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Full Length Research Paper

Variations among commercial cultivars of *Vetiveria zizanioides* in the photosynthetic and metabolic characters associated with essential oil accumulation

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Intraspecific variations in four widely cultivated cultivars of vetiver (*Vetiveria zizanioides* L) were analyzed for physiological basis of regulation of essential oil accumulation. The cultivars had the same biosynthetic route of oil accumulation but differed with respect to yield parameters. Among the four cultivars, Gulabi showed highest yield of oil while Kesari incorporated maximum ^{14}C into essential oil indicating efficient biosynthetic use of leaf assimilated metabolites transported to roots. Among the metabolite fraction in root, Gulabi had maximum ^{14}C content in ethanol soluble and ethanol insoluble fraction whereas in leaf sugar, amino acid and organic acid had higher content. The leaf photosynthetic rate showed significant positive association with root biomass. The ^{14}C label in metabolite sugar showed significant positive association with oil content in root, root biomass and oil yield. Efficient cultivar translocated a higher portion of leaf carbon assimilate towards the root and also towards the biosynthesis of essential oil. Thus, the physiological capacity of cultivars in terms of shoots photosynthetic capacity, assimilation of $^{14}CO_2$ and partitioning of metabolites between shoot and root regulate accumulation of essential oil.

Key words: $^{14}CO_2$ incorporation, primary metabolites, shoot-root partitioning, Vetiver.

Introduction

Vetiver (*Vetiveria zizanoides* L Nash) is an economically, pharmaceutically and ecologically important plant. Roots of vetiver yield essential oil that is used as a basic material for perfumery and cosmetics (Champagnet et.al. 2006, Maffei 2002, Massardo et.al. 2006). The biological activity of oil shows antioxidant (Kim et. al. 2005), insecticidal (Zhu et. al. 2001) and antibacterial property. Ecologically the plant has been extensively used for reclamation purpose and for restoring waste contaminated soil (Antiochia et. al. 2007). Vetiver oil is accumulated in secretory cells localized in roots (Maffei 2002). The terpenoid pathway produces the oil. The intermediary metabolites produced in the green leaves by the interaction between cytosolic (mevalonate) and plastid (DOX-P) route are transported to the roots (Giudice.et al.2008). While much less is known about subsequent biosynthesis, regulation and accumulation of oil biosynthesis in the roots (Guidice et.al. 2008).

Since root is the commercially important part, physiological investigation of metabolites partitioning shoot and root is very important. Maffei et.al. (1995) reported enzyme activities and concluded that vetiver has C_4 type of photosynthetic mechanism. Several extrinsic and intrinsic factors control accumulation of oil (Maffei et.al. 2002). Despite the economic importance of the crop, no information is available on the inter-relationship between the primary carbon metabolism and accumulation of oil. A large proportion of leaf

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photosynthate are required for root growth and development which in annual crops as vetiver could be about 30% of the total leaf photosynthate accumulated (Marschner 1986). No such information is available on photoassimilate partitioning to root and the simultaneous incorporation into essential oil.

Useful information for selection programmes can be obtained by studying various physiological and yield characters and their association in high yielding and low yielding cultivars. In the present paper, we report intraspecific variation in four widely cultivated cultivars of vetiver for carbon assimilates partitioning in terms of primary metabolite between shoot and root and in essential oil.

Materials and methods

Treatment

Treatments were four cultivars each replicated thrice. And experiment was in Randomized Block design.

Plant material

Slips of four cultivar of vetiver- KS-1, Kesari, Dharni and Gulabi were obtained from the farm nursery of Central Institute of Medicinal & Aromatic plants. The cultivar KS1 was released initially. One slip was planted in each pot (10L capacity) containing uniformly mixed soil and farmyard manure in a ratio of 3:1. The plants six of each cultivar were maintained in a glass house. Harvesting was done 12 months after planting.

Photosynthetic efficiency data

Carbon-di-oxide exchange rate (CER) - CER of the third leaf of the main stem of each cultivar was measured using a photosynthesis system model CI-310 PPS (CID Instrument, USA) (Srivastava and Srivastava 2007).

Chlorophyll content – A known weight of third leaf tissue of main stem of each cultivar was extracted with 80% acetone and absorbance recorded on a Helios spectrophotometer (Thermoelectron Corporation, UK) and chlorophyll content calculated according to method of Arnon (1949).

