Advance on Research and Dissemination of the Vetiver System in Guangzhou, China in the Past Two Years

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China is one of the best nations conducting study and dissemination to the Vetiver System (VS). As early as 1950s, China began to introduce and utilize vetiver for the purpose of refining essence oil. Since 1988 when Mr. Richard G. Grimshaw, the Chairman of the Vetiver Network introduced vetiver into China for soil conservation purpose, studies on vetiver were conducted widely in dozens of institutions, and the vetiver technique was spread and applied rapidly throughout South China. At the Third International Conference on Vetiver (ICV-3) held in 2003 in Guangzhou, the research and application achievements of China gave a deep impression on participants (Truong and Xia, 2003). Now it has two years passed since ICV-3, some new research and dissemination progresses have been gained again in Guangzhou, China.

Research Advance

1 Study on breeding new cold tolerant vetiver cultivar through biotechnology

In the past 15 years, vetiver has been widely used through South China for various purposes, such as erosion control, slope stabilization, land rehabilitation, sediment trapping, and pollution purification. Almost all utilizations in South China were successful. Due to its miraculous characteristics, vetiver has been strongly expected to introduce into North China. However, almost all tries and applications in the northern China failed owing to vetiver's poor resistance to cold. It seems that the common vetiver is very difficult to take root in the northern regions of China; therefore it is necessary to breed new vetiver varieties using transgenic biotechnology in order to realize the goal.

Trehalose serves as a protection of enzymes and membranes in many microorganisms under adverse environment stress, such as drought, salt and cold (Crowe *et al.*, 1990; Strom and Kaasen, 1993). In order to acquire cold-resistant vetiver variety, a plant expression vector pCAMBIA 1301 containing trehalose-6-phosphate (*otsA*) gene was transferred to vetiver through mediation of *Agrobacterium tumefaciens*. As to vetiver, the variety Karnataka from India was chosen due to its some promising potentials, such as low height and keeping green in winter (Fig.

1). This work has acquired the following advances.

Fig. 1 Karnataka, a more promising vetiver cultivar because it assumes lower and greener appearance in winter than other vetiver cultivars (left) and its roots assume almost same characteristics as Sunshine in the aspects of amount and length (right)

1.1 Establishment of genetic transformation system of vetiver mediated by *Agrobacterium tumefaciens*

Before a genetic transformation system of vetiver mediated by *Agrobacterium tumefaciens* is established, parameters influencing transformation efficiency should be studied. Hygromycin (Hyg) is a common screening marker gene. Fig. 2 shows that 100 mg/L of Hyg was a perfect screening concentration for vetiver, which could not only control the growth of calli effectively but also prevent the quick death of calli. Through a series of experiments, other perfect parameters for establishment of genetic transformation system of vetiver were 1.0 OD *Agrobacterium* concentration, 200 μM Acetosyringone (AS) concentration, 1-4 subcultures of calli, 20-22℃ co-cultivation temperature and 3-5 days of co-cultivation.

1.2 Genetic transformation of vetiver mediated by *Agrobacterium tumefaciens* EHA105/1305.1

Firstly, strain of EHA105/1305.1 was selected through Triparental Mating (Wang and Fang, 2002); concretely speaking, supporter DH/5α containing pCAMBIA 1305.1 was first mixed with acceptor EHA105 under the help of helper HB101/pRK2013, and then colony EHA105/1305.1 was picked out from LB solid medium containing Kanamycin and Rifamycin. Secondly,

embryonic calli were immersed in *Agrobacterium* AAM supplemented with 200 μmol/L AS suspension for 10 min, and then transferred to co-cultivation medium (GP) for 3 days of cultivation in the dark at 25℃. Thereafter, some calli were randomly selected to immerse in the GUS assay buffer overnight at 37℃ and then were observed under a dissection microscope. The observation shows that parts of calli had already become blue color distinctly (Fig. 3), indicating that plasmids of the colony EHA105/1305.1 were transformed successfully into calli. The calli were then cultured on the screening medium (inducing medium and 100 mg/L Hyg and 500 mg/L Cephalothin) in dark at 25℃ for 4 weeks (Fig. 4). Surviving calli were transformed to similar screening medium (inducing medium and 50 mg/L Hyg and 250 mg/L Cephalothin) in dark at 25℃ for additional 4 weeks.

