The Vetiver Network Awards - 2006

Every three or four years the Vetiver Network makes cash awards to individuals and agencies for expanding the knowledge base and application of vetiver grass technology for environmental improvement purposes. The awards are to persons who have made a major contribution to vetiver research and development, not only for the actual work undertaken but also to its potential use and application by the wider public.

The 2006 awards are the fifth of a series of awards that have been made since 1990.

Vetiver Champion

- Winner: Dr. Tran Tan Van, Coordinator Vietnam Vetiver Network

Innovation

- Winner: Bingbing Yang, Guojiang Wu, Zhenrong Ma, Hanping Xia, " Study on efficient regeneration system and agrobacterium-mediated transformation of *Vetiveria zizanioides*" (South China Botanical Garden, Chinese Academy of Sciences)

Disaster Mitigation

- Winner: T.T. Van[1], L.V. Dung[2], P.H.D. Phuoc[3] and L.V. Du[3], Vetiver system for natural disaster mitigation and environmental protection in Vietnam, [1]Coordinator Vietnam Vetiver Network, Research Institute of Geology and Mineral Resources (RIGMR), Ministry of Natural Resources and Environment (MONRE), Thanh Xuan, Hanoi, Vietnam, [2]University of Can Tho, Can Tho City, Viet Nam, [3]Ho Chi Minh City Agro-Forestry University, Ho Chi Minh City, Vietnam.

Country Award

- Winner: Venezuela, Gerardo Yépez Tamayo, Coordinator of the Venezuela Vetiver Network and Dr. Oscar Rodriguez, President Latin America Vetiver Network

Vetiver Champion

Dr. Tran Tan Van, of the Research Institute of Geology and Mineral Resources (RIGMR), Ministry of Natural Resources and Environment (MONRE), Thanh Xuan, Hanoi, and Coordinator of the Vietnam Vetiver Network is selected as the 2006 worldwide Vetiver Champion by The Vetiver Network due to his outstanding work over the past 5 years. He has been a driving force behind the rapid expansion of Vetiver technology and its adoption in much of Vietnam. Over three quarters of the all provinces in Vietnam are now using Vetiver technology in some form for: erosion control, riverbank stabilization, infrastructure protection, wastewater management and disaster mitigation. He provides not only the guidance needed for technical activities, but he has inserted into this program an unlimited amount of energy and spirit that inspires others to move forward disseminating information on the multitude of Vetiver uses. Dr. Van is a team player and has been able to effectively mobilize not only government resources, but those from the donor community (Dutch government, the Wallace Genetic Foundation, the Australian government, and the William Donner Foundation) as well. We, at the Vetiver Network are pleased to announce this award to Dr. Tran Tan Van and hope that his efforts and those of his colleagues continue to accelerate across the entire country.

Country Award

The Vetiver Network is pleased to announce that the 2006 country award for outstanding performance in disseminating and promoting the use of Vetiver technology goes to Venezuela. Under the guidance of Gerardo Yépez Tamayo, Coordinator for the Venezuela Vetiver Network and the regional Latin American Vetiver Network, Vetiver use in Venezuela has expanded greatly over the past several years. We would like to also thank the Polar Corporation Foundation, Faculty of Agronomy, Central University of Venezuela and Soil Science Society of Venezuela for their technical and financial support to introduce and promote Vetiver technology in their country. A movement to apply appropriate technology requires the support and coordination of a wide range of people across various landscapes and needs to be sustained over time. Evidence of this kind of effort is found in the Venezuela program. We are confident that this program will serve as a model to others in the region and be a stimulus to motivate others to study and adopt Vetiver systems as solutions to the wide range of problems facing them Latin and South American countries.

Innovation

Bingbing Yang et. al., under the guidance of Hanping Xia in China has for the first time undertaken basic research on developing a cold-tolerant variety of Vetiver. The implications of ultimately selecting a cold tolerant variety is very high given the need for effective soil and water conservation (erosion control) in temperate regions of the world. The ability to withstand winter temperatures would extend the reaches of Vetiver into North American, Northern Europe and Russia as well as China and mountainous regions of the Middle East. Their research represents the first steps on the path to select promising plant materials that have been genetically engineered to withstand cold temperatures. Using an agrobacterium-mediated gene transfer system, Bingbing Yang successfully demonstrated not only its feasibility, but developed protocols that allow for explant regeneration via somatic embryrogenesis. This methodology lays the foundation for screening and selecting for cold tolerance among potential new cultivars of *Vetiveria zizanioides*. This innovative approach to plant breeding is laying the groundwork that will someday see the selection of Vetiver cultivars capable of thriving in regions that currently do not have Vetiver as a tool to fight erosion, pollution and to stabilize infrastructure. The initial work for this research was funded by the Wallace Genetic Foundation and The South China Institute of Botany (Academica Senica)

Disaster Mitigation

Dr. Van and his colleagues have accomplished something special in Vietnam over the past 5 years with respect to disaster mitigation and environmental protection. This has been especially true with respect to protecting sea dykes when conventional systems have failed. The coastal areas of Vietnam are constantly being menaced by seasonal heavy storms linked to the annual typhoon season. Despite some early setbacks they persisted and showed the Vietnamese government particularly the Dyke Department how a bioengineered solution offered a long-term, cost effective solution for coastal cities, industries, and agriculture when faced with strong storms. Indeed in 2003, the Dyke Department issued state decrees 436 and 438 that mandated Vetiver hedge use on riverbanks and on sea dykes. In September 2005 when typhoon 7 hit Hai Hau District in Nam Dinh province, the government and Vietnamese people were able to see first hand how even young Vetiver hedges could provide effective protection when conventional systems failed. Dr.Van and his colleagues were assisted by the Chaipattana Foundation, both technically and financially to further this effort and create future projects dealing with coastal protection. Equally impressive has been the work his team has done with respect to riverbank protection and sand dune stabilization within Central Vietnam with support from the Dutch government. Many communities in this region are located directly along riverbanks and eroding banks have caused much damage due to the wave action from motorized boats plying the various river systems. This effort has led other provinces to adopt Vetiver technology. Finally the work implemented along the Ho Chi Minh Highway is also an example of successful bioengineering along long stretches of this new highway system. By 2005 more than 250 km had Vetiver hedges protecting drains and road cuts and by 2006 more sections were being installed. Ultimately the entire 3000 km highway will be protected by Vetiver making it probably the single largest use of Vetiver to prevent landslides in the world.

STUDY ON EFFICIENT REGENERATION SYSTEM AND AGROBACTERIUM-MEDIATED TRANSFORMATION OF VETIVERIA ZIZANIOIDES

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Abstract: Vetiver (*Vetiveria zizanioides*) has been widely used in South China for the purpose of erosion control and ecological restoration. However, vetiver cannot be applied to northern regions of China due to its poor resistance to cold, although there is a huge demand to it in north China. To improve its cold resistance, regeneration system of vetiver was established first. Axillary buds and aseptic adventitious buds of vetiver were used as explants to investigate the factors that affected its somatic embryogenesis and plant regeneration. Explants were cultured on MS medium supplemented with 2,4-D or 6-BA. The results showed that 2,4-D was an important factor to induce somatic embryogenesis, for explants could be regenerated via somatic embryogenesis as long as the medium contained 2,4-D without or with 6-BA. Cytology observation, moreover, proved that embryonic calli originated from epidermal cells and parenchyma cells of vetiver, which had the typical embryonic structure of monocotyledon. Regeneration ability of embryonic calli could be maintained for almost two years, and regeneration frequency kept over 80% regardless of subculture times from 0 to 24. The whole process from callus induction to green plantlets transplanted to soil needed about 3-4 months.

Efficient regeneration system is the foundation of genetic transformation. *Agrobacterium*-mediated transformation system was also established. Plant expression vector p1301UN-*ots*A was constructed by inserting the *ots*A gene digested with *Sac* I/*Kpn* I into MCS of binary vector pCAMBIA1301UN. The freeze-thaw method was used to mobilize the recombinant plasmid into DH5a. Restriction analysis and DNA sequence analysis confirmed that the construction of plant expression vector p1301UN-*ots*A was successful. This recombinant plasmid contains an Ubi promoter, a *hpt* gene, an *ots*A gene, a km^r gene and a *gus* gene, so it is very useful and efficient for gene transformation of monocotyledon. Moreover, an efficient genetic transformation system of vetiver was described, embryonic calli were infected with *A. tumefaciens* EHA105/pCAMBIA1301 (OD_{600}=0.4-0.5) for 20 min and then transferred to CIM in the dark at 25℃ for 3-4 d; infected calli were then selected on SIM in the dark at 25℃ for 4 weeks. Using the optimized protocol, 18% of the infected calli were Hyg B resistant. Transient integration and expression were confirmed by GUS assay. However, Hyg B resistant calli and plantlets showed obvious growth retardation and phenotypic alterations compared to the control. On the whole, this paper initiated the study on gene engineering of vetiver, and established its regeneration system and *Agrobacterium*–mediated transformation system, which formed a good fundament for screening cold tolerant cultivar of vetiver.

Key words: somatic embryogenesis, embryonic calli, *Agrobacterium*-mediated transformation, plant expression vector, resistant calli

Abbreviations: 2,4-D – 2,4-dichlorophenoxyacitic acid; 6-BA – 6-benzyladenine; AS – acetosyringone; Cef – cefotaxine; CIM – co-cultivation induction medium; CM – co-cultivation medium; DM – differentiation medium; GTE – GUS transient expression; GUS – β-glucuronidase; *gus* – β-glucuronidase reporter gene; *hpt* – hygromycin phosphotransferase gene; Hyg B– hygromycin B; IBA – indole-3-butyric acid; IM –

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induction medium; Kan – Kanamycin; *km*^{r} – kanamycin selection gene; KT – Kinctin; MCS – Multiple Colony Site; NAA – naphthaleacetic acid; otsA – trehalose-6-phosphate synthase; PCR – Polymerase chain reaction; PP_{333} – poclobutrazol; Rif – Rifampicin; SDM – screening differentiation medium; SIM – screening induction medium; SRM – screening rooting medium

1 Introduction

Vetiver (*Vetiveria zizanioides*), a magical perennial monocotyledon in the tropical and subtropical regions, has been widely used in South China for the purpose of erosion control and ecological restoration. However, the plant is poorly resistant to low temperature that it is completely restricted for its dissemination and application in north China. In order to improve its cold tolerance, gene engineering opens new avenues to meet the specific demand.

Genetic transformation mediated by *Agrobacterium tumefaciens* was first reported in the 1980s (Block *et al.*, 1984). Since then *Agrobacterium*-mediated transformation has become the standard method to genetically modify dicotyledonous plants. Repeatable and efficient *Agrobacterium*-mediated transformation of monocotyledons was demonstrated first in rice a decade ago (Hiei *et al.*, 1994). The key factors in this method are a "super-binary" vector and the addition of AS to CIM. Subsequently, *Agrobacterium*-mediated transformation of many plant species, such as maize (Zhao *et al.*, 2002), barley (Tingay *et al.*, 1997) and Italian ryegrass (Bettany *et al.*, 2003) were reported.

Efficient regeneration system is the foundation of genetic transformation. Somatic embryogenesis is an effective approach to enhance regeneration frequency. There have been well documented on somatic embryogenesis in many plants, such as sugarcane (Ahloowalia and Maretzki, 1983), corn (Vasil *et al.*, 1984), bermudagrass (Li and Qu, 2004) and alfalfa (Szucs *et al.*, 2006), etc. However, very few related studies on vetiver, up to now, have been conducted except for calli induction and culture *In vitro* of vetiver (Mucciarelli *et al.*, 1993; Ruth *et al.*, 2000; Ma *et al.*, 2000).

Trehalose, a non-reducing disaccharide synthesized by *ots*A gene, is widely used as protectant of enzymes and membranes in many microorganisms under adverse environment stress, such as drought, salt and cold, so it can enhance resistant ability of plants (Crowe *et al.*, 1990; Strom and Kaasen, 1993; Drennan *et al.*, 1993). In recent years, transgenic plants of tobacco, potato and sugarcane containing *ots*A gene have been obtained through application of some new bio-techniques, including electroporation, microprojectile bombardment, *Agrobacterium*-mediated, and so on (Goddijn *et al.*, 1997; Yeo *et al.*, 2000; Wang *et al.*, 2003). Among these methods, *Agrobacterium*-mediated transformation is often preferred over other plant transformation systems because of the simplicity, low cost, high transformation efficiency and lower transgene copies integrated into plant genome (Ishida *et al.*, 1996; Matzke *et al.*, 2001; Dong and Qu, 2005).

According to above information, the present study aims at enhancing cold-resistant ability of vetiver by transforming *ots*A gene into vetiver cells through establishing a regeneration system, a plant expression vector system and an *Agrobacterium*-mediated transformation system.

2 Materials and Methods

2.1 Plant materials and medium

All vetiver samples used in this study were collected from the nursery of South China Botanical Garden. Axillary buds of vetiver were sterilized with 70% ethanol for 2 min, and then rinsed 3 times with distilled water and sterilized with 20% $NaClO_3$ for 10 min and with 0.1% $HgCl_2$ for 20 min. MS basal medium (Murashige and Skoog, 1962) supplemented with different concentrations of growth regulators was used in the study (Table 1); the solid medium contained 8.0% agar, pH was adjusted to 5.85 with 1 N NaOH or 0.1 N HCl, then autoclaved them at 121℃ for 15 min.

Table 1 Medium composition of bacterial culture, tissue culture and transformation for vetiver

Medium	Composition
YEP	10 g l^{-1} Tryptone, 10 g l^{-1} Yeast Extract, 5 g l^{-1} NaCl and 1.5% agar. pH7.0
IM	MS supplemented with 2.0 mg l^{-1} 2.4-D and 0.5 mg l^{-1} KT. pH5.8
CIM	IM supplemented with 200 μmol AS. pH 5.4
SIM	IM supplemented with 50 mg l^{-1} Hyg B and 500 mg l^{-1} Cef. pH5.8
DM	MS supplemented with 1.0 mg l^{-1} 6-BA. pH5.8
SDM	DM supplemented with 25 mg l^{-1} Hyg B and 250 mg l^{-1} Cef. pH5.8
SRM	Half strength MS supplemented with 0.1 mg l^{-1} IBA, 0.1 mg l^{-1} PP_{333}, 25 mg l^{-1} Hyg B and 250 mg l^{-1} Cef. pH5.8

2.2 Bacterial strains, plasmids and vectors

E. coli DH5a/ pWY (Wang *et al.*, 2000) contains a 1.431 Kb *ots*A gene, provided by Beijing Institute of Microbiology, the Chinese Academy of Sciences. *E. coli* DH5a/1301UN (Fig. 1) was provided by Beijing Agriculture University. The binary vector p1301UN, derivatives of pCAMBIA1301 (CAMBIA, Canberra, Australia), was used in this transformation experiment. The T-DNA of the binary vector includes Ubi-1 promoter, *hpt* selectable marker gene and *gus* reporter gene (Ohta *et al.*, 1990).

