BRIEF COMMUNICATION

Utilization of exogenously supplied primary precursors for essential oil synthesis in *Cymbopogon* species

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Abstract

The relative efficiency of incorporation of the exogenously supplied primary precursors [2-^{14}C]acetate, [U-^{14}C]glucose and [U-^{14}C]sucrose into essential oil was determined in the immature leaves of three *Cymbopogon* species (*C. martinii*, *C. winterianus* and *C. flexuosus*). Acetate was most efficiently incorporated into essential oil in *C. winterianus* and *C. flexuosus*, whereas glucose was the best precursor in *C. martinii*. The observations are consistent when expressed as radioactivity [Bq] of essential oil per leaf, as percent incorporation or moles of precursors utilized for essential oil synthesis. Thus, there is selectivity in the efficiency of precursor utilization for the oil synthesis in *Cymbopogon* species.

Essential oils are commercially important secondary plant products owing to their use in pharmaceutical, flavour and perfumery industries and *Cymbopogon* species are some of the widely exploited aromatic grasses. Considerable attention was paid to biosynthesis of essential oils (*e.g.* Croteau 1987, Singh *et al.* 1989, 1990, 1991, Singh-Sangwan *et al.* 1993). Radiotracers are important tools to study various aspects of essential oil biogenesis. Many labelled compounds auch as CO_2, sucrose, glucose, acetate and mevalonate are routinely employed for such purposes (Banthorpe *et al.* 1972, Charlwood and Banthorpe 1978). Although mevalonate is an obligate precursor of terpenoids, glucose has been reported to be the most efficient precursor for monoterpenoid synthesis, whereas, mevalonate is the best precursor of sesquiterpenes (Croteau *et al.* 1972). Furthermore, Groeneveld *et al.* (1982) have observed that sucrose, as compared to glucose, xylose and acetate, is maximally

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Received 21 September 1991, *accepted* 3 September 1992.
Acknowledgements: We are grateful to Dr. R.S. Thakur, Director, CIMAP for encouragement, providing facilities and helpful suggestions. CIMAP communication number 990.
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incorporated into triterpenoids. This indicates that selectivity in precursor utilization is governed by the class of terpenoids to be synthesized. In the present investigation relative efficiency of *in vivo* incorporation of labelled precursors acetate, glucose, and sucrose into essential oil (consisting of $\geq$ 95 % monoterpenoids) was assessed to discern selectivity in the utilization of primary precursors for oil biogenesis in *Cymbopogon* species.

Plants of lemongrass (*Cymbopogon flexuosus* Stapf.), citronella (*Cymbopogon winterianus* Jowitt.) and palmarosa (*Cymbopogon martinii*) were raised at experimental farm of CIMAP, Lucknow, India, following standard agronomic practices. Partially (60 - 65 % of maximal leaf area) expanded leaves, from the tillers of same age (one month), were used for the tracer studies.

[2-^{14}C]acetate (specific activity 2112.7 GBq mol^{-1}), [U-^{14}C]glucose (9435 GBq mol^{-1}) and [U-^{14}C]sucrose (21460 GBq mol^{-1}) were obtained from Bhabha Atomic Research Centre, Trombay. Six immature leaves were transferred to a tube containing an aqueous soution (1 cm^3, pH 7) of labelled precursor (185 GBq mol^{-1}) and dipped the cut ends in the solution. The tubes were kept in sunlight (800-1000 μmol m^{-2} s^{-1}) and after the uptake of precursor 4 $\times$ 0.5 cm^3 of half strength Hoagland solution was added. The leaves were further maintained in above nutrient medium for 24 h at ambient temperature and then the oil was isolated.

Leaves fed with labelled precursors, were chopped and subjected to micro-scale steam distillation using mini Clevenger's apparatus as described previously (Singh *et al.* 1989). Essential oil was recovered by ether extraction and dehydrated with anhydrous sodium sulphate.

Radioactivity in oil aliquot was monitored in a liquid scintillation counter (*LKB Rack Beta 1215*) using PPO-POPOP-toluene cocktail. Efficiency of the equipment for ^{14}C was 89 %.

Amount of labelled precursors utilized was calculated as the ratio between radioactivity [Bq] in the essential oil and specific activity [GBq mol^{-1}] of precursor multiplicated in the case of glucose and sucrose by 1800 and in the case of acetate by 1000 and was expressed in pmoles. From labelled carbons in 3 molecules of [U-^{14}C]glucose or 1.5 molecules of [U-^{14}C]sucrose only 10 are incorporated into monoterpenes. However, with the use of [2-^{14}C]acetate, there is no loss of the label and, therefore no correction factor is required in the above calculations.

