

Partitioning of photosynthetically fixed $^{14}\text{CO}_2$ into oil and curcumin accumulation in *Curcuma longa* grown under iron deficiency

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Abstract

Changes in leaf growth, photosynthetic efficiency, and incorporation pattern of photosynthetically fixed $^{14}\text{CO}_2$ in leaves 1 and 2 from plant apex, in roots, and rhizome induced in *Curcuma* by growing in a solution culture at Fe concentration of 0 and 5.6 g m^{-3} were studied. ^{14}C was incorporated into primary metabolites (sugars, amino acids, and organic acids) and secondary metabolites (essential oil and curcumin). Fe deficiency resulted in a decrease in leaf area, its fresh and dry mass, chlorophyll (Chl) content, and CO_2 exchange rate at all leaf positions. The rate of $^{14}\text{CO}_2$ fixation declined with leaf position, maximum being in the youngest leaf. Fe deficiency resulted in higher accumulation of sugars, amino acids, and organic acids in leaves at both positions. This is due to poor translocation of metabolites. Roots and rhizomes of Fe-deficient plants had lower concentrations of total photosynthate, sugars, and amino acids whereas organic acid concentration was higher in rhizomes. $^{14}\text{CO}_2$ incorporation in essential oil was lower in the youngest leaf, as well as incorporation in curcumin content in rhizome. Fe deficiency influenced leaf area, its fresh and dry masses, CO_2 exchange rate, and oil and curcumin accumulation by affecting translocation of assimilated photosynthates.

Additional key words: amino acids; Fe; leaf position; organic acids; primary and secondary metabolites; sugars; turmeric.

Introduction

Iron is an important micronutrient involved in various photosynthetic processes, namely chlorophyll (Chl) biosynthesis (Morales *et al.* 1990), photosynthetic electron transport system (Terry 1980), formation of chloroplast ultrastructure (Nishio and Terry 1983), photosynthetic enzyme activity and carotenoid content (Abadia *et al.* 1991). Structure and function of the whole photosynthetic apparatus is affected by iron deficiency (Marschner 1986, Abadia 1992).

Turmeric (*Curcuma longa* L. syn. *C. domestica* Valen.) is cultivated for its underground rhizome which is widely used as condiment, dye stuff, cosmetic, flavour, in food industry, and for religious and auspicious occasions (Govindarajan 1980). The active substances are synthesised in leaves, and translocated and present in leaf and rhizome. The major constituents are essential oil, curcumin, and oleoresins stored in rhizome. Growth and development of leaves and rhizome are dependent on several factors such as cultivation practices (Randhawa and Mahey 1988) or genotype (Rao and Rao 1994). Despite the economic importance of plant, little is known

about micronutrient influence on growth and physiology. Growth of turmeric plant is influenced by deficiencies of Fe and Zn (Rethinam *et al.* 1994). A large proportion of photosynthates accumulated by plants are required for root growth and metabolism which in annual crops such as turmeric could be 30 % of total photosynthate accumulated (Marschner 1986). The rhizome development and subsequent accumulation of curcumin depend on the translocation of photosynthates from the leaf blades. During the growing season, carbon fixed by the leaf is transported to the growing rhizome for storage. The amount of photosynthate produced by the leaves and the proportion translocated to the rhizome greatly influence size and yield of rhizome and also accumulation of curcumin and oleoresins. Hence photosynthate partitioning to the sink-rhizome may control productivity. Since curcumin is biosynthesised in leaves, the inherent photosynthetic capacity could be additional controlling factor. Thus under Fe deficiency there might be alteration in the availability and the content of translocated photosynthate which might be

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Abbreviations: Chl – chlorophyll; P_N – net photosynthetic rate; PPO-2,5 - diphenyl-oxazole; POPOP - 1,4-di-2(5-phenyl oxazolyl) benzene.

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utilised in curcumin accumulation and rhizome development.

