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Changes in $^{14}CO_2$ assimilate partitioning into primary photosynthetic metabolites in flowering and non-flowering rose (*Rosa damascena* Mill) plants

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Abstract

Changes in $^{14}CO_2$ assimilation into primary photosynthetic metabolites in developing leaves of flowering and non-flowering plants of rose have been investigated. Total ^{14}C assimilated increased up to 4.5 h and then declined in flowering plants, while in non-flowering plants maximum assimilation was observed at 1.5 h and thereafter it steadily declined. First and second leaf (from apex) in flowering shoot assimilated maximum photosynthate which continued to increase with time, whereas second leaf of non-flowering plants assimilated maximum CO_2 which decreased with time. Maximum photosynthate translocation to flower occurred at 3 h after feeding. A significant portion of photosynthate remained untranslocated in stems of non-flowering plants. Within leaves, maximum metabolite labeled was sugars in flowering shoot at 1.5 h, which declined later, whereas amino acids and organic acids level increased upto 4.5 h. Very little metabolite remained untranslocated in stem. In non-flowering plants, level of these metabolites decreased with time, however high level of metabolites remained in stem. Contrasting and reverse pattern of carbon assimilation was observed between flowering and non-flowering shoots. In non-flowering shoot, sugars and amino acids contents were significantly negatively correlated, whereas a significant positive correlation was observed in flowering shoot. The flowering and non-flowering shoots significantly differed in partitioning of primary photosynthetic metabolites between leaf, stem and flower.

Key words : carbon partitioning, *Rosa damascena*, rose.

Introduction

Rose (*Rosa damascena* Mill), is an important essential oil yielding crop. The rose oil obtained from flowers after hydrodistillation is extensively used in perfumery and cosmetic industry. Flower bud atrophy and irregular flowering are important constraints in rose productivity (Farooqi *et al.* 1994). Among other factors, atrophy of flower buds has been attributed to a reduced translocation of assimilates to the apices in the early stage of shoot development and to the alterations in the distribution of assimilates among the various parts of the plant (Mor *et al.* 1981). Flower development is dependent on several factors like nutrient supply, which affect phytohormone balance and source sink relationship through the transport of photoassimilate. In perennial plants, the ability of flowering depends largely on the mobility pattern of the assimilates in the shoot (Marschner 1986). Thus, one of the possible reasons controlling

flower development could be assimilation capacity and or translocation pattern within the plant (Khayate & Zieslin 1986; Mor & Halevy 1979).

The photosynthetic capacity of individual leaf in rose vary with age (Bozarth *et al.* 1982). In Samantha rose (*Rosa hybrida*), all leaves on the flowering shoot had similar photosynthetic capacity (Jiao *et al.* 1989). The flowering and non-flowering plants differ in carbohydrate content also (Mor & Halevy 1979; Halevy 1974). However, the differences with respect to the concentration of the primary photosynthetic metabolic pool consisting of sugars, amino acids and organic acids between flowering and non-flowering plants are not known.

In rose petals, glandular structures on upper epidermal cells are the sites of oil accumulation. Radiotracer studies have shown that metabolites such as sugars (products of CO_2 assimilation) are best precursors for oil biosynthesis (McGarvey & Croteau 1995; Banthorpe *et al.* 1975) and there is a positive association between photosynthetic capacity and oil accumulation in many essential oil bearing plants (Srivastava 1991). The precursors for the oil biosynthesis are produced in the leaves, whereas oil biosynthesis/accumulation occurs in petals. Rose petals being carbon heterotrophic, have to depend on leaves for biosynthetic precursors. The leaf growth and its photosynthetic capacity thus seem to be the key determinants of flower productivity. Therefore, it is important to understand the differences between the translocation pattern of freshly fixed $^{14}CO_2$ between flowering and non-flowering rose plants at a stage when flower buds start to develop. The objective of the present study was to find out the contribution of photoassimilates in shoots at different time intervals towards flower growth in flowering and non-flowering rose plants.

Materials and methods

Rose (*Rosa damascena* Mill) plants (more than 4 years old) maintained in CIMAP field were used for this study. Shoots from flowering and non-flowering plants were chosen when the flower buds started to appear on flowering plant.

