THE VETIVER AGRONOMIC RESEARCH PROGRESS IN TAIWAN FOR THE PAST DECADE

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Abstract

The vetiver has been introduced into Taiwan three times in the past century. The first two introductions did not make significant impact on the ecosystem and almost extinct after the introductions. The third introduction started in the 1997, the author found seven local accessions and received 20 worldwide representing germplasm through the Vetiver Network in four batches. Fourteen accessions of 'wild' seedy vetiver germplasm were also received in 1998 from the NGPS, USDA.

The acquired vetiver germplasm was investigated in a series of agronomic, physiological, genetic and breeding studies. From the agronomic aspect, the regional adaptability of vetiver in the diversified environmental conditions of Taiwan was found it can grow well in any kind of soil and rainfall pattern of Taiwan below the altitude of 1000 meter and can not over 2000 meter. The region between altitude 1000 and 2000 meter with full sunlight will support the growth of vetiver but not the shading condition. The weedy potential was also evaluated using all the seedy and three cultivated accessions and found the seedy accessions are prolific seed producers exhibiting invasive potential and thus was recommended to be restricted to the general public for use in Taiwan. The growth characteristics of vetiver were studied in the phytotron. The maximum growth of vetiver was found in the 25°C treatment of the Ohito accession with root extension rate at 3.96 cm per day. On average, 3 cm of daily extension rate was exhibited in the temperature above the 25°C treatments, while the 15°C treatment did not show visible above-ground growth also recorded with average 0.52 cm of root extension daily. The morphological distinctiveness between the seedy and cultivated vetiver was found in the width of leaf and number of bristles and cilia on the glume. Significant difference was also found in the thousand-grain seed weight ranging from 0.78g to 1.47g with respect to the harvesting month and accessions. The optimum germination condition for vetiver was found in the combination of eight hours of light in 23^{o}C and 16 hours of dark in 13^{o}C. The RAPD and AFLP DNA marker system were used to estimate the genetic distance between all the accessions and founf to be 36.9% and 18.9% was estimated among the cultivated accessions, suggesting breeding efforts to increase the genetic diversity of the cultivated vetiver. The seedy and the cultivated accessions were separated into two distinct groups by the genetic markers. The DNA profiles for each of the cultivated accessions were also developed using RAPD and AFLP markers. Eight RAPD markers were converted to SCAR markers for unambiguous identification of the seedy accessions from the cultivated accession and also the non-sunshine accessions from the sunshine ones. The photosynthetic characteristics including light saturation point, light-saturated photosynthetic rate, light compensation point and dark respiration rate, were also found significantly different among cultivated accessions revealed the shade treatment, but the difference was not consistent with the genetic relationship estimated by the DNA markers. The high level of CO_2 concentration was found to be effective in promoting the growth of vetiver and difference among accessions was also found in respects to the amount of enhancement and the partition of biomass between above- and below-ground tissues. A field study of the emission gas discharged from power plant as a supply of CO_2 fertilizer indicated higher amount of discharge of the emission gas supported high rate of growth in the order of 8 times of gas discharge to 2.24 times of veitver growth. The method of reproduction of vetiver was found to be self-incompatible and the difference of fertility between seedy and cultivated vetiver accession was significant, the fertility of the cultivated accessions estimated to be 1.11%. The rate of germination of the seed was estimated to be 86.48%. A hybrid population consisting from five half-sib families containing 251 seedlings was evaluated in the field for two years displayed significant difference in the reproductive and vegetative growth characteristics. The results from the biomass production and several morphological traits suggested an ample potential for the breeding of vetiver.

Introduction

The vetiver was known to the author since 1992 after reading the impressive and almost unbelievable characteristics of its environmental adaptability in the Vetiver Network Newsletters. In 1997, the author seeking plant species for erosion control in vulnerable landslide zones in Taiwan, vetiver was the primary candidate after reviewing the features of these sites. The majority of the vetiver related literatures is focusing on the engineering aspects. Although agronomic information been published and sited as in the promotion materials from several counties and organizations, many questions remained unanswered and the local data in Taiwan was not available. The awareness of the potential challenge from the local environmentalists considering the magnitude of land coverage when vetiver used as an erosion control measure, the priority of acquiring local information of the vetiver is second to none. The vetiver was subjected to the regional adaptability evaluation in confined plots in the Experiment Farms of the National Taiwan University. The key issue addressed in the study was the weedy potential of both the seedy and non-seedy vetiver. The regional adaptability was next issue addressed which led to the recommendation of site application of vetiver. Vegetative propagated slips of vetiver are the primary planting material for the regions outside the native countries. The homogeneity of the genetic background possesses potential vulnerability to pest damage. The development of new varieties is the solution to the situation. The vetiver breeding works aiming at the release of new variety, including the analysis of the genetic relationship of the germplasm, mating systems, and the development of the target traits screening techniques, were initiated in 1998 after enough germplasm received. The works related to the study of regional adaptability and breeding were reported in this paper.

