In vitro study on production and histochemical localization of essential oils produced in root plantlet of java vetiver (*Vetiveria zizanioides*)

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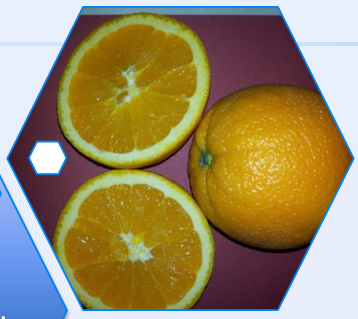
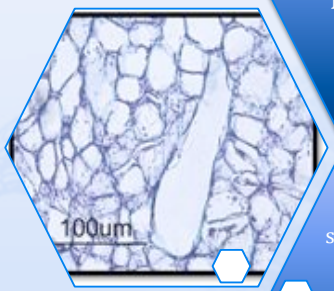
Plants as Chemical Factories

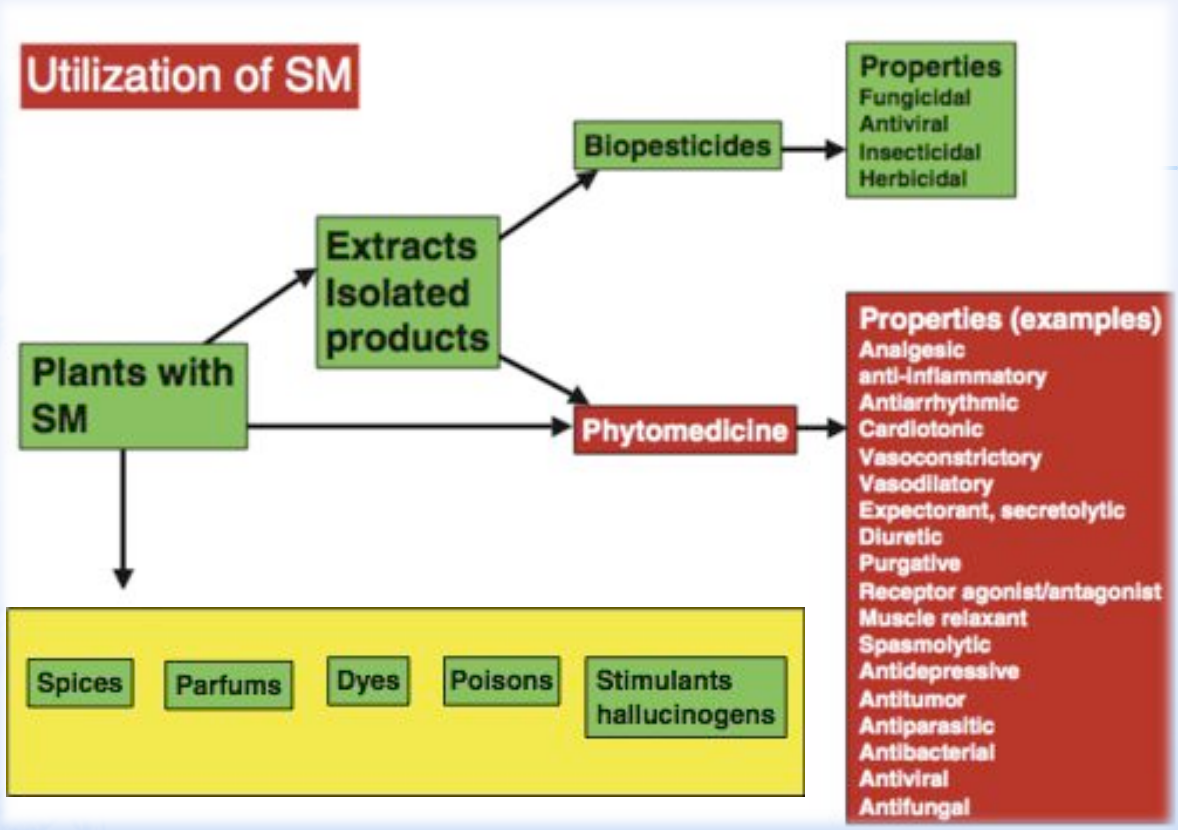


Food, beverages, paper, timber, textiles, oils etc.