Extraction of essential oil

For determining ^{14}C in essential oil, a known weight of $^{14}CO_2$ fed root were subjected to hydro distillation in a Clevenger's apparatus (Clevenger 1928). The volatile oil was eluted from the Clevenger apparatus and was further extracted by ether to remove traces of oil which may have remained attached to the glass. The ^{14}C label in the oil was determined in a scintillation counter (Wallac 1409, USA) using a PPO-POPOP toluene cocktail (Srivastava and Luthra 1994).

Tracer studies

For $^{14}CO_2$ feeding, plants of each cultivar (with intact roots) were placed in a Plexiglass chamber (20L capacity) around a central vial containing $Na_2{}^{14}CO_3$ solution (activity 1.85 MBq, specific activity- 1.628 GBq/mol) obtained from the isotope division of Bhabha Atomic Research Center, Mumbai, India. $^{14}CO_2$ was liberated by injecting 4ml of $2MOL.H_2SO_4$ into the carbonate solution through a PVC inlet tube and uniformly distributed within the chamber using a small electric fan. Plants were initially exposed to $^{14}CO_2$ for 4h. After 4 h, a saturated solution of KOH was run into the central vial to absorb remaining $^{14}CO_2$. The pots were then removed from the chamber and the plants allowed assimilating $^{14}CO_2$ for next 20h.

After the exposure time, plants were carefully uprooted from the soil and separated into shoots (total aerial parts) and roots. Each of the parts i.e. shoots and roots were processed for determining ^{14}C label into major primary metabolic fractions such as Ethanol Soluble (ES), Ethanol Insoluble (EIS) and Chloroform Soluble (CS). Simultaneously the biosynthetic capacity to utilize currently assimilated metabolites into essential oil by roots was determined by quantifying ^{14}C label into essential oil in roots.

A known weight of shoot and root biomass was fixed immediately in boiling ethanol to maintain the metabolic status. The fixed material was ground in ethanol, filtered and dried. The dried material was extracted in a known volume of distilled water and this aqueous phase was termed as ES fraction. The unfiltered ground material was further hydrolyzed by enzyme diastase in 0.05M acetate buffer (pH 5.2) at 50ºC and the fraction termed as EIS fraction. The aqueous phase was further extracted with an equal volume of chloroform and this fraction termed as CS fraction. ^{14}C content in ES and EIS fraction were measured using Bray's scintillation fluid while in CS fraction using PPO-POPOP toluene cocktail in a liquid scintillation counter. ES fraction was further separated into metabolic pool through amberlite ion exchange column chromatography into neutral (sugars), acidic (organic acids) and basic (amino acids) fractions. The ^{14}C contents in eluates after column chromatography were measured using Bray's scintillation fluid in a liquid scintillation counter (Wallac 1409, USA) (Srivastava et al. 2004, Srivastava and Luthra 1994). Actually there are many similar analyses into leaves and roots so it appears that many experiments are lumped together which actually are not.

Table 1: The oil content, root biomass and oil yield of four cultivars of Vetiver.

Cultivar	Oil content (%)	Root biomass (g/plant)	Oil yield (g/plant)
KS 1	0.81	122.5	0.99
Kesari	0.92	150.0	1.27
Dharni	1.07	225.0	2.36
Gulabi	1.65	245.0	4.04
CD 5% CD1%	0.38 0.64	50.35 83.32	0.48 0.80

CD 5% and CD1% are statistical terms defining values at 5% and 1%

Table 2 Leaf chlorophyll content and CO_2 exchange rate of four cultivars of Vetiver.

Cultivar	Chlorophyll (chl.) content (mg/g leaf Fresh wt)			CO_2 exchange rate (µmole $CO_2/m^2/s$)
	Total	chl. a	chl. b	
KS 1	1.22	0.92	0.29	6.25
Kesari	1.33	1.01	0.31	7.82
Dharni	1.28	0.96	0.29	8.03
Gulabi	1.27	0.96	0.30	7.93
CD 5% CD 1%	0.29 0.49	0.26 0.43	0.07 0.13	1.06 1.70

CD 5% and CD1% are statistical terms defining values at 5% and 1%

Statistical analysis

The mean data collected for various parameters were subjected to simple statistical analysis. Mean and correlation (simple) were done using statistical software available at Genetics, Plant breeding and statistical department (Singh and Chaudhary 1979).

