Sucrose mobilization in relation to essential oil biogenesis during palmarosa (*Cymbopogon martinii* Roxb. Wats. var. *motia*) inflorescence development

VINOD SHANKER DUBEY†, RITU BHALLA* and RAJESH LUTHRA**

Neurobiotechnology Center, The Ohio State University, Columbus, Ohio 43210, USA
**Plant Microbes Interaction Lab, Department of Biological Sciences, National University of Singapore, Singapore 117604*
***CSIR Complex, Dr K S Krishnan Marg, Pusa, New Delhi 110 012, India*

†*Corresponding author (Fax, 407-823-0956; Email, vdubey@mail.ucf.edu)*

Palmarosa inflorescence with partially opened spikelets is biogenetically active to incorporate [U-^{14}C]sucrose into essential oil. The percent distribution of ^{14}C-radioactivity incorporated into geranyl acetate was relatively higher as compared to that in geraniol, the major essential oil constituent of palmarosa. At the partially opened spikelet stage, more of the geraniol synthesized was acetylated to form geranyl acetate, suggesting that majority of the newly synthesized geraniol undergoes acetylation, thus producing more geranyl acetate. *In vitro* development of palmarosa inflorescence, fed with [U-^{14}C]sucrose, resulted in a substantial reduction in percent label from geranyl acetate with a corresponding increase in free geraniol, thereby suggesting the role of an esterase in the production of geraniol from geranyl acetate. At time course measurement of $^{14}CO_2$ incorporation into geraniol and geranyl acetate substantiated this observation. Soluble acid invertase was the major enzyme involved in the sucrose breakdown throughout the inflorescence development. The activities of cell wall bound acid invertase, alkaline invertase and sucrose synthase were relatively lower as compared to the soluble acid invertase. Sucrose to reducing sugars ratio decreased till fully opened spikelets stage, concomitant with increased acid invertase activity and higher metabolic activity. The phenomenon of essential oil biosynthesis has been discussed in relation to changes in these physiological parameters.

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1. Introduction

Palmarosa (*Cymbopogon martinii* Roxb. Wats.) inflorescence is a good source of 'geraniol'-rich commercially important essential oil. Palmarosa oil is used to impart a rose-like aroma to a wide range of perfumes, soaps, cosmetics, toiletry and tobacco products (Dubey 1999). Essential oil metabolism is governed by the balance between photosynthesis and the utilization of photosynthate and/or growth-differentiation (Srivastava and Luthra 1994). Although sucrose is the major end product of photosynthesis in most plants, many other compounds including amino acids, lipids and secondary metabolites (such as terpenoids) are also synthesized (Sturm 1999). The synthesis of these compounds requires energy and carbon skeletons, which can be provided either from sucrose breakdown or directly from photosynthesis. Sucrose plays an important role in plant metabolism as an important storage sugar and the main form of reduced carbon translocated from source leaves to developing growth

Keywords. *Cymbopogon martinii*; essential oil; inflorescence; palmarosa; sucrose metabolism

Abbreviations used: GAE, Geranyl acetate cleaving esterase; GLC, gas liquid chromatography; MVA, mevalonate; TLC, thin-layer chromatography.

centres in the plant (Ranwala and Miller 1998). In tissues which are specialized for a particular storage or structural function, sucrose is diverted to an increasing extent away from respiration into a restricted range of products such as lipid, protein, starch or even storage as sucrose (Whittaker and Botha 1997). Two major enzymes are involved in the cleavage of sucrose in plants viz. invertase and sucrose synthase. Invertase hydrolyzes sucrose to glucose and fructose, whereas sucrose synthase cleaves sucrose in the presence of UDP to form UDP-glucose and fructose. Both enzymes are expressed in sink tissues, but their sucrolytic activity varies depending on the species, tissue type and development stage (Aloni *et al* 1997; Ranwala and Miller 1998). The products of sucrose cleavage are converted to hexose-phosphates which can enter the glycolytic and hexose monophosphate (HMP) pathways to provide substrates and reducing power for growth and storage product synthesis (King *et al* 1997). Furthermore, two intermediates of the glycolytic pathway, glyceraldehyde-3-phosphate (triose-phosphate) and pyruvate, are the known precursors for terpenoid biosynthesis in plants through newly discovered mevalonate-independent (non-MVA) pathway (Luthra *et al* 1999; Lichtenthaler 1999; Mahmoud and Croteau 2002), which is not only important in plants but also has a greater significance for human beings – since the same non-MVA pathway could be used as potential drug targets against some human pathogens (Dubey 2002).