Plant secondary metabolites

The cells of roots, stems, leaves, flowers, and fruits produce conspicuous resources : a vast array of potent chemicals that people use every day.



The use of plant secondary metabolites

(Wink, 2009)

Essential oils

- Essential oils are volatile aromatic compounds that are used in flavors, fragrances, and in aroma therapy for health purposes(Oyen and Dung 1999).
- The essential oil is named after the aromatic plant from which it has been derived.
- They are often stored in specialized storage and secretory sites like glandular hairs, resin canals or oil ducts and schizogenous glands (Banthorpe 1988; Charlwood and Charlwood 1991).
- Essential oils may be found in any part of a plant and can be extracted from roots, wood, bark, leaves, flowers, fruit and seed for commercial purposes (Oyen and Dung 1999).

Essential oil-producing plants

Lamiaceae (mint, basil, lavender)

Vetiver (*Vetiveria zizanioides*) One of plant species from Poaceae that can produce an essential oil

Rosaceae, and Poaceae (Kayser & Quax, 2002).

Vetiveria zizanioides (vetiver)

Perennial tropical grass, belongs to the family of Poaceae (Gramineae)

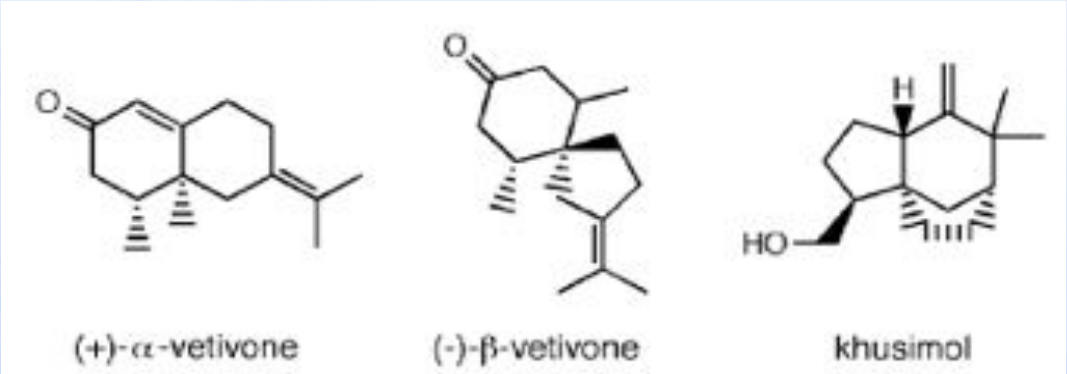
<u>Use of vetiver</u> *:*

- Protection against erosion →its densely packed, stiff and tough grass stems and the deeply penetrating root system
- Roots contain an essential oil, called vétiver oil.
- Vétiver is highly tolerant to arsenic, cadmium, chromium, copper, lead, nickel and zinc in the soil ➔ suitable for the rehabilitation of lands contaminated with these elements
- Medicinal applications. In medicine, both the plant and its essential oil are used. Vetiver oil - antifungi, antimicrobial, dan antioxidant

(Adams et ai. 1998; ; Chou et al., 2012; de Guzman and Oyen 1999; Devprakashet al., 2011; Kim et al., 2005; Vietmeyer and Ruskin 1993).

