Indian Journal of Experimental Biology
Vol. 38, November 2000, pp. 1086-1091

Review Article

Polyamines and plant alkaloids

Bharati Ghosh
Department of Botany, Bose Institute, Calcutta 700 009, India

Naturally occurring alkaloids are nitrogenous compounds that constitute the pharmacogenically active basic principles of flowering plants. Alkaloids are classified into several biogenically related groups . Tobacco alkaloids are metabolised from polyamines and diamines putrescine and cadaverine . N-methyl transferase is the first enzyme in alkaloid biosynthetic pathway which drives the flow of nitrogen away from polyamine biosynthesis to alkaloid biosynthesis. Arginine decarboxylase has been suggested to be primarily responsible for providing putrescine for nicotine synthesis. Tryptophan is the precursor of indole alkaloids . However, the biosynthetic pathway of tropane and isoquinoline alkaloids are not clear. Genes for several key biosynthetic enzymes like arginine decarboxylase, ornithine decarboxylase, putrescine N-methyl transferase and spermidine synthase, hyoscyamine 6 β hydroxylase,tryptophan decarboxylase etc have been cloned from different plant species. These genes are regulated by plant hormones, light, different kinds of stress and elicitors like jasmonates and their strong expression is primarily in the cultured roots. In view of this, the axenic hairy root cultures induced by *Agrobacterium rhizogenes* have been utilised to synthesise secondary metabolites. The current development in the knowledge of alkaloid biosynthesis, particularly molecular analysis, has been discussed in this review that may help to open up new avenues of investigation for the researchers.

Plants produce a wide range of natural products derived from secondary metabolic pathways which are useful for pharmaceutical,agrochemical, fragrance and food industries .These naturally occurring secondary metabolites are alkaloids which contain secondary, tertiary or quaternary nitrogen atoms in their molecules. These nitrogenous compounds constitute the pharmacogenically active 'basic principles' of flowering plants, and have traditionally been of interest due to their various physiological activities in animals and human . Alkaloids are important for defense of the plant against pathogenic organisms and herbivores, or protoxins for insects which further modify the alkaloids and incorporate them into their own defense/secretions[1]. Alkaloids have been isolated from diverse organisms . However, little information is available regarding the synthesis of complex structure of alkaloids which contain multiple asymmetric centers. Nevertheless the pharmacological effects of these compounds are well known. Nowadays the sophisticated analytical instrumentation, precursors labeled with stable isotopes, analysis of products by nuclear magnetic resonance spectroscopy and the use of plant cell culture as experimental system has helped in understanding the alkaloid biosynthetic pathway in different plants [2]. Alkaloids are classified into several biogenically related groups but the enzymes and genes have been characterised only in following groups [26]

- Nicotine and Tropane alkaloids
- Indole alkaloids
- Isoquinoline alkaloids

This review describes the biosynthesis and molecular aspects of tobacco alkaloids in detail and the rest of the groups in brief.

Polyamines as precursors of tobacco alkaloids

The ubiquitous polycationic polyamines, a class of aliphatic amines, are found in almost every cell compartment including the cell wall. They are deeply involved in a series of cellular events such as replication, cell division,protein synthesis[3] and plant growth and developmental processes[4,5] but the exact function of polyamines is not known [5]. In plants, diamine putrescine (Put) can be synthesized by two independent pathways which involve ornithine

Abbreviations used: ODC, Ornithine decarboxylase; ADC, Arginine decarboxylase; SPDS, Spermidine synthase; SPMS, Spermine synthase; SAMDC, S-adenosylmethionine decarboxylase; PMT, Putrescine N-methyl transferase; DAO, diamine oxidase; H6H, Hyoscyamine 6β hydroxylase; PAs, Polyamines; PUT, Putrescine; Spd, Spermidine; Spm, Spermine; SAM, S-adenosyl methionine; dSAM, decarboxylated SAM; MeJA, Methyl esters of jasmonates

decarboxylase (ODC) and arginine decarboxylase (ADC) and these two pathways regulate the growth, development and stress responses of plants[4,5]. Spermidine synthase (SPDS) transfers the aminopropyl moiety of decarboxylated S-adenosylmethionine (dSAM) to Put producing Spermidine(Spd). Spermine (Spm) is similarly synthesized by another enzyme Spermine synthase (SPMS). dSAM is formed from SAM by SAM decarboxylase (SAMDC).