History and sources of the Vetiver germplasm in Taiwan

According to the legend, vetiver was first brought into west coast of central Taiwan more than one hundred years ago by melon farmers from China used as wind break to help stabilize the sand. The second introduction of vetiver was during 1970's for the evaluation of species for erosion control by certain government agency, but the document was lost due to the abort of the project. When the author started the study of vetiver in 1997, botanical specimen of vetiver collected in 1943 was found in the herbarium of National Taiwan University and the sampling location of the specimen was revisited several times trying to find the live vetiver plant in the vicinity and the search was not rewarded. The first sample received by the author was from Mr. Diti Hengchaovanich from Thailand in 1998. The sample was collected in a farm located in Ohito, Japan and presumed to be cold tolerant for it was picked up when the ground was covered with snow. Fourteen accessions of 'wild' seedy vetiver germplasm were also received in 1998 from the NGPS, USDA. In 1999, total four live accessions of vetiver were received by the author. Two local accessions including the one from the 1970's introduction was retrieved from the botanical garden of Agriculture Research Institute of Taiwan by the advice of Prof. Young-Chen Tsai. The other local accession was given by Mr. Lien-Chin Chou who preserved the plant for more than 40 years in his garden and it was collected by his father from the melon farm where the vetiver first introduced a century ago. Another sample from China was given by Ms. Chin Chang. And the Huffman vetiver was purchased from the Horticulture System, Inc. FL. USA. Three more batches of foreign samples, including thirteen selected worldwide representing germplasm from Dr.

Robert Adam in 2000, three Southern Pacific region accession from Dr. Paul Truong in 2001 and two accessions from Mr. Mark Dafforn in 2001. Two more local accessions from central and northern Taiwan was identified and collected by the author from deserted farmlands in 2001. In 2002, another two local accessions, one from farmland and the other from tidal region of water dam in central Taiwan, were collected by the author. These accessions has been preserved in the confined germplasm nursery in the Experiment Farm of National Taiwan University (NTU) since the collection and listed in table 1.

Table 1. The sources and classification of the evaluated Vetiver grermplasm.

ID	Species	Source (other locations)	Type-'-
		South India Type	
VVZ009	*V. zizanioides* cv. 'Ohito'	Japan	U
VVZ010	*V. zizanioides* cv. 'Taiwan_'	Taiwan, Taichung	U
VVZ011	*V. zizanioides* cv. 'Huffman_'	USA, Florida	S
VVZ012	*V. zizanioides* cv. 'Ping-Tang'	China, Ping-Tang	S
VVZ013	*V. zizanioides* cv. 'Taiwan_'	Taiwan, Taichung	U
VVZ014	*V. zizanioides* cv. 'Sunshine'	USA, Louisiana	S
VVZ015	*V. zizanioides* cv. 'Huffman _'	USA, Florida	S
VVZ016	*V. zizanioides* cv. 'Capitol'	USA, Louisiana	S+
VVZ017	*V. zizanioides*	Sri Lanka, Colombo	SL
VVZ018	*V. zizanioides*	Malawi, Lilongwe	SL
VVZ019	*Vetiveria spp?*	Costa Rica, Puerto Viejo	CR
VVZ020	*Vetiveria spp?*	Panama, Western, site B (Costa Rica)	P
VVZ021	*V. zizanioides*	Malawi, Zomba	O
VVZ022	*V. zizanioides* cv. 'Malaysia'	Spain, Murcia (Malaysia)	O
VVZ023	*V. zizanioides* cv. 'AVC'	Spain, Murcia (Malaysia)	S-
VVZ024	*V. zizanioides* cv. 'Karnataka'	Spain, Murcia (Malaysia)	KM
VVZ025	*V. zizanioides* cv. 'Sabak Buntar'	Spain, Murcia (Malaysia)	I-
VVZ026	*V. zizanioides* cv. 'Parit Buntar'	Spain, Murcia (Malaysia)	O
VVZ027	*V. zizanioides* cv. 'Taiwan_'	Taiwan, Changhua	U
VVZ028	*V. zizanioides* cv. 'Monto'	Australia, Queensland	S
VVZ029	*V. zizanioides* cv. 'Fiji'	Australia, Queensland(Fiji)	S
VVZ030	*V. zizanioides*	Australia, Queensland	S
VVZ031	*V. zizanioides* cv. 'Taiwan_'	Taiwan, Jungli	U
VVZ032	*V. zizanioides* cv. 'Eurovetiver'	Germany, Kassel	U
VVZ033	*V. zizanioides* cv. 'Talofofo'	USA, Guam	U
		North India Type	
VVS001	*Vetiveria* sp.	India (USDA PI 536999)	W
VVS002	*Vetiveria* sp.	India, Motihari (USDA PI 504808)	W
VVS003	*Vetiveria* sp.	India, Motihari (USDA PI 504807)	W
VVS004	*Vetiveria* sp.	India, Gobardana (USDA PI 504802)	W
VVS005	*Vetiveria* sp.	India, Gobardana (USDA PI 504803)	W
VVZ001	*V. zizanioides*	India (USDA PI 271633)	W
VVZ002	*V. zizanioides*	India (USDA PI 538753)	H
VVZ003	*V. zizanioides*	India (USDA PI 213903)	W
VVZ004	*V. zizanioides*	India (USDA PI 538754)	H
VVZ005	*V. zizanioides*	India (USDA PI 538756)	H
VVZ006	*V. zizanioides*	India, Uttar Pradesh (USDA PI 538757)	W
VVZ008	*V. zizanioides*	India, Punjab, Simla (USDA PI 196257)	W
VVZ009a	*V. zizanioides*	India (USDA PI 302300)	W