Vetiver oil

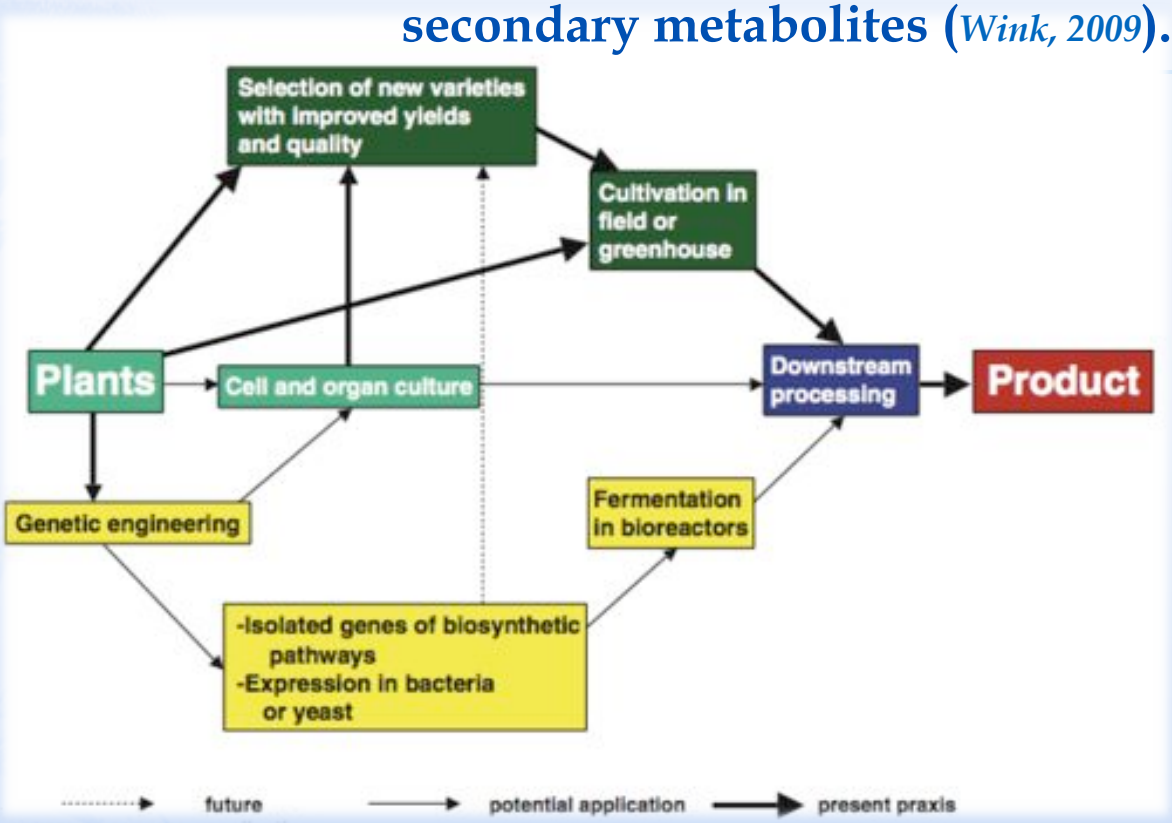
- Composed of 300 components ➔ cyclic dan bicyclic sesquiterpenoid, hydrokarbon, alcohol, keton, aldehyde and acidic substance (Cazasaussus *et al*., 1988; de Guzman dan Oyen, 1999; Vietmeyer dan Ruskin, 1993), monoterpen (Nikiforov *et al*., 1992) and phenolic compound (Shibamoto dan Nishimura, 1982).
- Main component that influence vetiver oil quality ➔ nootkatone, such as (+)-a-vetivone, (-)-ß-vetivone and khusimol (de Guzman and Oyen, 1999; Demole *et al*., 1995).



- Naturally, vetiver can be propagated using a rhizome ➔ long period of vegetative propagation
- In vitro culture of vetiver can be used as an alternative method for
  - plant improvement
  - secondary metabolite production

(Mucciarelli & Leupin, 2002).

Strategies for the production of secondary metabolites (*Wink, 2009*).



Research purposes

- To establish plantlet of *Vetiveria zizanioides*.
- To compare composition of extract of Vetiver oil in root of plantlet and root of *in vivo* plant
- To examine localization of vetiver oil in root cell of *V. zizanioides* by using histochemistry method.