Fig. 1 Plasmid p1301UN

2.3 Enzymes and reagents

All restriction enzymes, T4 DNA ligase and DNA Purification Kit were purchased from TaKaRa Biotechnology Co. Ltd., Japan; Hyg B was purchased from Roche Diagnostics Corporation Indianapolis, USA; Rif, Kan, Cef and other reagents were purchased from Ding Guo Biotechnology Corporation, China. PCR primers were synthesized by Shanghai Shengon Biology Coporation, China and DNA sequence analysis was identified by TaKaRa.

2.4 Callus induction, subculture and differentiation

Sterilized axillary buds were cut longitudinally and then incubated on IM supplemented with 30 g l^{-1} sucrose, 2.0 mg l^{-1} 2,4-D and 0.5 mg l^{-1} KT in the dark at 25±2℃ for about

2-4 weeks. Then induced calli were transferred into IM in the dark at 25±2℃ for subculture once a month. Six mediums containing different proportions of 2,4-D and 6-BA were used to induce embryonic calli from non-embryonic calli and aseptic adventitious buds. The general olefin slice method was used to observe the cytoarchitecture of embryonic calli.

4 weeks after incubation, light yellowish and compact embryogenic calli were transferred into DM containing 2.0 mg l^{-1} 6-BA and 2.0 mg l^{-1} NAA, and then illuminated with 1200 lx light for 12 h per day. The incubation temperature was about 25±2℃. After the regeneration plants were formed from embryonic calli, they were transferred into the rooting medium containing 0.1 mg l^{-1} IBA for about 2 weeks. The plantlets were subsequently grown in a greenhouse at 25±2℃ and then planted in the nursery when they were 30-40 cm high.

2.5 PCR amplification and construction of plant expression vector p1301UN- *ots*A

PCR was used to obtain *ots*A gene from the Plasmid pWY. According to the sequence of 5' and 3' terminus of *ots*A gene, specific primers were designed as follows: the forward primer included a recognition site for *Kpn* I at its 5' end, and the reverse primer included a recognition site for *Sac* I. PCR amplification system was 50 µl, and the reaction procedure was one cycle of 94℃ for 5 min; 30 cycles of 94℃ for 30 s (denaturation), 55℃ for 30 s (annealing), 72℃ for 2 min (extension); a final elongation at 72℃ for 10 min (one cycle).

Forward primer: 5' - GGGCCGGTACCATGAGTCGTTTAGTCGTAGTATC - 3' (*Kpn* I)
Reverse primer: 5' - GCTACCTTTCCAAAGCTTGCGTAGGAGCTCGCCT - 3' (*Sac* I)

To construct the plant expression vector p1301UN-*ots*A, 1.431 Kb PCR products of *ots*A fragments were digested with *Sac* I/*Kpn* I, and the binary vector p1301UN was also digested with *Sac* I/*Kpn* I, and then the target fragment was ligated into the MCS site of the binary vector p1301UN with T4 DNA ligase. The freeze-thaw method was used to mobilize p1301UN-otsA into EHA105 (An *et al.*, 1988; Hood *et al.*, 1993), The resulted EHA105/p1301UN-otsA was inoculated in YEP liquid medium at the presence of 50 mg l^{-1} Rif and 50 mg l^{-1} Km until OD_{600} reached about 1.0. The bacteria were collected by centrifugation (12,000 g for 1min) and re-suspended in AAM medium (Hiei *et al.*, 1994) supplemented with 200 µmol l^{-1} AS, to make the OD_{600} reach about 0.5 for co-cultivation with vetiver embryonic calli.

2.6 *Agrobacterium*-mediated transformation and selection

Embryonic calli were sliced into small pieces (1-2 mm in diameter), and then immersed in AAM supplemented with 200 µmol l^{-1} AS suspension for 20 min, then transferred to CIM in the dark at 25℃ for 3-4 d. The calli were then collected and rinsed with distilled water and 500 mg l^{-1} Cef for several times, blot dried, and then incubated on SIM in dark at 25℃ to inhibit *Agrobacterium* growth. 4 weeks later, the calli were subjected to two more rounds of selection. Calli growing vigorously under selection were then cultured on SDM for 4 weeks. The incubation temperature was kept at 25°C under a 12/12 h (day/night) photoperiod (cool white fluorescent light). Regenerated shoots were transferred onto SRM for rooting.

2.7 GUS histochemical assay

Assay for transient expression of the *gus* gene was performed by histochemical assays with X-gluc (Jefferson, 1987) as the substrate of the enzyme. Hyg B resistant calli were immersed in the GUS assay buffer overnight at 37℃ and then observed under a microscope.

3 Results and Discussion

3.1 Induction conditions of embryonic calli

Embryonic calli could not formed if no 2,4-D in the medium regardless how the 6-BA concentration was (Table 2); on the contrary, embryonic calli could formed if 2,4-D existed no matter how the concentration of 6-BA was. The best proportion 2,4-D:6-BA for aseptic adventitious buds was 1.0:0.5, with the embryonic calli induction frequency was up to 77.9%; the best proportion 2,4-D:6-BA for non-embryonic calli, however, was 0.5:0, with the induction frequency was up to 96.7%.

From above analysis, it ensured that 2,4-D played a key role in regeneration of vetiver. Explants regenerated via somatic embryogenesis when the medium contained only 2,4-D without or only a little 6-BA; explants, however, regenerated via organogenesis when the medium did not contain 2,4-D but only 6-BA.

Table 2 Effects of growth regulators on induction frequency of embryonic calli

2, 4-D (mg L^{-1})	6-BA (mg L^{-1})	Material	Number of materials	Number of E-calli	Induction frequency (%)
0	0.5	C	90	0	0
		B	72	0	0
0	1.0	C	93	0	0
		B	64	0	0
0.5	0	C	90	87	96.7
		B	70	0	0
1.0	0.5	C	97	83	85.6
		B	77	60	77.9
2.0	1.0	C	90	72	80.0
		B	78	30	38.5
4.0	2.0	C	92	50	54.3
		B	90	34	37.8

C=Non-embryonic callus; B=Aseptic adventitious bud; E-calli=embryonic calli.

3.2 Formation, characters and regeneration ability of embryonic calli

Axillary buds of vetiver began to expand about 10 d after inoculating. The epidermal cells were active, and their cytoplasm became thick and divided quickly (Fig. 2A-e). Moreover, some parenchyma cells stained deeply around vascular bundles also divided quickly (Fig. 2B-p). It indicated that the origin of calli came from both epidermal cells and parenchyma cells. In strong division areas, cells with typical embryonic structure could be found, including single cell (Fig. 2C-s), couple cells (Fig. 2C,D-t), four cells (Fig. 2C-f), and multiple cells (Fig. 2D-m).

Every embryonic callus of vetiver was irregular, white granulose, and compact (Fig. 2E), and had the typical embryo structure of monocotyledon, such as scutellum (Fig. 2F-cp), coleoptile (Fig. 2F-cr) and coleorhiza (Fig. 2F-sc), etc. Embryonic calli began to differentiate in the DM (Fig. 2G, H). Regeneration frequency of embryonic calli was 92.0% for 18 months, and 81.6% for 24 months; that is to say, regeneration ability of embryonic calli of vetiver was still very strong even two years later (Table 3).

Table 3 Effects of subculture duration on regeneration frequency of embryogenic calli

Subculture duration (months)	Number of E-calli	Number of regeneration E-calli	Regeneration frequency (%)
18	400	368	92.00
20	550	509	92.55
22	650	557	85.69
24	750	612	81.60

Data were collected every month in the last six months; E-calli = embryogenic calli

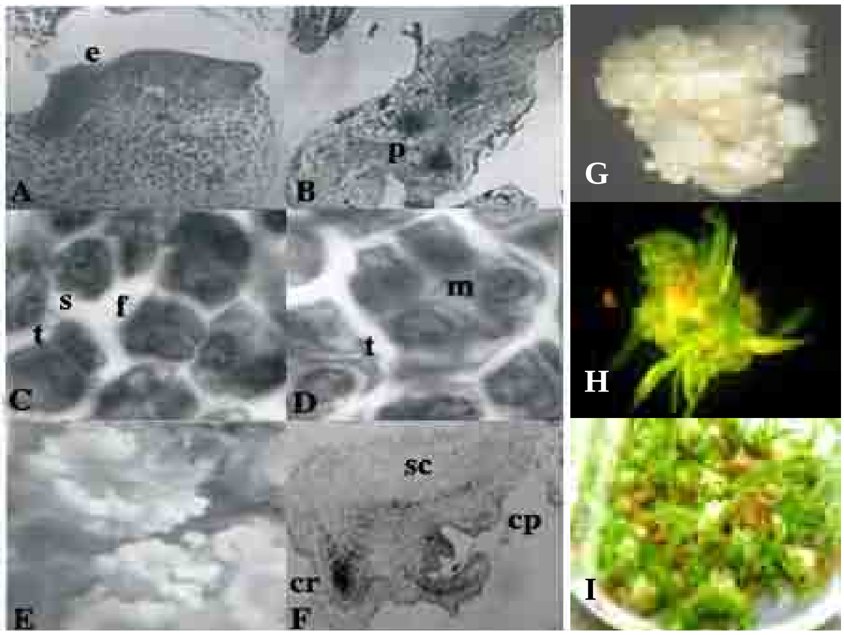

Fig. 2 Formation, characters and differentiation of embryonic calli

A: Epidermal cells of the explant 2 weeks after inoculation (×100); B: Vascular bundle and parenchyma cells 2 weeks after inoculation (×100); C: Different stages of embryonic calli: single cell (s), two cells (t), four cells (f) (×1000); D: Embryonic calli with multiple cells proembryo (m) (×1000); E and G: Visible embryonic calli (×30); F: Embryonic calli including coleorhiza (cr), coleoptile (cp), scutellum (sc) (×100); H and I: Differentiation of embryonic calli 2 weeks after inoculation

3.3 Constrcution of plant expression vector p1301UN-*ots*A

PCR amplification products were separated in a 1% agarose gel electrophoresis, estriction analysis and DNA sequence analysis showed that full length of *ots*A gene in Plasmid pWY is 1.431 Kb, coding 477 amino acids. Binary victor p1301UN was digested with *Sac* I/*Kpn* I and then recovered using DNA Purification Kit. Plant expression vector p1301UN-*ots*A was constructed by inserting the *ots*A fragment digested with *Sac* I/*Kpn* I into the MCS of binary vector p1301UN. The freeze-thaw method was used to mobilize the recombinant plasmid into DH5a. Restriction analysis and DNA sequence analysis confirmed that the construction of the plant expression vector p1301UN-*ots*A was successful (Fig. 3). T-DNA region of the recombinant plasmid p1301UN-*ots*A includes Ubi-1 promoter, *hpt* gene, *ots*A gene and *gus* gene (Fig. 4). One of this vector's advantages is that *ots*A gene inhabiting the vector is driven by Ubi-1 promoter, which could reduce the copy number of foreign genes in transgenic maize plants, so it might be useful in avoidance of gene silencing and highly active in monocotyledons (Christensen and Quail, 1996; Xu *et al.*, 2004).

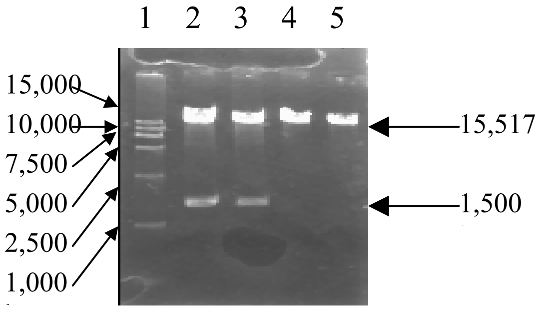

Fig. 3 Restriction analysis of recombinant plasmid p1301UN

Lane 1: Marker: DL15000; Lane 2-3: p1301UN-otsA double digested with *Sac I/Kpn* I; Line 4-5: p1301UN-otsA digested with *Sac* I

Fig. 4 Schematic structure of T-DNA region of p1301UN-*ots*A

3.4 Establishment of *Agrobacterium*-mediated transformation system of Vetiver

3.4.1 Screening press test to Hyg B

Hyg B, a strong cell growth inhibitor, has very high toxicity to many plants and, therefore, usually be used as selection marker of gene. Different concentrations (0, 25, 50, 75 and 100 mg l^{-1}) of Hyg B were added into IM respectively, and then embryonic calli of vetiver were cultured on these mediums for 4 weeks (Fig. 5). As a result, 50 mg l^{-1} Hyg B was perfect selection concentration for screening transgenic calli (Fig. 5C), as 50 mg l^{-1}selection pressure could not only inhibit calli's growth effectively, but also avoid their quick death.

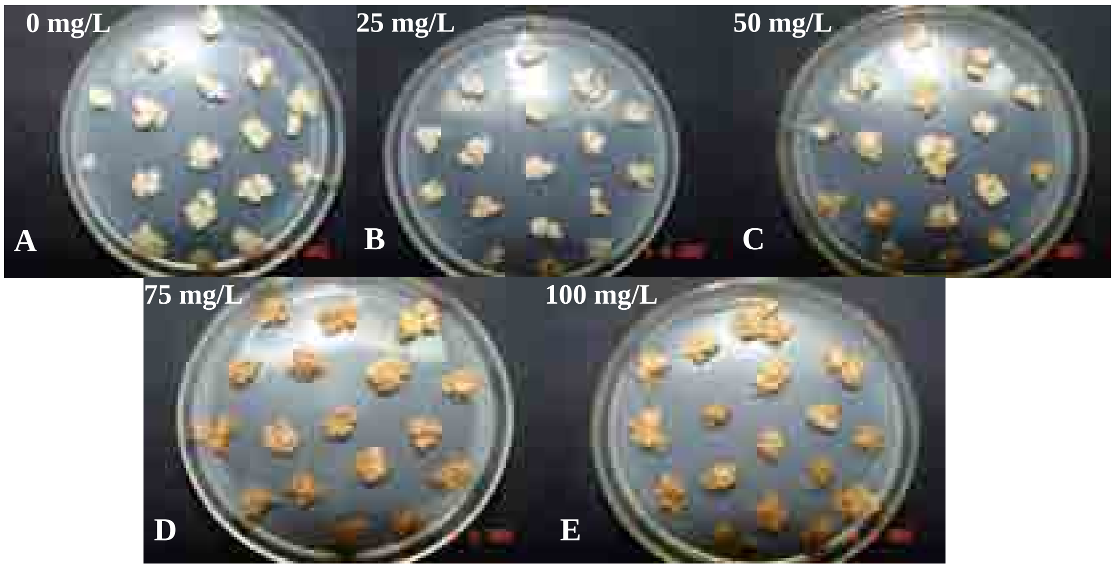

Fig. 5 The sensibility test to Hyg B

A and B: Embryonic calli grow well in 0 and 25 mg l^{-1} Hyg B; C: Embryonic calli stop growth in 50 mg l^{-1} Hyg B; D, E, and F: Embryonic calli are killed in 75 and 100 mg l^{-1} Hyg B

3.4.2 Concentration of *Agrobacterium tumefaciens*

Agrobacterium tumefaciens EHA105/pCAMBIA1301 was inoculated in YEP liquid medium in the presence of 50 mg l^{-1} Rif and 50 mg l^{-1} Km until OD_{600} reached about 1.0. The bacteria were collected by centrifugation (12,000 g, 1 min) and re-suspended in AAM supplemented with 200 μmol l^{-1} AS. The value of OD_{600} of AAM was observed one time per hour, and then embryonic calli were inoculated in the AAM for about 20 min. After 3 days of co-cultivation on CIM in dark at 25℃, the frequency of GUS transient expression (GTE) was evaluated by number of GUS-expressing calli over number of calli infected (Table 4). The result showed that high expression frequency could be obtained when the OD_{600}=0.416 (about 4 h), then the expression frequency declined as the concentration of EHA105 increased.