Earlier studies with lemongrass (*Cymbopogon flexuosus*) leaves revealed that the oil biosynthesis takes place mainly in young and expanding leaves. In these leaves metabolism of sucrose and transient starch is most rapid and oxidative pathways operate at elevated levels (Singh and Luthra 1988; Singh *et al* 1990, 1991), ensuring an efficient supply of carbon precursors/cofactors/energy and reducing power for biosynthetic purposes, including terpenoid synthesis (Bouwmeester *et al* 1998). Compartmentation of terpenoid metabolism, and apparent carbon and/or energy deficiency, are regarded as some of the possible factors in the regulation of essential oil biosynthesis (Gershenzon and Croteau 1990; McGarvey and Croteau 1995). The partitioning of assimilate among various metabolic pathways has also been suggested to regulate monoterpene production in aromatic plants (Croteau *et al* 1972; Srivastava and Luthra 1991, 1994).

Monoterpenoids, the major constituents of the essential oils, are known to be synthesized through the plastidic non-MVA pathway (Luthra *et al* 1999; Mahmoud and Croteau 2002). The synthesis of terpenoids is largely a fermentative process, requiring sugar as an energy source at energy deficient sites (Singh *et al* 1989; McGarvey and Croteau 1995). Owing to the non-photosynthetic nature of oil biosynthetic sites, the primary metabolic pathways have a greater significance. Secondary metabolic processes have been studied largely in isolation and relatively little is known about its integration with primary metabolism (Singh and Luthra 1988; Singh *et al* 1990, 1991). Primary and secondary metabolic processes are intimately interconnected, as the later derives precursors from the primary metabolic reactions. Palmarosa inflorescence, apart from being an important sink tissue, seems to be an ideal system to study this relationship. In the present investigation, the relationship between sucrose mobilization and essential oil biosynthesis was explored by following [U-^{14}C]sucrose incorporation into essential oil and its major constituents (geraniol and geranyl acetate) together with activities of various sucrose catabolizing enzymes during palmarosa inflorescence development. For the first time, the metabolism of sucrose in floral tissues, especially in relation to essential oil biosynthesis, is being explored.

2. Materials and methods

2.1 *Plant material and chemicals*

Palmarosa (*Cymbopogon martinii*, Roxb. Wats. var. *motia*) plants were raised from seedlings at the experimental farm of Central Institute of Medicinal and Aromatic Plants (CIMAP), Lucknow, India. The samples were taken at eight day intervals, representing five developmental stages viz. stage I, unopened spikelets; stage II, partially opened spikelets (anthers partially visible); stage III, fully opened spikelets (yellow anthers fully visible); stage IV, partially mature spikelets (anthers brown and yellow); stage V, fully mature spikelets (brown inflorescence) (Dubey *et al* 2000). Since fresh weight and dry weight of the spikelets vary with inflorescence development (Dubey *et al* 2000; Dubey and Luthra 2001), all results were discussed on a spikelet basis to avoid ambiguity.

Geraniol and geranyl acetate were purchased from Sarsynthase (France) and biochemicals from Sigma Chemical Co. (USA). Solvents and other chemicals used were of high purity (analytical reagent grade). The radiochemicals [U-^{14}C]sucrose (0·1 mCi, 300 mCi/mmol) and sodium [^{14}C] bicarbonate (0·5 mCi, 47·9 mCi/mmol) were procured from Bhabha Atomic Research Centre (BARC), Trombay, Mumbai, India.

2.2 *Incorporation of [U-^{14}C]sucrose into essential oil and its constituents*

For *in vivo* incorporation studies, palmarosa inflorescence at different developmental stages were cut under water and placed in vials with the cut ends dipped in an aqueous solution of 5 μCi radiolabelled [U-^{14}C]sucrose.

The vials were kept filled with half-strength Hoagland solution (Hoagland and Arnon 1938), and after 24 h of incubation, the spikelets were removed from the inflorescence, weighed, counted and subjected to microscale steam distillation using a mini-Clevenger apparatus. The volatile essential oil was recovered by ethyl ether extraction. Aliquots were taken for determination of total radioactivity in essential oil, and for thin-layer chromatographic (TLC) isolation of its major constituents.

The major essential oil constituents, geraniol and geranyl acetate, were separated by preparative TLC on silica gel G plates (20 × 20 cm, 0.5 mm) using a solvent system; toluene : ethyl acetate (96 : 4, v/v) (Dubey 1999). The concentrated essential oil samples were applied along with authentic samples of geraniol and geranyl acetate. The plates were dried at room temperature to remove solvent and visualized with iodine vapours. The spots/streaks, corresponding to the authentic geraniol and geranyl acetate, samples were scraped off directly into the scintillation vials or eluted with ethyl ether and used for determination of ^{14}C-radioactivity (Snyder 1964). Purity of the geraniol and geranyl acetate, separated through TLC, was checked by gas liquid chromatography (GLC) as previously described (Dubey *et al* 2000).