Materials and methods

Explants

Root culture

Callus culture

Shoot induction

Shoot multiplication

Plantlet regeneration

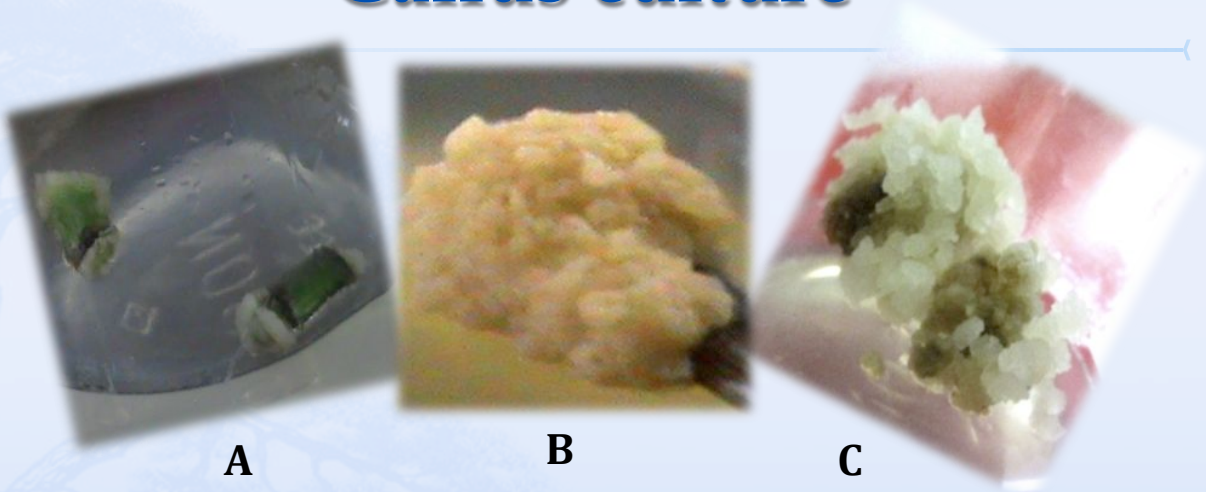
Acclimatization

root

Metabolite analysis

- GC-MS
- Histochemistry

Callus culture

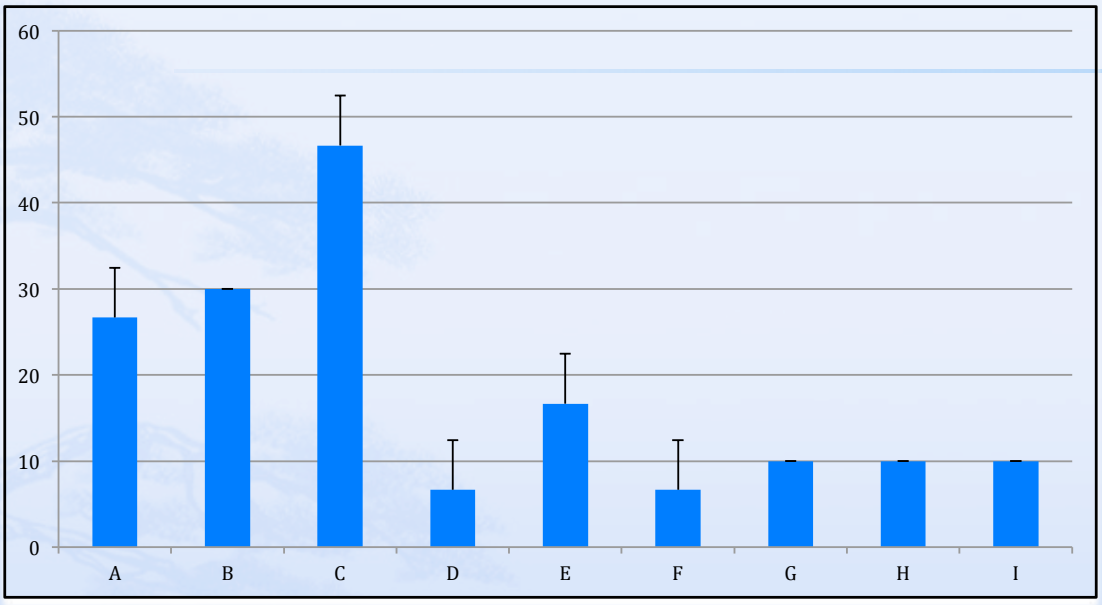


A B C

Callus regeneration from explant of vetiver following culture in MS media supplemented with 2,4-D, IAA and kinetin. A. Callus developed at cutting edge area of explant. B. Yellowish friable callus. C. White friable callus.

