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Full Length Research Paper

Allocation of different ^{14}C substrates into primary metabolites in relation to total alkaloid accumulation into *Catharanthus roseus* plants

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Studies were performed using ^{14}C radiolabel to elucidate the extent to which carbon from four sources (CO_2, sucrose, glucose and acetate) contribute to the primary metabolites and alkaloids produced by the roots, stems and leaves of *Catharanthus roseus* plant. $^{14}CO_2$ derived metabolites were most preferred for biosynthesis of leaf and root alkaloids. Roots were the major accumulators of metabolites accompanied by higher ^{14}C content into total alkaloids. ^{14}C-glucose derived metabolites were the second most preferred substrate for leaf and stem alkaloid accumulation and the stem showed highest incorporation into alkaloids. ^{14}C-sucrose was the third most preferred carbon source for biosynthesis of leaf, stem and root alkaloids. Among these substrates, ^{14}C-acetate was the least preferred by the plant for biosynthesis of alkaloids of leaf, stem and root. There were variations in the metabolite mobilization of sugars, amino acids and organic acids between leaf, stem and root when different C sources were supplied. A floating metabolic pool seems to influences the alkaloid biosynthesis occurring in leaf, stem and root. The relative higher incorporation of $^{14}CO_2$, ^{14}C-sucrose and ^{14}C-glucose suggests the preferential utilization of metabolites from MEP/terpenoid pathway that contribute to alkaloid accumulation as compared to ^{14}C acetate suggesting lower contribution by mevalonate/terpenoid pathway derived metabolites.

Key words: ^{14}C-Sources, primary assimilate partitioning, *Catharanthus roseus*, leaf, stem, root, sugars, amino acids, organic acids, total alkaloids.

INTRODUCTION

Catharanthus roseus (L) G. Don of family Apocynaceae is an industrially important medicinal plant. The alkaloids derived from the plant are highly valued as the leaves and the stems are source of vinblastine and vincristine that are indispensable components in certain type of cancer treatment. The roots accumulate ajmalicine and serpentine that is of important ingredients of medicines controlling high blood pressure and other cardiovascular diseases. Because of medicinal value, high price and low alkaloid content the plant is target of the extensive studies by the researchers all over the world (Verpoorte et al., 1997, 1999; van der Heijden et al., 2004; Misra and Kumar, 2000; Facchini and DeLuca, 2008). Wide spread research using techniques as-strain improvement/ selection, medium and culture conditions modification, two-phase system and two-stage system have been carried out in cell cultures for deeper understanding of factors controlling alkaloid production. These include feeding of various carbon sources as sucrose, glucose and fructose (Jung et al., 1992). Co-feeding of sucrose with secologanin also reported (Merillon et al., 1986). Feeding of intermediary metabolic building blocks of terpenoid pathway as geraniol and loganin (Hong et al., 2003). Precursor feeding as mevalonic acid, geraniol, secologanin, tryptophan, tryptamine (Krueger and Carew, 1978). Time course feeding of secologanin (Naudascher et al., 1989a) and loganin (Naudascher et al, 1989b). Feeding of 10-hydroxygeraniol and loganin (Morgan and Shanks, 2000). Even feeding of ^{15}N has been reported (Schripsema and Verpoorte, 1992; Schripsema et al., 1991). Metabolic building blocks as sugars, amino acids

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and organic acids have been reported as signals for alkaloid accumulation (Aerts et al., 1996). Presupposition of feeding studies is that, high level of intermediate/ precursor metabolites availability of the pathway will lead to higher accumula-tion of alkaloids. Experimental investigations on the other hand reveal that mere presence of high concentration of intermediary metabolites does not result into its greater biosynthetic utilization. In fact, high content of certain metabolites as tryptamine and geraniol in cell culture media proved toxic. This was ascribed due to lack of tissue differentiation, direct exposure to metabolites, or easy membrane permeability but most important aspect not considered was lack of storage capability of the synthesizing tissue.