For hydrolysis of ^{14}C-labelled geranyl acetate, the extracted geranyl acetate was taken into a round bottom flask containing 10% ethanolic KOH in excess and fitted with a guard tube packed with fused calcium chloride. The mixture was incubated at room temperature with constant stirring for 24 h. After incubation, the reaction mixture was extracted with ethyl ether to obtain geraniol. HCl was added to the aqueous phase to neutralize KOH. An aliquot from each of the ethyl ether and aqueous phase containing geraniol and acetate moiety, respectively, released by the hydrolysis of geranyl acetate, was directly taken in a scintillation vial for radioactivity counting. The radioactivity in ethyl ether and aqueous aliquots, and TLC isolates was determined using a *b*-liquid scintillation counter (LKB Wallace 1409, Pharmacia Biotech.). The counting efficiency for [^{14}C] was 95%. ^{14}C-radioactivity incorporated (pmol) into geraniol and geranyl acetate of palmarosa oil was calculated from total incorporation into oil, and their relative percentage incorporation values (Singh *et al* 1991).

2.3 In vitro *development of palmarosa inflorescence*

Palmarosa inflorescence with partially opened spikelets (fed with [U-^{14}C]sucrose, 5 μCi/μmol) were cultured for 10 days in test tubes with the cut ends dipped in half strength Hoagland solution containing 2% sucrose and 0·05% sporidex (an antibiotic). The incubation solution was changed after every third day. For essential oil accumulation during *in vitro* culture, a similar experiment was performed without using radiolabelled precursor. The spikelets (inflorescence) were harvested on every second day for essential oil extraction and TLC analysis of geraniol and geranyl acetate. The ^{14}C-radioactivity incorporated into essential oil and TLC isolates were also determined, as described above.

2.4 *Time course of $^{14}CO_2$ incorporation into essential oil*

The experiment involving $^{14}CO_2$ incorporation into essential oil in palmarosa inflorescence was performed as previously described (Srivastava and Luthra 1994). Briefly, palmarosa inflorescence with unopened spikelets (stage I) was cut under water and placed in small vials containing half-strength Hoagland solution. The inflorescence was carefully tested before the exposure to ensure that they were able to take up Hoagland solution properly. The vials containing inflorescence were kept in a sealed plexiglass chamber (45 × 45 × 61 cm) around a central vial containing $NaH^{14}CO_3$ (0·5 mCi) solution. $^{14}CO_2$ was generated by injecting 2 M H_2SO_4 into the bicarbonate solution through a teflon inlet tube and CO_2 was uniformly distributed with the help of an electric fan. The inflorescence was allowed to assimilate $^{14}CO_2$ in sunlight (800 μE $m^{-2}S^{-1}$) for one hour. At the end of this period, a saturated solution of KOH was run into the central vial to absorb the remaining $^{14}CO_2$. The exposure chamber was then opened and vials containing the inflorescence were kept in a growth chamber (temperature 25°C) for remaining experimental period. At intervals of 12 h, until 48 h, and then at six days, the spikelets were removed and analysed for ^{14}C-radioactivity in the essential oil and its major constituents viz. geraniol and geranyl acetate.

2.5 *Extraction and assay of sucrose metabolizing enzymes*

Palmarosa inflorescence was harvested at different developmental stages and spikelets were removed and used for enzymological studies. All enzyme extractions were carried out at 0–4°C in a cold room. The spikelets were homogenized in the specific extraction medium described for each enzyme. The extraction of invertases was carried out as described by Singh and Luthra (1988). The spikelets were ground in 10 mM Tris-maleate buffer (pH 7·0) and centrifuged at 20,000 *g* for 30 min. The supernatant fraction was used to assay soluble acid and alkaline invertases. The pellet obtained was washed thrice with the extraction buffer and finally resuspended in a fresh aliquot of the same buffer. The suspension was used to

determine insoluble (cell wall bound) acid invertase activity. The enzyme preparations were dialyzed overnight in the same buffer to remove the endogenous sugars. The extraction of sucrose synthase was done according to the procedure described by Suwignyo *et al* (1995). The spikelets were homogenized in 50 mM HEPES-NaOH (pH 7·5) buffer containing 5 mM $MgCl_2$, 1 mM EDTA-Na_2, 2·5 mM DTT, 2% (W/V) polyethylene glycol (PEG) 8000 and 1% (W/V) bovine serum albumin (BSA). The slurry was centrifuged at 20,000 *g* for 30 min. The supernatant was dialyzed overnight against the homogenization buffer and used for the assay of sucrose synthase activity.

