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Research Paper

# Partitioning of $^{14}Co_2$ Photosynthetic Assimilate Into Primary Metabolites, Roots and Into Total Saponins in Medicinal Herb *Chlorophytum Borivillanum*

Biology

**KEYWORDS :** amino acids, $^{14}CO_2$ incorporation, organic acids, primary and secondary metabolites, roots, sand culture, sugars, total saponins.

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## ABSTRACT

*Relationship between14CO2 photosynthetic assimilate partitioning between shoot and root and subsequent utilization of assimilate for saponins accumulation has been reported for the first time in Chlorophytum borivilanum. Time dependent distribution (12, 24 and 36h) showed maximum content of 14C in sugars and amino acids after 24h of feeding in leaves. Maximum translocation of leaf assimilates as sugars and amino acids to roots occur at 36h while organic acids were highest at 12h after feeding. In roots the total saponins content received maximum 14C assimilate up to 36h after feeding indicating that assimilate is biosynthetically utilized. The results of this study suggest that selection of Chlorophytum plants with higher photosynthetic capacity will result in higher saponins content.*

## 1. Introduction

Chlorophytum borivillanum (common name Safed Musli) is an important medicinal plant having industrial, pharmaceutical and nutraceutical properties. Dry roots are the important part that contains active constituent's saponins. Recent pharmacological studies on roots have indicated antiviral, anticancer, antioxidant, antibiabetic, antistress, aphrodisiac, antimicrobial and immunomodilatory activities (Mousumi et al. 2011 and other references cited therein). The nutraceutical properties are due to presence of Carbohydrate (42%), protein (9%), fibers (4%), sodium (0.04mg/g), potassium (0.08mg/g), calcium (6.6mg/g), magnesium 1.9mg/g), phosphorus (3.2mg/g), zinc (0.002mg/g) and copper (0.149mg/g) (Bourdia et al. 1995). The restricted distribution coupled with overexploitation has led to endemic nature of the plant (Biswas et al. 2003) and it has also been declared as one of the prioritized plant species by the National Medicinal Plant Board, New Delhi, India. In international scenario the cropped area under organic farming is about 26.4 million ha. The major producers and importers of organic products are European Union, U. S. A. and Japan. In national Indian agricultural scenario organic manures have been used since Vedic period. According to survey of IFOAM and Stiftung Oekologie & Landban (SOEL) Feb 2005 India had about 76,326 ha land under organic management, which was only 0.05% of total agricultural land. According to the survey there were about 5147 certified organic farms in India. The Indian organic farming industry is estimated at US $20 million and is mostly export oriented. As per APEDA report annually about 6792 t of organic products worth Rs. 72 million are exported from India. The data on area and production of Safed musli both under organic and non organic mode is not available. However Safed musli is cultivated in most states of the country prominent amongst them being Madhya Pradesh, Maharashtra, Punjab; Andhra Pradesh etc. (website-http//www.nabard.org/modelbankproject/musliasp) viewed on 25.4.2013.

The active principles or the precursors of active principles are synthesized in the leaves, translocated, biosynthesized and ultimately stored in specialized cells in roots. Growth of leaves and development of roots depend on factors such as nutrition, cultivation practices and genotype (Bordia et al. 1995). Despite the economic importance of the plant very little is known about the interrelationship between carbon (precursors) metabolism and precursor-product utilization for saponins accumulation-a solution for development of improved cultivars. A large proportion of leaf photosynthetic assimilates are required for root growth and development which in annual crops can be about 30% of leaf photosynthetic accumulation (Marschner, 1986). Besides requirements of assimilates for root growth, assimilates are required for production of saponins also. During the growing season carbon fixed by the leaf is transported to the roots. Hence the rate and amount of photo-assimilate produced and the proportion of assimilate transported greatly influence size, yield development and growth of roots as well as saponins accumulation. Since the precursors are produced in leaves, the inherent photosynthetic capacity may possibly be an additional controlling factor. No information is available on the rate of assimilate partitioning from shoot to root and its biosynthetic utilization. In the present paper we report time dependent $^{14}C$-photosynthate assimilation by leaves and the distribution of assimilate in to various primary metabolites and biosynthetic utilization for saponins in roots.