A wide variety of secondary metabolites of biological interest can be metabolized from Put through largely unelucidated pathways. The pyrrolidine rings of tobacco alkaloids (nicotine, nornicotine), tropane alkaloids (hyoscyamine, hyoscine, metelodine), pyrrolizidine (retronecine) are Put derivatives [6]. Cadaverine and Spd are precursors of quinolizidine while lunarine alkaloids are derived from Put[7].

Nicotine, the biologically active component of tobacco leaves, is found in the genus Nicotiana and other genus of *Solanaceae* though it occurs in many other plants including lycopods and horsetails[7]. It is the predominant alkaloid in most commercial cultivars of tobacco, highly toxic and serves as a very effective insecticide and fumigant. The tropane alkaloids that include the medically important anticholinergic compounds, hyoscyamine and scopolamine, have been used traditionally for their medicinal, hallucinogenic and poisonous properties. In addition to the Solanaceae family, tropane alkaloids are also found in the *Convolvulaceae, Cruciferae* and *Rhizophoraceae*[8]. Both these alkaloids are synthesized in tobacco roots from Arg and/or Orn by the action of ADC and ODC which involves the symmetric diamine Puts that is converted to N-methyl Put with the help of Put N-methyl transferase (PMT, EC 2.1.1.53). This is the first committed step in alkaloid biosynthesis which drives the flow of nitrogen away from PA biosynthesis to alkaloid biosynthesis. N-methyl Put can also be produced through direct methylation of Put or decarboxylation of l-methyl Orn. N- methyl Put is then oxidatively deaminated by diamine oxidase (DAO, EC 1.4.3.6) and cyclised spontaneously to the 1-methyl-Δ-pyrrolinum via 4-methylaminobutanol[9,10]. It is then condensed with nicotinic acid or its derivatives by an unknown enzyme to form nicotine[10]. SPDS transfers the aminopropyl moiety of dSAM to Put whereas PMT transfers the methyl moiety of SAM to Put. Substrate specificity studies and inhibition kinetics by amines have predicted that SPDS [11] and partially purified PMT[12] may have similar active sites for enzyme catalysis. Previously conflicting hypotheses have been proposed for the source of Put in nicotine synthesis in tobacco. It was believed that the ODC pathway is a major source of Put as accumulated nicotine showed a good correlation with the increase in ODC activity in roots of decapitated tobacco seedlings[13]. In later experiments ADC pathway has been suggested to be primarily responsible for providing Put for nicotine synthesis, as nicotine production in tobacco callus is effectively inhibited by DFMA, a specific inhibitor of ADC, and incorporation of ^{14}C into nicotine was more efficient from the uniformly labeled Arg than that from the uniformly labeled Orn[14].

The biosynthetic pathway of tropane alkaloids is not yet clear. Recently activities of two enzymes of tropane alkaloid biosynthesis, PMT and hyoscyamine 6β hydroxylase (H6H), 2-0xoglutarate-dependent enzyme, (EC 1.14.11.11), have been shown to be high in cultured roots but very low in cultured cells and cultured shoots. H6H catalyse the first oxidative reaction in the biosynthetic pathway leading from hyoscyamine to scopolamine and requires alkaloid substrate 2-oxoglutarate, ferrous ion, ascorbate etc.[15]. H6H not only hydroxylates various hyoscyamine derivatives but also epoxidizes 6,7-dehydrohyoscyamine, a synthetic alkaloid to scopolamine[16]. H6H has been purified to homogeneity from cultured roots of *Hyoscyamus niger* L[17]. Four monoclonal antibodies were raised against purified H6H and Western blot analysis showed that H6H is abundant in plant roots, but absent in leaf, stem, calyx, cultured cells and cultured roots. Immunohistochemical studies using monoclonal antibody and immunogold-silver enhancement studies detected H6H only in the pericycle cells of the young root in several scopolamine-producing plants[18]. Hartmann[1] demonstrated a close relationship between the biosynthesis of PAs and senceionine N-oxide, the major pyrrolizidine alkaloids produced in root cultures of *Solanum vulgaris*.