The reaction mixture of invertase (soluble or cell wall bound fraction) consisted of 0·2 M sodium acetate buffer (pH 4·8) containing 10 mM sucrose (for acid invertase) or 0·2 M phosphate-citrate buffer (pH 7·5) containing 10 mM sucrose (for alkaline invertase) with the enzyme extract in a final volume of 1·0 ml. The contents were incubated for 30 min at 30°C. The reaction was stopped by the addition of Nelson's reagent and the liberated invert sugars were estimated by Nelson's method (Nelson 1944) at wavelength 620 nm. The amount of sucrose hydrolysed was calculated by multiplying the value of invert sugars by 0·95. Controls for the assay consisted of reactions carried out with inactivated (boiled) enzyme. One unit of enzyme activity was defined as the amount catalysing the cleavage of 1 µmol of sucrose per min at 30°C. The reaction mixture for sucrose synthase contained 100 mM HEPES-NaOH (pH 7·5), 5 mM UDP, 50 mM sucrose, 5 mM NaF, and enzyme extract in a total volume of 500 µl. The mixture was incubated at 30°C for 30 min and the reducing sugar (as glucose equivalent) released was measured at 620 nm (Nelson 1944). One unit of enzyme activity was defined as the amount required to produce 1 µmol reducing sugar (as glucose equivalent) per min at 30°C. In all enzyme extracts, total soluble protein was estimated by Bradford method (Bradford 1976) using bovine serum albumin (BSA) as a standard.

2.6 *Extraction and estimation of free sugars*

The procedure for extraction of sugars was same as previously described (Singh and Luthra 1988). Sugars were extracted from dry palmarosa spikelets twice with hot 80% (v/v) ethanol followed by a complete extraction with hot 70% (v/v) ethanol. The pooled alcoholic extracts were concentrated, and sugar syrup was deproteinized by basic lead acetate and excess lead ions were precipitated with sodium oxalate crystals. The clear extract was used for the estimation of sucrose (Van Handel 1968) and reducing sugars (Nelson 1944).

3. Results

3.1 *Biogenesis of essential oil and its major constituents using [U-^{14}C]sucrose as precursor*

The incorporation of the [U-^{14}C]sucrose metabolite into essential oil, geraniol and geranyl acetate were determined at various stages of the inflorescence development. The biogenetic capacity of the inflorescence to synthesize essential oil increased nearly 3·5-fold from the unopened spikelets (stage I) to the partially opened spikelets (stage II) and then decreased substantially, until the fully mature spikelets (stage V). The percent incorporation of ^{14}C-radioactivity into geranyl acetate in oil was substantially more as compared to that in geraniol at all stages of palmarosa inflorescence development, except fully mature spikelets (stage V), where incorporation in geraniol was little more than that in geranyl acetate (figure 1). ^{14}C-labelled geranyl acetate was hydrolyzed by 10% ethanolic KOH to determine the relative distribution of label in geraniol and acetate moiety of geranyl acetate. The distribution of label in geraniol and the acetate moiety of geranyl acetate indicated that approximately 60 to 80% of the label incorporated into geranyl acetate was present in the acetylated geraniol moiety. The acetate moiety contributed just 20% to 40% of the label incorporated in geranyl acetate, depending upon the developmental stage (figure 2).

The distribution of label in the geraniol and acetate moiety of geranyl acetate at the partially opened spikelets (stage II) indicated that 83% of the label incorporated into geranyl acetate was present in the acetylated geraniol

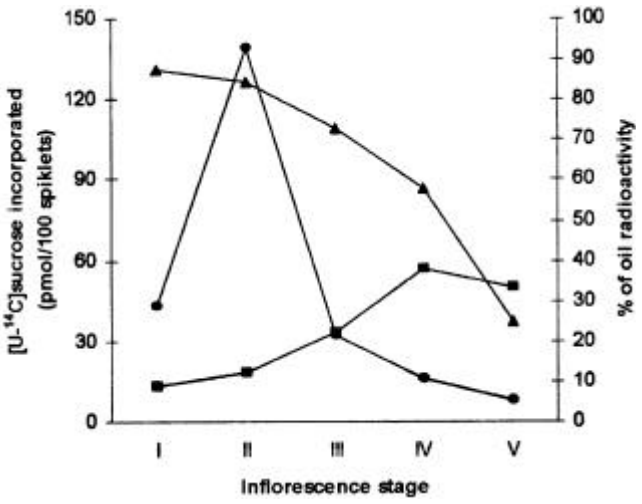

Figure 1. Incorporation of [U-^{14}C]sucrose into essential oil (●), and percent distribution of oil radioactivity into geraniol (■) and geranyl acetate (▲) during palmarosa inflorescence development. LSD value for [U-^{14}C]sucrose incorporated into essential oil at $P \leq 0{\cdot}01$ was 8·60.

moiety. The acetate moiety just contributed 17% of the label incorporated in geranyl acetate (figure 2).

The overall ^{14}C-radioactivity incorporated into free geraniol and acetylated geraniol at each stage of inflorescence development indicated that the incorporation into total geraniol increased until the partially opened spikelets (stage II) and then decreased substantially (figure 3). The radioactivity in acetylated geraniol was relatively greater as compared to that in free geraniol, except at the partially and fully mature spikelets (stage IV and V). The percentage of the newly synthesized geraniol that undergo acetylation decreased until fully mature spikelets (stage V) (figure 3).

