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Partitioning of ^{14}C-photosynthate of leaves in roots, rhizome, and in essential oil and curcumin in turmeric (*Curcuma longa* L.)

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Abstract

Incorporation of photosynthetically fixed ^{14}C was studied at different time intervals of 12, 24, and 36 h in various plant parts—leaf 1 to 4 from apex, roots, and rhizome—into primary metabolites—sugars, amino acids, and organic acids, and secondary metabolites—essential oil and curcumin—in turmeric. The youngest leaves were most active in fixing ^{14}C at 24 h. Fixation capacity into primary metabolites decreased with leaf position and time. The primary metabolite levels in leaves were maximal in sugars and organic acids and lowest in amino acids. Roots as well as rhizome received maximum photoassimilate from leaves at 24 h; this declined with time. The maximum metabolite concentrations in the roots and rhizome were high in sugars and organic acids and least in amino acids. ^{14}C incorporation into oil in leaf and into curcumin in rhizome was maximal at 24 h and declined with time. These studies highlight importance of time-dependent translocation of ^{14}C-primary metabolites from leaves to roots and rhizome and their subsequent biosynthesis into secondary metabolite, curcumin, in rhizome. This might be one of factors regulating the secondary metabolite accumulation and rhizome development.

Additional key words: amino acids; $^{14}CO_2$ incorporation; leaf age; organic acids; primary and secondary metabolites; rhizome; root; stem; sugars.

Introduction

Turmeric (*Curcuma longa* L. syn. *Curcuma domestica* Valen) is cultivated extensively for its underground rhizome that is widely used as condiment, dye stuff, drug, cosmetic, flavour, in food industry, and in religious and auspicious occasions (Govindarajan 1980). The major constituents are essential oil and curcumin that are present in leaves and rhizome, respectively. The active principles or precursors are synthesised in the leaves, translocated, biosynthesised, and stored in rhizome. Growth of leaves and development of rhizome depend on several factors such as nutrition (Rethinam *et al.* 1994), cultivation practices (Randhawa and Mahey 1988), and genotype (Rao and Rao 1994). Despite economic importance of the plant very little is known about the inter-relationship between carbon metabolism and/or precursor-product relationship of curcumin accumulation. A large proportion of leaf photosynthate are required for root and rhizome growth and development which in annual crops such as turmeric could be about 30 % of total leaf photosynthate accumulated (Marschner 1986). Simultaneously biosynthesis and accumulation of a secondary product, curcumin, also occurs. During the growing season carbon fixed by the leaf is transported to the new growing rhizome. Thus the rate and amount of photosynthate produced by the leaves and the proportion of photosynthate which is translocated greatly influence size, yield, development, and growth of rhizome as well as secondary metabolite accumulation. Thus transport and partitioning of leaf assimilate to the sink rhizome is one of the important factors controlling productivity. Since the precursors of curcumin are produced in leaves, the inherent photosynthetic capacity could be an additional controlling factor. No information is available about the rate of assimilate partitioning towards oil biosynthesis and curcumin accumulation. In the present paper we report time-dependent ^{14}C-photosynthate assimilation by leaves and the partitioning of ^{14}C-photosynthate in various plant parts, *i.e.*, between different leaves, roots, and rhizome, in relation to curcumin and essential oil accumulation.

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Materials and methods

Plants: Uniform mother rhizomes of turmeric (*Curcuma longa* L.) were planted in ceramic pots (10 000 cm^3 capacity) filled with silica sand previously cleaned by hot acid digestion (Agarwala and Sharma 1961). The plants received complete Hoagland solution (Hoagland and Arnon 1938). These plants after 2-3 weeks were transplanted and grown in 5 000 cm^3 amber coloured glass pots in solution culture and were maintained in a glasshouse at an ambient temperature (30-35 °C) and under irradiance of 800-1000 µmol m^{-2} s^{-1}. The leaves were numbered from top to the base of the shoot, with the uppermost leaf representing the youngest unexpanded leaf. There is a pseudostem in turmeric. Six-months-old turmeric plants in which fresh rhizomes had just begun to appear were harvested.