__According to Adams and Dafforn, 1997. S =Sunshine type ; S+ =Sunshine pattern with one additional band; S- = Sunshine pattern with one missing band; O =Other *V. zizanioides* banding patterns (various banding, each of which is different); SL =Sri Lanka clone; CR = Costa Rica clone; KM =Karnataka; P = Panama clone(Possible other *Vetiveria* species); I- =Ganges type with one missing band.

__W =Wild type; H =Hybrid; U =Unclassified

The evaluation of weedy potential of vetiver

The weedy potential of vetiver can be defined as invasiveness and persistence in a new region after its introduction. A plant species can invade into new territory by the dispersal of seeds or by the extension of stolens or rhizomes. The vetiver was evaluated in three locations in different altitudes to study the characteristics of vegetative and reproductive growth in response to different environment conditions. Two Southern India type accessions (VVZ009 and VVZ010) and 253 plants from the 14 Northern India type accessions were propagated to produce enough slips and plant into three plots in Experiment Farms of NTU located in sea-level, 1000 m and 2100m altitude in the June 31 of 1998. The survival rate, maturity, seed set and seed germination was observed and recorded in table 2.

Table 2. The vegetative and reproductive characteristics observed in sea-level, 1000 m and 2100 m among the selected vetiver germplasm.

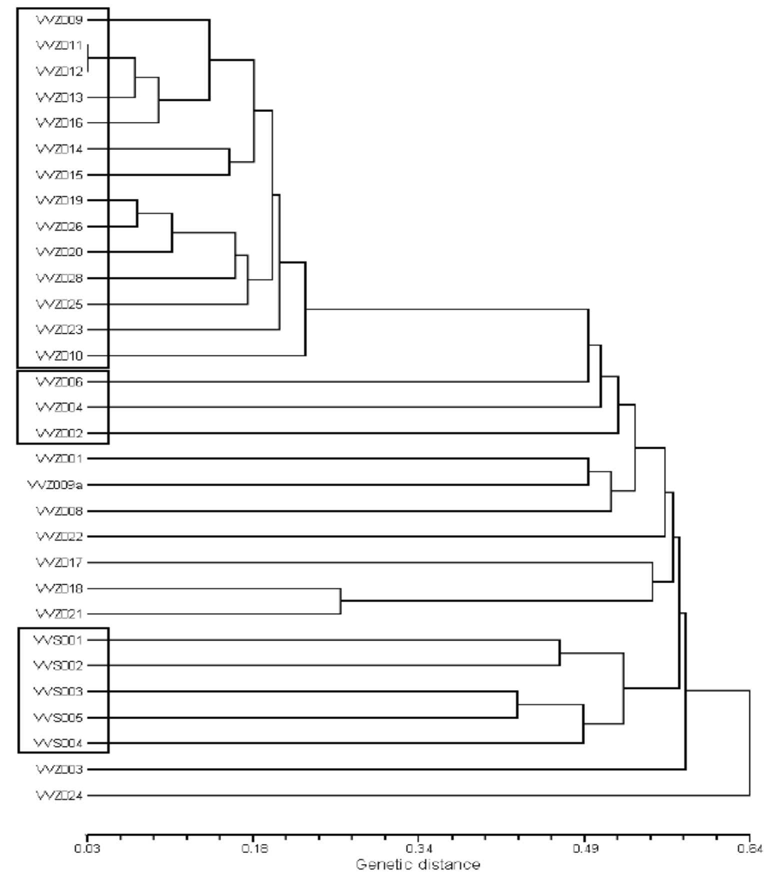
	Sea-level	1000 m Altitude	2100 m Altitude
		Rate of survival %	
Northern India type	99	30	52
Southern India type	99	85	80
		Maturity	
Northern India type	Seedhead produced	Vegetative stage	Vegetative stage
Southern India type	Seedhead produced	Vegetative stage	Vegetative stage
		Stolen or rhizome growth	
Northern India type	None	None	None
Southern India type	None	None	None
		Fertility %	
Northern India type	73	N/A	N/A
Southern India type	1.2	N/A	N/A
		Seed germinability %	
Northern India type	65	N/A	N/A
Southern India type	90	N/A	N/A