3.2 In vivo *hydrolysis of ^{14}C- labelled geranyl acetate, synthesized from [U-^{14}C]sucrose, during* in vitro *culture*

Biosynthetic studies using [U-^{14}C]sucrose indicated that the palmarosa inflorescence with partially opened spikelets was biogenetically most active to synthesize geranyl acetate (figure 2). Since majority of the label incorporated into geranyl acetate was present in the geraniol moiety at this stage, it was therefore an ideal system to study the *in vivo* hydrolysis of ^{14}C-labelled geranyl acetate. Palmarosa inflorescence with partially opened spikelets (stage II), fed with [U-^{14}C]sucrose, were cultured for 10 days in Hoagland solution containing 2% sucrose to study the hydrolysis of labelled geranyl acetate. The relative distribution of ^{14}C-radioactivity into acetylated geraniol (geranyl acetate) and free geraniol during the culture period showed a substantial loss of ^{14}C label from acetylated geraniol (geranyl acetate) with a corresponding increase in free geraniol (figure 4). An *in vitro* culture experiment without radiolabelled precursors for the same period of time showed a similar result, as the proportion of geranyl acetate decreased with a corresponding increase in the geraniol proportion in palmarosa oil (data not shown). Furthermore, we also detected a geranyl acetate hydrolyzing esterase activity in palmarosa inflorescence – both during *in vitro* culture and in the intact plants – at various stages of development. This substantiated the above observations (unpublished results).

3.3 *Time course of $^{14}CO_2$ incorporation into essential oil and its constituents*

Palmarosa inflorescence with unopened spikelets (stage I) was allowed to fix $^{14}CO_2$ for one hour and the samples were taken at 12 h intervals until 48 h, and then at six days, during the chase period. The incorporation of ^{14}C-radioactivity into essential oil (pmol/100 spikelets) decreased substantially after 12 h of chase period. The relative percent incorporation into geranyl acetate and geraniol during different times of the chase period showed that the label incorporated into acetylated geraniol (geranyl acetate) decreased substantially after 12 h and was accompanied by a corresponding increase in free geraniol (table 1). After 12 h, approximately 90% of the label incorpo-

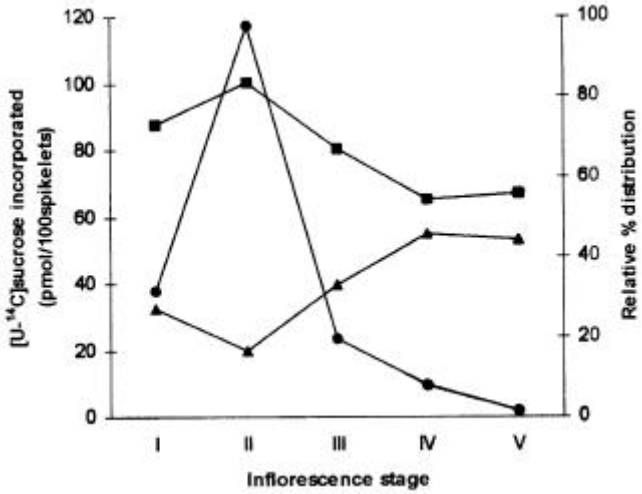

Figure 2. Incorporation of [U-^{14}C]sucrose into geranyl acetate (●), and relative percent distribution of radioactivity incorporated into geraniol (■) and acetate (▲) moiety of geranyl acetate during palmarosa inflorescence development. The pmol incorporated into geranyl acetate has been calculated from ^{14}C-label incorporated into essential oil and relative percent distribution of ^{14}C-radioactivity into geranyl acetate in oil.

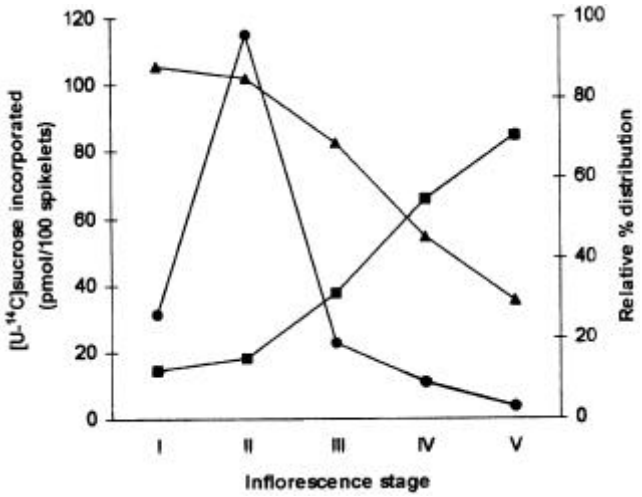

Figure 3. Incorporation of [U-^{14}C]sucrose into total geraniol (●), and percent distribution of radioactivity incorporated into free geraniol (■) and acetylated geraniol (▲) during palmarosa inflorescence development. The pmol incorporated into total geraniol represents sum total of the ^{14}C-label incorporated into free and acetylated geraniol.

rated into geranyl acetate was present in the acetylated geraniol moiety (data not shown). These results were analogous to that with [U-^{14}C]sucrose and [2-^{14}C]acetate (unpublished results) as precursors for essential oil biosynthesis. We also observed an increased ^{14}C-labelling in unidentified components in mature palmarosa inflorescence during *in vitro* development (data not shown). The ratio of the label incorporated into geraniol and geranyl acetate during six days of chase period was increased substantially (table 1).