Isolation of essential oil: The essential oil from a known mass of ^{14}C-fed leaves was isolated by steam distillation technique using the mini-apparatus of Clevenger (1928). The isolated essential oil was extracted by diethyl ether. Direct extraction by organic solvent or steam distillation yielded the same results.

Determination of curcumin in rhizome: A known mass of fresh rhizome was ground in ethanol and further diluted in ethanol. A standard curve was prepared at different concentrations from pure curcumin and absorbance was recorded on a *Spectronic 21D* (*Milton Roy & Co*, USA) spectrophotometer at peak absorption of 425 nm (American Spice Trade Association 1968, Prasad and Suresh 1997).

Tracer studies: Plants were harvested in the morning about 5 h after the beginning of light period. For $^{14}CO_2$ incorporation studies pots containing plants having four leaves with attached roots and rhizomes were placed in a sealed plexiglass chamber (20 000 cm^3 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, Trombay, India. $^{14}CO_2$ was generated by injecting 2 M H_2SO_4 into carbonate solution through a PVC tube and uniformly distributed throughout the chamber with the help of small electric fan. The plants were allowed to assimilate $^{14}CO_2$ for 12, 24, and 36 h under an irradiance of 800-1000 µmol m^{-2} s^{-1} and photoperiod of 11/13 h. At the end of respective time period, saturated solution of KOH was run into the central vial and left for 15 min to absorb excess $^{14}CO_2$. The plants were removed from the chamber and harvested. The leaves were removed along with the pseudostem, rhizomes were separated from the roots and processed as quickly as possible. Different time periods were used to investigate the rate of assimilate transport and partitioning and its subsequent utilisation in secondary metabolite accumulation into rhizome. For determining ^{14}C incorporation in oil, a known mass of leaves was subjected to micro-scale steam distillation as explained above. The volatile oil was recovered by ether extraction. The radioactivity in ether aliquot was determined in a scintillation counter (*Wallac 1409*) using PPO-POPOP-toluene cocktail (Srivastava and Luthra 1991a).

Freshly developed rhizome and roots were separated from leaves to determine primary metabolite concentrations at 12, 24, and 36 h in various plant parts. A known mass of tracer-fed leaves, rhizome, and root were extracted in boiling 80 % ethanol. The ethanol-soluble material was separated into neutral (sugars), basic (amino acids), and acidic (organic acids) fractions by *Amberlite* ion exchange columns (Srivastava and Luthra 1994). Ethanol-insoluble material was hydrolysed by diastase in 0.05 M acetate buffer (pH 5.2) at 50 °C. Total ^{14}C assimilated was calculated as the sum of label incorporated in ethanol-soluble and ethanol-insoluble fractions and expressed on fresh mass basis. The radioactivity in hydrolysed alcohol insoluble material and in eluates after ion exchange separation was measured using Bray's scintillation fluid in a liquid scintillation counter (*Wallac 1409*) (Srivastava and Luthra 1994). For determining ^{14}C incorporation in curcumin, a known mass of rhizome was ground and extracted in ethanol. The radioactivity in curcumin (alcohol fraction) was measured using Brays scintillation fluid.

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

Results and discussion

Position of the leaf from apex to base of shoot indicates a change in leaf physiological capacity. Plants were harvested when fresh rhizomes had just started to develop. This is the time when maximum photosynthates are needed for rhizome development. During leaf development the contribution of photosynthetic assimilates to biosynthetic system, viz. secondary metabolites, depends on its physiological status. After 12 h of feeding, plant leaves had highest fixation [53.4 MBq kg^{-1}(F.M.)] followed by developing rhizome [3.1 MBq kg^{-1}(F.M.)] and roots [2.8 MBq kg^{-1}(F.M.)] (Table 1). The 1st leaf fixed maximum ^{14}C [19.1 MBq kg^{-1}(F.M.)], which declined with leaf age, and in the 4th leaf was significantly lowest [7.2 MBq kg^{-1}(F.M.)]. When analy-

Table 1. Partitioning of ^{14}C [MBq kg^{-1}(F.M.)] into various metabolic fractions at different time intervals in leaves (1+2+3+4), roots, and rhizome in turmeric. T - total incorporation, ES – ethanol-soluble, EIS – ethanol-insoluble, S - sugars, AA - amino acids, OA - organic acids.