In the present paper we report on distribution of photosynthetically fixed $^{14}\text{CO}_2$ in leaf positions 1 and 2 from apex, into essential oil and curcumin in relation to

Materials and methods

Plants: Uniform mother rhizomes of turmeric (*Curcuma longa* L.) were planted in ceramic pots (10 000 cm³ capacity) filled with silica sand previously cleaned by hot acid digestion in steam for Fe removal (Agarwala and Sharma 1961). The steam digestion process was repeated to remove even traces of Fe impurity until its concentration was extremely low when measured by an atomic absorption spectrophotometer. The salts used in preparation of the nutrient solution were purified against Fe by dithizone solution (Hewitt 1966). Nutrient solution of Hoagland and Arnon (1938) was used except Fe which was supplied as Fe-EDTA (5.6 g m⁻³) for control plants and completely omitted in Fe deficient plants. Six pots each of control and deficient plants were kept inside a glasshouse at ambient temperature of 30-35 °C and under irradiance of 800-1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$. With the onset of deficiency, leaf growth, photosynthesis, and tracer studies were performed.

Leaf growth: Leaf area was measured on automatic leaf area meter (*Li-3000*, *Licor*, Lincoln, USA). Dry matter was determined by oven drying at 80 °C until a constant mass (48 h).

Chl content and CO_2 exchange rate: A known mass of leaf tissue was extracted with 80 % acetone. Chl absorbance was recorded on a *Spectronic 21D* spectrophotometer (*Milton Roy and Co.*, New York, USA) according to Arnon (1949). The net photosynthetic rate (P_N) was measured with the portable photosynthesis system (*Li-6000*, *Licor*, Lincoln, USA) (Srivastava and Luthra 1991a).

Essential oil was isolated by steam distillation technique using the apparatus of Clevenger (1928). The isolated essential oil was extracted by diethyl ether. The direct extraction by organic solvent and the steam distillation process 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 recorded on a *Spectronic 21D* (*Milton Roy & Co.*, USA) spectrophotometer at peak absorption of 430 nm (American Spice Trade Association 1968, Prasad and Suresh 1997).

primary metabolite contents in developing leaves, rhizome, and roots subject to Fe deficiency. Simultaneously changes in CO_2 exchange rate, Chl content, leaf area, and fresh and dry matter accumulation were determined.

$^{14}\text{CO}_2$ incorporation: Pots with plants were placed in a sealed plexiglass chamber (20 000 cm³ capacity) around a central vial containing $\text{Na}_2^{14}\text{CO}_3$ solution (1.85 MBq, 1.78 TBq mol⁻¹) obtained from the isotope division of Bhabha Atomic Research Centre, Trombay, India. $^{14}\text{CO}_2$ was generated by injecting 2 M H_2SO_4 into the carbonate solution through a PVC tube and uniformly distributed using a small electric fan. The optimum time for maximal $^{14}\text{CO}_2$ incorporation was found to be 24 h, beyond which incorporation into oil and curcumin decreases. The plants assimilated $^{14}\text{CO}_2$ for 6 h under an irradiance of 800-1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$. At the end of this period, a saturated solution of KOH was run into the central vial and left for 15 min to absorb excess $^{14}\text{CO}_2$. The chamber was then opened for the remaining incorporation period of 24 h. To determine $^{14}\text{CO}_2$ incorporation in oil, a known mass of leaves was subjected to micro-scale steam distillation. The volatile oil was recovered by ether extraction. The radioactivity in ether aliquots was determined in a scintillation counter (*Wallac 1409*) using PPO-POPOP-toluene cocktail.

Freshly developed rhizome and roots were separated from leaves to determine primary metabolite concentrations in various plant parts. A known mass of tracer-fed leaves, rhizomes, and roots of +Fe and -Fe plants was extracted in boiling 80 % ethanol. The ethanol-soluble material was separated into neutral (sugars) basic (amino acids) and acidic (organic acid) 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 accumulated was calculated as the sum of label incorporated in ethanol-soluble and -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 Brays 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, and radioactivity measured in alcohol fraction using Brays scintillation fluid.

Statistics: The results are means from three separate extractions. Variation in the treatments was statistically analysed by paired *t*-test.