3.4 *Changes in activities of sucrose metabolising enzymes and carbohydrate profile*

To understand the co-ordination between primary and secondary metabolism we monitored the activities of various sucrose metabolizing enzymes viz. acid invertase (soluble and cell wall bound), alkaline invertase and sucrose synthase, during palmarosa inflorescence development. Soluble acid invertase was found to be the major enzyme involved in sucrose breakdown. Its activity (per 100 spikelets basis) increased from the unopened spikelets (stage I) to the partially opened spikelets (stage II), and then decreased until the fully mature spikelets (stage V). However, substantial activity was present even after the partially opened spikelets (stage II) (figure 5). Cell wall-bound acid invertase exhibited a similar pattern, but its activity was relatively lower as compared to that of soluble acid invertase. The activities of alkaline invertase and sucrose synthase were much less compared to that of acid invertase (figure 5). The enzyme activities expressed on gFW^{-1} and mg^{-1} protein basis followed a similar trend with maximum activity at partially opened spikelets (stage II) (data not shown).

Changes in the carbohydrate profile, such as sucrose and reducing sugars were also determined during inflorescence development. The level of sucrose increased until partially opened spikelets (stage II) and then decreased, whereas the reducing sugars content increased from the unopened spikelets (stage I) until the partially mature spikelets (stage IV). The ratio of sucrose to reducing sugars decreased significantly from the unopened spikelets (stage I) till the fully opened spikelets (stage III) and then remained constant (figure 6).

4. Discussion

Sucrose is the most likely physiological source of carbon utilized in terpenoid biosynthesis (Singh and Luthra 1988; Luthra *et al* 1993; Bouwmeester *et al* 1998). Biosynthetic studies using [U-^{14}C] sucrose indicated that palmarosa inflorescence with partially opened spikelets is active to incorporate [U-^{14}C]sucrose metabolite into essential oil substantially. Related studies with flowers of essential oil-rose indicated that the essential oil radioactivity increased 300-fold during the transition from the onset of calyx opening to the half opening flower stage (Pogorel'skaya *et al* 1980). The overall incorporation of [U-^{14}C]sucrose radioactivity in developing fruits of caraway was significantly higher at the younger stages than in the older stages, and decreased from 3–6% of administered sucrose at 5–15 days after pollination (DAP) to less than 1% after 16–20 DAP (Bouwmeester *et al* 1998). Thus, only immature tissues were found biogenetically most active to synthesize essential oil substantially. At partially opened spikelets (stage II), most of the geraniol synthesized was acetylated to form geranyl acetate, suggesting that majority of the newly synthesized geraniol undergoes acetylation. In *Cymbopogon* species, acyclic monoterpenyl acetates (geranyl acetate and citronellyl

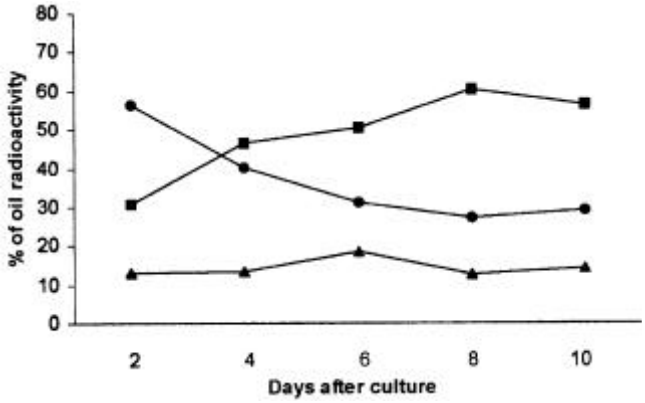

Figure 4. Relative percent distribution of ^{14}C-label into geranyl acetate (●), geraniol (■) and unknown constituents (▲) during *in vivo* hydrolysis of geranyl acetate (synthesized from [U-^{14}C]sucrose) accompanying palmarosa inflorescence development under *in vitro* conditions. The experiment was repeated three times and the representative data from a single experiment has been given.

Table 1. Time course measurement of labelled $^{14}CO_2$ incorporation into palmarosa inflorescence oil and its major constituents, geraniol and geranyl acetate.