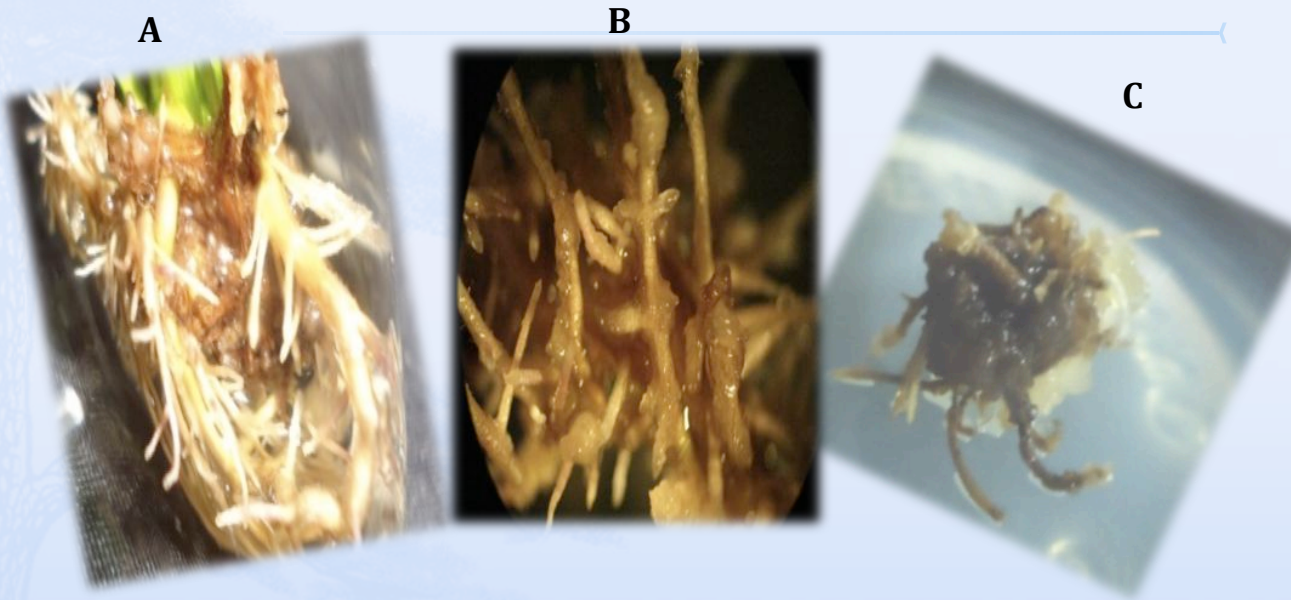
Morphology of callus developed on MS media supplemented with several combination of growth regulators

Growth regulators (ppm)			Percentage explant producing callus (%)	Callus morphology
2,4-D	IAA	Kinetin		
1	1	1	26,67	Friable
1	2	2	30	Friable
2	1	1	46,67	Friable
2	2	2	6.67	Compact
1	4	4	16.67	Friable
2	4	4	6.67	Compact
4	1	1	10	Compact
4	2	2	10	Friable
4	4	4	10	Compact