Results and discussion

The leaf position from apex to the base of the shoot represents developmental changes in the tissue age and leaf physiological capacity (Šesták 1985). By the time fresh rhizomes started to appear in -Fe plants, only two upper leaves remained while the lower leaves, due to typical Fe deficiency symptoms such as chlorosis and necrosis at later stage fell down. In both +Fe and -Fe plants, leaf area and fresh and dry matters increased from 1st to 2nd leaf counted from top; in -Fe plants the values were significantly lower than in +Fe plants. In both +Fe and -Fe plants the total Chl content and P_N increased from 1st to 2nd leaf, in -Fe plants the values were significantly lower at both leaf positions (Table 1). Fe deficiency reduces Chl content, Chl *a/b* ratio, and leaf P_N in a variety of plants by reducing leaf photochemical capacity either by preferential photodestruction of Chl *b* or by an increase in Chl *a* containing reaction centres relative to Chl *b* (Terry and Abadia 1986). Thus under Fe deficiency there was an overall decrease in P_N of turmeric plants. This reduction in P_N should be reflected in concentration of internal leaf photosynthates as well as in translocation to rhizomes and roots, and in secondary metabolism pathways.

The total concentration of ^{14}C -photosynthates in

Table 1. Changes in net photosynthetic rate (P_N), leaf parameters, $^{14}\text{CO}_2$ incorporation [$\text{MBq kg}^{-1}(\text{f.m.})$] in ethanol soluble and insoluble fractions, sugars, amino acids, and organic acids in developing leaves of turmeric subjected to Fe deficiency. */**—mean values significant at 5/1 % level of significance by paired *t*-test.

	Fe levels	Leaf position from top 1	2
Leaf area [cm^2 per leaf]	+Fe	102.52	142.21
	-Fe	49.53**	68.26**
Leaf fresh mass [g per leaf]	+Fe	2.37	2.74
	-Fe	1.16*	1.36*
Leaf dry mass [g per leaf]	+Fe	0.40	0.72
	-Fe	0.16*	0.26*
Chl (<i>a+b</i>) [$\text{g kg}^{-1}(\text{f.m.})$]	+Fe	0.61	0.81
	-Fe	0.33**	0.42**
P_N [$\text{mg}(\text{CO}_2) \text{ m}^{-2} \text{ s}^{-1}$]	+Fe	0.11	0.16
	-Fe	0.06**	0.08**
Total ^{14}C incorporation	+Fe	23.04	17.61
	-Fe	56.54**	30.08**
Ethanol-soluble fraction	+Fe	18.76	13.73
	-Fe	36.27**	23.69**
Ethanol-insoluble fraction	+Fe	4.28	3.40
	-Fe	20.27**	6.39**
Sugars + sugar phosphates	+Fe	12.52	5.31
	-Fe	29.58**	21.78*
Amino acids	+Fe	0.81	0.60
	-Fe	26.93**	2.44**
Organic acids	+Fe	1.28	1.08
	-Fe	3.92**	4.81**

youngest leaf was significantly higher than in the 2nd leaf in +Fe plants. In -Fe plants a similar trend was observed, however, the values were significantly higher than in +Fe plants. The ethanol-soluble and -insoluble fractions showed high incorporation in the youngest leaf that declined in the second leaf. However, -Fe plants showed significantly higher incorporation than +Fe plants. Among the primary metabolites, sugars had maximum incorporation followed by organic acids and amino acids at both leaf positions, with the youngest leaf more active than the second leaf. The concentrations of these metabolites in -Fe plants were significantly higher than in +Fe plants (Table 1). Thus the concentration of primary photosynthates in -Fe plants was significantly higher than in +Fe plants. This does not mean higher photosynthetic efficiency but possibly the photosynthates are not translocated to other parts of the plant and remain accumulated within the leaf as translocation is energy consuming process and Fe deficiency results in loss of energy-producing capacity. Further primary and secondary metabolism is the integration of several metabolic pathways that require linking of steps such as continuous production of precursors, their transport and translocation to the active site of synthesis, and finally accumulation. This sequence of steps depends upon normal functioning of associated metabolic pathways as carbon fixation, respiration (providing energy), and its link with secondary metabolic pathways. Any disruption in normal metabolic pathway will affect sequence of steps. Thus a plant may alter/adopt its metabolic pathways in response to a particular deficiency, *e.g.*, under Fe deficiency there might be alteration in the availability, utilisation of precursors, and availability of photosynthate. The fact that under Fe deficiency photosynthate translocation is affected is evident when its content is measured in roots, rhizomes, and in secondary metabolites.