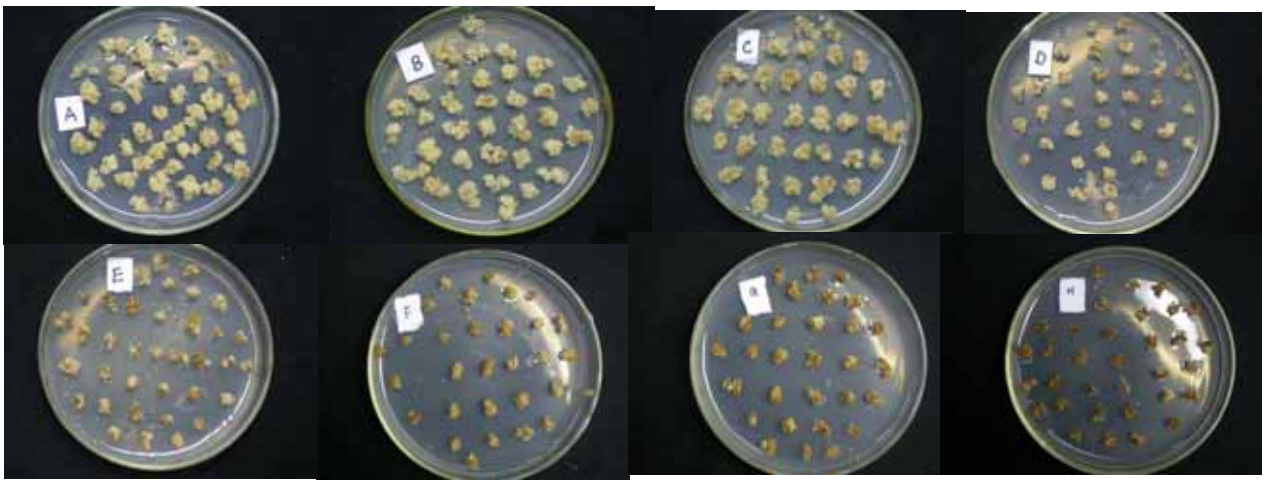

Fig. 2 The screening press test to hygromycin

Concentrations of Hyg from A to H are 5, 25, 50, 75, 100, 125, 150 and 175 mg/L respectively; A~C, embryonic calli grow well; D~E, embryonic calli grow slowly; F~G, embryonic calli stop growing; H, embryonic calli are killed

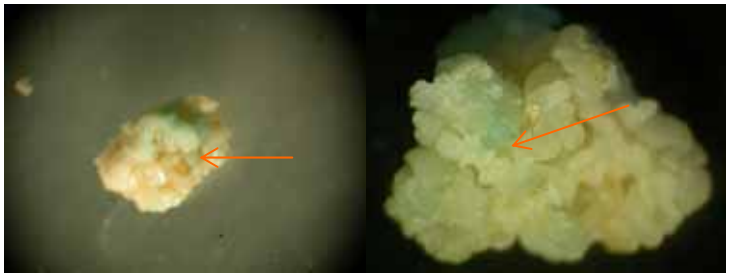

Fig. 3 Embryonic calli after 3-day co-cultivation

The arrows refer to blue parts that are successful transformation calli

1.3 Construction of plant expression vector

1.418Kb *otsA* fragment was acquired through PCR amplification first (Fig. 5), and then intermediate vector pWY1-*otsA* was constructed by inserting the *otsA* fragment into MCS site of pUC19 vector. After that, *otsA* fragment and plasmid pYH were ligated with T4 DNA ligase to acquire intermediate expression vector pWY3-*nos*; eventually plant expression vector pCAMBIA1301-*otsA* was constructed by inserting 3.2Kb DNA fragments containing promotor *P-35S*, gene *otsA* and terminator *nos 3'* into MCS site of binary vector pCAMBIA1301. Thereafter, recombinant DNA was conducted into *E. coli* competent cells DH5a to screen out white clones with characteristics of kanamycin-resistance, and then DNA was extracted to make PCR and restriction analysis (Fig. 6).