Despite these advances in cell culture, the Catharanthus plant remains the only source of these alkaloids. Since alkaloid, production in *C. roseus* is under strict developmental and environmental control and transport of pathway intermediates/substrates or precursors are compartmentalized; understanding the relationship between availability of primary metabolites in relation to alkaloid accumulation is fundamentally important (Verpoorte et al., 1997). *In situ* RNA hybridization immunocytochemistry studies have established that active synthesis of alkaloids takes place in epidermal tissues of leaves, stems and flower and in specialized lacticifer and idioblasts cells of these organs. In underground tissues, synthesis occurs in protoderm and cortical cells around apical meristems (Facchini and DeLuca, 2008). Such compartmentation strongly suggests movement of pathway and intermediary metabolite translocation. It has been suggested that vindoline as well as dimeric alkaloids are restricted to leaves and stems whereas catharanthine is distributed equally throughout above ground and underground tissues (St-Pierre et al., 1999). Biosynthesis of the Terpene indole alkaloids (TIAs) in *C. roseus* occurs from unification of two main streams of the pathway leading to the production of two key pathways intermediates:

1) Production of tryptophan derived from phosphoenol pyruvate and erythrose phosphate through shikimate pathway and its conversion to tryptamine.
2) Production of geraniol from the mevalonate or chloroplast operative MEP/terpenoid pathway, leading to production of secologanin. Recent evidence indicates that metabolite from triose phosphate pathway (MEP pathway, that operates in chloroplast) is the major contributor of carbon metabolites for monoterpenoid stream of alkaloid production pathway. This aspect has been hypothesized by precursor feeding experiments in cell cultures (Contin et al., 1998). Most of the feeding studies reported so far of different carbon/metabolite sources are limited to cell cultures only where membrane permeability, availability of nutrients, ease of metabolite availability and complexity of cellular functions are not limiting. These feeding effects under *in vitro* conditions may not translate with that at the whole plant level because at whole plant level metabolite transport from shoot to root and multi-cellular comparmentation of metabolites and its biosynthetic utilization occurs. Feeding of different carbon sources and its metabolic profiling into different plant parts and its biosynthetic utilization are lacking at whole plant level.

The objective of the present study was to monitor/ understand real time utilization capability of different metabolic carbon sources and how the mobilization of primary metabolites into different plant part occurs in relation to total TIAs accumulation. This was investigated by feeding of $^{14}CO_2$, ^{14}C- sucrose, ^{14}C-acetate and ^{14}C-glucose. ^{14}C label was chased and analyzed by it's partitioning into major primary metabolic fractions and distribution into major metabolic components as in sugars, amino acids, organic acids and the simultaneous utilization of these current assimilates for biosynthetic production into total alkaloids in different plant parts. For such studies, plants were grown in sand and hydroponics culture medium.

MATERIALS AND METHODS

Plant material

C. roseus (L.) G.Don (cv. Dhawal) seeds obtained from the gene bank of Central Institute of Medicinal and Aromatic Plants (CIMAP) were initially raised in 10, 000 cm^3 ceramic pots filled with acid washed with clean silica sand (Agarwala and Sharma, 1961). For growing plants in sand culture, 4 weeks old seedlings were transplanted in 5,000 cm^3 plastic containers.

For growing plants in hydroponics culture, 4 weeks old seedlings were transplanted to 2,500 cm^3 amber coloured glass containers filled with balanced nutrient solution. Modified nutrient solution of Hoagland and Arnon (1938) was used except Fe, which was supplied as Fe-EDTA in sand and hydroponics culture conditions. These pots were maintained in glass house at ambient temperature (30 to 35°C) and photosynthetic active radiation (PAR) (800 to 1000 µmol m^{-2} s^{-1}) measured by a light meter (model LI-188B, LiCOR Inc., USA). The nutrient medium was changed weekly and routine practice for maintaining plants in sand and hydroponics culture were observed.

Feeding of different ^{14}C substrates

Feeding of $^{14}CO_2$

For $^{14}CO_2$ feeding 4 months old sand culture grown plants were placed in a sealed plexiglass chamber (20 L capacity) around a central vial containing $Na_2{}^{14}CO_3$ solution (1.85 MBq, 1.78 TBq mol^{-1}) obtained from the isotope division of Bhabha Atomic Research Centre, Mumbai, India. $^{14}CO_2$ in the chamber (0.37 MBq) was liberated by injecting 2 M H_2SO_4 into the carbonate solution through a PVC inlet tube and uniformly distributed within the chamber using a small electric fan.