Fresh developing rhizomes of -Fe plants have significantly lower contents of total photosynthates. The content of ethanol-soluble fraction was much lower than that of ethanol-insoluble fraction, however, both were significantly reduced (Table 2). Among the metabolites, sugars and amino acid concentrations were significantly reduced whereas organic acid content was significantly higher in -Fe plants. (Table 2). Except for organic acid, there was lower translocation of photosynthate recently fixed. When analysed in roots, the total incorporation translocated was very little in -Fe plants and the difference was highly significant. Ethanol-soluble and -insoluble fractions in -Fe plants were significantly very minor ones as compared to those in +Fe roots. Among the metabolites, sugar content was significantly lower whereas amino acid content significantly higher in -Fe than +Fe roots; there was no significant change in organic acids translocated to -Fe and +Fe roots (Table 2). Thus

the translocation of primary photosynthates was higher in freshly developing rhizomes than in roots, and significantly very minor in roots under Fe deficiency.

Table 2. Variation in $^{14}\text{CO}_2$ incorporation [$\text{MBq kg}^{-1}(\text{f.m.})$] in ethanol soluble and insoluble fractions, sugars, amino acids, organic acids, in freshly developed rhizome and in roots of turmeric under Fe deficiency. */** - mean values significant at 5/1 % level of significance by paired t-test. ^{NS} - non-significant.

	Fe levels	Rhizome	Roots
Total ^{14}C incorporation	+Fe	4.81	3.05
	-Fe	1.82**	0.32**
Ethanol-soluble fraction	+Fe	3.93	2.68
	-Fe	1.08**	0.20**
Ethanol-insoluble fraction	+Fe	0.88	0.37
	-Fe	0.74**	0.11**
Sugars + sugar phosphates	+Fe	1.19	0.84
	-Fe	1.13**	0.74**
Amino acids	+Fe	0.06	0.07
	-Fe	0.05**	0.31**
Organic acids	+Fe	0.20	0.24
	-Fe	0.44**	0.23 ^{NS}

Among the secondary metabolite accumulation, $^{14}\text{CO}_2$ incorporated in essential oil in the youngest leaf was higher than in the 2nd developing leaf in +Fe plants. However, under Fe deficiency there was a significant decrease in oil accumulation in the youngest leaf, but the 2nd leaf accumulated significantly higher amounts than in +Fe plants. Similarly, the curcumin percentage in Fe-deficient plants was significantly lower. Thus the photosynthate translocation in the formation of curcumin in -Fe plants was reduced as well as the % content of curcumin: both were decreased. The ^{14}C incorporated in curcumin in -Fe plants was significantly lower (Table 3). Thus under Fe deficiency the photosynthetic capacity of leaves is reduced. Fe deficiency results in accumulation

of photosynthates as there is very little translocation of accumulated photosynthates towards roots and rhizomes and also towards secondary metabolites.

Table 3. Influence of Fe deficiency on $^{14}\text{CO}_2$ incorporation [$\text{MBq kg}^{-1}(\text{f.m.})$] in essential oils and percent curcumin content in turmeric. */** - mean values significant at 5/1 % level of significance by paired t-test.

Metabolite	Fe levels	Leaf 1	Leaf 2	Rhizome
^{14}C in essential oil	+Fe	0.00398	0.00265	-
	-Fe	0.00265**	0.00431*	-
Curcumin [%]	+Fe	-	-	0.46
	-Fe	-	-	0.14**

Studies on biosynthesis, precursor-product relationship, or photosynthate partitioning in spice crops are very scarce in the literature. Incorporation of ^{14}C -phenylalanine into capsaicin in *Capsicum* (Bennett and Kirhy 1968) and gingerol in ginger (Denniff and Whiting 1976) has been reported. In a 6-d feeding trial, ^{14}C -phenylalanine, ^{14}C -malonate, and ^{14}C -acetate were incorporated in different structural components of curcumin (Roughly and Whitling 1971, 1973). The present study highlights that metabolites from the photosynthetic pathway are incorporated in curcumin. In many essential oil bearing plants such as mints (Srivastava and Luthra 1991b) or citronella (Singh and Luthra 1988) there is a positive relationship between primary and secondary metabolism that is dependent on the nutritional status of plant (Srivastava and Luthra 1993). The development of rhizome and subsequent accumulation of curcumin depend on the translocation of photosynthate but under Fe deficiency there is reduced translocation to rhizome and roots which results in poor growth and curcumin accumulation in turmeric.

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