Table 4 The effect of *Agrobacterium* concentration on frequency of GTE in vetiver

Inoculation time（h）	Concentration of *Agrobacterium*	Number of calli treated	Number of GUS-expressing calli	Frequency of GTE (%)
1	0.050	31	0	0
2	0.115	37	2	5.4
3	0.224	41	5	12.2
4	0.416	40	8	20.0
5	0.560	38	5	13.1
6	0.737	37	3	8.1

3.4.3 Concentration of AS

Phenolics like AS are well-known virulence inducers for *Agrobacterium*, which plays an important role in the transformation process especially for monocotyledon (Dion *et al.*, 1995; Suzuki and Nakano, 2001). However, concentration of AS added into medium should be limited, otherwise calli would be killed due to *Agrobacterium*'s luxuriant growth. Different concentrations of AS were added into AAM and CIM; as a result, the frequency of GTE was highest when adding 200 μM AS into AAM and CIM (Table 5).

Table 5 The effect of AS concentration on frequency of GTE in vetiver

Conc. of AS (μM)	Number of calli treated	Number of GUS-expressing calli	Frequency of GTE (%)
0	40	0	0
100	36	3	8.3
200	38	7	18.4
300	41	6	14.6
400	40	4	10.0

3.4.4 Co-cultivation temperature

Lower temperature at 22℃ improves *Agrobacterium-mediated* gene transferring to plant cells (Dillen *et al.*, 1997). Low temperature also promotes pilus assembly leading to increase number of pili on the cell surface (Fullner *et al.*, 1996). To determine the influence of temperature during co-cultivation of vetiver, embryonic calli were co-cultivated at 18, 20, 22, 25, and 28℃, respectively. The highest frequency of GTE was observed at 22-25℃, in which 15.0-19.5% calli showed GUS activity (Table 6). However, frequency of GTE markedly decreased when the temperature was increased to 28℃, for some contaminations produced with the rapid reproduction of *Agrobacterium* restrained calli growth or even killed them if temperature became high.

Table 6 The effect of co-cultivation temperature on frequency of GTE in vetiver

Co-cultivated temperature (℃)	Number of calli treated	Number of GUS-expressing calli	Frequency of GTE (%)
18	37	2	5.4
20	38	3	7.8
22	40	6	15.0
25	41	8	19.5
28	37	3	8.1

3.4.5 Duration of co-cultivation

The duration of co-cultivation was observed by co-cultivated calli for different days of 1, 2, 3, 4 and 5 respectively. The frequency of GUS transient expression was only 2.7% after 1 day of co-cultivation, and was up to highest on day 3 or 4, and then decreased on day 5 due to abundant proliferation of bacteria (Table 7).

Table 7 The effect of co-cultivation time on frequency of GTE in vetiver

Days of co-cultivation	Number of calli treated	Number of GUS-expressing calli	Frequency of GTE (%)
1	37	1	2.7
2	38	2	5.3
3	40	5	12.5
4	41	6	14.7
5	37	3	8.1

Through the study on the five above-mentioned parameters, effective genetic transformation system of vetiver mediated by EHA105/ pCAMBIA1301 was established as follows: embryonic calli were immersed in AAM supplemented with 200 μmol l^{-1} AS until the OD_{600} was up to 0.4-0.5, then transferred to CIM containing 200 μmol AS in the dark at 25℃ for 3-4 d, the calli were then incubated on SIM containing 50 mg l^{-1} Hyg B and 500 mg l^{-1} Cef in dark at 25℃ to yield resistant calli.

3.5 *Agrobacterium*-mediated transformation

About one to two-month-old, light yellowish and compact embryonic calli were used for transformation. A total of 450 calli were co-cultivated with EHA105/p1301UN-*ots*A. After 4-day co-cultivation, embryonic calli were inoculated on SIM in dark at 25℃ for 4 weeks. During the selection period on Hyg B (50 mg l^{-1}), a majority of calli gradually turned brown or even died whereas some yellowish Hyg B resistant calli were observed 4 weeks after selection. These Hyg B resistant calli were subjected to a lower level selection (25 mg l^{-1} Hyg B) to the regeneration process. In the three transformation experiments, 18% calli showed resistance to the Hyg B selection. However, the differentiation and growth of Hyg B resistant calli and buds was commonly delayed for 10-15 d compared to those of the control, some of them showed multiple phenotypic alterations, such as yellow wrinkling leaves, slow growth, short and thin shape. Similar growth retardation phenomenon has also been found in transgenic tobacco, potato and sugarcane due to the over expression of *ots*A gene (Goddijn *et al.*, 1997; Yeo *et al.*, 2000; Wang *et al.*, 2003).

3.6 Analyses of Hyg B resistant calli

After 4-day co-cultivation, GUS expression was observed from resistant calli. Parts of calli had already become blue distinctly, indicating that T-DNA of vector EHA105/1301UN-*ots*A had been delivered into the plant cells (Fig. 6A), although the GUS expression levels varied among them. Fig. 7B showed Hyg B resistant calli after 4 weeks of cultivation on SIM. Fig. 6C-a showed Hyg B resistant buds after 4 weeks of cultivation on SDM, but no buds in controls (Fig. 6C-b).

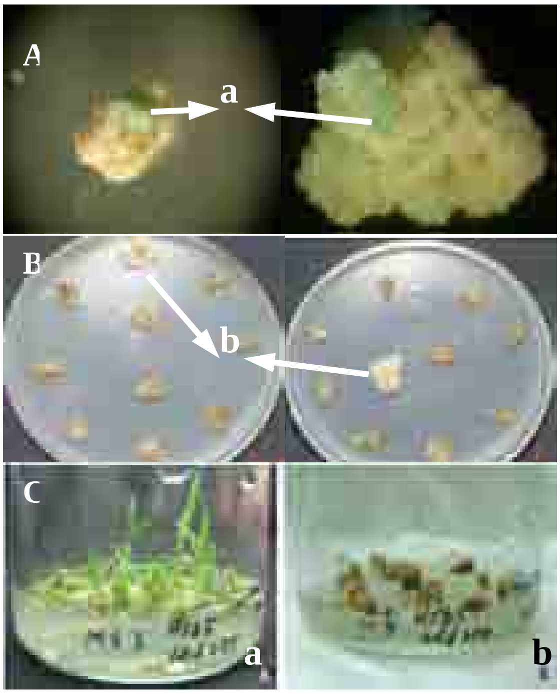

Fig. 6 Screening of resistant calli

A: GUS assay 3 d after co-cultivation (arrow a); B: Hyg B resistant calli (arrow b) 4 weeks after cultivation on SIM; C: (a) Hyg B resistant buds 4 weeks after cultivation on SDM; (b) control

4 Conclusion and Perspective

To our knowledge, this is the first report about gene engineering of vetiver. In this study, we gave a detailed observation on somatic embryogenesis and regeneration of vetiver, and established an effective and stable regeneration system for gene transformation. 2,4-D played an important role during the somatic embryogenesis. Explants could be regenerated via somatic embryogenesis when the medium contained only 2,4-D without or with 6-BA. Embryonic calli came from epidermal cells and parenchyma cells, which have the typical embryonic structure of monocotyledons, including racidele, embryonic bud, hypocotyl, scutellum, coleoptile and coleorhiza. Hence regeneration ability of embryonic calli of vetiver was very strong, and even could be maintained over two years. The whole process from callus induction to green plantlets transplanting to soil took about 3-4 months.

In the present study, plant expression vector p1301UN-*ots*A was constructed by inserting *ots*A gene digested with *Sac* I/*Kpn* I into the MCS site of binary vector pCAMBIA1301UN. Besides, an efficient *Agrobacterium*-mediated transformation system of vetiver was also established and five transformation parameters were optimized. Gus assay confirmed that the target gene was integrated into the Hyg B-resistant calli and plantlets, although some of them showed obvious growth retardation and phenotypic alteration.

So far, the obtained results have laid a good foundation for screening out transgenic cold-tolerant cultivars of vetiver. At present we are doing molecular analysis and cold resistance analysis to the Hyg B resistant plantlets, and will conduct further observation after they are transplanted to outside field and to northern regions of China with the objective of obtaining the true transgenic cold-tolerant cultivars of vetiver.

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References

Ahloowalia BS, and Maretzki A. 1983. Plant regeneration via somatic embryogenesis in sugarcane. Plant Cell Report, 2: 21-25.

An G, Ebert PR, Mitra A, *et al.* 1988. Binary vectors. In: Galvin SB, and Schilperoort RA (eds.) Plant Molecular Biology Manual. Kluwer, Dordrecht. pp. 1-19.

Bettany AJE, Dalton SJ, Timms E, *et al.* 2003. *Agrobacterium tumefaciens*-mediated transformation of *Festuca arundinacea* (Schreb.) and *Lolium multiflorum* (Lam.). Plant Cell Rep., 21: 437-444.

Block MD, Herrera EL, Vanmontagu M, *et al.* 1984. Expression of foreign genes in regenerated plants and in their progeny. EMBO J., 3: 1681-1689.

Christensen AH, and Quail PH. 1996. Ubiquitin promoter-based vectors for high-level expression of selectable and/or screenable marker genes in monocotyledonous plants. Transgenic Res., 5: 213-218.

Crowe JH, Carpenter JF, and Crower LM. 1990. Are freezing and dehydration similar stress vector? A comparison of modes of interaction of stabilizing solutes with biomolecules. Cryobiology, 27: 219-231.

Dillen W, DeClercq J, Kapila J, *et al.* 1997. The effect of temperature on *Agrobacterium tumefaciens*-mediated gene transfer to plants. Plant J., 12: 1459-1463.

Dion P, Belanger C, Xu D, *et al.* 1995. Effect of acetosyringone on growth and oncogenic potential of *Agrobacterium tumefaciens*. Methods Mol. Biol., 44: 37-45.

Dong S, and Qu R. 2005. High frequency transformation of tall frescue with *Agrobacterium tumefaciens*. Plant Science, 168: 1453-1458.

Drennan PM, Smith MT, and Goldsworthy D. 1993. The occurrence of trehalose in the leaves of the desiccation tolerant angiosperm *Myrothamnus flabellifolius.* Plant Physiol., 142: 493-496.

Fullner KJ, Lara JC, and Nester EW. 1996. Pilus assembly by *Agrobacterium* T-DNA transfer genes. Science, 273: 1107-1109.

Goddijn OJM, Verwoerd TC, and Voogd E. 1997. Inhibition of trehalase activity enhances trehalose accumulation in transgenic plants. Plant Physiol., 113: 181-190.

Hiei Y, Ohta S, Komari T, *et al.* 1994. Efficient transformation of rice (*Oryza sativa* L.) mediated by *Agrobacterium* and sequence-analysis of the boundaries of the T-DNA. Plant J., 6: 271-282.

Hood EE, Gelvin SB, Melchers LS, *et al.* 1993. New *Agrobacterium* helper plasmids for gene-transfer to plants. Transgenic Res., 2: 208-218.

Ishida Y, Saito H, Ohta S, *et al.* 1996. High efficiency transformation of maize (*Zea mays* L.) mediated by *Agrobacterium tumefaciens.* Nat. Biotechnol., 14: 745-750.

Jefferson RA. 1987. Assaying chimeric genes in plants: the GUS gene fusion system. Plant Mol. Biol. Rep., 5: 387-405.

Li, L, and Qu, R. 2004. Development of highly regenerable callus lines and biolistic transformation of turf-type common bermudagrass (*Cynodon dactylon* (L.) Pers.). Plant Cell Rep. 22: 403-407.

Ma GH, Xia HP, Xian YL, *et al.* 2000. Somatic embryogenesis and shoot formation from explants of *Vetiveria zizanioides*. Journal of Tropical and Subtropical Botany, 8: 55-59.

Mackenzie KF, Singh KK, and Brown AD. 1988. Water stress planting hyper sensitivity of yeast: protective role of trehalose in *Saccharomyces cerevisiae*. Gen. Microbiol., 134: 1661-1666.

Matzke M, Matzke AJM, Kooter JM, *et al.* 2001. RNA: guiding gene silencing. Science, 293: 1080-1083.

Murashige T, and Skoog F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol. Plant, 15: 473-497.

Mucciarelli M, Gallino M, Scannerini S, *et al.* 1993. Callus induction and plant regeneration in *Vetiveria zizanioides*. Plant Cell Tiss. Org. Cul., 35: 267-271.

Ohta S, Mita S, Hattori T, *et al.* 1990. Construction and expression in tobacco of a beta-glucoronidase (GUS) reporter gene containing an intron within the coding sequence. Plant Cell Physiol., 31: 805-813.

Ruth EL, Marianne L, and Charles E. 2000. Compact callus induction and plant regeneration of a non-flowering vetiver from Java. Plant Cell Tiss. Org. Cul., 62: 115-123.

Strom AR, and Kaasen I. 1993. Trehalose metalose metabolism in Escherichia coli: stress protection and stress regulation of gene expression. Mol. Microbiol., 8: 205-210.

Summerfelt ST, Adler PR, Gleen DM, *et al.* 1999. Aquaculture sludge removal and stabilization within created wetlands. Aquacultural Eng., 19: 81-92.