Chase period	Essential oil (pmol/100 spikelets)	Distribution of oil radioactivity (%)		Geraniol/ geranyl acetate
		Geraniol	Geranyl acetate	
12 h	98·50 ± 5·6	10·8	81·6	0·13
24 h	73·11 ± 4·8	15·4	63·3	0·24
36 h	57·49 ± 4·0	24·2	65·9	0·37
48 h	43·08 ± 2·8	32·9	53·0	0·62
6 days	52·93 ± 4·1	69·8	22·7	3·08

LSD value for $^{14}CO_2$ incorporated into essential oil at $P \leq 0{\cdot}01$ was 2·84.

acetate) are, in general, higher in the younger tissue and decrease significantly with maturity (Luthra *et al* 1991; Dubey *et al* 2000). This is in contrast to *Mentha* species where cyclic monoterpenyl acetate (menthyl acetate)

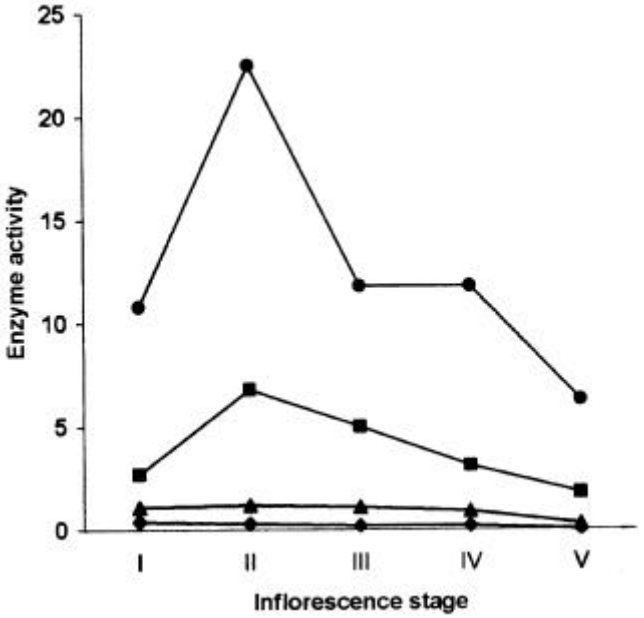

Figure 5. Activity profile of sucrose catabolizing enzymes viz. soluble acid invertase (●), cell wall bound acid invertase (■), alkaline invertase (▲) and sucrose synthase (◆) during palmarosa inflorescence development. The enzyme activity was expressed as μmol sucrose hydrolyzed/min/100 spikelets. LSD values at $P \leq 0{\cdot}01$ were 1·38, 0·19, 0·06 and 0·04, respectively.

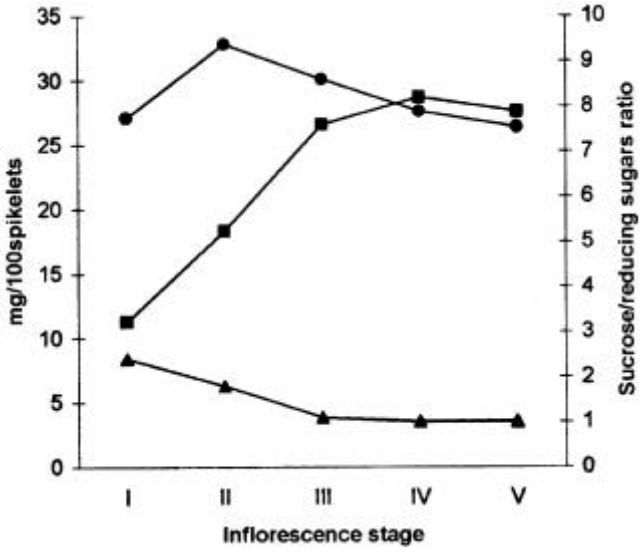

Figure 6. Levels of sucrose (●) and reducing sugars (■) and changes in sucrose to reducing sugars ratio (▲) during palmarosa inflorescence development. LSD values for sucrose and reducing sugars at $P \leq 0{\cdot}01$ were 0·58 and 0·76, respectively.

accumulate predominantly in mature tissues (Croteau and Hooper 1978; Srivastava *et al* 1990). Both *in vivo* and *in vitro* studies in palmarosa suggested that during inflorescence development the percent label in geranyl acetate decreased with concomitant increase in geraniol, thereby suggesting the possible role of an esterase in producing geraniol by hydrolyzing geranyl acetate during the process. These observations were very similar to what we observed in the intact palmarosa plants, i.e. the geranyl acetate proportion appreciably decreased with corresponding increase in geraniol proportion in the oil as the inflorescence matured (Dubey *et al* 2000). More recently, we have reported a geranyl acetate cleaving esterase (GAE) activity from immature palmarosa inflorescence (Dubey and Luthra 2001). We also observed the important role of GAE in geraniol production during inflorescence development (unpublished results).