Plants were initially exposed to$^{14}CO_2$ for 1 h in natural sunlight, which varied between 800 to 1000 µmol m^{-2} s^{-1} during the time of exposure. After 1 h, a saturated solution of KOH was run into the central vial to absorb the remaining $^{14}CO_2$. The plants were then removed from the chamber and allowed to assimilate $^{14}CO_2$ for next 6 h (Srivastava et al., 2004).

Feeding of other ^{14}C substrates

For the feeding of ^{14}C- sucrose, ^{14}C-acetate and ^{14}C-glucose rooted plants grown in hydroponics cultures were used. ^{14}C- sucrose, ^{14}C-acetate and ^{14}C-glucose were obtained from the isotope division of Bhabha Atomic Research Centre, Mumbai, India. A uniform dose of (0.175 MBq) of sucrose, acetate and glucose were given by keeping the plant in a glass vial dipped in 10 ml of Hoagland and Arnon's complete nutrient solution and the plants allowed to uptake ^{14}C in sunlight. After feeding of different ^{14}C substrates, plants were separated into leaves, stem and root. Each of the plant part was processed for determining the allocation of label into major primary photosynthetic metabolic fractions such as ethanol soluble (ES), ethanol insoluble (EIS) and chloroform soluble (CS) and into metabolic pools of sugars, amino acids and organic acids. Simultaneously the biosynthetic capacity to utilize currently assimilated metabolites into total alkaloids was determined by quantifying the label into total alkaloids of leaf, stem and roots. Thus, the separated plant parts leaf, stem and roots were divided in two portions:

1) A known mass of leaf, stem and root tissues was processed for determining the incorporation of current assimilate in total alkaloids as explained in "determination of total alkaloid content". The ^{14}C-content in total alkaloid was measured in a liquid scintillation counter using PPO-POPOP-Toluene cocktail (Wallac 1409, USA). The unit of expression was Bq/g.dry mass of tissue (leaf, stem and root).
2) A known mass of leaf, stem and root tissues were immediately fixed into boiling ethanol to maintain the tissue metabolic status. The fixed material was ground in ethanol, filtered, filtrate evaporated and diluted in a known volume of distilled water, this aqueous fraction termed as ethanol soluble fraction (ES). The unfiltered ground leaf tissue further hydrolyzed by enzyme diastase in 0.05 M acetate buffer (pH 5.2) at 50ºC was termed as ethanol insoluble fraction (EIS). The aqueous ES fraction was further extracted with an equal volume of chloroform, this fraction termed as chloroform soluble (CS) fraction, which contained pigments, and terpenoid pathway derived metabolites (Srivastava et al., 2004). The ^{14}C label in ES and in EIS fractions was measured using Bray's scintillation fluid and in CS fraction using PPO-POPOP-Toluene cocktail in a liquid scintillation counter (Wallac 1409, USA). The unit of expression was Bq/g.fr. mass of tissue (leaf, stem and root). The ES fraction was further separated into metabolites by column chromatography by passing through Amberlite ion exchange and separation into fractions consisting of neutral (sugar), acidic (organic acids) and basic (amino acids). The ^{14}C content in eluates after column chromatography was measured in a liquid scintillation counter (Wallac 1409, USA) using Bray's scintillation fluid (Srivastava et al., 2004; Dixit and Srivastava, 2000; Srivastava and Luthra, 1994).

Determination of total alkaloid content

Freshly harvested leaf, stem and root samples of ^{14}C fed plants were oven dried at 60°C for 48 h and powdered. A known mass of each plant material was extracted in 90% ethanol (3 times) filtered and concentrated to dryness. Dried residue was redissolved in ethanol diluted with equal volume of water and acidified with 3% hydrochloric acid. The mixture was extracted with hexane (3 times), hexane fraction discarded and aqueous extract cooled to 10°C and basified with ammonium hydroxide 3% to pH 8.5. This portion was further extracted with chloroform (3 times). The combined chloroform extract was washed with distilled water evaporated to dryness, and weighed (Uniyal et al., 2001). Counts in this alkaloid extract were determined in PPO-POPOP-Toluene cocktail in a liquid scintillation counter (Wallac 1409, USA). The unit of expression was Bq/g. dry mass of tissue (leaf, stem and root) (Srivastava and Srivastava, 2007).