Suzuki S, and Nakano M. 2001. *Agrobacterium*-mediated production of transgenic plants of *Muscari*

armeniacum Leichtl. ex Bak. Plant Cell Rep., 20: 835-841.
Szucs A, Dorjgotov D, Otvos K, *et al.* 2006. Characterization of three Rop GTPase genes of alfalfa (*Medicago sativa* L.). Biochem. Biophys. Acta, 1759 (1-2): 108-115.
Tingay S, McElroy D, Fieg S, *et al.* 1997. *Agrobacterium tumefaciens*-mediated barley transformation. Plant J., 11: 1369-1376.
Vasil V, Vasil IK, Lu CY, *et al.* 1984. Somatic embryogenesis long term cultures of *Zea mays* L. (Gramineae). Amer. J. Bot., 71: 158-161.
Wang YQ, Wang YX, and Dai XY. 2000. Cloning and expression of *otsA* gene in *E. coli*. Acta Microbiologica Sinica, 40: 470-474.
Wang ZZ, Zhang SZ, and Yang BP. 2003. Trehalose synthase gene transfer mediated by Agrobacterium tumefaciens enhances resistance to osmotic stress in sugarcane. Scientia Agricultura Sinica, 36: 140-146.
Xu ZQ, Gong LG, Huang X, *et al.* 2004. Transgenic Maize Plants with Low Copy Number of Foreign Genes were Produced with Maize Ubi-1 Promoter. Chinese Journal of Biotechnology, 20: 120-125.
Yeo ET, Kwon HB, Han SE, *et al.* 2000. Genetic engineering of drought resistant potato plants by introduction of the trehalose-6-phosphonte synthase (TPS1) gene from *Saccharomyces cerevisiae*. Mol. Cells, 10: 263-268.
Zhao ZY, Gu WN, Cai TS, *et al.* 2002. High throughput genetic transformation mediated by *Agrobacterium tumefaciens* in maize. Mol. Breed., 8: 323-333.

A Brief Introduction to the First Author

Bingbing Yang, a doctorate student, is studying at the South China Botanical Garden, Chinese Academy of Sciences. Since 2002, she has been engaged in study on the molecular biology and gene engineering of vetiver, and actively dedicated herself to the research and application and of vetiver in China. So far she has 5 academic papers in this aspect published.

VETIVER SYSTEM FOR NATURAL DISASTER MITIGATION AND ENVIRONMENTAL PROTECTION IN VIETNAM - AN OVERVIEW

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Keywords: Natural disaster, environmental protection, erosion, slope stabilization, pollution control.

ABSTRACT

The use of Vetiver grass for natural disaster mitigation in Vietnam has become very popular despite the fact it has been introduced into Vietnam for such purpose only 5-6 years ago and it was met with considerable skepticism at the beginning. However, thanks to the faith and efforts of some Vietnamese enthusiasts and believers, the grass is now known throughout the country and is in use practically in nearly 40 provinces (out of the total 64). It is planted in a very wide range of soil types and climatic conditions, from very cold winter in the North, very hot summer-cold winter, pure sand in Central Vietnam to acid sulfate soil, saline soil in the Mekong Delta.

The widest application of the VS is for river bank, irrigation canal, river and sea dyke erosion control, cut slope stabilization along highways. But it is also used for sand dune stabilization, reduction of soil erosion on sloping farm land due to surface runoff, reduction of flood damages etc. And very recently, several trials have been made in using VS for wastewater and pollution control, for reclamation of contaminated and toxic soils etc. Many lessons have been drawn out on the use of Vetiver grass, including its advantages and disadvantages as well as comparison with other, either rigid, ineffective, expensive, environmental-unfriendly structural measures or traditional bio-engineering approach. A plan to compile a manual on the use of Vetiver grass for natural disaster mitigation is now being implemented and it is expected to be complete by the end of 2006.

1.0 INTRODUCTION

The use of vegetation as a bio-engineering tool for land reclamation, erosion control and slope stabilization have been implemented for centuries and its popularity has increased remarkably in the last decades. This is partly due to the fact that more knowledge and information on vegetation are now available for application in engineering designs, but also partly due to the cost-effectiveness and environment-friendliness of this “soft”, bio-engineering approach.

Although Vetiver grass (*Vetiveria zizanioides*) has been used first by Indian farmers for various purposes more than 200 years ago, its real impact on land stabilization/reclamation, soil erosion and sediment control only started in the late 1980’s following its promotion by the World Bank. While it still plays a vital role in agriculture, the unique morphological, physiological and ecological characteristics of the grass including its tolerance to highly adverse growing conditions and tolerance to high levels of toxicities provide an unique bio-engineering tool for other, non-agricultural applications such as land stabilization/reclamation, soil erosion and sediment control.

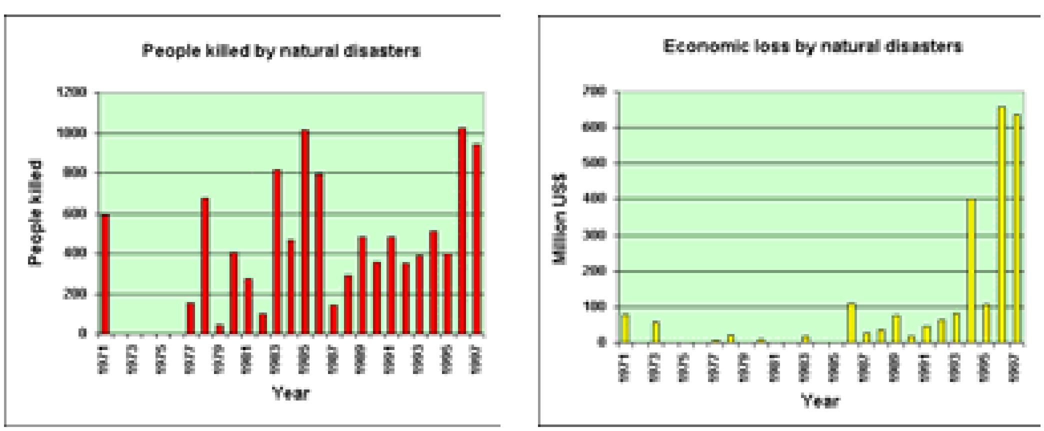
Although the concept of using Vetiver grass for various applications has only been introduced into Vietnam in 1999 by The International Vetiver Network (TVN) and since actively promoted by the Vietnam Vetiver Network (VNVN), Vetiver has become widely known throughout the country with numerous successful applications for natural disaster mitigation and environmental protection. Typical examples include road batter stability enhancement, erosion/flood control of embankments, dykes, riverbanks, sand dune fixation.

2.0 NATURAL DISASTERS IN VIETNAM

Vietnam is a natural disaster-prone country, where many types of natural disasters take place annually, causing a lot of losses of life, economic and environmental damages. Located in the tropical monsoon zone the country features a marked seasonal rhythm of rainfall of roughly 2,000 mm/year. However, about 75-80% of the rainfall takes place usually only during the 3 months of summer (starting from May in the North but becoming later in the South). And although there are about 200 rainy days a year, the major portion of the rain intensity falls in just about 10 days with more than 100 mm/day records. Coupled with the very diverse geological conditions, including many rock/soil types, active tectonic regime and the fast economic and demographic growth with its uncontrolled negative environmental degradation during the last two decades, such climatic extremes cause severe natural disasters, most frequent and destructive being floods, landslides and debris flows, flash floods, river bank and coastal erosion, sand storm/flow etc.

Total loss of life and properties by natural disasters has rapidly increased in 1990s. According to the "Second National Strategy and Action Plan for Disaster Mitigation and Management in Vietnam-2001 to 2020", about eight thousand people were killed, 2.3 million tons of foods were destroyed, and 6 million houses collapsed and washed away in the decade of 1991 to 2000. The total estimated economic loss was about USD2.8 billion, i.e. 1.8-2.3% of the national GDP or nearly USD 300 million yearly (Fig.1).

Fig.1. People killed and economic loss by natural disasters in Vietnam during 1974-1997 period (Source: UNDP Project VIE/97/002 - Disaster Management Unit).



A research project on natural hazards of geologic origin (geohazards) in 8 coastal provinces of

Central Vietnam was carried out recently (RIGMR, 2000-2002) which shows that river bank and coastal erosion, landslide, sand storm/flow are mainly caused by short but catastrophic storm/floods (Fig.2). A survey has been conducted for 850 km of banks of 25 main rivers and 900 km of the coastline in the region, identifying in each province tens of km of river bank or coastline under severe erosion. A landslide inventory has also identified 1600 landslides including nearly 100 large-sized ones. Many landslides occurred along important national and provincial routes. An example was the large landslide on the Hai Van Pass in 1999 that totally disrupted the North-South traffic for half a month and cost more than one million US dollars.

Fig.2. Major types of natural disasters in Vietnam.

Flood and innundation in the Red River Delta due to a recent storm No. 7 (Sept. 2005).

Flash flood and innundation in mountainous karst areas of NW Vietnam.

Coastal erosion in Hai Hau (Nam Dinh) due to storm No. 7 - a used to be nice walkway.

A guest house collapsed near Hoa Duan (Thua Thien-Hue) due to coastal erosion.

Right bank erosion along Tra Khuc River in Quang Ngai.

Bank erosion along Ganh Hao River (Bac Lieu), causing complete collapse of the jetty.

Landslide in the completely weathered granite along Ho Chi Minh Highway (Thua Thien-Hue).

Even concrete retaining wall did not help - a rock slide near Phong Nha (Quang Binh).

A garbage site in A Luoi (Thua Thien-Hue), leachate from which would move down to the A Sap River and thereafter … to Laos.

An AO-contaminated landfill at A So Airport (A Luoi, Thua Thien-Hue) (the AO content is 29 times higher than allowable).

Untreated waste water from a paper mill in Bac Ninh province - to be pumped directly into the Ngu Huyen Khe River just behind the dyke.

An environmental pond at the fertilizer plant in Bac Giang province which can’t help reducing suspended/soluble toxic elements.

Sand flow in Le Thuy (Quang Binh) in 1999, exposing the foundation of a pumping station.

And destroyed a 3-room brick house of this woman.

From what will be presented below, one would see that Vetiver grass can be applied effectively for reducing many types of natural disasters mentioned above.

3.0 TRADITIONAL REMEDIAL MEASURES AND THE NEED FOR NEW APPROACHES

The Ministry of Agriculture and Rural Development (MARD) and its provincial departments (DARD) are responsible for dyke management and "naturally" they undertake measures to protect river banks. Similarly, the Ministry of Transport (MOT) and its provincial departments (DOT) are in charge of road construction and road-related slope protection. Their concept is mostly to use structural, rigid protection measures e.g. concrete or rock riprap bank revetment, groins, retaining walls etc.

These measures are, however, very expensive and the State budget for such works can never be sufficient. For example, river bank revetment costs usually 200,000-300,000 USD/km, sometimes up to 0.7-1.0 million USD/km. An extreme case is the Tan Chau embankment in the Me Kong Delta which costs nearly 7 million USD/km. And it has been estimated that river bank protection in Quang Binh province alone would already require more than 20 million USD, while the annual budget for that is only 300,000 USD. Construction of sea dyke costs usually 0.7-1.0 million USD/km but more expensive sections costing up to 2.0-2.5 million USD/km are not rare. After the recent storm No. 7 in September 2005 that washed away many dyke sections, some dyke managers believe that even such rigorous dyke system is not rigid and strong enough (capable to withstand storms of up to 9^{th} level only) and they begin to talk about constructing stronger sea dykes (capable to withstand storms of up to 12^{th} level) that would cost about 7-10 million USD /km. In the mean time, budget constraint is always there and as a result, structural, rigid protection measures can only be very much localized, for the most acute sections, and can never be extended to the full length of the river bank/coastline that needs protection. The problem thus becomes much severe than the means to solve it, and one could conclude that this has seriously challenged the present concept of river bank and/or coastal protection using only localized, structural, rigid measures (Fig.3).

From the technical and environmental perspectives, one may notice the following concerns:

- Rock/concrete is mined/produced elsewhere, where it can cause environmental problems;
- Localized structural, rigid measures do not absorb flow/wave energy and tend to displace

erosion to another place, opposite or downstream. In so doing, they even aggravate the disaster, rather than really reduce it for the river as a whole. Typical examples of these can be found in several provinces in central Vietnam;

- Structural, rigid measures bring in considerable amount of stone, sand, cement into the river system, disposing considerable volume of bank soil into the river, all eventually causing the river to become full, changing, raising the river floor, thus worsening flood and bank erosion problems;

Fig.3. Traditional structural measures.

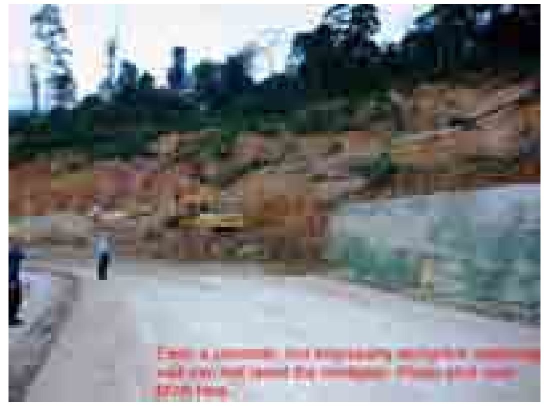

A rigorous rock riprap dyke along the Red River in Hanoi … And washed into the river.


Few of the many easily damaged sections of the salinity dyke system along Tra Bong River (Quang Ngai).

A very rigorously built sea dyke in Hai Hau (Nam Dinh) …

… and what is left from the previous dyke, about 200 m sea-ward and built 20 years ago.

Rock/concrete retaining walls built for road cut-slope stabilization …



… But they too, can not help in many places.

- Rigid structures are not compatible with the soft ground particularly on erodible soils. As the later is consolidated and/or eroded and washed away and undercut the upper rigid layer. This occurred in many places such as the right bank immediately downstream of the Thach Nham Weir (Quang Ngai province), where it cracked down and collapsed. Engineers try to replace concrete plates with rock rip-rap with or without concrete frame which, however, leaves the problem of subsurface erosion unsolved. A very typical example can be seen along the Hai Hau sea dyke, where the whole section of rock rip-rap collapsed as the foundation soil underneath was washed away;
- Rigid structures can only temporarily reduce erosion but they can not help stabilize the bank in case of big landslides with deep failure surface;
- Concrete or rock retaining wall is probably the most common engineering method applied for road slope stabilization in Vietnam. Most of these walls are, however, passive, waiting for the slopes to fail. When they do fail, they also cause the walls to fail as seen in many cases along the Ho Chi Minh Highway;
- Rigid structures like rock embankments are unsuitable for certain applications such as sand dune stabilization. They are, however, in some cases, still being built, as can be observed along the new road in central Vietnam.