For $^{14}CO_2$ incorporation studies, shoot terminals (six each with and without flower) having three compound leaves were cut under water and placed in vials with cut ends dipped in half strength Hoagland solution (Hoagland & Arnon 1938). These vials were then kept in a sealed plexiglass assimilation chamber (20 l) around a central vial containing $Na_2{}^{14}CO_3$ solution (1.85 MBq, specific activity 1.78 MBq mol^{-1}) obtained from the Isotope Division, Bhabha Atomic Research Centre, Trombay, India. $^{14}CO_2$ was generated by injecting 4 N H_2SO_4 into carbonate solution through a PVC tube and liberated $^{14}CO_2$ was uniformly distributed with the help of a small electric fan. The cuttings were allowed to assimilate $^{14}CO_2$ for one hour in $^{14}CO_2$ evolved within the assimilation chamber which was placed in sunlight (800-1000 µmol m^{-2} 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 $^{14}CO_2$ within the chamber. The chamber was opened and the shoots were harvested at different time intervals (1.5, 3.0, 4.5, 6.0 and 24.0 h from the start of $^{14}CO_2$ feeding). First, second and third leaf (from apex), stem portion between 1 to 3 leaves and flowers (in flowering shoot) were seperated.

For determining $^{14}CO_2$ incorporation into primary metabolites at different time intervals, a known weight of $^{14}CO_2$ fed sample (leaf, stem or flower) was extracted in boiling 80% ethanol. The ethanol soluble (ES) material was hydrolyzed by amyloglucosidase (Sigma) in 0.05 M acetate buffer (pH 4.5) at 50°C. The radioactivity in hydrolyzed alcohol insoluble material and in eluates after ion-exchange separation was measured by using Bray's scintillation fluid in a liquid scintillation counter (LKB Rack beta 1215). Total ^{14}C incorporated was calculated as sum of the label (DPG g^{-1} FW) incorporated in ethanol soluble fraction and expressed on fresh weight basis.

The results presented are the mean values from

three separate determinations and were statistically analysed by least significant difference test. Correlation coefficients were also calculated between sugars and amino acids and sugars and organic acids based on ^{14}C label incorporated in flowering and non-flowering shoots.

Results and discussion

The radioactive ^{14}C was fed when the floral buds just started to appear, because at this time assimilate portioning is most rapid and is one of the important factors determining flower biomass development and/or oil accumulation. The leaves of the flowering and the non-flowering shoots of *R. damascena* differed in their photosynthetic capacity and ^{14}C levels in primary photosynthetic metabolites namely, sugars, amino acids and organic acids. Initially (1.5 h) the non-flowering shoots had high incorporation level which later decreased (3 to 6 h). Thus, non-flowering shoots had higher photoassimilate accumulation than flowering shoot. The lower content of assimilated metabolites initially in flowering shoot indicated the possibility that the young developing flower bud is a strong sink. In non-flowering shoots, the higher assimilated metabolite level could be due to more vegetative buds. It must be emphasized that from the start of the bud to full development of the flower and simultaneously accumulation of oil, a continuous supply of assimilates is necessary. As a result the youngest leaf in flowering shoot might be contributing more to the flower development and had lower assimilate level than the leaf of non-flowering shoot. The label remaining in the stem at 1.5 h was highest and at later time it declined in non-flowering shoots (Table 1).

In contrast, the label remaining in the stem of flowering shoot increased from 1.5 to 6.0 h and thereafter declined (Table 1). The partitioning of assimilate towards the flower increased with time and was maximum at 3 h after feeding and thereafter it declined. However at 24 h there was again a higher translocation of photosynthate towards flower, which possibly could have been remobilized from stored photosyn-

Table 1. Ontogenic changes in total ^{14}C incorporation (combined ES+EIS) in various plant parts in flowering and non-flowering plants of rose

Time(h) after feeding	Plant part	^{14}C incorporation ($x10^5$ DPM $g^{-1}FW$)	
		Flowering plant	Non-flowering plant
1.5	Leaf-1	5.55	12.66
	Leaf-2	5.02	12.60
	Leaf-3	2.75	12.57
	Stem	5.69	14.27
	Flower	0.07	-
LSD 5%	0.06	0.06	
3.0	Leaf-1	5.73	3.48
	Leaf-2	6.40	7.90
	Leaf-3	3.50	7.04
	Stem	6.85	12.31
	Flower	0.24	-
LSD 5%		0.05	0.07
4.5	Leaf-1	6.57	3.80
	Leaf-2	6.64	5.56
	Leaf-3	3.94	4.65
	Stem	12.30	12.28
	Flower	0.14	-
LSD 5%		0.04	0.04
6.0	Leaf-1	6.83	0.90
	Leaf-2	7.30	4.72
	Leaf-3	2.29	3.17
	Flower	0.09	-
LSD 5%		0.01	0.13
24	Leaf-1	3.69	2.52
	Leaf-2	3.65	3.08
	Leaf-3	2.03	2.76
	Stem	4.78	9.83
	Flower	0.25	-
LSD		0.03	0.04

thate and used for flower biomass development and/or oil biosynthesis.