All the measurements were made in triplicate and the results are given as mean $\pm$ S.E.

The efficiency of incorporation of [2-^{14}C]acetate, [U-^{14}C]glucose and [U-^{14}C] sucrose into essential oil was determined in *C. martinii*, *C. winterianus* and *C. flexuosus*. The radioactivity incorporated into the oil was expressed as Bq per leaf as well as percentage of the exogenously supplied radioactivity incorporated into the oil (Table 1). Amount of labelled precursor utilized for essential oil synthesis was computed from pmoles of the precursor incorporated into oil by taking into account the loss of labelled carbon from precursor during its metabolism *en route* to essential oil (monoterpenoid) biosynthesis. The incorporation of [2-^{14}C]acetate into essential oil, as compared to that of [U-^{14}C]glucose and [U-^{14}C]sucrose, was considerable in *C. winterianus* and *C. flexuosus* whereas in *C. martinii* its incorporation was relatively meagre (Table 1). In *C. martinii* maximal incorporation into the oil was observed with [U-^{14}C]glucose. The incorporation of [U-^{14}C] sucrose was about 80 % of glucose. In *C. flexuosus* and *C. winterianus*, incorporation of labelled glucose into oil was only one-fourth and one-ninth, respectively, of that of acetate. The incorporation of sucrose in these plants was 2.0 and 2.7 fold higher, respectively, than that of glucose. The comparison of the percentage of incorporation reveals the same pattern (Table 1).

Table 1. Interspecific variation in the efficiency of precursor utilization for *in vivo* essential oil synthesis in *Cymbopogon* species. Six immature leaves were fed with an aqueous solution (pH 7.0) of the precursor (185 GBq mol^{-1}) and the radioactivity incorporated into essential oil was measured. The results are expressed on per leaf basis (mean of three independent determinations ± S.E.).

Cymbopogon spp.	Labelled precursor	Radioactivity incorporated into essential oil [Bq leaf^{-1}]	[%]	^{14}C precursor utilized for the oil synthesis per leaf [pmol]
C. martinii	[2-^{14}C]acetate	7.29 ± 0.49	0.024 ± 0.001	3.45 ± 0.23
	[U-^{14}C]glucose	40.54 ± 3.62	0.131 ± 0.012	7.74 ± 0.69
	[U-^{14}C]sucrose	32.65 ± 2.55	0.106 ± 0.008	2.74 ± 0.21
C. winterianus	[2-^{14}C]acetate	469.94 ± 26.55	1.524 ± 0.086	222.44 ± 12.57
	[U-^{14}C]glucose	52.76 ± 3.49	0.171 ± 0.011	10.06 ± 0.67
	[U-^{14}C]sucrose	103.72 ± 7.47	0.336 ± 0.024	8.69 ± 0.63
C. flexuosus	[2-^{14}C]acetate	309.29 ± 14.75	1.003 ± 0.048	146.40 ± 6.98
	[U-^{14}C]glucose	75.14 ± 6.84	0.244 ± 0.022	14.33 ± 1.30
	[U-^{14}C]sucrose	204.07 ± 13.26	0.662 ± 0.043	17.12 ± 1.11

Thus, among the primary precursors tested, acetate was most efficiently utilized for essential oil synthesis in *C. winterianus* and *C. flexuosus*. These results differed from those on other aromatic plants, where glucose was the best precursor for monoterpenoid syntesis in leaves (Croteau *et al.* 1972a). However, we also observed likewise maximum incorporation of glucose as compared to acetate and sucrose, in *C. martinii*. The variability in efficiency of precursor utilization for essential oil synthesis may be attributed to differential accessibility of precursors as a consequence of structural differences in the oil glands, which are presumed to be the sites of the essential oil synthesis and/or variation in the extent of competition for the precursor imposed by other metabolic pathways. Furthermore, energy status of the oil biosynthetic sites (Croteau *et al.* 1972b) and the level of the pertinent enzymes in channeling of the precursor to the mevalonate-isoprenoid pathway may also be important determinant of the extent of precursor utilization for oil synthesis. The reported poor incorporation of acetate into oil may also be due to the use of [1-^{14}C] acetate whose one-third label is lost as $^{14}CO_2$ during terpenoid synthesis (Banthorpe *et al.* 1972, Charlwood and Banthorpe 1978). With the use of [2-^{14}C] acetate there is no loss of label, hence, the use of [2-^{14}C] acetate gives a better estimate of the extent of incorporation as compared to [1-^{14}C] acetate. In summary, the studies suggest that there is a selectivity in the efficient utilization of the exogenously supplied precursor for essential oil synthesis in the leaves of *Cymbopogon* spp.

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Communicated by I. MACHÁČKOVÁ