Along with rigid structural measures, softer solutions, using vegetation have also been tried, though to a much less extent. For river bank erosion, the most popular bio-engineering method is probably the planting of bamboo, while for coastal erosion, mangrove, casuarinas, wild pineapple, nipa palm etc. are also being used (Fig.4). However, applications of these plants have shown some essential weak points, for example:

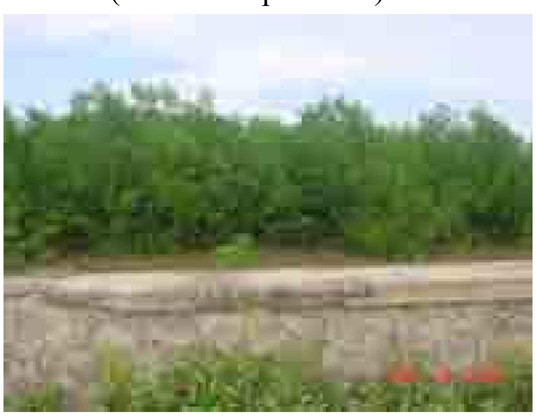
- Growing in clumps, bamboo can not provide closed hedgerows. The flood water tends to concentrate at gaps in-between clumps, where the water destructive power increases, thus causing more erosion to occur;
- Bamboo has only a shallow (1-1.5 m deep) bunch root system, not in balance with the high, heavy canopy, therefore clumps of bamboo put an additional heavy surcharge on a river bank, without contributing to the bank stability;
- With the bunch root system of bamboo, in many cases erosion undermines the soil below, creating conditions for larger landslides to take place. Examples of bank failure with extensive bamboo strips can be seen in several provinces in Central Vietnam;

– Mangrove trees, where they can grow, form a very good protection buffer zone for reducing wave power, thus reducing coastal erosion. However, establishment of mangrove is difficult and slow as its seedling is eaten by mice, and thus, of the hundreds of hectares planted, only a small part can develop to become forest. This has been reported recently in Ha Tinh province;
– Casuarinas trees have long been planted on thousands of hectares of sand dunes in Central Vietnam. Similarly, wild pineapple is also planted along banks of rivers, streams and other channels as well as along the contour lines of dune slopes. But they are good mainly for reducing wind power and respectively, sand storm but not sand flow as they do not form closed hedgerows and do not have deep root systems. Examples of building sand dykes along flow channels in Quang Binh province, with casuarinas and wild pineapple trees on top ended with obvious failure as the sand fingers continues to invade arable land. Moreover, experiences also show that casuarinas seedlings can hardly survive sporadic but extreme cold winter (less than 15^{o}C) while wild pineapple dies from extremely hot summer in North Vietnam;

Fig.4. Using endemic species.

Endemic species are used for river bank protection (Nam Dinh province) …

… But they too, can not help in many places (Tra Bong River, Quang Ngai province).



Mangrove is very good …

… But its establishment is difficult.

It seems, thus, that no appropriate engineering solutions for natural disaster reduction have been found yet in Vietnam. It is for this reason that the RIGMR research project recommends to fundamentally reconsider the present concept, economically and technically as well as environmentally.

4.0 INTRODUCTION OF THE VETIVER SYSTEM TO VIETNAM: INITIAL DIFFICULTIES AND SUBSEQUENT WIDE SPREAD

In 1999 at the request of the Ministry of Agriculture and Rural Development (MARD), Ken Crismier of The International Vetiver Network (TVN) invited two VS experts, Paul Truong and Diti Hengchaovanich, to visit Vietnam. A series of workshops were organized in Hanoi, Ho Chi Minh City and Nghe An province, which received great interests at all levels, from Ministries to farming communities. Several trial sites were set up following 3 truck loads of Vetiver grass donated by the Department of Land Development, Thailand. Some results were achieved by the National Institute for Soil and Fertilizer for erosion control in slope farming but it was not bold enough to move forward. On the other hand, due to the lack of good design, experience and care, some trials did not give good results in Central and North Vietnam when applied for river dyke protection and river bank erosion control. The grass was met with skepticism while the few first followers could not agree to cooperate in promoting the grass nation-wide.

4.1 The First Bold Results and Juridical Basis for Wider VS Application

Fortunately, almost at the same time in the South, with support from Paul Truong, Pham Hong Duc Phuoc from The Ho Chi Minh City University of Agro-Forestry carried out some trials in Central Highland for land slips control and slope stabilization. A private company, THIEN SINH, in Ho Chi Minh City was interested in using VS for land stabilization and environmental protection purposes. It requested Paul Truong and Pham Hong Duc Phuoc to advise and develop specifications for land slip control and slope stabilization on the HCM highway. Their good results convinced MARD and consequently in October 2001, MARD issued a Decree to allow for mass application of Vetiver grass for natural disaster reduction in Vietnam.

A significant importance is the change in attitude of the Dyke Department with respect to Vetiver grass. Being one of the few pioneers in Vietnam to try the grass (in 1999) for protecting its extensive dyke system, but due to bad design of their earlier trial, the Department unfortunately did not see encouraging results and since then was quite skeptical about this measure. Quite a few doubtful questions were raised with regards to the use of the grass on dyke, e.g. would it strengthen the soil or be rather harmful to the dyke body by increasing soil porosity and permeability, or would the grass root become harmful when dead etc. However, numerous successes at other sites, coupled with continuous failures of traditional “hard” measures gradually pushed the Dyke Department to accept the new solution. Although not entirely enthusiastic about it, the Dyke Department has silently issued Decrees No. 436 and 438/PCLB dated 12/09/2003 on the use of Vetiver hedgerows for river bank and coastline protection. Very recently the Dyke Department announced that it has approved the technology for protection of embankment on several rivers in North, Central and South Vietnam.

4.2 VS Application and Promotion in North Vietnam

In 2004, at the recommendation of Elise Pinners and Tran Tan Van, the Danish Red Cross funded a pilot project using Vetiver grass for sea dyke protection in Hai Hau district, Nam Dinh province (Fig.5). The project implementers came in and to their biggest surprise, they found out that Vetiver grass had already been planted 1-2 years earlier to protect several km of the inner side of the local sea dyke system. Although the planting design was not up to the standard recommended for such application, this planting has helped protecting the dyke system from erosion and the local people were already convinced of the effectiveness of the grass, asking for more mass planting. The effectiveness of Vetiver grass in reducing erosion of the sea dyke was even more remarkable after typhoon No. 7 in September 2005, which broke even rigorously rock rip-rapped sections.

Fig.5. VS application and promotion in North Vietnam.

Nam Dinh province - Vetiver grass and outer side of the newly built seadyke …

… Local Dyke Department planted Vetiver grass on the inner side of their seadyke.

Concerned with the wellbeing of the people living on the path of these typhoons in the Ha Long area, where their livelihood depends on the stability of this protective sea dike system, HRH ***Princess Maha Chakri Sirindhorn*** of Thailand, Patron of The Vetiver Network, has just approved a project for Chaipattana Foundation, a private foundation set up by the HM The King of Thailand, to assist VNVN both technically and financially to improve the stability of a sea dike at Hai Hau District, Nam Dinh Province, where the sea dike system was devastated by typhoons number 6 and 7 in September 2005. A group of Thai engineers and Vetiver experts will come to Hai Hau in July 2006 to finalize the project details with VNVN.

4.3 VS Application and Promotion in the Mekong Delta

In 2001 with financial and technical supports from the Donner Foundation and Paul Truong respectively, Le Van Du from the same university worked on Acid Sulfate Soil to stabilize canal and irrigation channels and sea dyke system in Go Cong province. Despite the poor embankment soil the grass grew rigorously in just a few months, helping to protect the sea dyke, preventing surface erosion and facilitating endemic species to establish.

Later on, during the period of 2003-2005, Le Van Du went on to try VS on the sodic-saline "desert" provinces of Binh Thuan and Ninh Thuan, where the annual rainfall intensity averages just 200-300 mm. In the topsoil a thin gypsum layer develops to effectively prevent the root system of many other species to penetrate to the more humid layer underneath. Du and his staffs developed a good technique to help Vetiver grass survive the initial stage. Once established, their trial showed that the grass root system reached 70 cm after 3 months, penetrating the gypsum layer. The grass grew 2-3 times faster than any other crops, yielding a fresh biomass of 25-30 tons during the first 2 months and up to 50 tons after 3 months (Fig.6).

Fig.6. VS application under extreme soil and climatic contitions in South Vietnam.


Planting Vetiver grass in Go Cong province, behind the natural mangrove on this Acid Sulphate Soil sea dyke. Surface erosion is reduced and local grass re-established.


75 day old sward produces 25 tons/ha (fresh weight) of biomass on this sodic-saline desert.

With financial support from Donner Foundation and technical support from Paul Truong, Le Viet Dung and colleagues from Can Tho University worked on river bank erosion control in the Me Kong Delta. The area features long lasting (up to 3-5 months) high water (flood) season, high (up to 4-5 m) difference in the water levels between high and low water seasons, and strong water flow during the high water (flood) season. In addition, river banks are mostly made up from alluvial, silt to loam soils, extremely erodible when wet. Due to the fast economic development in recent years, most boats traveling on rivers and canals now are motorized, in many cases with very powerful car engines. They even more aggravate the problem of river bank erosion by generating strong waves. However, inspite of these negative factors, Vetiver grass withstands well, protecting lots of precious land from erosion (Fig.7).

A very comprehensive Vetiver planting program has been carried out in An Giang Province, where annual flood can reach up to 6 m depth. The province 4932 km long canal system needs maintenance and repair every year. In addition, a network of dikes, 4600 km long, was built to protect 209957 ha of prime farm land from flood. The erosion on these dikes is about 3.75 Mm^3/year and required USD 1.3 M to repair. There are also 181 resettlement clusters built on dredged materials for people to live. These clusters also need erosion control measure from flood. Depending on the locations and flood depth Vetiver has been successfully used by itself or in combination with other vegetation. ***The total length of Vetiver planting for dyke protection from 2002-05 is 61 km using 1.8 M polybags.*** It is anticipated that for the next 5 years, 2006-2010, the 11 districts of An Giang province will plant ***2025 km of Vetiver hedges on 3100 ha of dike surface***. If unprotected by Vetiver, it is expected that 3750 Mm^3 of soil will be eroded and 5 Mm^3 will have to be dredged from the canals. Based on the current cost, the total maintenance cost over this period would exceed ***USD 15.5 M*** for this province alone. In addition, application of VS in this rural region will provide extra income to the local people: men to plant and women and children to prepare Vetiver polybags.

Fig.7. VS application and promotion in the Me Kong Delta.

Vetiver grass and river dyke in An Giang province …

… along natural river bank …

… and along the edge of flood resettlement centers.

The red line shows about 5 m of dry land is saved thanks to Vetiver grass.

As a result, extensive use of Vetiver grass is now seen along the rigorous sea and river dyke systems as well as along river bank, canals etc. in the Me Kong Delta.

4.4 VS Application and Promotion in Central Vietnam

In February 2002, with financial support from the Dutch Embassy Small Program and technical support from Elise Pinners and Pham Hong Duc Phuoc, Tran Tan Van from RIGMR tried to stabilize sand dunes and river banks in Central Vietnam. A sand dune was badly eroded by a stream that served as a natural boundary between farmers and a Forestry Enterprise. The erosion took place for several years, resulting in a mounting conflict between the two groups. Vetiver grass was planted in rows along the contour lines of the sand dune. After 4 months it formed closed hedgerows and the sand dune was stabilized. The Forestry

Enterprise was so happy that it decided to mass plant the grass in other sand dunes and even for the protection of a bridge abutment. The grass further surprised local people by surviving the coldest winter in ten year, when the temperature lowered down below 10^{o}C, forcing the farmers to replant twice their paddy rice and Casuarinas. After 2 years, local species such as Casuarinas, wild pineapple etc. re-established. The grass itself faded away under the shade of these trees but it has accomplished its mission. The project again proved that with proper care Vetiver grass could survive very hostile soil and climatic conditions (Fig.8).

Fig.8. VS application and promotion in Central Vietnam for sand dune fixation.

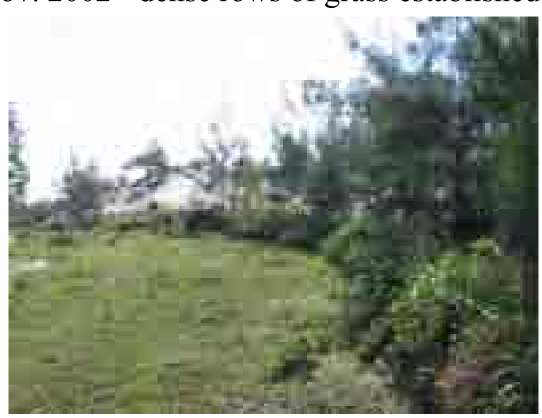
Trial application of VS for fixing sand dunes.

Early April 2002 - one month after planting.

Early July 2002 - four months after planting.

Nov. 2002 - dense rows of grass established.

Vetiver grass for protection of bridge abutment along the No. 1 National Highway.



Dec. 2004. Local species establish to fix the sand dune completely.

At the same time, Vetiver grass was planted to fix erosion of a river bank, bank of a shrimp pond and a road embankment in Da Nang City. Consequently, in October 2002 the local Dyke Department also decided to mass plant the grass on more bank sections of several rivers. Furthermore, the city authority decided to fund a project on cut slope stabilization using Vetiver grass along the mountainous road leading to the Ba Na resort (Fig.9).

Fig.9. VS application and promotion in Central Vietnam for river bank erosion control.

Da Nang city Dec. 2004 - Vetiver grass and river bank for already 2 seasons …

… Local farmers themselves planted Vetiver grass for protecting their shrimp ponds.

Following the success of this pilot project, a workshop was organized in early 2003 for more than 40 participants from local DARDs, DOSTEs, different NGOs and Universities of Central Vietnam's coastal provinces. The workshop helped both the authors of this paper and other participants draw useful lessons, especially on planting time, watering, fertilizing etc. After the event, also in 2003 World Vision Vietnam decided to fund another project for introducing Vetiver grass for sand dune fixation in the two Vinh Linh and Trieu Phong districts in Quang Tri province (Fig.10).

Fig.10. Workshops and field visits to promote the use of Vetiver grass.

Mid-February 2003 - Post-workshop field trip. The grass looks green even after the coldest in 10 years winter.

June 2003 - Nursery at home. A World Vision Vietnam-sponsored field trip for farmers from the neighbouring Quang Tri province.

Also as a result of this pilot project, Vetiver grass was recommended for use in another natural disaster reduction project in Quang Ngai province, which was funded by AusAID. With technical support from Tran Tan Van, in July 2003, Vo Thanh Thuy and his co-workers from the provincial Agricultural Extension Center planted the grass at 4 locations, for sea water intrusion protection dyke and irrigation canal in several districts. The grass grew well in all locations, and although at its young age, survived the flood in the same year (Fig.11).

Fig.11. VS application and promotion in Quang Ngai province.

Vetiver grass planted on river dyke along the Tra Bong River …

… On both sides of an anti-salinity estuary dyke along the same river.

… Another anti-salinity dyke section upstream, with the old-fashioned concrete rip-rap facing the river.

… And along a section of the irrigation canal. Note the poor shape of the opposite bank due to surface erosion.