Molecular cloning of polyamine and alkaloid biosynthetic genes

The cDNA clone representing ODC from *Datura* has been obtained recently[18]. ADC gene has been

cloned from oat[19], tomato[20], pea[21], *Arabidopsis*[22] and soyabean[23]. The cloning of cDNA encoding the enzyme nicotiana PMTs is generated by subtraction hybridization[24], the deduced aminoacid sequence of PMT is highly homologous to the sequence of SPDS from human (73% identical), mouse (70% identical) and *E.coli* (58% identical)[25]. Nicotiana PMT is more homologous to the peptide fragments (73% identity and 88% homology in 66 amino acid residues) encoded by a partial cDNA sequence of rice SPDS (GenBank accession No. D15401). The *Hyoscyamus niger* PMT cDNA was 1,350-bp long and encoded of 338 amino acids. The *Atropa belladonna* PMT cDNAs was 1,305 bp long and encoded of 336 amino acids. AbPMT cDNA was more similiar in nucleotide sequence to HnPMT cDNA (86% identity).AbPMTs and HnPMTs lacked the N-terminal tandem repeat arrays previously found in Nicotiana PMTs[26]. Very recently four cDNAs for SPDS were isolated from *Nicotiana sylvestris, Hyoscyamus niger* and *Arabidopsis thaliana*[27]. The plant SPDS has molecular mass of about 34 kDa, posses the cofactor binding motifs and are more homologous in amino acid sequence to tobacco PMT than to SPDS from mammals and *E. coli*. The SPDS gene is expressed in root, stem and leaf in N. *sylvestris* whereas the PMT gene is expressed only in root. From the phylogenetic tree constructed from a matrix of sequence similarities (UPGMA Tree Programme in Gene Works) it can be shown that PMT has evolved from SPDS in tobacco during the diversification of *Solanaceae*[28].

The cDNA clones encoding H6H were obtained from mRNA of the cultured roots of *Hyoscyamus niger*[29]. Nucleotide sequence analysis of the cloned cDNA revealed an open reading frame that encodes 344 aminoacids.RNA hybridization showed that mRNA of the hydrolase is abundant in cultured roots and present in plant roots but absent in leaves, stems and cultured cells of *Hyoscyamus niger*[29]. Expression of H6H cDNA clone in *E. coli* has demonstrated that H6H catalyses both reactions and that the hydrolase activity is about 40 fold stronger than epoxidase activity[30]. The amino acid sequence of H6H in *Hyoscyamus niger* is homologous to ethylene forming enzyme (ACC oxidase). Tropine reductase (EC 1.1.1.236) is another enzyme of biosynthetic pathway of tropane alkaloids which converts the 3-keto group of tropinone to the 3 hydroxyl of tropine. The cDNA of this enzyme was isolated from *Datura stramonium* which was expressed as a galactosidase fusion protein in *E. coli*[31].

Regulation of alkaloid biosynthetic gene expression

Like other plant genes, expression of the genes encoding biosynthetic enzymes for alkaloids are regulated spatially and temporally by plant hormones, light and stress.[2] Nicotine is synthesised in response to wounding. PAs are not only essential for cell division,but also play an important role in the development and stress responses in plants [5]. Salinity stress causes a rapid increase in the level of Put in sensitive rice seedlings [32] through activation of ADC and treatment of MeJA also increase accumulation of Put in rice leaves [33]. The levels of Put, Spd and Spm did not change significantly after treatment of tobacco cells with MeJA (methyl esters of jasmonates) but it caused accumulation of N-methyl Put and the nicotine [33]. MeJA treatment induces accumulation of not only mRNAs for ODC and SAMs but also mRNA for PMT. The expression of genes for ADC, an alternative enzyme for the synthesis of Put, and SAMDC involved in the synthesis of higher PAs (Spd and Spm) from Put, were not affected by MeJA[34]. The elicitors MeJA induces expression of genes coding for enzymes at various steps in the synthesis of nicotine by multiple regulatory mechanisms[33].

