VETIVERIM

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Editorial

Why and Why Not Vetiver?

Why Vetiver?

For soil and water conservation, stabilization (of backslope, sideslope, sand dune, river bank, embankment), environmental protection (pollution control, heavy metal removal), wastewater treatment, disaster mitigation, etc., vetiver has been chosen in lieu of so many other conventional methods and plants. This is because vetiver has many characteristics that lend itself to be used to do the job much better and cheaper than others. Above all, the vetiver system is a low cost, low-tech, and environmental friendly technique.

Why Not Vetiver?

The term 'vetiver grass' has been used in the literature from the very beginning, as this is a common practice in naming a plant under a group to which it belongs. Since vetiver belongs to the grass family (Poaceae), it is usually called 'vetiver grass', in the same way as other grasses such as napier grass, ruzi grass, Bermuda grass, etc., are called. There is a census of opinion, however, that the word 'grass' be dropped from the term to save space and time, and to change the tune of misconception in some countries where the word 'grass' has a notorious meaning of being useless. *So, why not just 'vetiver' (in stead of 'vetiver grass')?*

Another point to be raised is the capitalization of the word. Being used as a common name, it should not be capitalized (i.e. Vetiver), as is customarily written by some authors. In fact, simply writing (mainly typing) it as 'vetiver' is easier than 'Vetiver', as one does not have to shift the typing bar when typing the letter 'v'. *So, why not just 'vetiver' (in stead of 'Vetiver')?*

The last point to be raised is the employment of hard-engineering method in various activities such as embankment stabilization, road construction, wastewater treatment, etc. Hard engineering utilizes concrete and steel, aeration, and lots of energy. The approach is not environment friendly. Soft engineering through the use of vetiver is a low technology and environment friendly. *So, why not 'vetiver', a soft engineering (in stead of hard engineering method)?*

Interim Report on New Cold Tolerant Vetiver Cultivar through Biotechnology*

1. Introduction

In vitro culture is a basic-research means of genetic transformation, and is also a technology pre-condition of modifying any plant's species quality. Somatic embryogenesis is one of the ways of plant regeneration from culture *in vitro*, and is also an effective approach to improve regeneration frequency. In order to improve genetic quality of vetiver, especially in the aspect of cold tolerance, the author carried out research on somatic embryogenesis of vetiver and its cytology, especially the characters and formation conditions of somatic embryogenesis under the financial support of the Wallace Genetic Foundation through The Vetiver Network, the Natural Science Foundation of China (No.30370233), and South China Botanic Garden, the Chinese Academy of Sciences. In addition, the factors of somatic embryogenesis and plant regeneration of vetiver have also been investigated.

2. Materials and Methods

2.1 Materials: The experimental materials contained five cultivars, 'Kandy', 'Karnataka', 'Malaysia', 'Sunshine', and 'Zomba'; they are native to Sri Lanka, India, Malaysia, the US, and Malawi, respectively, and all are regarded as good ones for their locality. The five cultivars were first introduced into an experimental nursery in the US by Mr. Mark Dafforn and Dr. Robert P. Adams for a period of time, and then sent to the author in July 1999. Afterwards, they have been grown in the nursery of the South China Botanic Garden in Guangzhou, China. During the past years, 'Karnataka' was found to have the fastest tiller-forming speed and the shortest plant height, while 'Zomba' had the slowest tiller-forming speed in the nursery. (This result was reported in the Vetiverim 22:9-14.)

2.2 Explant: Two kinds of explants were used in this experiment for *in vitro* culture of vetiver. One was nodes with axillary buds of the plant sampled from the field and the other was aseptic adventitious buds from organogenesis of cultured materials in the test tube. When the former was used, the stalk with nodes was cut and its sheath shucked off. The nodes with axillary buds were intercepted and their surface was disinfected with 70% ethanol, 20% hypochlorous natrium and 0.1% chloridize mercury. Finally, the explants were inoculated onto the media. When the latter was used, adventitious buds were taken out from the test tube under axenic condition and then inoculated as the former.