Results and discussion

Root parameters

The cultivars showed differences among root biomass with cultivar 'Gulabi' having highest root biomass and cultivar 'KS1' showing lowest biomass. Similarly, root oil content was highest in cultivar 'Gulabi' and lowest in cultivar 'KS1' (Table 1). As a result, the oil yield was highest in cultivar 'Gulabi' compared to lowest in cultivar. The values given are of the highest and lowest yielding variety, the values of the other two are self explanatory in table below

Leaf characters

The lowest CO_2 exchange rate (CER) was found in 'KS1' and highest in 'Dharni' while 'Kesari' and 'Gulabi' had nearly similar values. Chlorophyll content (total as well as chl. a) was highest in 'Kesari' (Table 2). Thus, vetiver cultivars differed in photosynthetic efficiency and chlorophyll content.

$^{14}CO_2$ uptake, incorporation and distribution

The efficiency of photosynthetic carbon assimilation and the partitioning of photoassmilates to essential oil biosynthesis were estimated by measuring the uptake of $^{14}CO_2$ and analyzing the amount of tracer in number of metabolic pools in shoots, roots and in oils of roots. Determination of ^{14}C content in oil showed that all the three new cultivars have more label in oil. However, ^{14}C content in oil of Kesari is about five times more that KS1 indicating efficient biosynthetic utilization of leaf photoassimilate than other cultivars (Table 3).

Shoots of plant Gulabi fixed much more $^{14}CO_2$ than those of other cultivars and accumulated more in soluble than in insoluble photoassimilate fraction. Ethanol soluble fraction refers to fraction utilized in various metabolic pathways. Insoluble fraction (immobile portion) is that which forms the structural component and is not available for metabolic pathways. In the present study since enzyme diastase was used for hydrolysis only carbohydrates components could be identified.

Table 3: Incorporation of ^{14}C into essential oil in roots of four cultivars of Vetiver. All values in Bq/g root dry wt.

Cultivar	^{14}C in essential oil
KS 1	0.93
Kesari	4.93
Dharni	1.88
Gulabi	1.04
CD 5%	6.22
CD 1%	10.29

CD 5% and CD1% are statistical terms defining values at 5% and 1%

Table 4: The assimilation and partitioning of $^{14}CO_2$ into major metabolic fractions in shoots of four cultivars of Vetiver. All values in Bq/g shoot fresh wt.

Cultivar	Ethanol soluble fraction	Ethanol insoluble fraction	Chloroform soluble fraction
KS 1	69.36	22.78	43.43
Kesari	219.26	55.57	47.50
Dharni	213.51	61.94	105.24
Gulabi	453.49	83.84	33.97
CD 5%	338.43	51.41	154.66
CD 1%	560.00	85.08	255.92

CD 5% and CD1% are statistical terms defining values at 5% and 1%

Table 5: The distribution of ^{14}C assimilates into primary metabolic pool in shoots of four cultivars of Vetiver. All values in Bq/g shoot fresh wt.

Cultivar	Sugars	Amino acids	Organic acids
KS 1	5.45	4.17	4.23
Kesari	16.78	3.57	5.58
Dharni	13.31	3.72	6.54
Gulabi	48.56	4.05	7.85
CD 5%	36.63	1.50	3.02
CD 1%	60.61	2.40	5.00

CD 5% and CD1% are statistical terms defining values at 5% and 1%

(Srivastava et al. 2003). Chloroform soluble fraction (that contain pigment and terpenoid derived metabolites) was however highest in plant Dharni (Table 4).

Thus, the amount and proportion of label in these primary fractions differed among cultivars. Incorporation of ^{14}C label in metabolites into sugars was highest in plants of Gulabi followed by Kesari, Dharni and lowest in KS1. Incorporation into organic acids was also highest in Gulabi while label in amino acids was highest in KS1 (Table 5).

Incorporation of ^{14}C label translocated to roots indicate higher amount of distribution in plant Gulabi in ethanol soluble, ethanol insoluble, while chloroform soluble fraction was highest in plants of Dharni. In other plants the ^{14}C label in these fractions were lower (Table 6).

Incorporation of ^{14}C label in metabolites into roots was highest in sugars in plants of Gulabi while in amino acids and organic acids the label was high in plant of KS1 (Table 7).