Another section of the poorly eroded bank at Binh Thoi Commune, Tra Khuc River.

… And the traditional, primitive protection by local farmers using sand bag.

Vetiver grass comes in with local participation …

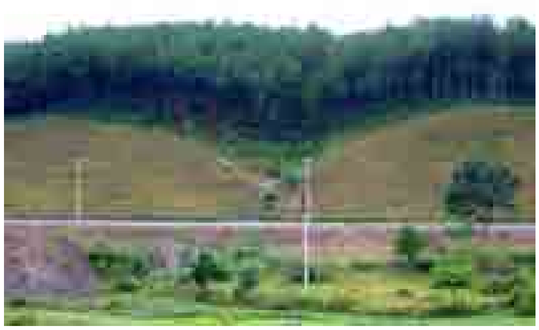
… And the bank stays intact after the flood season in Nov. 2005.

Following these successful trials, the project has decided to mass plant the grass on other dyke sections at 3 more districts, in combination with rock rip-rap measure. Some design modifications have been introduced to better adapt Vetiver grass to the local conditions. For example, mangrove and mangrove fern are planted on the lowest row to better withstand the saline water and effectively protect the embankment toe. The grass is further introduced to local communities so that the latters can protect themselves their own land.

4.5 VS Application and Promotion for Road Batter Stabilization

A particular bold move was made by the Ministry of Transport, following successful trials by Pham Hong Duc Phuoc and Thien Sinh Co. in using Vetiver grass for cut slope stabilization in Central Vietnam. In 2003, the Ministry of Transport allowed the wide use of Vetiver grass for slope stabilization in a series of National Highways, most notably along hundreds of km of the newly constructed Ho Chi Minh Highway (Fig.12).

Fig.12. VS application and promotion for road batter stabilization.



Vetiver grass for stabilizing cut slopes along the Ho Chi Minh Highway …

… either self or in combination with other traditional measures.

On a survey in January 2005 trip, a stretch of 500 km in the northern section of the Ho Chi Minh Highway was inspected to see that Vetiver planting was very extensive. In total more than half of this 500 km stretch has now been protected by Vetiver and more planting is in progress on the rest of the northern section as well as the southern section, and eventually most of this highway will be protected by Vetiver. This project is probably one of the largest VS applications in infrastructure protection in the world. The entire Ho Chi Minh Highway, over 3000 km long, is being and will be protected by Vetiver, planted on a variety of soils and climate: from skeletal mountainous soils and cold winter in the North to extremely acidic Acid Sulfate Soil and hot and humid in the South.

The extensive use of Vetiver grass for cut slope stabilization brings in very good results e.g.:

- Applied primarily as a slope surface protection measure, it greatly reduces run-off induced erosion, which would otherwise have caused many other hazards downstream (Fig.13);
- By preventing shallow failures, it greatly stabilizes cut slopes and consequently greatly reducing the number of deep slope failures;
- In some cases where deep slope failures do occur, it still does a very good job in slowing down the failures and reducing the failed mass; and
- It helps increase the environmental friendliness of the road etc.

Fig.13. Other effects of VS along the roads.

Improper rock/soil waste disposal.

… Which moves very far downstream.

Ugly and continuous failures of cut slopes as the vegetation cover is destroyed (Da Deo Pass, Quang Binh).

… But with Vetiver rows on top the slope very slowly squeezes down, considerably reducing the failed mass.

Pham Hong Duc Phuoc demonstrated clearly how VS should be used and its effectiveness and sustainability on a road leading to the Ho Chi Minh Highway. He carefully monitored the development of the VS in term of establishment (65-100%), top growth (95-160 cm after 6 months), tillering rate (18-30/plant) and root depth on the batter (Table 1).

Table 1. Vetiver root depth on Hon Ba road batters.

	Position on the batter	Root depth (cm)			
		6 month	12 month	1.5 year	2 years
	Cut Batter				
1.	Bottom	70	120	120	120
2.	Middle	72	110	100	145
3.	Top	72	105	105	187
	Fill Batter				
4.	Bottom	82	95	95	180
5.	Middle	85	115	115	180
6.	Top	68	70	75	130

Failures and successes with Vetiver grass protecting cut slopes along the Ho Chi Minh Highway show some further lessons:

- The slopes should first be internally stable as the effect of Vetiver grass does not come immediately and the slopes may fail before it really takes place. Stabilization may take place only after 3-4 months at the earliest; hence timing is also very important if slope failure during the forthcoming rainy season is to be avoided;
- Appropriate slope angle should not exceed 45-50^{o}; and
- Regular trimming is also important to ensure further growth of the grass to achieve good, dense hedgerows etc.

4.6 Application for Pollution Control

With financial support from the Wallace Genetic Foundation and technical input from Paul Truong the **Water Quality Improvement Project** was launched in all three regions of Vietnam, with the following project sites:

1. A seafood processing factory, CAFATEX, in Can Tho, Me Kong Delta
2. A small paper mill at Bac Ninh, north Vietnam
3. A small nitrogen fertilizer factory at Bac Giang, north Vietnam
4. A municipal landfill leachate disposal at A Luoi, Central Vietnam
5. A rural site contaminated with herbicides, pesticides and other toxic chemicals at A Luoi.

4.6.1 A seafood processing factory, CAFATEX, in Can Tho, Me Kong Delta

Due to the effectiveness of VS in treating wastewater and the current trend in water recycling around the world, the seafood processing factory, CAFATEX, was interested in testing VS as a method of phytoremediation to reduce the contaminant levels in the factory effluent. The emphasis is on the low cost and simple VS alternative instead of more costly chemical and engineering solutions. Can Tho City is the capital of the Mekong Delta, which is also the centre of several food processing industries. These seafood processing factories are the major sources of pollution to the region watercourses and farmlands nearby.

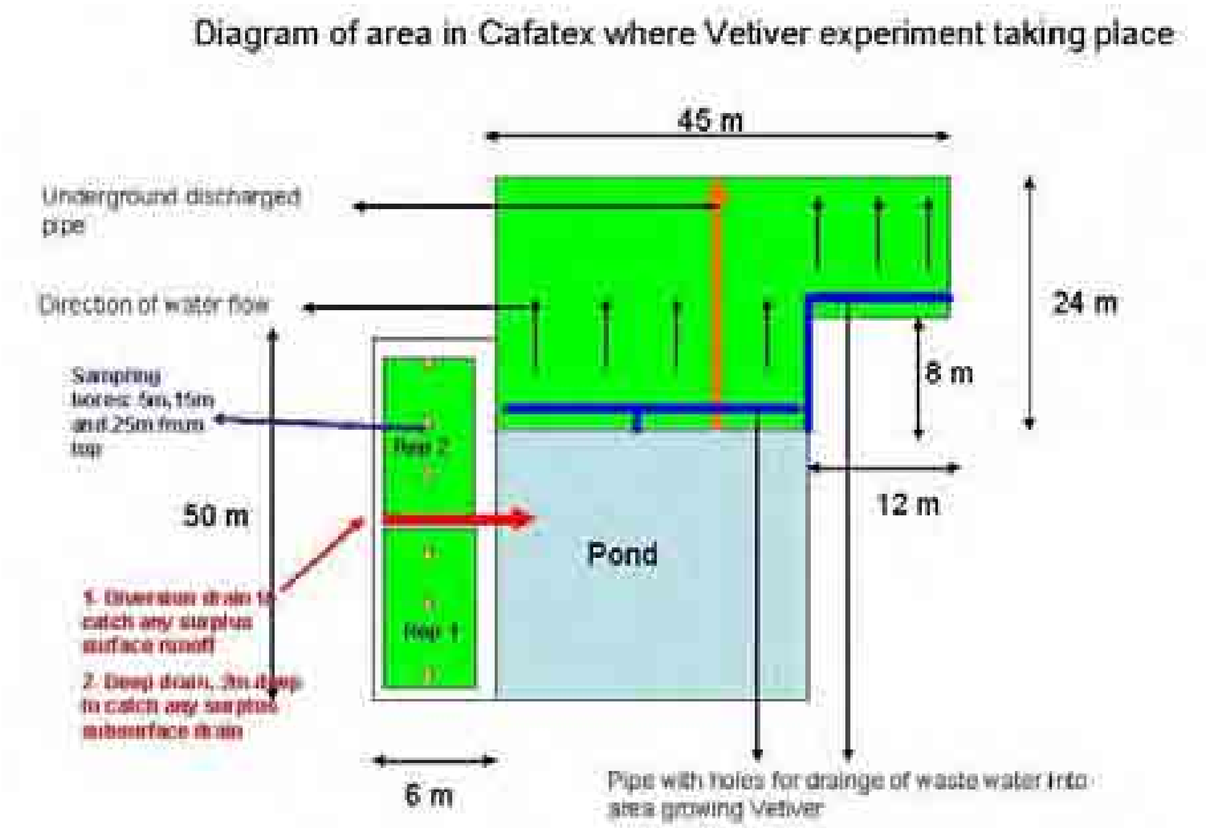
Two demonstration trials were set up at the factory. The first one was on an area of about 800 m^2, aiming at identifying which of the following methods would give the best result in the reduction of nitrate and phosphate in the wastewater:

- Subsurface flow; or
- Surface flow (flooding) over the Vetiver field

The second trial was 12 m long x 5 m wide, aiming at testing the ability of Vetiver grass in absorbing Nitrate and phosphate in wastewater.

Preliminary results have shown that both methods of irrigation are very effective in reducing nutrient load in the effluent (Fig.14).

Fig.14. Trial design and first results at Cafatex, Can Tho City.



Effluent pond and Vetiver planting in the background, ready for treatment.

4.6.2 A small paper mill at Bac Ninh, North Vietnam

This trial was established in July, 2005, at a small paper factory. The waste water from this factory is heavily polluted with acids and lime, as bad as landfill leachate, and is being discharged directly to a small river. As shown below, Vetiver could establish and grow well under these extremely polluted conditions (Fig.15).

Fig.15. VS trial application for pollution control at Bac Ninh paper mill, growing well along this highly polluted drain but could not survive when submerged in this muck.

4.6.3 A small nitrogen fertilizer factory at Bac Giang, North Vietnam

This trial was established in July, 2005 at a small nitrogen fertilizer factory. The waste water from this factory is highly polluted and is discharged to a small river after going through a chain of 3 environmental ponds which can't filter suspended and soluble wastes. The treatments consist of some rows on the banks of the holding ponds, where the soil is highly polluted from the ponds and on an adjacent wetland. Despite the highly polluted conditions, Vetiver established and grew well as shown in Fig.16 below.

Fig.16. VS trial application for pollution control at Bac Giang fertilizer plant, growing well both on the bank and in the polluted water.

4.6.4 A municipal landfill leachate disposal at A Luoi, Central Vietnam

A Luoi is a district in Central Vietnam mountainous region near the Laotian border and like most cities and towns in Vietnam it does not have any means to treat leachate from landfill depots. As a result the discharged leachate either drains into local streams and river or infiltrates to the underground water storage.

The leachate of A Luoi municipal landfill drains into a mountain stream, the water source for both domestic supply and agriculture use of several villages living along its course. Planting was carried out in February 2005, good establishment was achieved in July 2005 but due to the cold and wet weather growth has not been as good as in the low land (Fig.17).

Fig.17. VS trial application for leachate disposal control at A Luoi municipal landfill.

4.6.5 A contaminated site with herbicides, pesticides and other toxic chemicals at A Luoi

A Luoi was also the centre of very big battles during the Vietnam War. A So air base, in A Luoi district, was the main base of an Agent Orange (AO) spraying unit of the US Air Force in the northern section of South Vietnam. The billboard erected on the edge of the airfield warns local people to keep out of the airfield as it is heavily contaminated with Agent Orange and also has high level of Dioxin. ***Hatfield Consultant,*** a Canadian consulting group, carried out a site survey about 10 years ago and it has identified several "hot spots", which had very high level of herbicides and dioxin. A trial was set up in February 2005. The grass survives but the growth rate is slow which may require additional care. There is a plan to come back to the site, replant the grass and test its ability to adsorb/absorb the toxic chemicals (Fig.18).

Fig.18. VS trial application for control of toxic chemicals at A So air base.

5.0 VS APPLICATION AND PROMOTION

VS applications for natural disaster mitigation and environmental protection are now well known in Vietnam. In fact nearly 40 out of 64 provinces of Vietnam are now using Vetiver grass for various purposes. However, to further promote and convince any doubters, with the support of the Royal Netherlands Embassy and TVN, the Vietnam Vetiver Network organized a Regional International Conference entitled **"Vetiver System: Disaster Mitigation and Environmental Protection in Vietnam"** from 19-21 January, 2006 at Can Tho University, Can Tho city. 75 Vietnamese delegates from various governmental departments and private sectors in the Mekong Delta and Ho Chi Minh City participated in the conference and field trip. In addition, 18 international speakers from Australia, China, Indonesia, South Africa and Thailand presented papers on various topics covering Disaster Mitigation, Environmental Protection, and On-farm Applications and its Socioeconomic Impacts on local communities. Also as part of the Royal Netherlands Embassy Small Program, a manual on the use of VS for natural disaster mitigation and environmental protection is now being compiled and it is expected to be complete by the end of 2006.

6.0 SOME LESSONS, RECOMMENDATIONS AND CONCLUSIONS

Natural disasters are very diverse in origin, very wide-spread, affecting many communities. Some natural disasters have their causes originated from very far upstream. Therefore:

- Macro-scale, non-structural, basin-wide disaster management should be introduced. Rigid, localized, difficult to apply, expensive, environmental unfriendly structures should be avoided, minimized and replaced by cost-effective, soft and flexible, easy to apply, community-based and environmental friendly bio-engineering methods, such as using Vetiver grass;
- Vetiver grass doesn't compete with rigid, traditional structural measures but rather supplement them. For example, the later may be applied in combination with VS at critical sites, by the Government agencies, while the grass can be planted at other, less critical sites, by local communities. Successful examples of combining Vetiver grass with rigid rock riprap can be found around Da Nang city for river bank protection, in Hai Hau for sea dyke protection, or along the Ho Chi Minh Highway for cut slope stabilization etc.;
- Vetiver grass can also be used in combination with other local vegetation, such as Casuarinas or wild pineapple for sand dune stabilization in Quang Binh province, Nipa palm, mangrove and mangrove fern for river bank protection in brackish to saline water near the sea in Quang Ngai province;
- Experience with sand dune protection in Quang Binh province show that planting time is very important as watering is crucial for the grass to withstand the hostile climatic and nutrition conditions in sand dune areas. Planting at the end of the rainy season, i.e. about September, may ensure sufficient water. Likewise, planting during spring, i.e. January, with little but frequent rains would also be appropriate. However, planting right in the dry season, e.g. April-May would require a lot of watering work. But planting right in the rainy season may result in too much water to wash away the seedlings etc.