Statistical analysis

The results presented are mean values of three extractions and statistically analyzed for significance by analysis of variance.

RESULTS AND DISCUSSION

Metabolite partitioning and its incorporation into alkaloids in leaves

The analysis of distribution of current photo-synthate by leaves reflects the real-time utilization of assimilates by the biosynthetic pathway. When $^{14}CO_2$ was fed the biosynthetic utilization of assimilates into alkaloids as measured by ^{14}C content in total alkaloids was highest followed by ^{14}C-glucose then ^{14}C-sucrose and least from ^{4}C-acetate (Table 1). The distribution of ^{14}C into major metabolic fractions derived from $^{14}CO_2$ was highest in ES followed by ^{14}C-glucose then ^{14}C-sucrose and lowest from ^{14}C-acetate. However, the content of ^{14}C values in EIS showed a different trend of incorporation with maximum values from $^{14}CO_2$ assimilates followed by ^{14}C-acetate whereas from ^{14}C-sucrose and ^{14}C-glucose the label in EIS were nearly same. In CS fraction the maximum ^{14}C content were again derived from $^{14}CO_2$ assimilates followed by ^{14}C-glucose and ^{14}C-acetate and lowest from ^{14}C-sucrose (Table 2). Chromatographic separation of ES revealed maximum ^{14}C content from $^{14}CO_2$ assimilates into sugars, followed by organic acids and then lowest into amino acids. From ^{14}C-glucose assimilate content into sugars was highest, and then into amino acids and lowest into organic acids respectively. From ^{14}C-sucrose ^{14}C content into amino acids was maximum followed by sugars and then into organic acids. From ^{14}C-acetate, the label in amino acids was highest then in organic acids and lowest into sugars (Table 3). Thus, there were wide variations in metabolite contents in leaves when different carbon substrates were provided. Earlier studies on utilization of photo-synthetically fixed $^{14}CO_2$ into alkaloids in developing Catharanthus leaves revealed that of the total $^{14}CO_2$ assimilated the leaves at position 1 to 6 contained 8, 22, 25, 19, 13 and 8% of total assimilates, respectively.

The utilization of $^{14}CO_2$ assimilates into total alkaloids was maximum in the youngest leaves which declined with leaf age. ^{14}C content in ES increased up to third leaf and then declined. The ^{14}C content in metabolites as sugars and organic acids was also highest in third leaf (Srivastava et al., 2004).

It has also been reported that total alkaloid content is dependent on leaf age where with increasing leaf age (from younger to older leaves) contents of catharanthine and vindoline decrease (Westkemper et al., 1980; Deus-Neuman et al., 1987).

Table 1. Utilization of different ^{14}C substrates for the production of total alkaloids in different plant parts namely: leaf, stem and roots in Catharanthus and ^{14}C content in total alkaloids. All values in (Bq/g. dry mass tissue).

^{14}C Source	^{14}C content in total alkaloids of leaf	^{14}C content in total alkaloids of stem	^{14}C content in total alkaloids of root	Sum of leaf + stem + root alkaloids
$^{14}CO_2$	205.43	217.10	4857.30	5269.83
^{14}C-sucrose	128.53	1194.90	408.63	1732.06
^{14}C-glucose	133.60	1829.83	59.83	2023.26
^{14}C-acetate	58.76	188.53	81.80	329.09
SEM	31.00	497.98	470.12	
SED	43.84	704.25	664.85	
CD 5%	101.27	1626.83	1535.80	
1%	147.30	2366.31	2233.90	

Data are mean of three replicates.

Table 2. Metabolic profile of different ^{14}C substrates and ^{14}C content into primary metabolic fractions into leaves of catharanthus. All values in (Bq/g. F. mass leaves).