Negative effects of auxin on alkaloid biosynthesis have been known for a long time. It was shown recently that auxin down regulates several genes involved in alkaloid biosynthesis[34]. Auxin may be transported from the shoot to the root where it suppresses the genes for nicotine biosynthesis. Expression of nicotine converter gene which converts nicotine to nornicotine is induced with ethephon or ethrel[25].

Biotechnological manipulation of the alkaloid biosynthetic genes

By the application of in vitro culture techniques, efforts have been made to produce plant metabolism [35]. The axenic hairy root cultures induced by infection with *Agrobacterium rhizogenes* have the ability to synthesise secondary metabolites such as tropane alkaloids, tanin, and coumarins[35]. Higher accumulation of atropine and scopolamine was reported in hairy root cultures of *Atropa baetica* infected with *Agrobacterium rhizogenes*[36].

The synthesis of alkaloid is controlled at multiple steps by several enzymes[2]. The overexpression of

only one of the enzyme may not be enough in achieving significant amount of end product of the pathway. Metabolic engineering of the plants that serve as commercial sources of nicotine or scopolamine could enhance classical breeding in the effort to develop plants with optimal alkaloid patterns that serve as improved sources of pharmaceuticals. The exploitation of the biotechnological potential of the alkaloid biosynthesis has only just begun. Feeding low levels of Put (1 to 5 mM) can have a stimulatory effect of nicotine by transformed root culture of Nicotiana rustica[34].

Expression of yeast ODC gene into Nicotiana rustica roots resulted an increased accumulation of Put and nicotine[37] which first shows plant secondary product can be elevated by means of genetic manipulation. The 5' flanking region of the AbPMTgene was transferred to Atropa belladonna and further analysis showed that root is the main organ of PMT expression. Hyoscyamus niger H6H is expressed specially in root pericycle cells but absent in leaf, stem, calyx, cultured cells[17]. The H6H cDNA of Hyoscyamus niger was introduced into Atropa belladona; in which the enzyme was strongly and constitutively expressed in all parts of the plant [25]

Mutational analysis

Well defined mutants defective in alkaloid biosynthetic steps are valuable tools for the elucidation and regulation of biosynthesis of secondary metabolites. In the cured leaves of tobacco cultivars, nicotine is the predominant alkaloid though certain cultivars posses a gene that demethylates nicotine to nornicotine during curing. The nicotine converter gene was considered to be a means of lowering the nicotine content of tobacco but this approach was discarded due to poor smoking quality of tobacco products with high nornicotine contents[28]. In 1930, a genetically stable breeding line LA-Burley 21 was established by series of back crossing with Cuban cigar tobacco cultivars Burley 21. LA-Burley 21 and parental strain Burley are isogenic and have low nicotine biosynthesis loci NIC1 and NIC2 which regulates nicotine level in tobacco. Two mutants nic1 and nic2 with low nicotine levels accumulate proportionally more PAs, mostly Put and Spd in leaves and culture roots than the wild type probably because of the block of PMT (T. Hashimoto and S. Higashiguchi,unpublished information). Differential screening between the cultured roots of Burley 21 and LA Burley 21 resulted in isolation of two cDNA clones whose expression in the roots were repressed proportionally to the nicotine content (N. Hibi, unpublished information). Exogenous supply of auxin down regulated both genes in cultured tobacco roots which suggested that NIC1 and NIC2 are regulatory gene for nicotine biosynthesis (T. Hashimoto, unpublished information)