Leaves of single stemmed *R. hybrida* at different positions on the flowering shoot had similar photosynthetic and photorespiratory capacities (Jiao *et al.* 1989). The higher levels of ^{14}C in the leaves of non-flowering shoots compared with flowering shoots (in early stage, 1.5 h) may be due to nontranslocation, which, however, may occur rapidly in flowering shoot, where the flowering bud is the dominant sink and requires photosynthates for structural as well as oil biosynthesis. It was reported that in *R. hybrida*, 98 per cent of the fixed ^{14}C from the first leaf moved towards developing flower bud (Mor & Halevy 1979). Further, mobilization of photosynthates (starch) has been reported for essential oil biogenesis during leaf ontogeny in lemongrass (Singh *et al.* 1991).

The total ^{14}C fixed by flowering and non-flowering shoots was further analysed into metabolic pool as ethanol soluble, ethanol insoluble fractions and sugars, amino acids and organic acids in leaf, stem and flowers. These fractions serve as mobile components as precursors for various metabolic pathways. In the flowering shoot, the incorporation in ethanol soluble fraction was higher than in ethanol insoluble fraction at all leaf positions (1 to 3) and at different time intervals (1.5 to 24.0 h). Incorporation in ethanol soluble fraction increased from the youngest first leaf to maximum in second leaf and then declined in third leaf, whereas ethanol insoluble fraction had maximum incorporation in the youngest leaf which gradually declined in second and third leaf (Table 2). The trend of incorporation in these two fractions at 3.0, 4.5, 6.0 and 24.0 h was similar, though the incorporation values increased in leaves, being maximum at 6 h. Thus, in a flowering shoot, first two leaves fixed maximum CO_2. In stems, the incorporation in ethanol soluble and insoluble fractions was maximum at 3 h. Amongst the fractions, the youngest first leaf had maximum incorporation of sugars and amino acids, which declined in 2 and 3 leaves. However, 1 and 2 leaves had more or less similar incorporation of organic acid. The maximum translocation of ethanol soluble fraction and sugars towards flower was at 3 h after feeding (Table 2).

In the non-flowering shoot, the incorporation in ethanol soluble and insoluble fractions was maximum at 1.5 h which subsequently declined at 3.0, 4.5, 6.0 and 24.0 h (Table 3). This was just opposite to the incorporation pattern in flowering shoot where the incorporation at 1.5 h fraction was lowest and it increased progressively with increasing time. Among different leaves, incorporation in ethanol soluble fraction increased from leaf 1 to 2 and then declined. The incorporation of sugars increased from leaf 1 to leaf 3 (with maximum incorporation at 1.5 h) and decreased with time. The pattern of incorporation of ^{14}C into sugars in leaves of non-flowering shoot was again opposite to the incorporation pattern in leaves of flowering shoots, where youngest leaves had maximum incorporation. The pattern of incorporation of amino acids and organic acids showed similar trend up to 3 h and then did not show uniform trend (Table 3). Among the metabolites, sugars were most heavily labeled compared to amino acids and organic acids. In flowering shoots, it appeared to get translocated quickly as there was little concentration in stem, whereas in non-flowering shoots the concentration of these metabolites in stem was higher. The level and the transport of sugars were quick as these are also strong precursors of essential oil biosynthesis, which occurs in glandular structures situated in epidermal cells of rose petals. Sugars have been reported to have major ^{14}C concentration in Samantha roses (Jiao *et al.* 1989). Rate of sugar translocation towards oil was also time dependent, the maximum translocation being at 3 h after assimilation. However, 24 h later there was a heavy translocation towards flower. This could be possibly due to remobilization from stored photosynthate. In *R. hybrida*, the upper younger leaves supplied (maximum) assimilates to the flower bud at its initiation (Mor & Halevy 1979).

Table 2. Variations in $^{14}CO_2$ assimilation in primary photosynthetic metabolites in different plant parts in flowering plants of rose