Correlation analysis showed that leaf CER was significantly positively correlated with root biomass yield ($r = 0.766$). The ^{14}C content in ES fraction in leaf showed a significant positive association with root oil content ($r = 0.962$), root biomass ($r = 0.827$) and oil yield ($r = 0.931$). Carbon assimilation in leaf shows significant positive association with root oil content ($r = 0.971$), root biomass ($r = 0.739$) and oil yield ($r = 919$) (Table 8).

Abbreviations

TC-total chlorophyll, CA-chlorophyll a, CB-chlorophyll b,

Table 6: The distribution of ^{14}C assimilates into primary metabolic pool in roots of four cultivars of Vetiver. All values in Bq/g shoot fresh wt.

Cultivar	Sugars	Amino acids	Organic acids
KS 1	5.45	4.17	4.23
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CD 5%	36.63	1.50	3.02
CD 1%	60.61	2.40	5.00

CD 5% and CD1% are statistical terms defining values at 5% and 1%

Table 7: The distribution of ^{14}C assimilates into primary metabolic pool in roots of four cultivars of Vetiver. All values in Bq/g shoot fresh wt.

Cultivar	Sugars	Amino acids	Organic acids
KS 1	5.45	4.17	4.23
Kesari	16.78	3.57	5.58
Dharni	13.31	3.72	6.54
Gulabi	48.56	4.05	7.85
CD 5%	36.63	1.50	3.02
CD 1%	60.61	2.40	5.00

CD 5% and CD1% are statistical terms defining values at 5% and 1%

Essential oil biosynthesis and accumulation results from integration of different metabolic pathways. Each pathway requiring continuous production of precursors, their transport and translocation to the active site of synthesis. In vetiver, this metabolites transport has to take place from leaves to roots where oil is accumulated. Feeding studies revealed that improved cultivars Kesari, Dharni and Gulabi retained nearly 80% of fixed photosynthate in shoots and transported nearly 20% of assimilate to the roots. As a result assimilates were continuously available to the biosynthetic pathway in root. The other possibility is that biosynthetic efficiency of assimilate utilization is very efficient in these developed cultivars. Partitioning of ^{14}C-photosynthate of leaves in roots, rhizome and in essential oil and curcumin in turmeric (*Curcuma longa* L.) revealed that youngest leaves were most active in fixing ^{14}C at 24h. Fixation capacity into primary metabolites decreased with leaf position and time. The primary metabolite levels were maximal in sugars and organic acids and lowest in amino acids. Roots as well as rhizome receive maximum photoassimilate from leaves at 24h, this declined with time. The maximum metabolite concentrations in the roots and rhizomes were high in sugars and organic acids and least in amino acids. ^{14}C concentration into oil in leaf and into rhizome was maximal at 24h and then declined with time. These studies highlight importance of time dependent translocation of ^{14}C–primary metabolites from leaves to roots and rhizomes and their subsequent biosynthesis into secondary metabolite, curcumin in rhizome (Dixit and Srivastava 2000). Physiologically essential oil biosynthesis is highly dependent on the balance between carbon assimilation and utilization of photoassimilates as reported in other essential oil bearing plant as peppermint. In a previous report on peppermint, incorporation of $^{14}CO_2$ by leaves was highest into sugars followed by that in organic acids, amino acids and essential oil at all stages of leaf development (Srivastava and Luthra 1991a). Studies among variations in commercial cultivars of mints for metabolite characters associated with essential oil yield showed that efficient cultivars translocated a greater portion of metabolite towards essential oil (Srivastava et al. 2003). The photosynthetic capacity *per se* and translocation of assimilates to roots are important for growth and oil production but processes that follow photosynthesis namely respiration, photorespiration and biosynthetic efficiency to utilize assimilate can be major determinants of productivity.

CONCLUSION

Intraspecific variations in four widely cultivated cultivars

of vetiver (*Vetiveria zizanoides* L) were analyzed to understand the physiological basis of regulation of essential oil accumulation. Among the four cultivars, Gulabi showed maximum oil content, root biomass and hence oil yield. ^{14}C feeding studies showed that cultivar kesari efficiently utilized leaf-assimilated photoassimilate into oil biosynthesis. Considering the overall photosynthetic efficiency, shoot to root photo-assimilate translocation and their biosynthetic utilization are the major determinants of oil productivity.

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