2.3 Culture medium: The basal culture medium was MS medium. It was supplemented with 6-BA and IBA when inducing organogenesis; then further supplemented with 2,4-D and different confecting proportions of other growth regulators (such as auxin and cytokinin) when subculturing and inducing embryogenic calli; and again supplemented with kinetin, 6BA and NAA when inducing plants regeneration.

2.4 Differentiation, transplanting and planting of regeneration plants: Embryonic calli were transferred onto the differentiation medium and then illuminated for 12 h/d (1 200 lx). The incubation temperature was kept at 25±2°C. After developed from embryonic calli, the regeneration plants were transferred onto rooting medium for about two weeks, and then potted. Plantlets were planted on dry land when they were 20 cm high. They were irrigated and their growth performance was observed after they became green.

3. Results

3.1 Setting up effective regeneration system: Through *in vitro* culture of axillary buds, a perfect vetiver somatic embryogenesis and plant regeneration technology was set up. The results showed that MS + 2,4-D (2.0 mg/L) + KT (0.5 mg/L) are the proper inducing medium and subculture medium; MS + 6BA (1.0 mg/L) are the proper differentiation medium; and 1/2MS +

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IBA (0.1 mg/L) + MET (Poclobutrazol) (0.1 mg/L) are the proper rooting medium. Up to now, more than 10,000 cloning plants of vetiver have been obtained, and some mutants were found among them. The longest subculture time of embryogenic calli have reached 19 generations. Furthermore, a batch of newly-induced embryogenic calli (one generation) was also obtained. The establishment of vetiver regeneration system provides a staunch basis for its genetic transformation.

3.2 Exploring the factors of somatic embryogenesis and plant regeneration: In order to establish the vetiver regeneration system, a series of experiments have been conducted to study the factors of somatic embryogenesis and plant regeneration.

3.2.1 Somatic embryos inducing condition: Six media containing different proportions of auxin and cytokinin were used to induce somatic embryos of two culture materials (non-embryogenic calli and aseptic adventitious buds). When there was no 2,4-D added, somatic embryo was not developed on the medium regardless of the concentration 6-BA (0.5 or 1.0 mg/L) (Table 1). On the contrary, when 2,4-D was added, somatic embryos always developed whether or not 6-BA was added. Moreover, the medium without 6-BA had the highest inducing frequency (96.7%) for somatic embryos. When the content of 2,4-D reached 4.0 mg/L, 62.2% aseptic adventitious buds could not be induced to produce somatic embryos. The best proportion of 2,4-D and 6BA for induction of adventitious buds was 2:1 with inducing frequency of up to 79.5%. The quality of most somatic embryos was very good.

Integrating vetiver's reaction to growth hormone (2,4-D) and cell division hormone (6-BA) in the medium when it was in *in-vitro* culture, it could be inferred that explants (axillary buds) would form regeneration plants via organogenesis (adventitious buds) when the medium did not contain growth hormone but contained cell division hormone. On the contrary, explants would form regenerated plants via somatic embryogenesis when the medium contained only 2,4-D without or only a little 6-BA. This indicates that 2,4-D is really the important factor for graminaceous plants to induce somatic embryogenesis when cultured *in vitro.*

Table 1. Effect of different confecting proportions of auxin and cytokinin on inducing somatic embryos in vetiver

Culturing material		Code of medium	2,4-D (mg/L)	6-BA (mg/L)	Status of somatic embryos
Kinds	Amount (P)				
C	90	8	0	0.5	No E-callus
B	72	8	0	0.5	Only buds and leaves developed, no E-callus
C	93	9	0	1.0	No E-callus
B	64	9	0	1.0	Only buds and leaves developed
C	90	10	0.5	0	E-callus very well developed; only 3.3% of pieces of calli were not E-callus
B	70	10	0.5	0	Only 10% buds developed into calli, but none was E-callus
C	97	11	1.0	0.5	E-callus well developed, and 14.1% of pieces of calli had no E-callus
B	77	11	1.0	0.5	21.5% aseptic adventitious buds had no E-callus, and the majority of the others developed into very good E-callus
C	90	12	2.0	1.0	E-callus well developed; only 9.9% of pieces of calli had no E-callus
B	78	12	2.0	1.0	61.5% of buds had no E-callus
C	92	13	4.0	2.0	E-callus well developed; 15.4% of pieces of calli had no E-callus
B	90	13	4.0	2.0	62.2% of buds had no E-callus