Indole alkaloids

The indole alkaloids comprise about 1800 members of alkaloids of which the antimalaria drug quinine from *Cinchona .officinalis*, the antineoplastic drug camptothecin from *Cinchona .acuminata*, the homeopathic drug strychnin from *Strychnos nuxvomica* and the chemotherapeutic agents vincristine and vinblastine from *Catharanthus roseus* are isolated from plant materials [2]

Tryptophan as precursors of Indole alkaloids

Tryptophan decarboxylase catalyses the decarboxylation of L-tryptophan to tryptamine which can serve as substrate for strictosidine synthase . Strictosidine synthase catalyses tryptamine and secologanine to form the indole alkaloids [2,25] like ajmalicine,cantharanthine,vindoline etc .

The cDNA clone encoding tryptophan decarboxylase was isolated from *Catharanthus roseus* and amino acid sequence show similiarities with an aromatic L-aminoacid decarboxylase from *Drosophila melangoster*[39] .The cDNA encoding Strictodine synthase was isolated from *Rauvolfia serpentina* and *Catharanthus roseus*[40,41]. The gene for strictosidine synthase,str 1[42],has been isolated from *Rauvolfia serpentine* and the transcript accumulates primarily in the roots and mature leaves.

Isoquinoline alkaloids

The active members of this large and diverse class of alkaloids are emetine (an antiamoebic), colchicine (a microtubular disrupter and gout suppressant), berberine (an antimicrobial against eye and intestinal infections), morphine (a narcotic analgesic),codeine (a narcotic analgesic and antitussive and sanguinarine (an antimicrobal used in oral hygeine) which are largely isolated from plants[2]. Due to the complexity of structure only berberine is isolated and synthesised from cell suspension of *Coptis japonica*[43]

The complete picture of aligning 13 enzymes comprising methyl transferase and oxidases for berberine synthesis is not yet clear[2] .

Conclusion

Tobacco is the only commercial nonfood crop that enters the world trade as a leaf and is of great economic importance. More than 50% of world tobacco consists of flue-cured virginia tobacco (FCV). The ecochemical functions of nicotine have been thoroughly analysed in recent years and several interesting aspects of alkaloid biogenesis and its control are now emerging. The exploitation of the biotechnological potential of tobacco alkaloid biosynthesis has only just begun. But there is no information regarding the direct evidence of genes that regulate alkaloid metabolism. With the introduction of molecular biology into plant alkaloid field induction of alkaloid biosynthesis can be analysed at the level of gene activation and gene expression patterns which can be interpreted as a first indication of possible functions. The biological function of alkaloids can be studied by isolating specific regulatory genes that control multistep pathway. Molecular cloning of putative alkaloid specific master genes may require more advanced rDNA technology which one day may lead to biotechnological application.

References

1 Hartmann T, Alkaloids in herbivores: *Their interactions with secondary plant metabolites*, vol 1, 2nd edition (Academic Press, San Diego) 1991, 9.

2 Kutchen T M, Alkaloid biosynthesis-the basis for metabolic engineering of medicinal plant, *The Plant Cell*, 7 (1995) 1059.

3 Tabor C W & Tabor H, *Polyamines, Annu Rev Biochem*, 53 (1984) 749.

4 Evans P T & Malmberg R L, Do polyamines have role in plant development ? *Annu Rev Plant Physiol Plant Mol Biol*, 40 (1989) 235.

5 Kumar A, Atlabella T, Taylor M A & Tiburcio A F, Recent advances in polyamine research *Trends Plant Sci*, 2 (1997) 124.

6 Robinson T, in *The biochemistry of alkaloids*, vol 3, edited by Kleinzeller A , Springer, GF & Wittman, HG (Springer, Berlin) 1981, 35.

7 Flores H E, Protacio C M & Signs M W, Plant nitrogen metabolism, *Recent Adv Phytochem*, 23 (1991) 329.

8 Smith T A, in *The biochemistry of plants*, vol 7, edited by P K Stumpf & E E Conn (Academic Press) 1981, 249.

9 Leete E , Alkaloids derived from ornithine, lysine and nicotinic acid, in *Encyclopedia of Plant Physiol New Series*, vol 8,edited by E A Bell & BV Charlwood (Springer-verlag Berlin Heidelberg New York) 1980, 65.