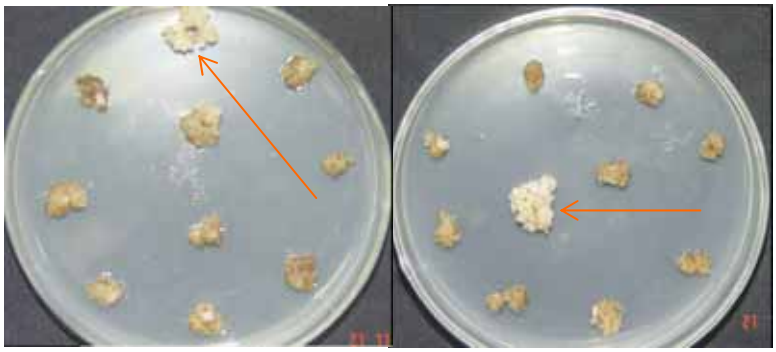

Fig. 4 Embryonic calli after screening for 4 weeks
The arrows refer to white parts that are resistant calli

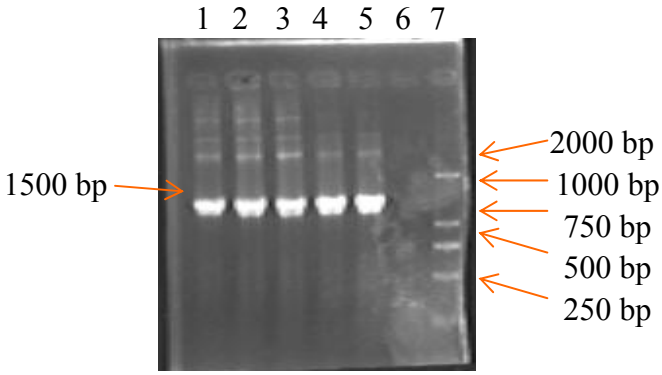

Fig. 5 Analysis of PCR products by gel electrophoresis
1~5, otsA fragments; 6, control; 7, DL2000 marker

As to the next-step work that is being conducted or will be done: after the plant expression vector pCAMBIA1301-*otsA* is constructed, transgenic plants of vetiver containing target gene *otsA* will be try to acquire through *Agrobacterium tumefaciens* mediation, then calli and leaves will be identified by the means of GUS histochemical assay, and Southern and Northern analysis. After that, cool tolerance test will be conducted between normal plants and transgenic plants to obtain truly new vetiver cultivar that can resist cold.