^{14}C source	Ethanol soluble fraction	Ethanol insoluble fraction	Chloroform soluble fraction
$^{14}CO_2$	2192.63	233.63	232.76
^{14}C-sucrose	155.10	148.16	41.13
^{14}C-glucose	268.00	150.10	93.63
^{14}C-acetate	126.53	177.23	62.73
SEM	115.99	31.40	20.20
SED	164.04	44.40	28.57
CD 5%	378.94	102.58	66.00
1%	551.19	149.21	96.00

Data are mean of three replicates.

Table 3. Metabolite profile of different ^{14}C substrates and ^{14}C content into primary metabolic pool into leaves of catharanthus. All values in (Bq/g. F. mass leaves).

^{14}C source	Sugars	Amino acids	Organic acids
$^{14}CO_2$	97.16	16.00	53.63
^{14}C-sucrose	35.90	39.53	34.30
^{14}C-glucose	42.86	33.23	20.30
^{14}C-acetate	16.63	29.50	26.60
SEM	3.63	7.82	6.11
SED	5.14	11.06	8.64
CD 5%	11.88	25.55	19.97
1%	17.28	37.16	29.05

Data are mean of three replicates.

Metabolite partitioning and its incorporation into alkaloids in stems

Green tissues of stem also accumulate alkaloid. Analysis after feeding of different carbon substrates revealed that highest ^{14}C content in alkaloids was obtained from ^{14}C-glucose, followed by ^{14}C-sucrose, from $^{14}CO_2$ assimilates and least from ^{14}C-acetate (Table 1). Distribution into major metabolic fraction in ES showed maximum ^{14}C label in assimilates from $^{14}CO_2$ followed by ^{14}C-glucose, from ^{14}C-sucrose and lowest from ^{14}C-acetate (Table 4). In EIS fraction ^{14}C content was highest from $^{14}CO_2$ assimilates followed by ^{14}C-glucose then from ^{14}C-acetate and lowest from ^{14}C sucrose In CS fraction

Table 4. Metabolic profile of different ^{14}C substrates and ^{14}C content into primary metabolic fractions into stems of catharanthus. All values in (Bq/g. F. mass stem).

^{14}C source	Ethanol soluble fraction	Ethanol insoluble fraction	Chloroform soluble fraction
$^{14}CO_2$	984.86	237.40	57.33
^{14}C-sucrose	304.63	175.40	49.10
^{14}C-glucose	848.13	203.53	91.13
^{14}C-acetate	229.33	198.23	57.83
SEM	142.43	36.10	16.91
SED	201.43	51.05	23.91
CD 5%	465.32	117.94	55.24
1%	676.83	171.55	80.35

Data are mean of three replicates.

Table 5. Metabolic profile of different ^{14}C substrates and ^{14}C content into primary metabolic pool into stems of catharanthus. All values in (Bq/g. F. mass stem).

^{14}C source	Sugars	Amino acids	Organic acids
$^{14}CO_2$	78.73	31.23	62.13
^{14}C-sucrose	48.93	35.60	34.60
^{14}C-glucose	91.33	59.30	42.73
^{14}C-acetate	51.93	44.13	27.20
SEM	4.27	10.34	17.12
SED	6.04	14.62	24.22
CD 5%	13.95	33.78	55.95
1%	20.30	49.14	81.39

Data are mean of three replicates.