Regarding the problem of pore water pressure inside the slope, there can be some good arguments as follows:

- Increase in water infiltration is one of the major effects of vegetation cover on sloping lands and there is concern that extra water will increase the pore water pressure in the soil which could lead to slope instability;
- However, field observations show much better counter-effects. First, planted on contour lines which would trap and spread runoff water on the slope, the extensive root system of Vetiver grass helps prevent localized accumulation of surplus water and distribute it more evenly and gradually;
- Second, increased infiltration is also balanced by a higher, and again, gradually rate of soil water depletion by the grass;
- Research in soil moisture competition in crops in Australia (Dalton *et al*, 1996) indicated that under low rainfall condition this depletion would reduce soil moisture up to 1.5 m from the hedges, thus increasing water infiltration in that zone, leading to the reduction of runoff water and erosion rate;
- Geotechnically, these conditions have beneficial effects on slope stability. On steep (30-60^o) slopes the space between rows at 1 m VI (Vertical Interval) is very close, this moisture depletion would be greater therefore further improve the slope stability.
- In high rainfall areas, to reduce this potentially negative effect of Vetiver grass on slopes, Vetiver hedges could be planted on a gradient of about 0.5% to divert extra water to stable drainage outlets (Hengchaovanich, 1998).

7.0 ACKNOWLEDGEMENTS

VNVN is grateful to The Vetiver Network (TVN), the Royal Netherlands Embassy in Vietnam Small Program, AusAid, Wallace Genetic Foundation, Donner Foundation, World Vision Vietnam, Danish Red Cross and many others for their kind supports. VNVN's steady growth would not also be possible without the continuous supports, inputs and advises of many individuals, in particular: Dr. Paul Truong, Director and Asia Pacific Representative, The International Vetiver Network, Ken Crismier, the first VNVN's coordinator, and Elise Pinners, Senior TVN Associate and other colleagues in Vietnam and overseas.

8.0 REFERENCE

1. Dalton, P. A., Smith, R. J. and Truong, P. N. V. (1996). Vetiver grass hedges for erosion control on a cropped floodplain, hedge hydraulics. Agric. Water Management: 31(1, 2) pp 91-104.
2. Hengchaovanich, D. (1998). Vetiver grass for slope stabilization and erosion control, with particular reference to engineering applications. Technical Bulletin No. 1998/2. Pacific Rim Vetiver Network. Office of the Royal Development Projects Board, Bangkok, Thailand.
3. Hengchaovanich, D. and Nilaweera, N. S. (1996). An assessment of strength properties of vetiver grass roots in relation to slope stabilization. Proc. First Int. Vetiver Conf. Thailand pp. 153-8.
4. Le Van Du and Truong, P. (2003). Vetiver System for Erosion Control on Drainage and Irrigation Channels on Severe Acid Sulfate Soil in Southern Vietnam. Proceedings 3rd International Conference on Vetiver grass (ICV3), Quangzhou, China, Oct., 2003.
5. Tran Tan Van et al. (2002). Report on geohazards in 8 coastal provinces of Central Vietnam -current situation, forecast zoning and recommendation of remedial measures.

Archive Ministry of Natural Resources and Environment, Hanoi, Vietnam.
6. Tran Tan Van, Elise Pinners, Paul Truong (2003). Some results of the trial application of Vetiver grass for sand fly, sand flow and river bank erosion control in Central Vietnam. Proceedings 3rd International Conference on Vetiver grass (ICV3), Quangzhou, China, Oct., 2003.
7. Truong, P. and Baker, D. (1996). Vetiver grass for the stabilization and rehabilitation of acid sulfate soils. Proc. Second National Conf. Acid Sulfate Soils, Coffs Harbour, Australia pp 196-8.
8. Truong, P. N. (1998). Vetiver Grass Technology as a bio-engineering tool for infrastructure protection. Proc. North Region Symposium. Qld Department of Main Roads, Cairns August, 1998.
9. Truong, P. N. and Baker, D. E. (1998). Vetiver Grass System for Environmental Protection. Technical Bulletin N0. 1998/1. Pacific Rim Vetiver Network. Office of the Royal Development Projects Board, Bangkok, Thailand.
10. Truong, P., Gordon, I. and Baker, D. (1996). Tolerance of Vetiver grass to some adverse soil conditions. Proc. First Int. Vetiver Conf., Thailand.

VETIVER HANDICRAFT PRODUCTION: AN INNOVATIVE FOCUS ON COMMUNITY DEVELOPMENT AND POVERTY ALLEVIATION IN RURAL VENEZUELA

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ABSTRACT

The Vetiver "Fundacion Empresas Polar" Project (VFEPP) has been conducted in an wide and diverse geographic zones, having trained approximately 11.000 people.

Five years ago, (VFEPP) started the Vetiver Project with the following general objectives: (i) to alleviate social disparities, (ii) to promote the making of vetiver handicraft as an initial step in the execution of an integral, economic ecological and social development project. (iii) to develop markets for vetiver handicraft in Venezuela. (iv) to increase participation of the poor population. (v) to stimulate the fight against the destruction of the natural resources and (vi) to enhance the spiritual and moral value through appropriate dynamics.

The innovative approach of the PVFEP is aimed at raising interest in vetiver handicraft in participants: women, young people, children and later, to the whole family. The activities begin through the conference *Why vetiver? An economic, ecological and social project.* Shortly after that the training of the vetiver handicarft begins and connecting the participants to the market, setting basic principles for quality; which occurs very quickly (in some cases in a month). It provides extra income to the families, the community develops a great interest in the Project, the reason why the planting of vetiver with the intention of producing fiber is promoted, on sites close to the houses where erosion, and contaminated waters are the common cological problems. The social aspect is focused on the principle of motivating the self-esteem, the community participation, the respect to their political, religious and cultural values. Music is a very important companion through sharing songs, directed readings and games, whose contents have been analyzed previously by professionals in the educational area. The groups consolidate through the local leaders and the position of the local coordinator is selected within the participants, by their personal conditions and disposition towards the handicrafts.

The participation of the PVFEP in each locality has a minimum of three years, and a maximum of five years, until the participants have been consolidated through businesses, companies, association or cooperatives. This methodology of work has been transferred to other government and private institutions, through strategic associations with the intention of promoting the use of vetiver in Venezuela. The development of the VFEPP is sustained by academic activities, supported too by national universities, and Research and Development projects.

Key words: Handicraft, communitarian development, erosion control, waste water tertiary treatment

1.0 INTRODUCTION

Although Vetiver has been in Venezuela for more than one hundred years, it is in the last 20 years that it has reached a very important height, and its presence has been expanded to many places of the country. The VFEPP has contributed to this expansion and dissemination of this plant in diverse geographic spaces, in the last five years. It has promoted and supported researches jointly with the Central University of Venezuela, Romulo Gallegos University and Simón Rodriguez University, "núcleo Canoabo" and with private companies; such as Pepsi Cola Venezuela-Agua Minalba, and Caucagua, Polar Brewery C.A, "Planta San Joaquin" and Polar Brewery C.A, "Planta Oriente".

2.0 MATERIALS AND METHODS

The scheme used by the VFEPP is focused on an community integral development plan, first to increase practice of the artisans and connecting the participants to the market to improve the economic income of the families; later, a program of planting in the areas affected by landslides and contaminated water associated with the houses of project's participants. The results, in trying to harness community interests, are to improve its quality of life as well as the self-esteem of many peoples.

2.1 Vetiver Planting System

The trainees are motivated to be independent by obtaining the raw material for themselves, that is to guarantee through easy methods of planting and the preparation of the treated fiber for vetiver handicraft. Once, the project begins, the treated fiber is donated to the communities. Once the artisan production has started and VFEPP reduced the amount of fiber gradually. Parallelly when the orders increased for artisan pieces, it would create more demand for raw material. At this time, the interest for planting of vetiver increased. An inspection is made to the sites where the people live, for identifying contaminated water and erosion problems. Also, VFEPP helps people in poor communities to use vetiver for land parcel demarcation, creation of "canteros" for production of family agriculture and orchards, and to teach how to protect houses in high risk areas, land parcels, irrigation channels and lagoons.

2.2 Formation of Staff for Project Vetiver of Polar Foundation Companies

From the beginning, the Project activities have been based on the program developed by the Pacific Rim Vetiver Network with the assistance of the Latin American Vetiver Network. The publications, The Vetiver a barrier against the erosion (1996) and "Vetiver Handicraft in Thailand (1999) were the precursors of VFEPP. Later, members were trained in design techniques and marketing of handicraft from course organized by the Technical Office for the Handicraft, from "Cooperación Española, and Fundación Empresas Polar-Cultural. The course of Negotiation of Harvard University, coordinated by the "Red Soc" (Social Network) Venezuela led to the systemization of VFEPP work methodology. The courses of Mental Maps of the "Organization Logros" gave excellent tools in the organizational schemes of VFEPP. A member of VFEPP staff assisted in the first artisan fair in Lausanne – Switzerland and presented a small sample of vetiver handicrafts made in Venezuela. This experience was very important for the training on how to make exposition. There was also a course for Design and Marketing of handicraft in "Cartagena de Indias", Colombia organized by the Office of Spanish

Cooperation. In addition, the VFEPP training course in Thailand through the "Bureau of Cottage and Handicraft Industries Development" in vetiver handicrafts and dyeing of fibers was invaluable. All these experiences have been transferred immediately to the participants. In the PVFEP the concept of social audit is practiced to implement control and systematization of the available information, transparent information to the communities, especially in relation to the commercialization of handicraft, relation with the national government and the presence in the universities to support researches of vetiver on specific topics.

2.3 Commercialization and Marketing

The different groups sell vetiver handicraft at national level based on a quality control system, label design, distribution and prices. Local, regional and national exhibitions, direct sale to companies, promotion of corporative gifts in companies were carried out. Advertising in newspaper, magazines, tourists guides, radio and television programs, sales in hotel and touristy place (along the route to Yaracuy), catalogs, bulletins, photos and movies were carried out. Face to face handling of internal conflicts to improve the interpersonal relations was carried out. In looking for directions to improve the quality, a very important contribution was received from Ing. Leopoldo Rodriguez Crespo, who is the expert in management of Polar quality of Companies. In this phase the opinions from the owners of the stores and the consumers were taken into account through soundings and direct product survey.

The expo-sales also have been a great incentive to improve the quality of the products. Other factor of importance was the quality of the fiber, especial the method and frequency of cut and the vegetative state of the plant. In this stage, Gregoria Sanchez, Jorge Romero and Jesus Gonzalez, contributed ideas that improved the knowledge on the fiber production. Also the company Vetiver Antierosión especially designed a very simple dryer to dry fiber in rainy time and for areas with high relative humidity. The exhibitions combined with sales have promoted a series of products from vetiver that involve the design and innovation. There is a very high acceptance of VFEPP once someone explains that the project concentrated on the fight against poverty, protection of environmental value and social development.

The PVFEP market is oriented by the following general principles:

- ***Innovation:*** Increase the types and production lines, emphasis on the artisan design.
- ***Permanent research***: Information of fashions and trends. Recovery and Restoration of pieces elaborated in other materials and application of vetiver fiber to add value.
- ***Continuous improvement and securing of the quality***: correction of faults, use of materials and redesign.
- ***Diversification:*** To create, from models of other products, conserving the raw material and techniques.
- ***Respect of the cultural traditions***: The PVFEP introduces vetiver as an alternative to substitute original artisan traditions, supporting them and combining the fiber similar to the traditional element with the advantages of raw material being readily available very quickly, little initial investment and without forcing the trainees to rely on many external materials to the artisan system.

3.0 RESULTS AND DISCUSSION

Experiences of the total indices of the PVFEP are summarized in the Table 1, which listed a total of 3.673 families directly involved. However, this does not take into account the multiple effects of the relatives and neighbors of the participants who had received the training, that means 11,019 persons altogether. The income to the communities of the Project recorded was US$ 188,198 from 2002 to July 2006. Until now it has been based on 75 cooperating stores in the whole country. The exhibitions are very important totaling 204 in all. The plantlets used were 780,323 units, for a total of 79,107.7 linear meters of barriers planted which is equivalent to 7.89 ha. It is noted the increase of the fiber packages produced by the communities from 97 units in the 2002 to 11,924 packages for July 2006, and a total of 39.037

TABLE Nº 1.

	2002	2003	2004	2005	2006	TOTAL
Families	381	1.023	643	528	1.098	3.673
Total people	1.143	3.069	1.929	1.584	3.294	11.019
Handicraft pieces	2.279	3.747	5.406	14.137	9.196	34.765
Income to communities US $	4.-047	10.342	23.544	52.771	97.492	188.198
Direct employments	381	1.023	643	528	1.098	3.673
Indirect employments	38	103	65	51	110	367
Shops	2	6	16	31	20	75
Exhibition	7	19	32	54	92	204
Vetiver plantlets planting	13.000	27-950	138.295	266.140	334.938	780.323
Lineal meters planting	1.800	2.686	14.414,7	26.714	33.493	79.107,7
Planting hectares	0,18	0,26	1,44	2,67	3,34	7,89
Familiar Agricultural	0	3	31	26	25	85
Fiber packets produced	97	460	7.936	18.620	11.924	39.037
Conferences	10	14	27	24	21	96

3.1 HOGARES SANTA ANA: Old People Homes. (HSA)-Aguirre Carabobo State

In March 2001, PVFEP initiated activities in HSA, located to the west of the Carabobo state, with the support of the old residents, and the participation of communities of Aguirre, Canoabo, Canoabito, Santa Ana, Montalban, Miranda, Bejuma, the Savannah of Canoabo. The support of the priest Oscar Monzon, through of the song in masses, was fundamental. The Agronomy Department of Venezuela Central University, Romulo Gallegos University, Simon Rodriguez University, "nucleo Canoabo", jointly with the "Alcaldia de Montalban", were the institutions that support the initiation of forming an ideal vetiver project, for the humble families of the region. The first handicraft pieces were created with much love that marked the passages in securing the quality and the guidelines for the development of the market.

3. 2 MIRALEJOS- La Guaira Vargas State

The incipient VFEPP, by initiation of the Red Soc. (Network Soc) of Venezuela and the Priest Mario Lizarazo from "La Guaira", Vargas state, started its activities in the 2002, as communities were very depressed by the tragedy, incalculable losses in La Guaira, that left a considerable number of destroyed houses and dead people. The sectors of Miralejos, La Vuelta in "Catia la Mar" were selected as pilots area to establish and planting vetiver interacting with young people and women. The zone was inhabited by 5,000 families, located in front of the International Airport of Maiquietia. With the support of Priest Mario Lizarazo, people started a project to weave with the fiber and root of vetiver and protecting fragile soils of the zone. For 5 years a group of women have been weaving, hand bags, baskets, with vetiver, and have maintained its presence in small "stand" at the International airport of Maiquetia.