## 2. Materials and methods

### 2.1. Plant material

Plant material (roots of cv.CIM OJ) was obtained from the farm nursery of Central Institute of Medicinal & Aromatic Plants. The roots were grown in acid-washed silica sand (Agarwala and Sharma, 1961). The plants were supplied with balanced solution of Hoagland and Arnon, 1938. Plants were maintained in a glasshouse at ambient temperature of (30-35$^0$ C) and irradiance of 800-1000µmol/m$^2$/s. Ten weeks old plants in which fresh roots had started to develop were harvested.

### 2.2. $^{14}CO^2$feeding, its distribution into metabolic fractions and primary metabolic pool in leaves and roots.

For $^{14}CO_2$ feeding studies plants were placed in a sealed Plexiglas chamber (20L capacity) around a central vial containing $Na_2{}^{14}CO_3$ solution (1.85 MBq; 1.7 TBq/mol) obtained from the Isotope Division of Bhabha Atomic Research Center, Mumbai, India. $^{14}CO_2$ in the chamber (0.37MBq) was liberated by injecting 2M $H_2SO_4$ into the carbonate solution through a PVC inlet tube and uniformly distributed within the chamber using a small electric fan. The plants were allowed to assimilate $^{14}CO_2$ for 12, 24 and 36h. After the end of respective time period of feeding, saturated solution of KOH was run into the central vial for 15 min to absorb leftover $^{14}CO_2$. The plants were then removed from chamber and harvested (Dixit and Srivastava, 2000). The leaves and roots were separated and the leaves were immediately fixed in boiling 80% ethanol to maintain metabolic status. Harvested roots were divided in to two portions 1- immediately fixed into boiling 80% ethanol for determination of primary metabolites, 2- to determine $^{14}C$ content in total saponins.

A known weight of fixed material (leaves and roots) was ground in ethanol, filtered, filtrates evaporated and diluted in a known volume of distill water, this aqueous fraction termed as Ethanol Soluble fraction (ES). The unfiltered ground tissue further hydrolyzed by enzyme diastase in 0.05M 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 Chloroform Soluble fraction termed as (CS), which contained pigments (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. Wt. of leaf/root. The ES fraction was further separated into metabolites by column chromatography by passing through Amberlite ion exchange column chromatography and separated 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 liq-

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uid scintillation counter (Wallac 1409, USA) using Bray's scintillation fluid. (Srivastava et al., 2004, Dixit and Srivastava., 2000, Srivastava and Luthra., 1994).

**2.3. Determination of total saponins**

A known mass (10g) of chlorophytum root powder was suspended in 50ml of 85% ethanol and left overnight (3 times). Ethanol was evaporated and soft portion was extracted with 50ml petroleum ether and solvent discarded. The soft portion was further extracted with 50ml of ethyl acetate followed by 50ml of chloroform for 30min each and solvent discarded from separating funnel. The soft portion was dissolved in 50ml of methanol, filtered and concentrated. To this concentrated extract was added drop by drop 50ml of acetone. A white precipitate was formed dried in oven at 60°C and collected as brown powder (Bourdia et al. 1995). $^{14}C$ counts in these total saponins extract were determined by a liquid scintillation counter (Wallac 1409, USA) in a PPO-POPOP-Toluene cocktail. The unit of expression was Bq/g dry wt of root.

**2.4. Statistical analysis**

The results presented are the mean values of three separate extractions and were subjected to LSD analysis.