Plant part	Time after feeding [h]	Fractions T	ES	EIS	S	AA	OA	CD 5 %	1 %
Leaf	12	53.44	39.72	13.71	27.77	1.36	5.39	5.31×10^{-6}	7.63×10^{-6}
	24	63.88	51.24	12.63	31.91	2.03	4.12	4.16×10^{-6}	5.97×10^{-6}
	36	37.12	27.42	13.88	18.09	1.44	6.55	4.93×10^{-5}	6.90×10^{-5}
Root	12	2.86	2.35	0.50	1.50	0.09	0.41	6.64×10^{-6}	9.29×10^{-6}
	24	3.03	2.66	0.37	0.83	0 .07	0.24	2.98×10^{-6}	4.31×10^{-6}
	36	1.26	1.01	0 .24	0.66	0.08	0.19	1.99×10^{-6}	2.82×10^{-6}
Rhizome	12	3.11	2.71	0.40	0.69	0.02	0.11	7.63×10^{-6}	1.06×10^{-5}
	24	4.61	3.73	0.87	1.19	0.06	0.20	1.32×10^{-5}	1.87×10^{-5}
	36	1.46	1.07	0.38	0.75	0.04	0.19	3.32×10^{-6}	4.64×10^{-6}

sed in fractions, the ethanol-soluble fraction had higher incorporation than ethanol insoluble fraction (Table 2). The sugars had highest ^{14}C incorporation followed by organic acids, lowest was in amino acids in leaves of all positions (Table 3). The 1st leaf had maximum incorporation in sugars that progressively decreased as the leaf matured (Table 3). The patterns of incorporation in amino acids and organic acids were similar (Table 3). The rhizome had higher content of the total ^{14}C [3.1 MBq kg^{-1}(F.M.)] fixed than root [2.8 MBq kg^{-1}(F.M.)] (Table 1). The pattern of incorporation in metabolites was highest in sugars, followed by organic acids, and least in amino acids both in rhizome and roots, with roots having higher incorporation than rhizome (Table 1).

Table 2. Partitioning of ^{14}C into total, ethanol-soluble and ethanol-insoluble fractions [MBq kg^{-1}(F.M.)] in leaves of turmeric at different time intervals.

Fraction	Time after feeding [h]	Leaf position from apex 1	2	3	4	CD 5 %	1 %
Total incorporation	12	19.07	15.85	11.13	7.20	0.014	0.023
	24	22.94	18.55	16.05	6.49	0.018	0.028
	36	13.06	12.16	9.16	6.40	0.023	0.034
Ethanol-soluble fraction	12	13.91	12.35	8.06	5.31	0.006	0.011
	24	18.80	14.69	12.74	5.04	0.099	0.149
	36	8.43	7.91	6.54	3.98	0.016	0.023
Ethanol-insoluble fraction	12	4.11	3.86	3.30	1.42	0.009	0.014
	24	5.17	3.50	3.03	1.87	0.006	0.009
	36	4.61	4.24	2.62	2.42	0.008	0.016

After 12 h of feeding leaves contained 0.009 MBq kg^{-1}(F.M.) in essential oil and 0.013 MBq kg^{-1}(F.M. rhizome) in curcumin (Table 4). At 24 h after feeding, the total incorporation in leaves increased and the trend of total incorporation in leaves, roots, and rhizome was similar as at 12 h (Table 1). With respect to leaf position the youngest leaf had significantly higher ^{14}C incorporation than leaves 2, 3, and 4, however, these values were higher at 24 h than at 12 h (Table 2). The incorporation into ethanol-soluble fraction was the highest at all leaf positions (Table 2). As concerns metabolites, the first leaf had always peak incorporation in sugars, organic acids, and amino acids and it progressively declined in leaves 2, 3, and 4 (Table 3). Rhizome had always higher ^{14}C incorporation [4.6 MBq kg^{-1}(F.M. rhizome)] than root [3.0 MBq kg^{-1}(F.M. root)] (Table 1). Simultaneously, rhizome had higher incorporation in ethanol-soluble fraction than root (Table 1). The pattern of ^{14}C incorporation in metabolites was highest in sugars followed by organic acids, and least in amino acids both in rhizome and roots (Table 1) with rhizome having higher contents than roots. Partitioning of assimilated ^{14}C towards essential oil [0.012 MBq kg^{-1}(F.M. leaf)] and curcumin [0.016 MBq kg^{-1}(F.M. rhizome)] was maximum at 24 h (Table 4).