Legend: P = piece; C = non-embryogenic callus; B = aseptic adventitious bud; E-callus = embryogenic callus

3.2.2 Difference on inducing frequency of embryonic calli of different cultivars: Table 2 shows that there were prominent differences among the inducing frequency of embryonic calli of different vetiver cultivars. 'Zomba' had the highest inducing frequency, up to 96.7%, followed by 'Karnataka', 93.1%, while 'Malaysia' assumed the lowest frequency of less than 30%. However, all cultivars could be induced to form calli at different levels from nearly 30% to 93% (Plate I: Figs.1-5). This result offers the basis to conduct further experiments using different cultivars.

Table 2. Inducing frequency of calli among vetiver cultivars*

No.	Cultivar	No. of explant	No. of calli (piece)	Frequency of calli induction (%)
1	'Kandy'	117	84	71.7
2	'Karnataka'	188	175	93.1
3	'Malaysia'	102	28	27.4
4	'Sunshine'	100	69	69.0
5	'Zomba'	237	229	96.7

** Same medium for all cultivars; data were from two replicates and investigation was conducted 15 days after inoculation.*

3.2.3 Effects of low temperature condition on plant regeneration ability of embryonic calli: In order to breed and screen out cold-resistant material, three groups of embryonic calli of different generations, all coming from 'Karnataka', were put into incubators with 3-7^{o}C and then incubated in the sun for 12 h/d (1 200 lx) with different low temperature times of 98, 82 and 82 days (Table 3).

Table 3. Effects of low temperature treatment (3-7℃) on differentiation frequency of embryogenic calli in vetiver 'Karnataka'

Generation	Duration (day)	No. of calli (piece)	Status of differentiation		
			No. of differentiation calli	Differentiation frequency (%)	Regenerated plantlets (cluster)
6th	98	84	34	40.5	78
13th	82	98	49	50.0	90
16th	82	280	135	48.2	235

Table 3 shows that parts of embryogenic calli would be no longer differentiated or even died, but some 40.5-50.0% of them still kept regeneration power under the 3-7°C low temperature condition (Plate I: Fig. 6). Further test should be done to confirm whether or not these embryogenic calli stressed by low temperature have a stronger ability to tolerate cold.

3.2.4 Plant regeneration of somatic embryos and their regeneration ability that may be kept longer: After embryogenic calli were transferred to the differentiation medium, they began to turn green and gradually germinated in two weeks, and then developed into plantlets. The embryonic structure and its regeneration ability can be kept longer under the subculture condition. The pictures (Plate I: Figs. 7-9) show that the growth status of regeneration plants grew quite well from the non-subculture (0 generation) to the 15th generation, which is impossible for other graminaceous plants, such as rice. Table 4 shows that embryogenic calli subcultured for two years still had a very strong regeneration ability.

Table 4. Effect of subculture duration on regeneration frequency of embryogenic calli in 'Randy'*

Subculture duration (month)	No. of E-calli (piece)	Regenerated plantlets (cluster)	Regeneration frequency (%)
18	400	368	92.0
20	750	695	92.7
22	350	300	85.7
24	750	612	81.6

** Data are from count every two months in recent six months; clustered plantlets regenerated from same callus are calculated as one cluster.*

3.3 Preliminary observation of somaclonal variation: So far, many variations have been observed among over 10,000 clonal plants, including plant type (from erect to creeping one), plant height (during the middle seedling phase), leaf color (from green to purple), leaf shape (from lance shape to curle shape), etc. (Plate I: Figs. 10-11). All these above-mentioned differentiations are beneficial, thus further research will be done to ascertain if these variations derive from gene mutation and what kind of gene mutation it is.

