide linked subunits: acidic and basic of 32—34 kDa and 22—24 kDa respectively. The acidic subunit is the candidate for the modification of 11S globulins. In order to improve the functionality of this protein, the primary structure of this subunit was modified in two sites; in the globulins 11S III variable region were inserted eight aminoacids (Val—Tyr—Val—Tyr—Val—Tyr—Val—Tyr) and in the C terminal of the acidic subunit were inserted four aminoacids (Arg—Ile—Pro—Pro). Plasmids were constructed using the amarantin cDNA cloned in the pET 32b(+) expression vector, and utilizing site directed mutagenesis to insert the modified encoding sequences. There were constructed three plasmids containing the encoding sequences to produce the modified proteins: (a) amarantin acidic subunit, (b) internal modification of amarantin acidic subunit and (c) C-terminal modified amarantin acidic subunit. They were expressed in *E. coli* Origami (DE3); it was observed that the acidic subunit was expressed in a higher quantity than the modified proteins and when we monitored the growth kinetic, the *E coli* harboring the plasmids of modified proteins, seems to grow slower than that non modified one, that in the protein modified with the RIPP peptide at C terminus in the western blot assay, appear bands of higher than expected molecular weight, this may indicate that the prolines inserted interact in some way in the proper protein folding generating a less stable protein.

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