10 Hibi N Fujita T, Hatano M Hashimoto T & Yamada Y, Putrescine N-methyltransferase in cultured roots of *Hyoscyamus albus*, *Plant Physiol* , 100 (1992) 826.

11 Shirahata A, Morohoshi T, Fukai M , Akatsu S & Samejima K, Putrescine or spermidine binding site of aminopropyltransferases and competitive inhibitors, *Biochem Pharmacol*, 41 (1991) 205.

12 Slocum R D & Galston AW in *Inhibition of polyamine metabolism*, edited by McCann PP, Pegg, A E & Sjoerdsma A (Academic Press) 1987, 3055.

13 Tiburcio A F & Galston AW, Arginine decarboxylase as the source of Putrescine for tobacco alkaloids, *Phytochemistry*, 25 (1986) 107.

14 Hashimoto T& Yamada Y, Hyyoscyamine 6β-hydroxylase, a 2-oxoglutarate dependent dioxygenase in alkaloid producing root cultures, *Plant Physiol*, 81 (1986) 619.

15 Yamada Y & Hashimoto T, *Proc Jpn Acad Scr B Physiol Biol Sci*, 66 (1986) 619 (original not cited).

16 Yamada Y, Okabe S & Hashimoto T, *Proc Jpn Acad Scr B Physiol Biol Sci*, 66 (1990) 73 (original not cited).

17 Hashimoto T, Hayashi A, Amano Y, Kohno J, Iwanari H, Usuda S & Yamada Y, Hyoscyamine 6β-hydroxylase,an enzyme involved in tropane alkaloid biosynthesis,is localised at the pericycle of the root, *J Biol Chem*. 266 (1990) 4648.

18 Michael A J, Furze J M , Rhodes M J C & Burtin D, Molecular cloning and functional identification of a plant ornithine decarboxylase cDNA, *Biochem J*, 314 (1996) 241.

19 Bell E & Malmberg R L, Analysis of cDNA encoding arginine decarboxylase from oat reveals similarity to the E coli arginine decarboxylase and evidence of protein processing, *Mol Gen Genet*, 224 (1990) 431.

20 Rastogi R, Dulson J & Rothstein S J, Cloning of tomato (Lycopersicon esculentum Mill) arginine decarboxylase gene and its expression during fruit ripening, *Plant Physiol* , 103 (1993) 829.

21 Perez-Amador M A, Carbonell, J & Granell A, Expression of arginine decarboxylase is induced during early fruit development and in young tissues of Pisum sativum (L), *Plant Mol Biol*, 28 (1995) 997.

22 Watson M B & Malmberg R, Regulation of Arabidopsis thaliana (L) Heynh arginine decarboxylase by potassium deficiency stress, *Plant Physiol*, 111 (1996) 1077.

23 Nam K H, Lee S H & Lee, J H, Differential expression of ADC mRNA during development and upon acid stress in soybean hypocotyl, *Plant Physiol* , 110 (1996) 774.

24 Hashimoto T, Hibi N & Yamada Y, Subtractive hybridization, *Plant Tissue Cult Lett*, 10 (1993) 307.

25 Hashmoto,T & Yamado Y, Alkaloid biogenesis: Molecular aspects Annu Rev Plant Physiol, *Plant Mol Biol*, 45 (1994) 257.

26 Suzuki K, Yun D J, Chen X-Y, Yamada Y & Hashimoto T, An Atropa belladona hyoscyamine 6 B-hydrooxylase gene is differentially expressed in the root pericycle and anthers, *Plant Mol Biol*, 40 (1999) 141.

27 Hashimoto T, Tamaki K, Suzuki K& Yamada Y, Molecular cloning of plant spermidine synthase, *Plant Cell Physiol*, 39 (1998) 73.