3.4 Cytology observation on somatic embryogenesis: In order to ascertain the cyto-architecture of somatic embryos, they were taken out from the medium and then dipped in the fixation liquid containing carbinol-ice acetic acid (3:1). Thereafter they were sliced up according to general methods of olefin slice, whose thickness was 8 μm, and then stained with Ehrich-hematoxylin. After mounting, they were observed and taken pictures under microscope to systematically observe cytological feature of somatic embryogenesis (Plate II).

3.5 Allopatric planting of the somaclonal variants: Through cooperation with the Changzhou Biological Produces, Co. Ltd. in Hebei Province, North China, vetiver seedlings obtained from the above experiment were planted at the company's nursery in last August. Their growth status and process have been recorded and observed carefully. A detailed investigation will be made after the northern winter is over.

4. Further Plan of Action

The following activities will be conducted in the future:

- Continue to establish and preserve the regeneration system of embryonic calli of vetiver.
- Continue to observe and record the existing materials, especially the mutants, and set up an information data bank.
- Chemical induction: investigate the treatment effects of different concentrations of EMS on lethality rate, regeneration frequency and mutation frequency of embryogenic calli, etc.
- Establish genetic transformation technology applying to vetiver: extrinsic genes (trehalose-6-phosphate synthase) are introduced into vetiver by *Agrobacterium*-mediated; gene gun is also considered to be used simultaneously.
- Transplant and plant the regeneration seedlings that have been treated and transformed genetically in the nursery of South China Botanic Garden and in a cold region (Changzhou, Hebei Province) simultaneously, and then observe their growth habits and record them.
- Set up a demonstration project using the newly-bred vetiver cultivar that has a stronger ability to resist cold.

Plate I. Regeneration and variation in vetiver:

1-5. Induction for somatic embryogenic calli; E-calli are induced from #2, #3, #5, #7 and #10

6. Effect of low temperature treatment on plant regeneration of embryogenic calli; some embryogenic calli still maintain differentiation ability under 3-7°C

7-9. Embryogenic calli subcultured for long-term still maintain very strong regeneration ability; the growth status of regeneration plants are quite good all along from non-subculture (0 generation) to the 15th generation

(Space for Plates 1-2)

10-11. Somaclonal variations; many types of variation are observed: plant type (from erect type to creeping type), plant height (during middle seedling time), leaf color (from viridescence to purple), leaf shape (from lance-shape to curled-shape), etc.

Plate II. Formation of somatic embryos and regeneration of vetiver plants

1. Epidermal cells *(e)* of the explant begin to initiate division after two weeks of inoculation
2. Vascular bundle and parenchyma cells *(p)* begin to divide after two weeks of inoculation
3. Somatic embryo with pro-embryo at different stages: single cell *(s)*, two cells *(t),* four cells *(f)*
4. Somatic embryo with multiple cells of pro-embryo *(m)*
5. Visible embryonic callus
6. Single somatic embryos consist of coleorhiza *(cr),* coleoptile *(cp)* and scutellum *(sc),* vascular bundles joined between coleorhiza and coleoptile
7. Embryonic callus in the differentiation period; each somatic embryo is budding
8. A large number of regenerated plants from somatic embryogenesis

9-10. Abnormal somatic embryo with torpedo pro-embryo that exists only in Dicotyledoneae.

ICV-3 Post Conference Proceedings

Thanks to Paul Truong, Xia Hanping and Frank Mason who volunteerd to undertake a hard job in assembling all relevenat information of the Third International Conference on Vetiver held in Guangzhou, China, 6-9 October 2003. The ICV-3 Post Conference Proceedings are assembled in the CD-ROM, reproduced and distributed by the Office of the Royal Development Projects Board of Thailand to all participants. The Contents of the Proceedings include 15 topics, viz. (1) General Report, (2) Opening Session, (3) Technical Papers, (4) Award Winners, (5) Open Discussion, (6) Closing Session, (7) Study Tour, (8) Social Events, (9) Exhibition and Posters, (10) Vetiver Video, (11) Feedback, (12) Business Meeting, (13) Participant List, (14) Author Search, and (15) ICV-2 Proceedings.