**3. Results and discussion**

Plants were harvested at a stage when fresh roots had started to develop. This is the time when maximum photo assimilate are needed for root growth. During leaf development the contribution of photo assimilates depends on metabolic demand. With increase in time the total $^{14}C$ content in leaves increased from 12h (7138Bq/gfwt) to 24h (20976Bq/gfwt) with decline at 36h (20079Bq/gfwt). Decline at 36h may perhaps be due to factors such as due to partial closure of stomata, changes on $CO_2$ concentration due to respiratory release or photorespiration as the plants are enclosed in the chamber for long time. When the total content was analyzed into major metabolic fractions the ES and EIS fraction had highest content (29024Bq/gfwt) and (1419Bq/gfwt) respectively. However the $^{14}C$ content in CS was highest at 36h (1056Bq/gfwt). The ES fraction was further analyzed by column chromatography to determine the label in metabolic pool of sugars, amino acids and organic acids. With increase in time $^{14}C$ content in sugars (2074Bq/gfwt), amino acids (97Bq/gfwt) and organic acids (201Bq/gfwt) were highest at 24h feeding. At 12h and 36h the $^{14}C$ content in these metabolites were lower (Table 1).

**Table 1**

Assimilate distribution of $^{14}C$ (Bq/gfwt) in to various metabolic fractions at different time intervals in leaves. T-total (ES+EIS+CS), ES-Ethanol Soluble Fraction, EIS-Ethanol Insoluble Fraction, CS-Chloroform Soluble Fraction, S-Sugars, AA-Amino Acids and OA-Organic Acids.

| Plant part | Time after feeding | T | ES | EIS | CS | S | AA | OA |
|---|---|---|---|---|---|---|---|---|
| Leaves | 12hr | 7138.0 | 6449.0 | 237.0 | 451.0 | 469.0 | 37.0 | 73.0 |
| | 24hr | 20976.0 | 29024.0 | 1419.0 | 849.0 | 2074.0 | 97.0 | 201.0 |
| | 36hr | 20079.0 | 17929.0 | 1102.0 | 1056.0 | 1072. | 52.0 | 91. |
| SEM | | 5968.0 | 13.0 | 1.0 | 6.4 | 6.3 | 1.6 | 2.6 |
| SED | | 8441.0 | 19. | 1.3 | 9.1 | 8.9 | 2.3 | 3.6 |
| LSD 5% | | 23435.0 | 52. | 3.8 | 25.0 | 24.8 | 6.4 | 10.2 |

Photo assimilated metabolites translocated from leaves to roots were also determined. With increase in time the total $^{14}C$ content in roots increased from 12 (477Bq/gfwt) to 36h (1181Bq/gfwt). In major metabolic fractions as ES (904Bq/gfwt), EIS (101Bq/gfwt) and CS (177Bq/gfwt) highest content were observed at 36h of feeding. The ES fraction was further analyzed by column chromatography to determine the label in metabolic pool of sugars, amino acids and organic acids. With increase in time the flow and $^{14}C$ content in sugars (29Bq/gfwt) and amino acids (27Bq/gfwt) increased up to 36h of feeding. However, $^{14}C$ content in organic acids was higher initially at (123Bq/gfwt) and then decline. Thus the flows from leaves to roots of sugars and amino acids increase with time while in organic acids were higher initially (Table 2).The $^{14}C$ in total saponins continue to increase from 12h (210Bq/gfwt) to 36h (514Bq/gfwt). This indicated that assimilate transported from leaves are biosynthetically utilized for production of total saponins (Table 2).

**Table 2**

Assimilate distribution of $^{14}C$ (Bq/gfwt) in to various metabolic fractions at different time intervals in roots. T-total (ES+EIS+CS), ES-Ethanol Soluble Fraction, EIS-Ethanol Insoluble Fraction, CS-Chloroform Soluble Fraction, S-Sugars, AA-Amino Acids and OA-Organic Acids, SP-Total saponins (Bq/gdrywt)