highest ^{14}C label was from ^{14}C-glucose nearly equal label from $^{14}CO_2$ and ^{14}C-acetate and lowest from ^{14}C-sucrose (Table 4). Further chromatographic separation of ES fraction into metabolic pool into sucrose showed maximum ^{14}C content from ^{14}C-glucose followed by $^{14}CO_2$, from ^{14}C-acetate and minimum from ^{14}C-sucrose. In amino acid, fraction highest label was from ^{14}C-glucose followed by ^{14}C-acetate and ^{14}C sucrose and lowest from $^{14}CO_2$. In organic acid fraction highest label was from $^{14}CO_2$ assimilates, and then from ^{14}C-glucose and from ^{14}C-sucrose and lowest from ^{14}C-acetate (Table 5). Thus, phloem transport of metabolites is different when carbon substrates are different. In earlier experiment phloem transport of $^{14}CO_2$ leaf assimilates in Catharanthus showed that top portion of stem had higher ^{14}C content in ES, EIS, sugars and alkaloid content while basal portion had higher content in CS, amino acid and organic acid content (Srivastava et al., 2004). A comparative analysis involving utilization of ^{14}C-sucrose by rooted and non-rooted (twigs) of Catharanthus was reported. Twigs revealed significantly higher ^{14}C label in alkaloids and in sugars in stem than in leaves. In contrast root contained higher ^{14}C label in ES, EIS, CS and in metabolite sugars, amino acids and organic acids than that present in stem and leaves. This was accompanied by higher ^{14}C content in alkaloids (Srivastava and Srivastava, 2006). Organ specific localization and metabolite movement have shown that vindoline as well as dimeric alkaloids are restricted to leaves and stems where as catharanthine is distributed equally in above and underground plant parts (St-Pierre et al., 1999).

Metabolite partitioning and its incorporation into alkaloids in roots

Roots are the major sink of leaf-assimilated photo-assimilates accounting for nearly 30% utilization of leaf assimilates (Marschner, 1986). These assimilates also provide metabolites for biomass and alkaloid production. When $^{14}CO_2$ was fed, the ^{14}C content in root alkaloid was highest followed by ^{14}C-sucrose, ^{14}C-acetate and lowest from ^{14}C-glucose (Table 1). Distribution into major metabolic fractions revealed that maximum ^{14}C in ES was from ^{14}C- acetate followed by ^{14}C-glucose, then ^{14}C-sucrose and least from $^{14}CO_2$. However, ^{14}C content in EIS was highest from ^{14}C-glucose, then from ^{14}C-sucrose, from ^{14}C-acetate and lowest from $^{14}CO_2$. In CS

Table 6. Metabolic profile of different ^{14}C substrates and ^{14}C content into primary metabolic fractions into root of catharanthus. All values in (Bq/g. F. mass root).

^{14}C source	Ethanol soluble fraction	Ethanol insoluble fraction	Chloroform soluble fraction
$^{14}CO_2$	593.70	124.20	56.73
^{14}C-sucrose	1739.70	1248.60	816.93
^{14}C-glucose	1935.20	1577.46	2900.30
^{14}C-acetate	2738.60	909.00	3241.63
SEM	354.17	123.30	312.39
SED	500.88	174.38	441.78
CD 5%	1157.03	402.82	1020.53
1%	1682.96	585.93	1484.41

Data are mean of three replicates.

Table 7. Metabolic profile of different ^{14}C substrates and ^{14}C content into metabolic pool into root of catharanthus. All values in (Bq/g. F. mass root).

^{14}C source	Sugars	Amino acids	Organic acids
$^{14}CO_2$	52.03	28.50	32.46
^{14}C-sucrose	88.10	19.73	24.43
^{14}C-glucose	115.03	26.60	32.93
^{14}C-acetate	172.80	49.23	42.63
SEM	14.14	4.55	5.07
SED	20.00	6.44	7.18
CD 5%	46.22	14.87	16.59
1%	67.23	21.64	24.13

Data are mean of three replicates.

fraction, highest ^{14}C label was from ^{14}C-acetate, and then from ^{14}C-glucose, from ^{14}C-sucrose and lowest from $^{14}CO_2$ (Table 6). Subsequent chromatographic separation of ES into metabolites showed maximum ^{14}C label into sugars from ^{14}C-acetate, then from ^{14}C-glucose, from ^{14}C-sucrose and minimum from $^{14}CO_2$. In amino acids, highest ^{14}C label was from ^{14}C-acetate, from $^{14}CO_2$, from ^{14}C-glucose and lowest from ^{14}C-sucrose. In organic fraction, maximum ^{14}C label was from ^{14}C-acetate, nearly similar label from ^{14}C-glucose and $^{14}CO_2$ and minimum from ^{14}C-sucrose (Table 7). Upon comparing the ^{14}C label in ES, CS, sugars, amino acids and organic acids, there content is higher as compared to that obtained from other ^{14}C sources but its utilization for alkaloid production is lowest. In contrast, ^{14}C label in root alkaloid was maximum when $^{14}CO_2$ was fed. Therefore, there is selectivity in utilization of substrate for alkaloid biosynthesis.