When analysed after 36 h of feeding, the total incorporation in leaves, roots, and rhizome was lower than that at 12 or 24 h of feeding (Table 1). In leaves the incorporation in ethanol-soluble fraction declined without significant effect in insoluble fraction (Table 1). Label content in sugars and amino acids declined but increased in organic acids (Table 1). Label content in sugars and

Table 3. Distribution of ^{14}C into sugars, amino acids and organic acids [MBq kg^{-1}(F.M.)] in leaves of turmeric at different time intervals.

Fraction	Time after feeding [h]	Leaf position from apex 1	2	3	4	CD 5 %	1 %
Sugars	12	9.26	8.01	6.17	3.54	0.009	0.012
	24	12.42	8.50	7.81	3.78	0.011	0.016
	36	5.05	5.12	4.70	3.27	0.009	0.013
Amino acids	12	0.47	0.35	0.31	0.23	0.005	0.008
	24	0.82	0.58	0.40	0.22	0.005	0.007
	36	0.56	0.36	0.28	0.23	0.004	0.006
Organic acids	12	1.29	1.06	1.12	0.07	0.006	0.009
	24	1.76	1.66	1.40	0.07	0.004	0.006
	36	1.96	1.88	1.53	1.23	0.038	0.005

amino acids declined but increased in organic acids (Table 1). With respect to leaf position, leaf 1 had significantly higher total incorporation than leaves 2, 3, and 4 although their values were less than the total incorporation at 24 h (Table 2). Incorporation in ethanol-soluble fraction declined at 36 h at all leaf positions whereas there was little variation in ethanol-insoluble fraction (Table 2). The first leaf had highest incorporation in sugars, organic acids, and amino acids that progressively declined with leaf age in leaves 2, 3, and 4. The organic acid fraction had maximum incorporation at 36 h at all leaf positions as compared to the incorporation at 12 or 24 h (Table 3). Total incor-poration at 36 h in root and rhizome was lower than at 24 h, and the rhizome had higher incorporation than the root (Table 1). The pattern of incorporation in metabolites was highest in sugars followed by organic acids and least in amino acids both in rhizome and roots (Table 1). Here again rhizome had higher values than root. With respect to secondary metabolites, incorporation in essential oil was 0.004 MBq kg^{-1}(F.M. leaf) and in curcumin 0.012 MBq kg^{-1}(F.M. leaf) at 36 h which was lower than incorporation at 24 h (Table 4).

Table 4. Distribution of assimilated $^{14}CO_2$ into essential oil [MBq kg^{-1}(F.M. leaf)] and curcumin [MBq kg^{-1}(F.M. rhizome)] at different time intervals in turmeric.

Secondary metabolite	Time [h] after feeding 12	24	36	CD 5 %	1 %
Essential oil	0.009	0.012	0.004	9.620×10^{-4}	1.470×10^{-3}
Curcumin	0.013	0.016	0.012	0.034×10^{-5}	0.005×10^{-4}

With regard to percent distribution of assimilated ^{14}C among leaves, roots, and rhizome at different time intervals maximum percentage was incorporated in leaves followed by rhizome and least in roots (Table 5). 12 h after feeding 90 % of assimilated ^{14}C was incorporated in leaves, 4.8 % in root, and 5.2 % in rhizome (Table 5). After 24 h of feeding, 89 % of the total ^{14}C incorporated by the plant was fixed by leaves, 4.2 % by roots, and 6.4 % by rhizome (Table 5). Leaves assimilated 93 %, roots 3.1 %, and rhizome 3.7 % after 36 h of feeding (Table 5).