| Plant part | Time after feeding | T | ES | EIS | CS | S | AA | OA | SP |
|---|---|---|---|---|---|---|---|---|---|
| Roots | 12hr | 477.0 | 423.0 | 27.0 | 27.0 | 27.0 | 20.0 | 123.0 | 210.0 |
| | 24hr | 720.0 | 623.0 | 55.0 | 42.0 | 41.0 | 20.0 | 25.0 | 308.0 |
| | 36hr | 1181.0 | 904.0 | 101.0 | 177.0 | 53.0 | 29.0 | 27.0 | 514.0 |
| SEM | | 8.4 | 7.6 | 0.2 | 2.0 | 0.6 | 0.2 | 2.1 | 3.7 |
| SED | | 11.9 | 10.7 | 0.3 | 2.9 | 0.8 | 0.2 | 3.0 | 5.3 |
| LSD 5% | | 33.0 | 30.0 | 0.9 | 8.0 | 2.4 | 0.6 | 8.4 | 14.8 |

C-metabolism is the centre stage metabolic pathway that provides intermediary building blocks to cellular metabolism including secondary metabolic pathway. Such studies pertaining to relationship between primary carbon metabolism and secondary metabolite accumulation are very few in medicinal plants. In the first report of this kind a relationship has been explored in this important medicinal herb Chlorophytum. Carbon metabolic activity of each leaf changes during the course of its ontogeny. Differences in the utilization pattern of C-assimilates into secondary metabolites have been observed in many plants of secondary metabolite importance. In developing peppermint leaves the incorporation of $^{14}CO_2$ into sugars was maximal followed by organic acids, amino acids and essential oil at all stages of leaf development. The incorporation into sugars and amino acids declined as the leaf matured whereas that in essential oil and organic acids increased with leaf expansion and then decreased (Srivastava and Luthra, 1991a). In different species of mint, $^{14}CO_2$ incorporation increased from 1-6 of feeding. Most of the $^{14}C$-label appeared in metabolites sugars and amino acids. At the same time there were also significant changes with time in $^{14}C$ label in to essential oil (Srivastava and Luthra, 1661b). The present study does not quantify the contribution of individual leaf or clusters for its own growth and development and trsportation towards roots and total saponins biosynthesis/production and accumulation. Whereas lower leaves which are physiologically mature may require less for photo- stasis and transport more assimilate towards the root. In onion with increasing leaf growth lower leaves (leaf 7) exported 93% of fixed carbon towards the growing bulb. Roofs, however, were a weak sink and their $^{14}C$ supply was from basal leaves Khan, 1961). In turmeric (Curcuma longa, spice bearing plant), the youngest leaves were most active in fixing $^{14}CO_2$. Roots and rhizome received highest floe of $^{14}C$ from leaves after 24 hours after of feeding which later declined with time (Dixit and Srivastava, 2000).

This is in contrast to essential oil accumulation were assimilate production and oil accumulation occurs in the leaves (in either peltate or glandular oil glands). In Samantha rose 60% of $^{14}C$ fixed by youngest leaves was localized in sugar component in petals that accumulate in essential oil. (Jiao.et al., 1989).This pattern of essential oil accumulation being highest in younger leaves are also found in other essential oil bearing plants such as Ociminm (Dey and Choudhry, 1983, Cymbopogan (Singh and Luthra, 1988).

**4. Conclusions**

This study demonstrates for the first time feeding of $^{14}CO_2$ and analyzing the profile of primary metabolites giving a real time picture of mobility of metabolites from leaves to roots and its biosynthetic utilization for total saponins production. The flow of metabolites towards roots continues to increase up to 36h of feeding. At the same time $^{14}C$- content in total saponins also continue to increase up to 36h. Which of these three primary metabolites are preferentially utilized cannot be specified. Incidentally however, sugars and amino acids continue to increase up to 36h. It is imperative to understand that a continuous supply of metabolic building blocks coupled with the utilization of assimilates by efficient biosynthetic pathway together generate best results.

$^{14}CO_2$ feeding reflects photo-synthetic efficiency which can be also measured by carbon-di-oxide exchange rate (CER).Hence plants having higher CER will be higher producers of total Saponins. Thus this could be a criterion for future selection of crops.

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