In order to help the viewers who may not be aqauinted with the process of viewing the CD-ROM, instructions have also been provided. Although the presentation in the CD-ROM requires Adobe Reader® 6, or Adobe Acrobat® 6, there are links to install them either for Microsoft Windows 98SE, ME, XP, NT4; or for Macintosh OSX.

Each foreign participant of ICV-3 will receive this CD-ROM through surface mail by the end of May 2004. Xia Hanping will take care of all Chinese participants; anyone who does not receive it by the end of April, please contact him directly. Those who did not participate ICV-3 and wish to have access of the CD-ROM may write to the Office of the Royal Development Projects Board for a free copy, provided that they are still available.

Vetiver Grass Particle Boards

Forest resource is one of the most important natural resources of the country and humanity. It has profound effect on the equilibrium of the ecosystem. The deterioration of the ecosystem has resulted in increasing crisis from natural disasters. The rapid decline of natural forests instigated by people's invasion for living, and clearing of the forest for timber and other forest products, has prompted the initiation of a research project by a private firm in Thailand for the development of particle boards on a commercial scale in order to be used as a wood substitute.

The objective of this venture is to make use agricultural waste materials such as the leaves of vetiver grass, lemongrass, aromatic pandanus; together with by-products of rice cultivation, i.e. rice hull and rice straw; as well as wood chips, as raw materials for producing of particle boards. These raw materials are either agricultural wastes or by-products that have to be destroyed by burning or other forms of elimination, which often cause environmental problem. This is true for vetiver leaves, which have to be pruned every few months, as well as rice hull, wood chips, and lemongrass leaves. These boards will be used in place of natural wood as an approach for the conservation of the forest, and thus save the nation's precious environment.

The Golden Vetiver Grass Board Industry Co. Ltd., a pioneer firm in the development of particle boards from vetiver and other plants, is under the management of Ms. Parinda Tarevichitsilp. She has an inspiration to produce vetiver grass board on industrial scale for sale in the markets, both in Thailand and abroad. After a considerably long period of research and investigation to produce such boards, she was finally successful. Thus, she started to produce the vetiver grass boards and other products for use as home appliances. Within a period of a short time, the feed back from her customers who use her boards and other products have been pretty good. The company is so proud that it is the first company in Thailand that produces vetiver grass board for sale in Thailand and for export worldwide.

The Golden Vetiver Board Industry will concentrate on developing its products and invent new products from various by-products for producing the boards in order to reduce the load on natural wood obtained from the forest, thereby conserving our precious forest for our children.

Vetiver grass board consists of cellulose, fiber, and lignin, similar to natural wood. The quality of vetiver grass board is specified under JIS A 5908, Level 18. Its density is 750 - 850 kg/m^3. Vetiver grass board has high strength, is enduring and water resistant, with a mild fragrance of the vetiver, and has beautiful ornament pattern. It does not have chemical odor that would injure the eyes and nose. Its property is similar to natural wood. Vetiver grass board can be used to make furniture, or as interior design material such as wall, floor, or ceiling.

The board is easily used in construction. Simply cut the board with normal saw, or preferrably precision saw, then smoothen with sandpaper and coat with lacquer. For decorative use, the board should be smoothened with fine-grain sandpaper, and coated with urethane lacquer once again. The boards can be joined together with screw or latex glue.

As for the raw material procurement, the company buys dry leaves of vetiver at the farm gate price of Baht 1-4/kg, depending on the distance from the factory in Samut Prakan Province. This is considered a fair price for the farmers who could earn an extra income from cutting the vetiver leaves, which they have to trim off every few months to rejuvenate the plants. It is estimated that an average dry-weight yield of 1,000 kg per harvest would be obtained from a linear length of 1 km of the vetiver hedgerows, or 1 kg/m. This would give the farmers an extra income of Baht 1,000 to 4,000/km (*ca.* US$ 25 to 100) of vetiver hedgerows, or double or triple this amount annually if the plant is harvested twice or thrice a year, which is a suggested method for proper maintenance of the vetiver hedgerows.