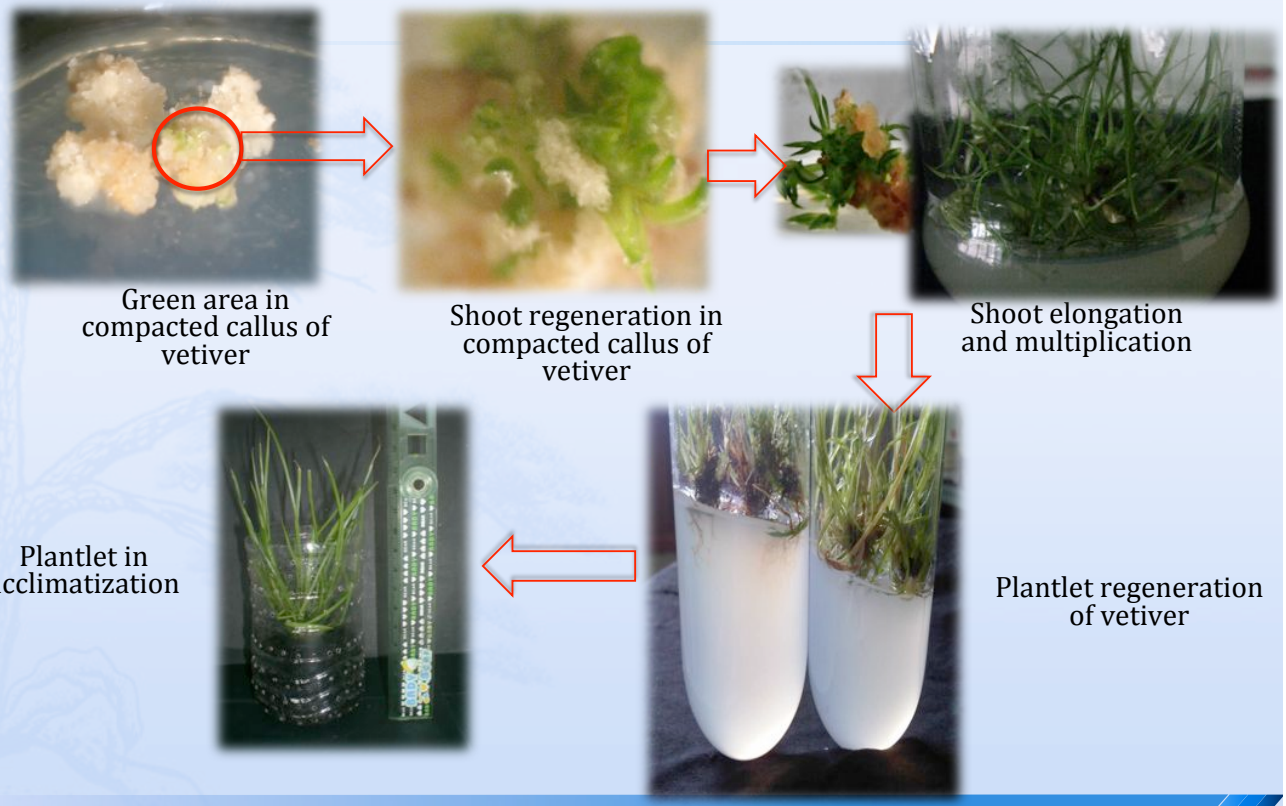
Percentage of callus forming-explants in MS media supplemented with combination of growth regulators (2,4-D; IAA; KIN)

Root culture

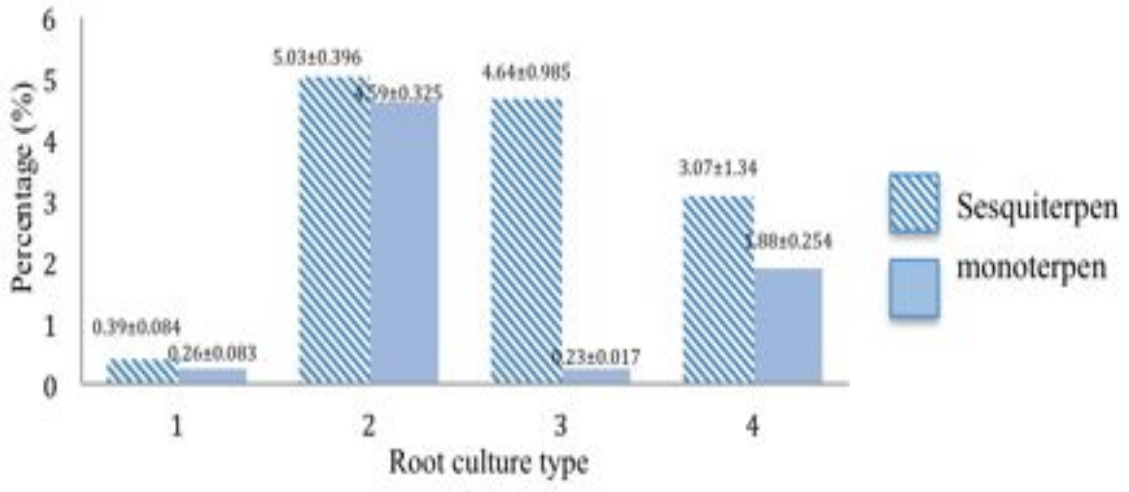


Adventitious root formation at the basal shoot explant (A & B) and rooted callus (C) of vetiver following cultured in MS medium supplemented with 1 ppm NAA or 0.5 ppm NAA