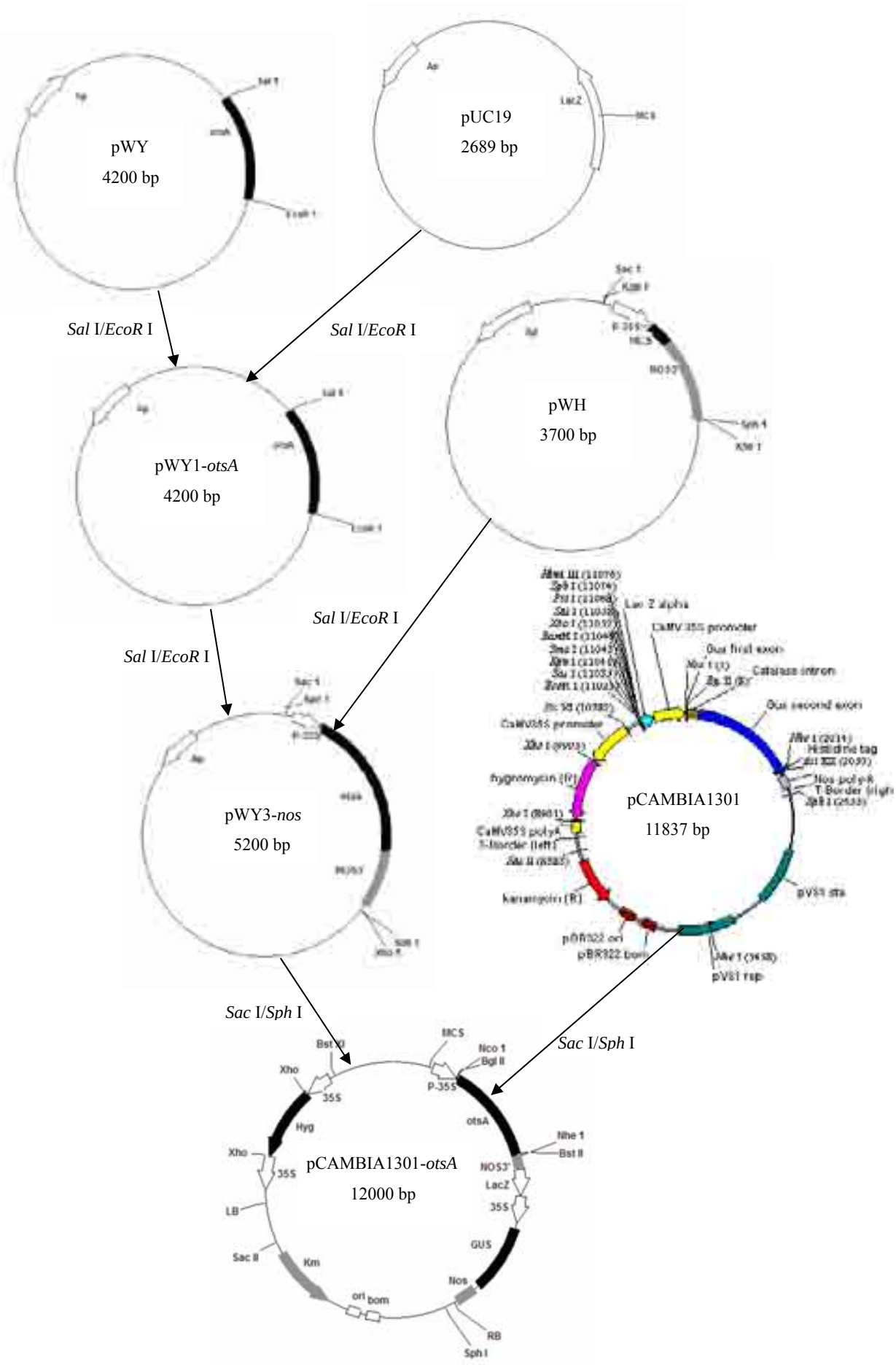

Fig. 6 The whole process of construction of plant expression vector p 1301-otsA

Dissemination Advance

Since the Guangzhou Hongri Landscape Engineering Co. Ltd. first ran vetiver market in China in 1997, so far there have been at least 20 companies, enterprises and institutions in Guangzhou alone entering this field or doing vetiver business exclusively, which has made Guangzhou become one of the most active cities around the globe in the aspect of running vetiver market. For example, there were several entrepreneurs and companies appearing at the venue of ICV-3 and financially supporting the conference (Truong and Xia, 2003). It was also they who made the vetiver technique go into the market and serve the society directly (Xia, 2002). In the past two years after ICV-3, these entrepreneurs and companies still flourished actively in the vetiver circle, and furthermore, some new companies joined in it and further activated the vetiver undertakings. For example, the Guangzhou Peifeng Environmental Protection Co. Ltd. is just newly established one running vetiver exclusively.