The number of viable seeds from a single plant after one growth season was estimated to be 2500 for the Northern India type vetiver and 20 for the Southern India type vetiver.

The number of viable seed produced by the Northern India type is enormous and will make significant population after its invasion thus should not allowed to be used in Taiwan. The result of the Southern India type vetiver observed in this study exhibited contradictory with the rest of the world, which was investigated in the following method of reproduction study. Even though the Southern India type vetiver did produce viable seed, the number could be considered insignificant to make ecological impact.

The genetic relationship between the Northern and Southern India type vetiver(Liu,2001)
Cultivated vetiver (southern India type) is propagated through vegetative cloning of the tiller and thus expected to be less diverse genetically. The threat of outbreaks of epidemic of pest might destroy the entire population has been brought to attention with the historical examples such as the southern blast of corn. The northern India vetiver with prolific seed production ability has been excluded in the application of erosion control outside the vetiver native distribution regions to prevent it from becoming weed. But the presumed source of cultivated vetiver is by domestication of northern India vetiver; make the northern India vetiver a major source for the breeding of new cultivated varieties. Understanding the genetic relationship between the northern and southern India vetiver will help the utilization of the germplasm in terms of breeding.
The genetic diversity among the northern and southern India types vetiver was studied using RAPD markers. Thirteen of the twenty-five cultivated southern India type vetiver collections were received from Dr. Adams' collection, which had been surveyed for the worldwide representation of the cultivated vetiver germplasm. Two local collections from Taiwan and one from Japan as well as twelve seedy northern India vetiver accessions received from NPGS, USDA were included in the study.

Seven informative RAPD primers were chosen in the experiment to amplify characteristic DNA fragments of each sample. Sixty-seven unambiguous polymorphic DNA fragments were identified. The information of the presence and absence of the amplified DNA fragments was used to estimate the genetic distance among the samples. The estimated



genetic distance between each sample was further analyzed by principle coordinate analysis to disentangle the genetic relationship of the collection. The estimated genetic distances between the cultivated vetiver collections were closer to each other than to the northern India vetiver samples. (Figure 1) Fourteen of the cultivated vetiver samples were also shown as a small compact group in the PCO analysis, the other five cultivated vetiver samples was scattering in between the compact cultivated vetiver group and the northern India vetiver samples. The northern India vetiver collections were distantly related to each other and spread out in the PCO plot representing a much larger diverse genetic resource. (Figure 2) The result indicated unambiguous population differentiation between the northern and southern India type vetiver. The five cultivated vetiver samples transiting between the other 'sunshine' vetiver and wild seedy vetiver provide the evidence of domestication.

Fig. 1. Dendrogram of the genetic distance among vetiver accessions. Dendrogram of cluster analysis using genetic distance of RAPD analysis for 19 South India type vetiver accessions, 12 North India type vetiver accessions.