Plantlet regeneration

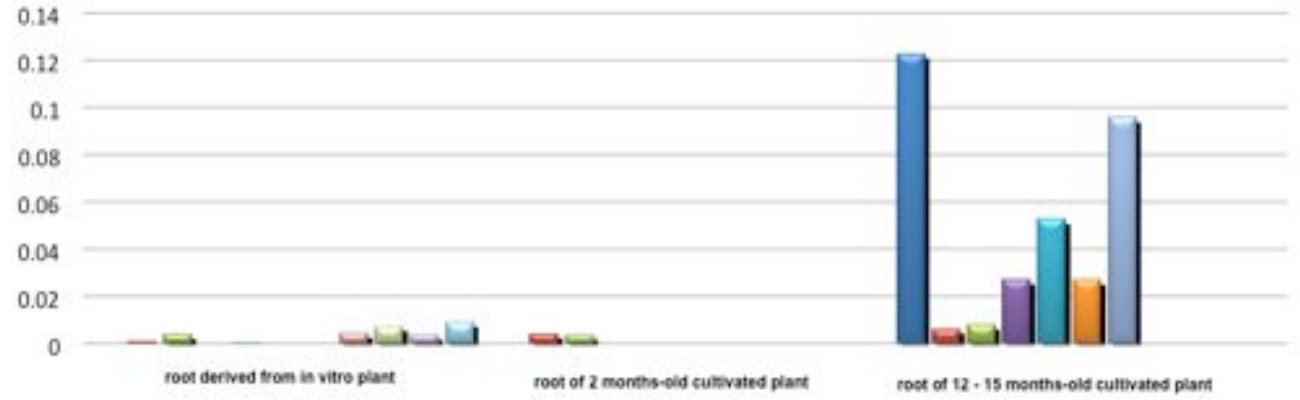


METABOLITE ANALYSIS



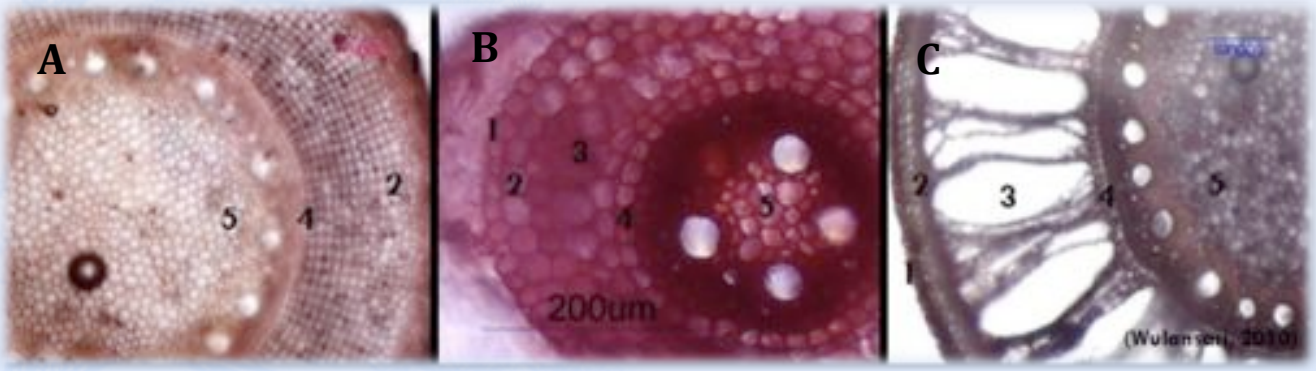
Sesquiterpen and monoterpen concentration on root culture of *Vetiveria zizanioides.* (1) root tip-derived root culture. (2) shoot basal-derived root culture. (3) callusing root culture. (4) adventitious root culture.