For further information and photographs of the products, please look in the website: <http://www.golden-board.com>

ICV-4 to be held in Caracas, Venezuela, October 2006

During the Business Meeting of ICV-3 held in Guangdong Hotel, Guangzhou on 7 October 2003, the issues of the venue, theme and date of ICV-4 were discussed. As was reported in Vetiverim-27, there were four nominees, namely: (i) the Philippines, by Ms. Noah Manarang, (ii) Venezuela, by Dr. Oswaldo Luque, (iii) China, by Ms. Wen Zhu Li, and (iv) South Africa, by Mr. Jon McCosh. It was agreed that all nominees should go back and prepare a proposal containing necessary information which should include the sources of funding, organizational support, plan of operation, the venue and other facilities, etc. The proposal should be finalized and submitted to the Chairman of the Continuing Committee (CC) for ICV-4 within six months.

The Editor, as the Chairman of CC/ICV-4, has received, by the deadline (end of March 2004), only one proposal from Dr. Oswaldo Luque of Venezuela. A reminder has been sent to the other three candidates. It turned out that none is in a position to provide the proposal due to some technical problems within their own countries. Thus, the CC/ICV-4 has agreed that ICV-4 be held in Caracas,Venezuela in October 2006 with the theme, “Vetiver and People: A Green Investment for Sustainable Development”. Details of ICV-4 will be made available in Vetiverim-29.

Karyomorphological Study of Vetiver Germplasm in Thailand

The above article has been published in the AU Journal of Technology, Vol. 7, No. 2, pp. 75-80, October 2003. The authors are P. Kongprakhon, N. Sangduen and K. Namwongprom of the Faculty of Science, Kasetsart University, Bangkok, Thailand. A reprint of the article can be requested from Dr. Nitsri Sangduen, Genetics Department, Kasetsart University, Bangkok 10903, Thailand. The Abstract of this article is presented below:

A cytological technique was developed to display chromosome morphology from the root tip cells of 15 ecotypes of vetiver collected from all over Thailand. They were classified as eight *Vetiveria zizanioides* and seven *V. nemoralis* ecotypes. Humic acid solution was applied to obtain healthy root tips used for conventional slide preparation. The appropriate times for cutting the root tips were between 11 a.m. to 1 p.m. for further fixation. Enzyme mixture treatment appeared to be a promising way to separate groups of cells and to clear the cytoplasm. It was found that all ecotypes studied had the same chromosome number, $2n = 2x = 20$. Three B-chromosomes were observed only in one ecotype. The variation in satellite chromosomes in terms of number and morphology was evident in six ecotypes. The total chromosome length varied from 1.8-8.4 μm. The karyotype consisted of metacentric and submetacentric chromosomes, all of which were nearly symmetrical. Localization of the heterochromatic regions on the chromosome of vetiver was achieved by: (a) modified C-band, and (b) N-band. It was possible to characterize each pair of the vetiver chromosomes. All ten pairs of chromosomes were identified by these banding patterns, along with their length and arm ratios. They can be used as the basis for reproducible karyotypes and for detecting chromosomal relationships among ecotypes. Each ecotype showed at least one chromosome with a whole dark band, indicating a constitutive heterochromatin. The remainder indicated variation patterns from ecotype to ecotype of some faint and dark bands.

Ameliorative Potential of Vetiver for Reclamation of Sodic Soils*

Soil sodicity as such causes marked imbalance in nutrient absorption by plants. A high concentration of sodium restricts potassium and calcium accumulation in plant tissues. The great ability of Vetiver to limit sodium entry into shoot tissues and to maintain sufficient potassium and calcium concentration at high sodicity levels indicate tolerance of vetiver to sodic stress. Significantly greater accumulation of Na^+ in the root than in the shoot tissues indicates that vetiver regulates the Na^+ concentration by restricted translocation of Na^+ from root to shoot tissues.