The physiological source of carbon in green plants is photosynthetically fixed CO_2. The palmarosa inflorescence is green and is able to fix labelled $^{14}CO_2$. $^{14}CO_2$ in the light has been found to be a good precursor of monoterpene biosynthesis in several aromatic plants (Croteau *et al* 1972; Singh *et al* 1991; Srivastava and Luthra 1991). The incorporation into palmarosa oil decreased substantially after 12 h of chase period, suggesting the rapid metabolic turnover of monoterpenoids. Terpenes are commonly believed to undergo rapid metabolic turnover in plants (Croteau *et al* 1972; Srivastava and Luthra 1994). However, few studies showed that the rapid turnover of the monoterpene is found only in detached plant parts/system, and it does not occur in rooted plants (Gershenzon *et al* 1993). The authors, however, further suggested that turnover of terpenes probably did occur in intact plants, but was masked because the loss of radioactivity from the terpene pool and was precisely compensated by the radioactivity of newly synthesized terpenes generated from ^{14}C-labelled substrates whose carbon atoms were fixed during the initial pulse chase period (Gershenzon *et al* 1993). This may also could explain that the detached organs/plant parts have higher rates of turnover than the intact plants – since, detached organs/plant parts are expected to have minimal amounts of carbon reserves for additional terpene synthesis, and therefore would be less able to compensate for any apparent losses of terpenes (Gershenzon *et al* 1993). Moreover, apparent terpene turnover may also result from non-degradative conversions or any other chemical reactions that transform terpenes into some unknown product (like the formation of water-soluble terpene glycosides) which are commonly found during the catabolic processes occurred predominantly in mature plant parts (Gershenzon and Croteau 1990).

In developing palmarosa inflorescence, the incorporation of ^{14}C-radioactivity from [U-^{14}C]-labelled sucrose

into essential oil increased until partially opened spikelets (stage II) (figure 1), which indicates that substantial photosynthate is diverted towards essential oil biosynthesis during this period. Thus, immature palmarosa inflorescence is expected to have a high sink capacity; i.e. it has the ability to metabolize incoming sucrose. The capacity of a sink tissue to import is a function of its ability to metabolize incoming photosynthate arriving mainly in the form of sucrose, thereby maintaining an appropriate source to sink gradient of transported photosynthate (Schaffer *et al* 1987). Sucrose synthase and acid invertase are present in both source and sink tissues, and their activities in the sink tissue can be taken as reflection of the mobilization strength of the sink (Khayat and Zieslin 1987). The present study suggested that the soluble acid invertase was the major enzyme involved in sucrose breakdown at all the stages of inflorescence development. The predominant sucrolytic activity varies depending on the species, tissue type, and developmental stage (Aloni *et al* 1997; Ranwala and Miller 1998). High levels of acid invertase have been predominantly found in rapidly growing tissues that undergo active cell division and expansion, such as young seedlings (Arai *et al* 1991), elongating internodes (Zhu *et al* 1997), young leaves (Schaffer 1986; Singh and Luthra 1988), developing fruits (Whittaker and Botha 1997; Drier *et al* 1998) and flower petals (Hawker *et al* 1976; Paulin and Jamain 1982; Woodson and Wang 1987). The high activity of acid invertase (soluble and cell wall bound) during the initial phases of inflorescence growth appears to be necessary in providing metabolites and energy to growing tissues for inflorescence (spikelets) opening and various other biosynthetic (primary and secondary metabolic) processes including essential oil biosynthesis. The sharp increase in soluble acid invertase activity at partially opened spikelets (stage II), where the essential oil biosynthesis (as determined by [U-^{14}C]sucrose incorporation into essential oil) was maximal, emphasizes its role as the principal route by which sucrose is made available for oil biosynthesis. The import of sucrose into petals in cut carnation flowers was high during flower opening and subsequently decreased during petal senescence (Paulin and Jamain 1982). Large amounts of sugars are utilized as substrates for respiration, synthetic purposes and as an osmolyte for maintaining osmotic potential to expand carnation petal cells (Ichimura *et al* 1998), suggesting that invertase probably plays an important role in determining the patterns of assimilate distribution in flowers. Soluble acid invertase activity has also been correlated with dry weight gain in most of the floral organs of Easter lily (*Lilium longiflorum*) (Clement *et al* 1996; Ranwala and Miller 1998).

In palmarosa inflorescence, soluble acid invertase enzyme was thus identified as the major sucrose catabolizing enzyme playing an important role in providing carbon involving the process by which sucrose is being utilized for various biosynthetic purposes including essential oil biosynthesis. However, the variability in the hexose levels may be attributed to the changes in the turnover rates of hexoses, determined by the metabolic requirements of the organ and the growth stage (Singh and Luthra 1988; Ranwala and Miller 1998). As the level of sucrose in palmarosa inflorescence depends upon synthesis, import, hydrolytic activity, respiration and utilization rate of hexoses in other biogenetic pathways, the decrease in sucrose to reducing sugar ratio till fully opened spikelets (stage III) may be attributed to the increased acid invertase activity and higher metabolic activity. Furthermore, the decline in essential oil biogenesis after the partially opened spikelets stage, despite the availability of photosynthates, suggests the involvement of some control mechanisms in essential oil biosynthesis.

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