1 Slope stabilization of highways

In the past 5 years, China's highway construction has made a huge progress. Taking Guangdong an example, the driven mileage of highways has been up to 2900 km in the province by the end of 2005, increased by 1050 km or 56.8% compared with the year 2002. However, the construction and maintenance of highways has become more and more expensive. This is because many newly built highways go through mountain regions, which not only dig up mountains and excavate tunnels for their construction, but also must conduct slope stabilization work, usually using the "hard method" (Fig. 11). As a result, the construction fee has been up to RMB 40~50 million Yuan/km (about US$5~6.2 million/km), or even higher. Hence, how to decrease the cost has been an important issue that highway departments concern very much. The Vetiver System is just such a technique or "soft method" that can effectively abate construction fees of highways, especially those of their slope stabilization projects.

Fig. 11 A section of highway being constructed in northern hilly region of Guangdong
The huge up-slope is covered by cement wall that is very expensive and short of ecologic benefit

For example, the Guangzhou Peifeng Environmental Protection Co. Ltd., one company established just in 2004, has done over 100,000 m^2 of vetiver's slope stabilization projects along highways. These projects concentrated in the Zhangjiang section of the newly-built Zhangjiang-Chongqing highway. Zhangjiang lies in southwest Guangdong, nearly 500 km to Guangzhou, and belongs to tropical monsoon climate. Soils in here are mainly strong acidic laterite and very infertile kaolin. The vetiver projects began in the spring of 2005, and parts of projects are still under construction. All finished projects have obtained very good results, and vetiver formed intact and dense hedgerows in 2-3 months. Especially deserving to mention, Mr. Lin Bing, the General manger of the company, adopted the author's advice using the lower and greener vetiver variety, Karnataka. As a result, the spot's landscape was distinctly ameliorated while the effect for slope stabilization was not influenced compared with the common variety, Sunshine. As a matter of fact, our observation shows that roots of Karnataka and Sunshine have almost same growing speed and length (Fig. 1), while the former tiller formation speed seems to be quicker (Xia and Liu, 2003). Therefore, it would be better to use Karnataka as the material of the vetiver eco-engineering (Fig. 12~15).

Fig. 12 A large scaled vetiver nursery with the total area of 8 ha established by Guangzhzou Peifeng Environmental Protection Co. Ltd.

Fig. 13 A section of slope on the Zhangjiang section of Zhangjiang-Chongqing highway that is ready, dug up and manured, to be planted with vetiver

Fig. 14 A vetiver project in the section of the highway one month after planting vetiver

Fig. 15 Another vetiver project in the same section three months after planting vetiver. Please note, vetiver cultivar in here is Karnataka

Guangzhou Green Sunshine Garden Engineering Co. Ltd. has also done lots of work in this aspect in the past two years. For instance, the company finished a huge slope stabilization project with a total area of 98,000 m^2 alone the Ganzhou-Chongyi Highway of Jiangxi Province in the end of 2003. At first, the project was determined to use the stone-wall measure by the local highway department; later on, the vetiver measure was adopted under the persuasion of the company. As a result, the project saved RMB 800,000 Yuan (about US$ 100,000) for the highway department. In the process of construction, the company also adopted different measures according to different slope gradients and positions. In gentle down-slopes, the main measure was the “soft method”, namely vetiver was planted along contour; in steep up-slopes, the main measure was the “hard method” combined “soft method”, namely stone and cement panes were constructed first, and then vetiver was planted in the panes (Xu *et al.*, 2005). Due to high quality of cultivation and maintenance, the survival rate of vetiver was higher than 98%.

Five months later, the slope stabilization and erosion control of the whole project assumed a quite good efficiency, and the local highway department gave a satisfying acknowledgment to the company (Fig. 16~19).