Conclusions

By feeding different ^{14}C substrates and analyzing profile of primary metabolites a real time illustration of mobility of metabolite, its distribution into different plant parts and the biosynthetic utilization for TIAs production was monitored. At whole plant level, the capability to utilize different carbon sources depends upon the metabolic status and availabity/requirement of metabolic pool that together control biosynthetic potential to accumulate TIAs. Considering only different carbon substrates are utilized for alkaloid production; when $^{14}CO_2$ is fed maximum ^{14}C content was found in root and then in stem and leaf alkaloids. After $^{14}CO_2$, ^{14}C-glucose shows maximum ^{14}C content in stem and root alkaloids. ^{14}C-sucrose shows higher content in stem and root alkaloid. Among these four substrate tested, ^{14}C-acetate is least preferred substrate considering the ^{14}C label in leaf, stem and root alkaloids (Table 1). Hence, the $^{14}CO_2$ photosynthetically derived metabolites are most efficiently utilized and incorporated into biosynthetic pathway. In cell culture, studies it has been shown by feeding of labeled glucose that MEP pathway and not the mevalonate pathway was the major route for biosynthesis of secologanin (Contin et al., 1998). The present study indicates that “in planta”, the $^{14}CO_2$ assimilates, ^{14}C-sucrose, ^{14}C-glucose are preferentially utilized through the MEP pathway because of high ^{14}C content in alkaloid when these substrates are fed as compared to ^{14}C acetate feeding. Which of these metabolites from different carbon substrates are preferentially utilized

cannot be specified and what are the changes in the spectrum of alkaloids produced cannot be defined. Our efforts are continuing for determination of change of ^{14}C in spectrum of alkaloids so that a clear picture of biosynthetic utilization of primary metabolites could be established.

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REFERENCES

Aerts RJ, Schafer A, Hesse M, Bauman TW, Slusarenko A (1996). Signaling molecules and the synthesis of alkaloids in *Catharanthus roseus* seedlings. Phytochemistry, 42: 417-422.

Agarwala SC, Sharma CP (1961). The standardization of sand culture technique for the study of macro and micro (trace) element deficiencies under Indian conditions. Curr. Sci., 11: 427.

Contin A, Van DHR, Lefeber AWM, Verpoorte R (1998). The iridoid glucoside secaloganin is derived from novel triose phosphate/pyruvate pathway in *Catharanthus roseus* cell cultures. FEBS Lett., 434: 413-416.

Deus NP, Stockigt J, Zenk MH (1987). Radioimmunoassay for the quantitative determination of Catharanthine in *Catharanthus*. Planta Med., 53: 184-188.

Dixit D, Srivastava NK (2000). Distribution of photo-synthetically fixed $^{14}CO_2$ into curcumin and essential oil in relation to primary metabolites in developing Turmeric (*Curcuma longa*) leaves. Plant Sci., 150: 165-171.

Facchini PJ, DeLuca V (2008). Opium poppy and Madagascar periwinkle: model non-model systems to investigate alkaloid biosynthesis in plants. Plant J., 54: 763-784.

Hong SB, Hughes EH, Shanks JV, Ka YS, Gibson SI (2003). Role of Non-Mevalonate pathway in indole alkaloid production by *Catharanthus roseus* hairy roots. Biotech. Prog., 19: 1105-1108.

Hoagland DR, Arnon DI (1938). The water culture method for growing plants without soil. Calif. Agric. Exp. Stat. Circ., 32: 347.

Jung KH, Kwak SS, Kim SW, Lee H, Choi CY, Liu JR (1992). Improvement of catharanthine productivity in hairy root cultures of *Catharanthus roseus* by using monosaccharide as a carbon source. Biotech. Lett., 14: 695-670.