Time(h) after feeding	Plant part	Fraction(x 10^4DPM g^{-1}FW)		Fraction(x10^4DPM g^{-1}FW)		
		Ethanol soluble	Ethanol insoluble	Sugars	Amino-acids	Organic acids
1.5	Leaf-1	40.20	15.36	22.91	3.03	2.29
	Leaf-2	44.18	6.07	19.17	1.32	2.02
	Leaf-3	23.29	4.27	12.14	1.09	2.56
	Stem	56.34	0.62	4.30	1.92	1.62
	Flower	0.72	0.05	0.52	0.08	0.11
LSD 5%		0.55	0.18	0.29	0.01	0.03
3.0	Leaf-1	50.12	7.23	16.11	2.05	2.68
	Leaf-2	60.23	3.78	11.13	1.56	2.65
	Leaf-3	33.32	1.82	12.44	1.35	2.14
	Stem	67.96	0.53	6.42	2.23	2.22
	Flower	2.32	0.09	1.05	0.27	0.69
LSD 5%		0.63	0.12	0.09	0.02	0.03
4.5	Leaf-1	59.44	6.37	14.13	7.82	8.38
	Leaf-2	60.33	6.11	35.34	3.74	6.66
	Leaf-3	37.32	2.17	24.52	4.75	4.76
	Stem	122.27	0.79	4.79	3.91	3.94
	Flower	1.36	0.08	0.72	0.42	0.29
LSD 5%		0.35	0.09	0.20	0.08	0.05
6.0	Leaf-1	63.19	5.17	38.15	3.85	6.15
	Leaf-2	67.62	5.41	32.80	2.47	5.54
	Leaf-3	21.37	1.21	13.80	1.88	3.44
	Stem	36.80	0.31	2.60	2.36	18.60
	Flower	0.94	0.05	0.65	0.08	0.09
LSD 5%		.11	0.02	2.76	0.04	0.10
24	Leaf-1	35.10	1.87	24.74	1.35	3.77
	Leaf-2	35.40	1.17	25.04	1.87	4.38
	Leaf-3	19.55	0.83	10.38	1.11	1.98
	Stem	47.48	0.36	3.95	1.92	3.36
	Flower	1.59	0.15	1.51	0.22	0.51
LSD 5%		1.18	0.01	0.06	0.05	0.07

Correlation coefficients among metabolites namely, label incorporation into sugars and amino acids; and sugars and organic acids indicated that sugars and amino acids had a significant negative correlation in non-flowering shoots (-r=0.606=p=0.5), whereas in flowering shoot the correlation was positive and significant (r=0.739=p=0.05). In conclusion in

Table 3. Variations in $^{14}CO_2$ assimilation in primary photosynthetic metabolites in different plant parts in non-flowering plants of rose

Time(h) after feeding	Plant part	Fraction(x 10^4DPM g^{-1}FW)		Fraction(x10^4DPM g^{-1}FW)		
		Ethanol soluble	Ethanol insoluble	Sugars	Amino-acids	Organic acids
1.5	Leaf-1	10.42	2.24	49.74	3.84	8.73
	Leaf-2	10.91	1.69	75.32	5.14	11.05
	Leaf-3	10.33	2.24	78.23	4.08	10.28
	Stem	14.15	0.11	40.24	9.99	5.90
LSD 5%		0.05	0.03	0.25	0.06	0.13
3.0	Leaf-1	3.01	0.46	14.25	1.96	2.83
	Leaf-2	7.35	0.55	21.69	3.30	4.53
	Leaf-3	6.45	0.59	50.61	4.53	3.51
	Stem	12.23	0.08	22.47	37.42	9.71
LSD 5%		0.07	0.01	0.34	0.26	0.04
4.5	Leaf-1	3.52	0.28	30.79	2.09	2.04
	Leaf-2	5.27	0.29	34.30	2.31	2.10
	Leaf-3	4.46	0.19	34.37	2.07	2.10
	Stem	12.23	0.05	65.71	3.74	3.09
LSD 5%		0.03	0.02	0.11	0.02	0.06
6.0	Leaf-1	.81	0.09	1.91	0.69	0.73
	Leaf-2	4.43	0.29	1.74	1.06	1.07
	Leaf-3	3.03	0.13	3.36	1.05	1.21
	Stem	6.05	0.07	7.73	1.71	2.01
LSD 5%		0.13	0.01	0.07	0.02	0.02
24	Leaf-1	2.25	0.27	10.67	1.67	6.63
	Leaf-2	2.91	0.17	12.13	1.63	3.05
	Leaf-3	2.45	0.30	11.91	1.94	3.63
	Stem	9.75	0.07	11.53	1.50	1.62
LSD 5%		0.03	0.01	0.28	0.07	0.04

R. damascena the shoot tips containing the flower primordia had a higher requirement of assimilated photosynthate and showed relatively higher rate of translocation. Thus, once flower initiation/buds developed in a shoot, continuous supply of photosynthate was essential for flower biomass development as well as accumulation of essential oil. The results of the study emphasize that though assimilate partitioning is a dominant regulatory factor once the flower primordia have emerged, other regulatory processes also control flowering.

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