Fig. 16 A section of up-slope along the Ganzhou-Chongyi Highway that was just dug up

Fig. 17 Vetiver was being planted on contours of the above highway in the beginning of 2003

Fig. 18 The appearance of the section of down-slope of the highway two months after planting vetiver

Fig. 19 The appearance of the section of up-slope of the Highway a year and a half after planting vetiver

2 Slope protection of reservoirs and hydraulic power stations

As a typical representative in slope protection of reservoirs and hydraulic power stations, the Guangzhou Vetiver Grass Environmental Science and Technology Co. Ltd. has done lots of work in this aspect. In the past two years, this company carried out at least 5 vetiver projects in 5 different reservoirs and hydraulic power stations, which are Hezhou Hydraulic Power Station, Longtan Hydraulic Power Station, and Guigang Wusijiang Reservoir in Guangxi Autonomous Region, Tianshengqiao Hydraulic Power Station in Guizhou Province, and Jingping Hydraulic Power Station in Sichuan Province.

For instance, Wusijian Reservoir, a large-typed reservoir of Guigang City, Guangxi, plays a very important role in the local agricultural irrigation and resident life (Fig. 20). Due to

highly-weathered and extremely incompact granite matrix however, many cut side-slopes of the reservoir were badly eroded and some of them even had severe landslip, which threatened the reservoir's longevity and safety of passerby (Fig. 22, 23). Before 2003, many a measure had been tried by the local government to control erosion and landslide, but all failed (Fig. 21).

Fig. 20 The main dam of Wusijiang Reservoir located in the suburb of Guigang, Guangxi

Fig. 21 A failed project of planting grass in lattices set on a slope of the reservoir conducted several years ago

Fig. 22 A slope of the reservoir assuming severe collapse, which would be reined with the vetiver eco-engineering

Fig. 23 Another slope of the reservoir assuming severe landslide, which would also be reined with the vetiver eco-engineering

From October 2003 to October 2005, I am allocated to Guigang as a temporary vice-mayor. As soon as I arrived in Guigang, I immediately introduced the vetiver technique to the government and pertinent departments. Very soon, they adopted my advice and determined to try the technique first in the Wusijiang Reservoir. In order to guarantee success, I specially invited Guangzhou Vetiver Grass Environment Science and Technology Co., Ltd. to conduct the project. Under the auspices of the Water Conservancy Department of Guangxi, and Guigang Municipal Government, the project was conducted in August 2004, and two above slopes with a total area of 6400 m^2 were executed with the Vetiver System. During the period of construction and maintenance in the second half of 2004, it appeared very high temperature and long-term drought,

which was extremely infrequent in Guigang's history. For example, the monthly rainfalls from September 2004 through February 2005 were only 25.2, 0, 21.1, 10.9, 19.2, and 26.5 mm, respectively, while the mean monthly rainfalls in the same period for past 30 years are 95.6, 63.3, 43.7, 28.4, 45.4, and 57.0 mm; the former only accounted for 30.9% of the latter! Although manual irrigation was conducted almost everyday during that period, the efficiency of water utilization was quite poor owing to too high and steep slopes and too slack soil groundmass. As a result, almost all other species planted simultaneously with vetiver, such as *Ficus microcarpa*, *Hibiscus rosa-sinensis*, *Paspalum notatum*, and *Cynodon Dactylon*, were killed by high temperature or drought in spite of that almost all seedlings were prepared in polybags (Fig. 24), but vetiver showed extremely strong vitality, and its survival rate exceeded 95%. However, from planting in August 2004 to February 2005, vetiver grew very poor all the time due to extremely execrable weather and infertile soil (Fig. 25). Since April 2005, the situation began to produce change radically with the advent of rainy season that vetiver assumed a rapid and luxuriant growing trend and the two slopes were completely covered and stabilized by vetiver very soon. In June 2005, a severe flood disaster took place in many places of Guangdong and Guangxi, including Guigang. According to the meteorological record set in the dam of Wusijiang Reservoir, the rainfall in June alone was 366.4 mm, twice of the same period of past years, and furthermore the highest day-rainfall occurred on June 5th, up to 79.8 mm. As a result, many slopes along the reservoir and nearby it were severely eroded or even washed away, but the two vetiver-protected slopes were hardly influenced by storms. On August 10th, Mr. Zhang Huode, the deputy director of Water Conservancy Department of Guangxi, and his staff members gave a special visit to the project, and they were deeply impressed by the vetiver and its miraculous efficiency (Fig. 26, 27). Mr. Zhang made a decision on the spot that the Water Conservancy Department of Guangxi will continue to support the Vetiver System and make it spread out throughout Guigang and then the whole Guangxi.