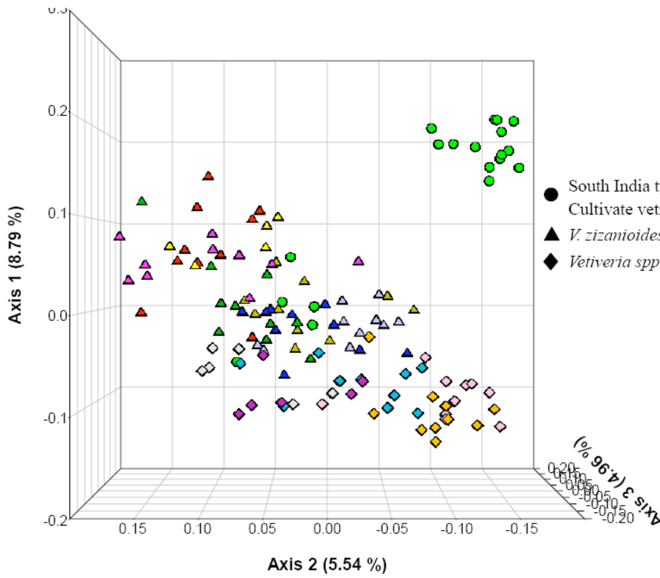


Fig. 2. The genetic distance among South India type and North India type vetiver accessions. Principal coordinate analysis of 12 North India type accessions and 19 South India type accessions using genetic similarity of RAPD analysis.

The seed characteristics of the cultivated vetiver (Yang, 2002)

Although the cultivated vetiver has been considered to be "non-fertile", open-pollinated seeds were harvested from five cultivated lines in the nursery of vetiver germplasm in the Experiment Farm of National Taiwan University, Taipei, Taiwan 2001-2002. The seeds were studied for the characteristics with respect to morphology, fertility and germination with comparison to the "wild" seedy vetiver.

The caryopsis of wild vetiver appears to be rougher and has more bristles and cilia than cultivated one under stereomicroscope and scan electron microscope (Figure 3).

All the involucres on the florescence were hand harvested every month during December to March then processed to collect pure seeds according to the ISTA (International Seed Testing Association) protocol. The average seed fertility of the cultivated vetiver is 1.11% and is affected by month of collection therefore by temperature. The average thousand-grain-weight is 1.35gm; the non-sunshine line (Ohito) is lighter than the sunshine lines, but not significant statistically. The optimum temperature for vetiver seed germination is 23 degree of centigrade with light on for 8 hours and 13 degree with light off for 16 hours. The average rate of seed germination is 86.48% but the lines and month of harvest also affecting the germination with interaction. All the germinated seeds had been grown in the nursery and appeared to be normal. The non-germinated seeds had been treated with TTC seed viability test and concluded to be dead rather than dormant. The five cultivated vetiver lines are capable of producing viable seeds even though the rate is low and no dormancy of the seed been concluded.

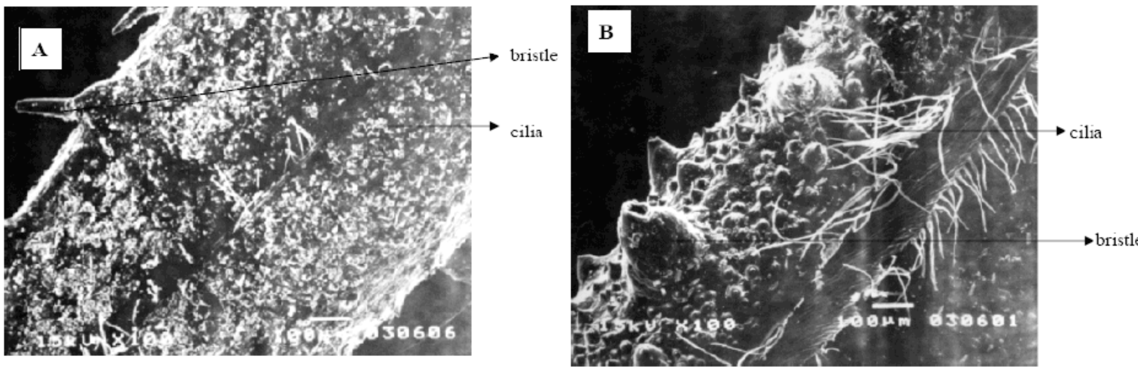


Fig. 3. Scanning electron micrographs of caryopsis of cultivated and wild vetivers (A_cultivated vetiver VVZ009_B_wild vetiver VVZ004).