3. 3 HATO VIEJO, Yaracuy State

A poor community of 600 families is located between Carabobo and Yaracuy states. Its main source of income is working in orange farms as laborers. Many settlers work in the clay factory in the school "Manos de Barro". And for two years a group of women has been dedicating to creating vetiver handicraft, developing a line of production of roosters and hens in different versions. Additionally, the artisan activities are motivated and supported by the Elmaisanco property, neighboring to the community.

3. 4 CUICAS, Trujillo State

Cuicas together with two Andean towns "Hill of Bonilla and the Conception", with an average of 1200 families of average poverty, opened the doors to PVFEP four years ago. Their main sources of income are the coffee plantations, cattle raising and the maintenance of property. Many families have informal candy sales and fruits shops along the highway. The weaves of “cola de mula” (*Sporobolus indicus*). the Palmiche (*Roystonea regia B.H.K*) and the clay factory are the recognized crafts of these towns. They have been working with vetiver in the last four years, forming groups of women and men from neighboring communities in the schools; the children complement their basic education with the weave of vetiver. All are developing a line of production of traditional cloths in manual loom, making chairs, wrist bands and carpets in different designs. This project is a leader in school community relation, with its participation in five Bolivarianas Schools in the zone.

3. 5 BIRONGO, Estado Miranda

It is a coastal town of 2600 families of average poverty. Their main income sources is the cocoa cropings and the preparation of the chocolate as well as other crops. Some families process the cocoa and are recognized by the artisan chocolate "Flor de Birongo". They use “la tapara” (*Crescentia cujete*) for vegetarian recipes, and “totumas” species to make soup (“sanchocho”) along the river banks. For the last three years a group of women has been dedicated to the creation of pieces of vetiver handicrafts, developing several lines of production, where they emphasize on fruits, hats, animals, purses and carpets in different versions.

3.6 SANTA CRUZ DE ARAGUA, Estado Aragua.

Estado Aragua is a traditional town in the center of the country. It has around 5000 families of average poverty. The main industry is the use and the culture of banano (*Musa sapientum L.*). The more outstanding handicarfts are wood carvings, clay pottery, handicraft of woven banano and enea fibers, and some candies. Groups of women, men and children have been dedicated to vetiver handicraft since two years ago, developing several lines of production: fruits, portfolios and diverse figures of animals. This project attracts people from other parts of the country, among them are from “El Sombrero, Guarico State, Guigue, Carabobo state, Malpica the Toro, Aragua state. Also they have transfered activities to government organissations, such as the Environmental and Resource Ministry, Mission Robinson, and Agricultural and Land Ministry.

3.7 SANTA ROSA DEL SUR, Estado Carabobo.

This area is formed by dispersed houses in the confluence of three states (Aragua, Carabobo and Guarico), characterized by the High River Basin of the Guarico River, a zone of about 200,000 square kilometers with serious ecological problems, mainly by the susceptibility of the land to water erosion. Its importance is highlighted as it is the water catchment basin of the Metropolitan Aqueduct of Caracas. Three years ago, the Romulo Gallegos University, Inparques in association with producers Asolasco and Asobena, were the institutions that motivated the introduction of the VFEPP to 40 families of extreme poverty, who farm by shifting cultivation on very high slopes. The VFEPP has been taking part in the recovery of 2 ha of land with vetiver barriers planting on contours to fortify and protect the soils, annual crops and roads against erosion and to filter runoff waters. Additionally, with the leaves, a group of women spends their free time making two lines of production of utilitarian pieces (place mat and baskets). It has been a great positive impact in the economic, ecological and social areas at this pilot zone.

3. 8 MALPICA EL TORO, Aragua State Sub Project.

The population is in extreme poverty. The participants have protected their soils with vetiver barriers, and use the plant to border their cultivations, mainly bananos to improve the soil humidity and the protection against erosion. The principal handicraft they produce are made from corn leaf; and candies. The vetiver handicraft has been developed with the participation of students, men and women; they are developing an important line of production of furniture with wrought iron weaving with vetiver fibers.

3.9 LA MARROQUINA, Yaracuy State Sub Project.

This is a community of 300 families of average poverty, whose main activity is sugar cane cropping and other works on properties. The young people practice ecological activities by giving special attention to the slash and burn cultivations on the mountainous areas. The sale of vetiver handicrafts produced in the zone by a group of women would benefit the eco-tourism on the property. They manufacture figures of birds, mammals of different sizes and forms. The craftswomen receive support from near by property, La Marroquina, for the direction, the provision of raw material and some contacts for the marketing of the crafts produced. The Güaquira farm supports the commercialization of the pieces in shops and stores in Caracas.

3. 10 PALMONAGAS, Monagas Sub Project.

Dra. Maria Bellorin, Ing Armando Hernandez from “Fundacion Empresas Polar” and Lic. Haidee Centeno from Palmonagas conducted an integral project where VFEPP is part of this community strategy. The project has selected vetiver in the communities of “La Hormiga”, “Vuelta Larga” and “El Zamuro”; they are members of family groups of average poverty. The growing and extraction of Oil Palm have been practiced for many years; and this work had become the main source of income for the inhabitants of these communities. Many families raise chickens in their house, but this economic activity is disappearing. A group of women and men have been motivated with the idea of the VFEPP. They have created important pieces and obtained their first considerable income. The project is only three month old.

3.11 GÜIGUE, Carabobo State Sub Project

It is a traditional town of 300 families with middle level poverty. Their main employment sources are agriculture and handicraft production. They are supported by San Jose's Benedictine Abbey (Catholic Church). They maintain some traditional customs such as cock fighting. A group of women has been devoted to the vetiver handicraft, developing two production lines: handbags and special fabric in manual loom, which have a positive impact to the income of some families dedicated to this project since two years ago.

3.12 PAYARES, Yaracuy State Sub-Project.

This Sub-project, under the responsibility of Dra. Maria Bellorin from Polar Foundation and Dr. Carlos Acosta, includes a community of 1500 families of half poverty. Their activities are supported by Trainer Luzmila de Velarde as a PVFEP consultant. They have their basic revenues from various forms of agriculture and plantation of sugar cane. The communities used corn leaf to make handmade objects and sell within the group. A small group of women has been devoted to the making of vetiver place mat and hats since two years ago.

3.13 SAN PEDRO DE LOS ALTOS, Miranda State, Sub-proyect. Strategic Association (SA)

This community located in the High Cuenca of the River San Pedro, with an approximate population of 3000 families of half poverty. The community produces flowers and other agriculture produces. Among the typical handicrafts made in the area are basket and doll. With support of Pepsi Cola – Venezuela, Agua Minalba; a group of women has been devoted to the design and making of vetiver pieces for three years, developing several production lines such as animals, angels and handbags in different versions. This project underlines the concept of sustainable development, establishing forests that will consolidate the lands owned by the company over a 20 years period, in which vetiver is a fundamental precursor to tree planting, and integrating the neighboring communities with this sustainable development through the vetiver handicraft system.

3.14 POLAR BREWERY, BARCELONA, Anzoátegui State Sub-Project, SA

This group of participants develops the idea of transferring the methodology of vetiver work of the Project Vetiver from Polar Foundation to the social area of private companies. From the East of the country, 25 people from near by communities are sponsored to come to this organization each semester. The training focus on the economic, ecological and social aspects and two lines of Vetiver handicraft products have been developed such as animals and fruits, which has gained a very positive response from the local market.

3.15 POLAR BREWERY, San Joaquín, State Plants Carabobo, SA

An experiment was initiated by VFEPP for the Treatment of Residual Wastewater, with the idea of gaining appropriate knowledge to understand the physical, chemical and biological processes that occur in biological tertiary treatments of residual waters, and at the same time to obtain low cost technologies that can be transferred to poor communities affected by these problems, which are very common in Venezuela.

3.16 TOTAL & GAS Co.Venezuela, Jusepin Edo. Monagas, Sub-project, SA

It is a very dynamic community group that has been supported by oil companies. Their main income source is based on the oil industry activity, the "Oriente" University and plantation of passion fruits. They use the fiber of "enea" (*Typha latifolia*) and some families made hats and baskets. Leaders and people, mainly women of diverse communities take charge in the production of vetiver handicraft since one year. They are concentrating on three important production lines: handbags of braids, hats and fruits.

3. 17 C.V.G Bauxilum The Pijiguaos. Bolívar State, Methodological Transfer

The production of handmade goods is an integral part of the ethnic cultural roots and identity of the Venezuelan aboriginal tribes. The VFEPP started a methodology transfer program to several indigenous communities of this region. Each community makes containers, baskets, hats and other crafts with fibers coming from "moriche" (*Mauritia flexuosa*) and other common palms in the area, which have notably affected the ecosystem by over exploitation. Today, most of them work with vetiver fiber with an age group focus: the children learn as apprentices and the adults as artisans teaching their ancestral techniques to new generation. The vetiver fiber is an excellent solution because the plantation is easy to plant and near their houses, it is ready to be harvested when the leaves are at least three months old, and then every month.

4.0 ACTIVITIES OF RESEARCH AND DEVELOPMENT, TRANSFER OF EXPERIENCES AND STRATEGIC ASSOCIATION OF VFEPP.

This activity is important because it generates the knowledge, through research in cooperation with several national universities and public and private companies (Polar Cervercería C.A., Plantas San Joaquin y Oriente, Pepsi Cola Agua Minalba and Vetiver Antierosión C.A., CVG Bauxilum The Pijiguaos, Ministry of the Environmental Nirgua, State Yaracuy):

Central University of Venezuela
Initiating the following investigation projects:
Thesis: "Study of different systems of planting vetiver"
Master Thesis: "Treatment of residual waters with vetiver" Ing Mónica Scavo
Master Thesis: Study of the treatment of waters polluted with fluorine with vetiver. Ing Yazmín Ruiz.

Romulo Gallegos University
Participation in conferences, transfer of experiences gained from VFEPP to the inhabitants from the High Cuenca of the River Guárico
Simon Rodriguez University

Consolidation of nurseries on University lands and transfers the PVFEP to the community Santa Ana-Canoabo-state Carabobo.

Private companies:
Brewery Polar-plant San Joaquín.
Study of treatment of residual waters in drums, lagoons and humedales. Responsibility of Dr. Oswaldo Luque M, Technician Associated to the Investigation: Edgar Ceballos.

Pepsi Cola Venezuela-Agua Minalba.
Motivation of a sustainable project for the consolidation of a forest in the springs of Agua Minalba, then involved the neighboring communities to plant vetiver for the handicraft industry.

Project Vetiver Polar Foundation Companies and Vetiver Antierosión C.A.
Study of the behavior of the vetiver under flood conditions in property Carmen Teresa Farm. Aragua, State. Responsibility of Dr. Oswaldo Luque M, Tec. Rafael Luque, TAI Edgar Ceballos and TSU Grace Rivero.

*Public Enterprise*s
CVG-Bauxilum The Pijiguaos. Consultant to Vetiver Antierosión C.A in the rehabilitation work in the Bauxite mines. Transfer to the Project Vetiver CVG Bauxilum The Pijiguaos, State Bolívar to indigenous communities

5.0 CONCLUSION

It is feasible for vetiver projects to contribute to an integral community development, and poverty alleviation through generating short term economic resources, which motivates to reach ecological and social objectives that are effective and easy to implement. The integral focus of the VFEPP to benefit the quality of life of the participating communities is a common characteristics at all places where VFEPP are involved. In other sense, the introduction of the vetiver handicraft to communities - specially in the indigenous area - is a solution that can supplement ancestral customs, and it contributes to supply of scarce fibers.

In respect, to wastewater management by using vetiver grass, it could solve the disposal problem and improve quality of discharges from communities. Vetiver could be integrated to erosion control systems, in infrastructures protection (roads and agricultural lands) and production of plants and fiber for handicrafts. Up to now, the sales of vetiver handicrafts have been carried out through exhibitions, cooperative stores, promotional gifts of companies and direct sales by the artisans.

Finally, we agreed with Chomchalow´s conclusion (2004) when he visited the VFEPP:

That by utilization of vetiver as a source of raw material for handicraft making, the poor people in the community not only earn extra income - most often the only income -, but are united in their mutual activity in the community. This has culminated in more and more vetiver is being planted for soil and water conservation in the farmlands and elsewhere. This is in contrast with the conventional approach in which the farmer are encouraged to grow vetiver to protect their soil, and to use vetiver as a *by-product- for handicraft-making and other utilization, which often ends up with little or no planting at all as the farmers earn no income from such planting. Judging from the look at the smiling faces with starry eyes of the poor villagers as they sang the vetiver song or while they made the handicrafts, and even the young children who were brought to involve in such activities. The author could say that they were quite happy with their involvement in the vetiver project".*

Finally, the handicraft processes with vetiver are carried out with very little investment from the community that could be summed up as: **MIND, HEART AND HANDS**.

6. BIBLIOGRAPHY

Industrial Promotion, Dep. of. 1999. Vetiver Handicrafts in Thailand, Tech. Bull. Nº 1999/1, PRVN / RDPB, Bangkok, Thailand.

Chomchalow, N. 2000. Techniques of Vetiver Propagation with Special Reference to Thailand. Tech. Bull. Nº 2000/1, PRVN / ORDPB, Bangkok, Thailand.

Chomchalow, N. 2003. Vibrant Versatile Vetiver. An Archive of Useful Information on Vetiver. Special Bull. Nº 2003/1, PRVN / ORDPB, Bangkok, Thailand.

Chomchalow, N. 2004. From Venezuela with Love. Vetiverim, A Quarterly Newsletter of the Pacific Rim Vetiver Network. Nº 29, ISSN 0859-8878.

National Research Council, 1993. Vetiver Grass: A Thin Line Against Erosion. National Academy of Science, Washington, D. C.

Truong and Baker. 1998. Vetiver Grass System for Environmental Protection, Tech. Bull. Nº 1998/1, Pacific Rim Vetiver Network (PRVN), Office of the Royal Development Projects Board (RDPB), Bangkok, Thailand.

Truong, P.; and Smeal, C. 2003. Research, Development and Implementation of the Vetiver System for Wastewater Treatment. PRVN, Tech. Bull. Nº 2003/3. ORDPB, Bangkok, Thailand.

A Brief Introduction to the First Author

Dra. Graciela Pantin, has degrees in Sociology at the "Andres Bello" Catholic University, Caracas, Venezuela, and a Master of Arts, New York University, New York, USA. She has occupied a range of executive positions in public sector working with National Gallery of Art and Ministry of the Culture. In the private sector, she worked as General Director of the Caracas Athenaeum. For fourteen years, since 1992 she has been General Manager of "Fundacion Empresas Polar".

She has participated in several executive meetings, at the University Institute of Superior Studies of Plastics Arts "Armando Reveron" (Caracas) and at the International Center of Tropical Agriculture (CIAT), Cali, " Colombia.