Ability of Vetiver to withstand higher pH and water-logging makes it suitable for cultivation in sodic lands which have low infiltration capacities. We conducted several experiments under glasshouse, microplots and field condition. Our results from experiments indicate that Vetiver could withstand high soil alkalinity upto pH 10.0. However, the performance of the crop is not that satisfactory under saline condition. Soil and irrigation water salinity beyond 10.0 and 12.0 dSm^{-1}, respectively decreased the root yield to a great extent. On the other hand shoot and root yields of Vetiver were not significantly affected with the high sodium adsorption ratio (SAR) of 31.9 me/L(with EC 12.5 dS/m) and residual sodium carbonate (RSC) of 2.0 me/L (EC 3.0) of irrigation water. The soil sodicity did not have an adverse effect on the quality of the oil as this was found to be at par with the market specifications; in some cases it is even better.

Growing of Vetiver in sodic soil resulted in significant reduction in pH, EC and ESP and increase in organic carbon content in soils after harvest of crop due to biological action of

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roots. The result of an experiment indicate that the soil pH was reduced from 10.5 and 9.5 to 9.5 and 9.0, respectively with growing of Vetiver for a period of eighteen months.

Utilization of sodic soils for cultivation of Vetiver will create a tremendous employment generation (estimated to be 167 man-days/ha/year) in rural India. Thus, the benefits of cultivation of Vetiver in barren sodic lands are quite encouraging in terms of higher crop productivity to meet the demands of raw materials in national or international market, high economic return, bio-reclamation of sodic soils and employment generation in the rural areas. However, the oil yields are sometimes lower. Therefore,there is a need to develop the varieties which can withstand high salinity and sodicity without reduction in the oil yield.

Phytoextraction of Lead from Contaminated Soil by Vetiver

Paul Truong has recently reviewed a paper for publication in the *Science Asia*, the official Journal of the Science Society of Thailand. The paper entitled: "Phytoextraction of Lead from Contaminated Soil by Vetiver Grass (*Vetiveria* sp.)" by a group of scientists from Mahidol, Burapha and Kasetsart Universities in Thailand.

This paper reported the findings of a soil-culture study, which investigated the phytoextraction of lead in two species of vetiver grass (*Vetiveria zizanioides* and *V. nemoralis*) irrigated with increasing levels of lead. Based on the results, *V. zizanioides* best tolerated and accumulated the greatest amount of lead. The vetiver system offers a potential avenue for soil phytoremediation. It is a very cost effective, environmental friendly and practical tool for the control and attenuation of heavy metal pollution when appropriately applied.

This paper will be published in the next issue of the Journal; if you are interested in this work, you can contact the Journal <scjss@mahidol.ac.th> for more details.

Esk Shire Council Shares Grand Prize in Healthy Waterways Awards

Esk Shire Council has won a $5,000 share in grand prize money for its Toogoolawah Vetiver Grass Wetlands System which saw the installation of innovative technology developed by Queensland Department of Natural Resources, Mines and Energy to upgrade the existing sewage treatment plant. Representatives from Esk Shire Council were presented with their trophy and prize for winning the Sinclair Knight Merz Government Award at a ceremony held at Brisbane's Customs House on 6 April 2004. Esk Shire Council shared half of the $10,000 grand prize with Bulimba Creek Catchment Coordinating Committee and four other prize-money winners who were honored for their outstanding contributions to the improvement, protection and management of the waterways and catchments of Southeast Queensland throughout 2003.

Relevant information about the 'Toogoolawah Vetiver Grass Wetlands System':

- Instead of undertaking a traditional and costly upgrade of the Toogoolawah sewage treatment plant, Esk Shire Council installed an innovative natural technology recently developed by Queensland Department of Natural Resources and Mines and Energy.
- The Vetiver Grass Wetlands System treats sewage effluent in two stages: The main treatment involves the effluent passing through a vetiver grass wetlands which has been constructed over 3 ha in rows, following the contours of the land to allow good contact between the grass and the effluent.
- Vetiver is a grass species which takes up the water and filters it. It has traditionally been used on land but this is the first time that a vetiver grass wetland has been used to treat sewage effluent. This innovation has been globally recognized and Esk Shire Council presented a paper on the project at the Third Interntional Confernce on Vetiver held in Guangzhou, Guangdong, China, 6-9 October 2003.