28 Hibi N, Higashiguchi S Hashimoto T & Yamada Y, Gene expression in tobacco low nicotine mutants, *Plant Cell*, 6 (1994) 723.

29 Matsuda J, Okabe S, Hashimoto T & Yamada Y, Molecular cloning of hyoscyamine 6β-hydroxylase,,a 2-oxoglutarate dependent dioxygenase from cultured roots of Hyoscyamus niger J Bio Chem , 266 (1991) 9460.

30 Hashimoto T, Matsuda J & Yamada Y, Two step epoxidation of hyoscyamine to scopolamine is catalysed by bifunctional hyoscyamine 6β hydroxylase, FEBS Lett, 329, (1993) 35.

31 Hashimoto T & Yamada Y, Tropane alkaloid biosynthesis: regulation and application, In Proc 7th Annu Penn State Symp, *Plant Physiol*, (1992) 122.

32 Basu R & Ghosh B Polyamines in various rice (*Oryza sativa L*) genotypes with respect to sodium chloride salinity, *Physiol Plant*, 82 (1991) 575.

33 Imanishi S Hashizume K, Nakakita M, Kojima H, Matsubayashi Y, Hashimoto T, Sakagami Y & Nakamura K, Differential induction by methyl jasmonate of genes encoding ornithine decarboxylase and other enzymes involved in nicotine biosynthesis in tobacco cell cultures, *Plant Mol Biol*, 38 (1998) 1101.

34 Chen C T & Kao C H, Osmotic stress and water stress have opposite effects on putrescine and proline production in excised rice leaves, *Plant Growth Regul*, 13 (1993) 197.

35 Mizusaki S, Tanabe Y, Noguchi M & Tamaki E, Changes in the activities of ornithine decarboxylase,putrescine N-methyl transferase and N-methylputrescine oxidase in tobacco roots in relation to nicotine biosynthesis, *Plant Cell Physiol*, 14 (1973) 103.

36 Zarate R, Tropane alkaloid production by *Agrobacterium rhizogenes* transformed hairy root cultures of *Atropa baetica* Wilk (Solanaceae) *Plant Cell Rep*, 18, (1999) 418.

37 Hamill J D, Robins R J, Parr A J , Evans D M , Furze J M & Rhodes M J C, Overexpressing a yeast ornithine decarboxylase gene in transgenic roots of *Nicotiana rustica* can lead to enhanced nicotine accumulation, *Plant Mol Biol*, 15 (1990) 27.

38 Yun D J, Hashimoto T& Yamada Y, Metabolic engineering of medicinal plants: transgenic Atropa belladonna with an improved alkaloid composition, *Proc Natl Acad Sci USA*, 89 (1992) 11799.

39 De Luca V, Marineau C, & Boisson N, Molecular cloning and analysis of cDNA encoding a plant tryptophan decarboxylase:comparison with animal DOPA decarboxylase, *Proc Natl Acad Sci USA*, 86 (1989) 2582.

40 Kutchan T M, Hampp N, Lottspeich F, Beyrenther K & Zenk M H, The cDNA clone for strictosidal synthase from Rauwolfia serpentina: DNA sequence determination and expression in *E coli, FEBS Lett*, 257 (1988) 40.

41 Mc Knight T D, Roessner C A, Devagupta R, Scott A I & Nessler C L, Nucleotide sequence of a cDNA encoding the vacuolar protein strictosidine synthase from Catharanthus roseus, *Nucleic Acids Res*, 81 (1990) 4939.

42 Bracher D & Kutchan T M, Strictosidine synthase from R serpentina: analysis of a gene involved in indole alkaloid biosynthesis, *Arch Biochem Biophys*, 294 (1992) 717.

43 Zenk M H, Rueffer,M, Kutchan T M & Galneder E, Future trends of exploration for the biotechnological production of isoquinoline alkaloids, In application of plant cell and tissue culture edited by Y Yamada (John Wiley and Sons) 1988, 213.