Krueger RJ, Carew DP (1978). *Catharanthus roseus* tissue culture: The effects of precursors on growth and alkaloid production. Llyodia, 41: 327-330.

Marschner H (1986). Yield and source-sink relationship. In: The Mineral Nutrition of Higher Plants. Marschner H (ed.) Academic Press, New York, pp. 115-154.

Merillon JM, Doireau P, Guillotm A, Cheniux JC, Rideau M (1986). Indole alkaloid accumulation and Tryptophan decarboxylase activity in *Catharanthus roseus* cultures in three different media. Plant Cell Rep., 5: 23-26.

Misra P, Kumar S (2000). Emergence of periwinkle *Catharanthus roseus* as a model system for molecular biology of alkaloid: Phytochemistry, pharmacology, plant biology and *in vivo* and *in vitro* cultivation. J. Med. Arom. Plant Sci., 22: 306-337.

Morgan JA, Shanks JV (2000). Determination of metabolic rate-limitations by precursor feeding in *Catharanthus roseus* hairy root cultures. J. Biotech., 79: 137-145.

Naudascher F, Doireau P, Guillot TM (1989a). Time course studies on the use of secologanin by *Catharanthus roseus* cells cultured *in vitro*. J. Plant Physiol., 134: 608-612.

Naudascher F, Doireau P, Guillot TM (1989b). Time course studies on the use of loganin by *Catharanthus roseus* cells cultured *in vitro*. J. Plant Physiol., 134: 366-368.

Schripsema J, Petenberg LA, Erkelens C, Verpoorte R (1991). Nitrogen metabolism in cultures of *Tabernemontana divaricata*. Phytochem. 30: 3951-3954.

Schripsema J, Verpoorte R (1992). Search for factors involved in alkaloid production in cell cultures of *Tabernemontana divaricata.* Planta Med., 58: 245-249.

Srivastava NK, Luthra R (1994). Relationship between photosynthetic carbon metabolism and essential oil biogenesis in peppermint under Mn-stress. J. Exp. Bot., 45: 1127-1132.

Srivastava NK, Misra A, Srivastava AK, Sharma S (2004). Utilization of photo-synthetically fixed $^{14}CO_2$ into alkaloids in relation to primary metabolites in developing leaves of *Catharanthus roseus*. Photosynthetica, 42: 469-472.

Srivastava NK, Srivastava AK (2006). Utilization of exogenously supplied ^{14}C- sacharose into primary metabolites and alkaloid production in *Catharanthus roseus*. Photosynthetica, 44: 478-480.

Srivastava NK, Srivastava AK (2007). Influence of gibberellic acid on $^{14}CO_2$ metabolism, growth and production of alkaloid in *Catharanthus roseus*. Photosynthetica, 45: 156-160.

St-Pierre PB, Felipe A, Vasquez F, DeLuca V (1999). Multicellular compartmentation of *Catharanthus roseus* alkaloids biosynthesis predicts intracellular localization of pathway intermediates. Plant Cell, 11: 887-900.

Uniyal GC, Bala S, Mathur AK, Kulkarni RN (2001). Symmetry C_{18} column: a better choice for the analysis of indole alkaloids of *Catharanthus roseus*. Phytochem. Anal., 12: 206-210.

Van der Heijden DHR, Jacobs DI, Snoeijer W, Hallaed D, Verpoorte R (2004). The *Catharanthus* alkaloids: pharmacognosy and biotechnology. Curr. Med. Chem., 11: 607-628.

Verpoorte R, Van DHR, Moreno PRH (1997). Biosynthesis of terpene Indole alkaloids in *Catharanthus roseus* cells. In: Cordell G.A. (ed). The Alkaloids. Vol. 49. Academic Press. pp. 221-299.

Verpoorte R, Van DHR, Ten HHJG, Memelink J (1999). Metabolic engineering of plant secondary metabolic pathways for the production of fine chemicals. Biotech. Lett., 21: 467-479.

Westkemper P, Wieczorek V, Gueritte F, Langlois Y, Poteir P, Zenk MH (1980). Radioimmunoassay for the quantitative determination of Vindoline in *Catharanthus*. Planta Med., 39: 34-37.