• This project relied on simplified technology used in an incredibly innovative way to produce excellent outcomes, including helping to restore the ecosystem in the local creek that feeds into Wivenhoe Dam.

• Esk Shire Council has shown itself to be a local government leader, demonstrating that high-tech and costly upgrades may not be the best solution for every situation. This project has shown how small sewage treatment plants throughout the region could benefit from inexpensive methods for protecting the environment.

Healthy Waterways is a unique regional collaboration between State and local government, industry and communities across Southeast Queensland. The Healthy Waterways Awards program is an annual event to celebrate and recognize the contributions and achievements made by organizations and individuals to the health and sustainability of our waterways, livelihoods and lifestyles. The Healthy Waterways Vision for the future is: "By 2020, our waterways and catchments will be healthy ecosystems supporting the livelihoods and lifestyles of people in Southeast Queensland. This will be managed through collaboration between community, government and industry."

Letters to the Editor

Roots of *Vetiveria zizanioides* and *V. nigritana*

Cris Juliard sent me a few slips of *Vetiveria nigritana* during summer of 2002. The slips survived one full month in postal transit, and the etiolated slips on arrival were recovered by placing them in water for over 15 days when they turned green suitable for field planting. That material is fully grown up now and has also bore seeds. Perhaps this species does not form seeds in Senegal (to be confirmed by Cris). I am forwarding here the photographs of the root clump of *V. nigritana vis-a-vis V. zizanioides* (from north Indian wild type khus) for your perusal.

I notice one clear cut difference in the physiography of the roots of the two species. Whereas *V. zizanioides* type roots are thick and smooth (with little fibrous secondary roots), the one from *V. nigritana* are thin and more fibrous. Also, the slips in *V. zizanioides* have axillary buds close to nodes imparting spreading plant habit, but in *V. nigritana* the nodes are sparse with little axillary buds. As has been discussed earlier in our publications, the thicker and smooth roots are prefered for perfume quality oil. However, I plan to distill oil from roots of *V. nigritana* to find out its quantitative concentration and qualitative differences, if any. On preliminary examination I notice that the roots of *V. nigritana* are profusely infested by black ants, which is not the case with *V. zizanioides*, indicating there may be some qualitative differentiation in the oil of two species, or there is some sugary substance present therein.

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(space for photo of roots of *V. zizanioides* and *V. nigritana*)

Root Physiology of *V. zizanioides* and *V. nigritana*

The information on the roots provided by Umesh Lavania is terrific; the lack of roothairs and fine ramification have always seemed to me signs of selection, and may coincidentally help

account for their great penetrating power as well as strength. Assessing the morphology of axillary buds, and how it affects the caespitose habit of above-ground portions of vetiver, cries out for documentation. It fits with all I know about the short, curled rhizomes from which tillers arise, except I thought it helped keep the plant compact! My impression has been that both wild North India vetiver and *V. nigritana* were more lax than the cultivated 'nonflowering' types. I don't know if there is any photoperiod involved in *V. zizanioides* flowering; it seems sporadic among the cultivated types and more-related to plant maturity. Yes, *V. nigritana* is generally considered fully fertile. I wonder if it could cross with *V. zizanioides*.

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Confirmation on Seed Setting of *V. nigritana*

It is always a pleasure to hear from you and to read of the multiple vetiver activities you coordinate in the editions of Vetiverim. Regarding the research that Dr. Lavania is conducting in India on *V. nigritana* sent to him from Senegal, I can confirm that while the species does produce seed panicles, we have not found any confirmation that these are fertile. In fact, *V. nigritana* seems to be dissapearing from the landscape in at least two countries of West Africa, namely Senegal and Mali. The root of the plant is harvested from the wild and used to purify drinking water, for incense and for medicinal purposes. However, there are, as yet, no effort to multiply the plant on a commercial basis. For the same reasons that Dr. Lavania found a difference in the physiography of *V. nigritana* to *V. zizanioides*, the former is not as well suited for erosion control as the latter.

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