Composition of vetiver oil in root from *in vitro* culture and cultivated plant of *Vetiveria zizanioides*

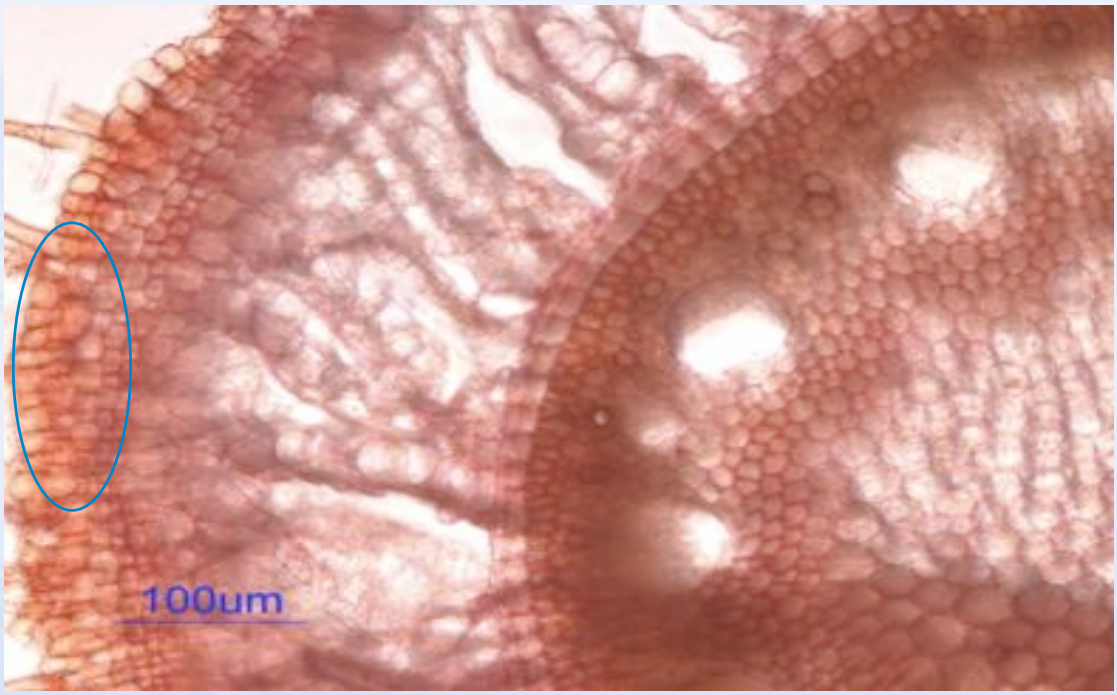


- phenol,2,4,6-tris (1-methylethyl)
- 2-Naphtalenemethanol,decahydro-alpha.,.alpha.,4-trimethyl-8-methylene, [2R(2.alpha.,4a.alpha.,8a.beta.)]
- 2-Naphthalenemethanol, 1,2,3,4,4a,8a-hexahydro-.alpha.,.alpha.,4a,8 tertramethyl-, [2R-(2.alpha.,4a.alpha.,8a.alpha.)]-
- 1H-3a,7-Methanoazulene-7(1H)-one,octahydro-4,8,8-trimethyl-6-methylene-
- 1,4-methanoazulen, octahydro-3,8,8-trimethyl-6-methylene-,[3R(3.alpha.,3a.beta.,7.beta.,8a.alpha.)]
- isoledene
- beta.vatirene
- Ylangene
- Cedrene
- Junipene
- bicyclosesquiphellandrene

Histochemistry



Root cross section of *Vetiveria zizanioides.* A. A month-old of cultivated plant. B. 5-month-old plant derived from *in vitro* culture. C. 12-15 month-old cultivated plant.



Root cross section of *Vetiveria zizanioides* showing positive reaction with neutral red, a reagent for terpenoid detection

Conclusions

- Plantlet of *Vetiveria zizanioides* has been successfully regenerated from basal shoot explant following cultured in MS media supplemented with 2,4-D, IAA and kinetin for callus induction, MS + BAP for shoot induction and multiplication and MS + IBA for root induction. Plantlet showed high survival rate during acclimatization
- Quantitative and qualitative analysis of metabolite showed that the component of vetiver oil could be detected in root culture
- Root from 2-month old *in vitro* plant of *Vetiveria zizanioides* could produce vetiver oil.
- *In vitro* culture is a potential method for plant regeneration, as well as for essential oil production of *Vetiveria zizanioides*