Fig. 24 Parts of vetiver seedlings, prepared in polybags on the spot, ready for the project of Wusijiang Reservoir slope stabilization

Fig. 25 Due to abnormal heat and subsequent long drought, vetiver grew very poor in winter, and other species all dies out in spite of the fact all plants were prepared with polybags and watered 1~2 times per day

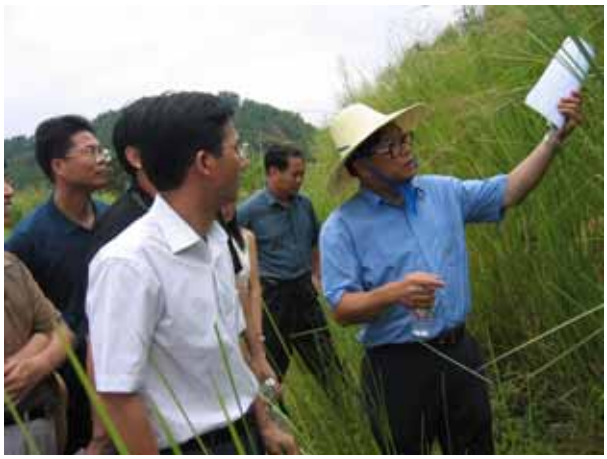

Fig. 26 Mr. Zhang Huode (right one), the deputy director of Water Conservancy of Guangxi, and his understrapper gave a full acknowledgment to vetiver's miraculous effect in here

Fig. 27 Vetiver became mature 14 months after planting, which meant that the vetiver technique took roots, blossomed out and produced fruits

In addition, an observation to growing speed of vetiver roots was conducted in the reservoir spot. As a result, a quickest growing speed so far was obtained that vetiver roots grew 1.6 m within 65 days. It is really a miracle! Maybe the extreme hot and dry weather promoted the growth of vetiver roots.

Fig. 28 An observation box for investigating growth of vetiver roots was set up on the spot on August 25th, 2004

Fig. 29 It is really incredible that the vetiver roots grew up to 175 cm from 10 cm within 65 days, from August 25th to November 1st!

3 Revegetation of quarries

Revegetation for quarries, especially for rock headwalls, is always a very thorny thing, and even a world-class difficult problem. In recent years, a newly typed, complex vetiver technique used for revegetation of quarries eventually came out, which was invented jointly by Mr. Zhang Ping, the General Manger of Guangzhou Eco Environment Science and Technology Co. Ltd. and the author. Combining the traditional vetiver technique with special cement troughs and construction methods, the new technique successfully solves the problem of headwall restoration (Zhang and Xia, 2003). For example, the Litchi Hill Quarry lied in the Guangzhou Science Town was constructed for its revegetation in autumn of 2003. It was still under construction when ICV-3 participants visited it on October 2003. Although it was the case, it still made a deep impression on all participants. This is because: 1) the headwall is up to 90 m at the highest points, and furthermore the whole headwall is very steep, almost perpendicular to the ground; and 2) what's more, there is no platform at the foot of the headwall but a deep and huge pit full of water formed by excavation of stone. Therefore it was regarded by Mr. Richard Grimshaw as one of the most difficult revegetated quarries around the globe. Two years have passed, how is it today? The hedgerows have become more and more stable, and the whole headwall has become greener and greener (Fig. 30, 31). It is really unbelievable!