Physiological studies including factors regulating curcumin accumulation, biosynthetic studies including precursor-product relationship, or photosynthate partitioning studies in spice crops are very rare. Incorporation of specific compounds such as ^{14}C-phenylalanine into capsaicin in *Capsicum* (Bennett and Kirhy 1968) and gingerol in ginger (Denniff and Whiting 1976) have been reported. In unusually long 6-d feeding trial, precursors ^{14}C-phenylalanine, ^{14}C-malonate, and ^{14}C-acetate were incorporated in different structural components of curcumin in 4- to 5-month-old plants (Roughly and Whiting 1971, 1973).

Table 5. Percent distribution of ^{14}C assimilated [MBq kg^{-1} (F.M.)] into different plant parts at different time intervals in turmeric.

Time [h] after feeding	Total ^{14}C assimilated	% of total leaf	root	rhizome
12	59.41	90	4.8	5.2
24	71.52	89	4.2	6.4
36	39.84	93	3.1	3.7

The $^{14}CO_2$ incorporation studies highlight the important relationship between primary and secondary metabolism in essential oil and curcumin. Metabolic

activity of each leaf changes in the course of its ontogenesis. The youngest turmeric leaves were most active in $^{14}CO_2$ assimilation and this capacity declined with leaf position and time. The maximum translocation towards secondary metabolite accumulation, *i.e.*, curcumin and essential oil, occurred at 24 h after feeding. Thus there is a continuous flow of photosynthates towards roots and rhizomes. Differences in the incorporation of $^{14}CO_2$ into secondary metabolites were observed in several plants of secondary metabolite importance. In different species of mints, $^{14}CO_2$ incorporation increased from 1 to 6 h and most of the label appeared in sugars and organic acids; there were also significant changes with time in $^{14}CO_2$ incorporation in oil (Srivastava and Luthra 1991b). 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). The present study does not quantify the contribution of individual leaf photosynthate towards secondary metabolite contribution. But it is possible that young leaves, though having higher fixation capacity, may retain greater proportion of assimilate for its own growth and developmental process and contribute less towards secondary metabolism. Whereas lower leaves, which are physiologically mature, fix less and may transport more assimilate towards rhizome and root. In onion with increasing growth period, lower leaves (leaf 7) exported 93 % of the fixed carbon towards the growing bulbs. Roots, however, were a weak sink and received their ^{14}C supply only from basal leaves (Khan 1981). The curcumin accumulation occurs in rhizome and assimilate from leaf has to be transported through pseudostem to roots and then to developing rhizome. This rate of flow is thus an important regulatory factor controlling both the growth of rhizome and accumulation of curcumin. This is in contrast to essential oil accumulation, where assimilate production and oil accumulation occur in leaves. In rose 60 % of ^{14}C fixed was localised in sugar fraction in petals that accumulate essential oil (Jiao *et al.* 1989). The $^{14}CO_2$ incorporation into leaf and essential oil are similar to essential oil accumulation in other aromatic plants such as *Majorana* (Croteau 1977), *Ocimum* (Dey and Choudhury 1983), and *Cymbopogon* (Singh and Luthra 1988) where oil accumulates in leaves. With proceeding time (at 36 h) there was a decline in the leaf ^{14}C assimilation. This could be due to factors such as closure of stomata, changes in CO_2 concentration in chamber due to respiratory release, or photorespiration because the plants were enclosed in the chamber for a long time.

In conclusion, the youngest leaves in turmeric are most active in fixing $^{14}CO_2$ at different time intervals, and this capacity decreases with leaf position and time. The primary metabolite concentrations are maximum in sugars and organic acids and least in amino acids. Organic acid content increased with time in all leaves whereas sugar and amino acid concentrations increased up to 24 h and then declined. Roots and rhizome received maximum photoassimilate during 24 h from leaves and this declined with time. Rhizomes received more photoassimilate than roots. The primary metabolite concentrations were high in sugars and organic acids and low in amino acids. Maximum photoassimilates transported from leaves were incorporated in secondary metabolites, in oil and curcumin, at 24 h and then the icorporation declined with time.

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