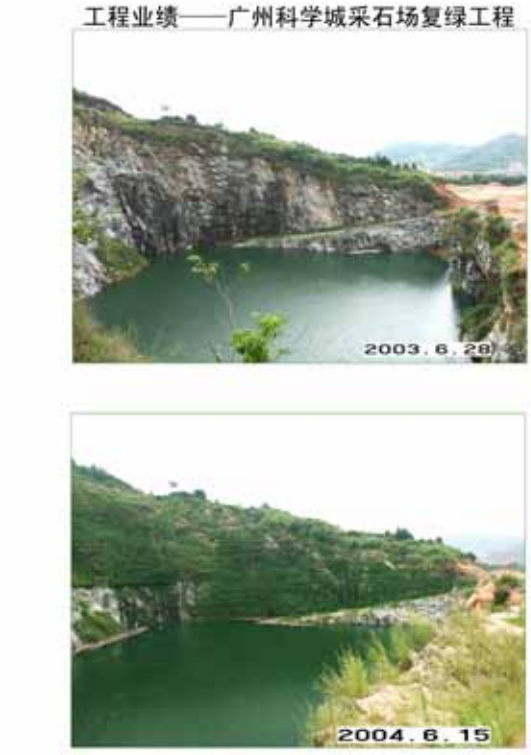

Fig. 30 Up: the original whole appearance of Lichi Hill Quarry before revegetation. Please note the huge water pit quarried
Down: the rehabilitated whole appearance 8 months after the revegetation project was over

Fig. 31 Up: the original one corner of Lichi Hill Quarry before revegetation. Please note the headwall is almost perpendicular to the ground
Down: the rehabilitated corner half a year after the revegetation project was over

At the ICV-3, the paper “Revegetation of quarry using a complex vetiver eco-engineering technique” (Zhang and Xia, 2003) was conferred the first prize by The Vetiver Network and the Certificate of Excellence by the Chaipattana Foundation of Thailand; now the project is applying to the Guangzhou Municipal Government for the Prize of Guangzhou Excellent Construction Projects. There are tens of thousands of construction projects in Guangzhou each year, and at least $10 billions are invested in them. If the “mini” project could win the government prize, it would be absolutely miracle!

After wining success in Zhuhai Baitengshan project and Guangzhou Litchi Hill project, Mr. Zhang Ping and his company immediately became a noted entrepreneur and enterprise for quarry rehabilitation throughout Guangdong province. In the past two years, he was invited once and again to conduct nearly 50 similar items in Zhongshan, Dongguan, Panyu as well as Guangzhou for the same purpose; the revegetated areas were up to 1,000,000 m^2, and the construction funds exceed RMB 100 million Yuan (Fig. 32, 33). Moreover, the originally devised technique has been further ameliorated: 1) cement slabs are cast directly on headwalls, not be precast nearby any more; and 2) the depth of cement troughs is increased to 80 cm from original 40~50 cm to provide more spacious room for growth of plants. These ameliorations have made newly finished projected produce better benefits and efficiency.

Fig. 32 An original panorama of Daxing Quarry in Dongguan City, approximately 100 km southeast to Guangzhou

Fig. 33 Another completely different landscape exhibited in front of us after the complex vetiver eco-engineering was finished for 4 months

On the whole, no matter what aspect of VS, research or dissemination, has made considerable progress in the past two years in China, particularly in Guangzhou. The vetiver eco-engineering is increasingly becoming one of the most important means for stabilization of highway slopes, rehabilitation of quarries and minelands, and protection of river canals, reservoirs and lakes in southern regions of China.

Acknowledgments

The basic research work mentioned in the paper was supported partly by the Natural Science Foundation of China (No. 30370233) and The Vetiver Network. The author wishes to thank the Guangzhou Peifeng Environmental Protection Co. Ltd., the Guangzhou Green Sunshine Garden Engineering Co. Ltd., the Guangzhou Eco Environment Science and Technology Co. Ltd., the Guangzhou Vetiver Grass Environmental Science and Technology Co. Ltd., and the Guangzhou Hongri Landscape Engineering Co. Ltd. for their excellent work with special regard to the VS, and for their help rendered to the author in the preparation of the manuscript.

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