The method of reproduction of vetiver (Yang, 2002)

The cultivated vetiver has been presumed selections from its wild seedy relatives with human intervene. The lack of fertility is based on field observation where no seed has been collected from cultivated vetiver worldwide. Unexpected fertile seeds were found in the nursery of Experiment Farm of National Taiwan University, Taipei, Taiwan. The method of reproduction of vetiver was studied to answer the phenomenon. Thirteen out of 171 seedy vetiver genotypes were selected randomly in the nursery to study the difference of fertility between open- and self-pollination treatments. Field observation indicated the vetiver is protogyny with pistil extrudes out of the floret about three days before stamens for both cultivated and wild vetiver in temperature 25-35 degree of centigrade. The open pollinated inflorescence has almost 100% of seed set while the self-pollinated inflorescence has less than 1% of fertility with no difference to zero statistically. This traditional experiment provided an evidence for the out-crossing and self-incompatibility nature of the pollination behavior of vetiver. Molecular markers were applied to the half-sib populations of the seeds harvested from the five cultivated vetiver with seedy vetiver within distance for open pollination to concur the conclusion. RAPD markers were used to identify the source of pollen donor of each half-sib progeny plant. All the progenies were shown to be hybrid.

The development of DNA profiles and SCAR markers for the cultivated vetiver (Yang 2003)

The lack of invasive ability through seed and vegetative organs is the primary trait to

convince conservatives to adopt vetiver into a new environment. The finding of the self-incompatibility of seed production in vetiver has posed a dilemma in the application of vetiver: there should be only one genotype planted in a location to prevent seed production and consequently risk the vetiver from outbreaks of epidemic of pest. The risk will increase with the expansion of application of vetiver. Although the risk has been aware, before new variety of vetiver released to the public, the greater threat of vetiver becoming weed through seed production is obvious and can be avoid. The only way to avoid the seed production based on the self-incompatibility is to plant only one genotype in a region. The worldwide germplasm of cultivated vetiver is morphologically similar make the use of molecular marker to distinguish among the genotypes a tool of choice.
Twenty-five cultivated vetiver samples received worldwide and 171 genotypes from 13 accessions of seedy vetiver from NPGS, USDA were included in the study. RAPD and AFLP marker systems were used in the development of DNA profile to compare their stability and efficiency. Three of the four selected AFLP primer combinations can distinguish the 25 cultivated vetiver alone based on the profiles of the amplified polymorphic DNA fragments. (Table 3) Relatively fewer number of polymorphic DNA fragments been amplified by RAPD system, the results indicated it requires at least three of the 12 informative RAPD primers to distinguish the 25 cultivated vetiver genotypes. (Table 4) The experiment also concluded the AFLP marker is effective and stable, yet the cost and facility requirement is higher than RAPD. In order to increase the stability and reduce the cost, SCAR markers were developed from the DNA fragments amplified by RAPD. Eight SCAR markers were developed to separate wild and cultivated vetiver and also found to be able to identify each one of the six non-sunshine type cultivated vetiver. More SCAR markers are expected to be developed to distinguish the sunshine type vetiver.

Table 3. DNA profile of the vetiver lines revealed by *Mse*I-CAA / *Eco*RI-AC primers combinations in AFLP analysis.

Molecular weight (bp)	VVZ 017	VVZ 018	VVZ 021	VVZ 022	VVZ 024	VVZ 032	VVZ 009	VVZ 010	VVZ 011	VVZ 012	VVZ 013	VVZ 014	VVZ 015	VVZ 016	VVZ 019	VVZ 020	VVZ 023	VVZ 025	VVZ 026	VVZ 027	VVZ 028	VVZ 029	VVZ 030	VVZ 031	VVZ 033
102.6	◆	◆	◆	◆	◆	◆	◆	◆	◆	◆	◆	◆	◆		◆	◆	◆	◆	◆	◆	◆		◆	◆	◆
103.9	◆			◆	◆	◆		◆				◆		◆			◆					◆			
117.4		◆				◆					◆		◆											◆	
139.5	◆					◆										◆		◆							
141			◆				◆	◆			◆		◆		◆					◆	◆				
151.7							◆																	◆	
184.9		◆	◆	◆	◆	◆	◆	◆				◆	◆		◆		◆	◆	◆	◆	◆	◆	◆	◆	◆
197			◆		◆	◆	◆	◆					◆							◆	◆		◆	◆	◆
255.6					◆		◆	◆	◆	◆	◆		◆		◆	◆	◆	◆	◆	◆	◆	◆	◆	◆	◆
272.7	◆			◆																					
281.6		◆	◆							◆	◆					◆		◆			◆		◆		

Table 4. DNA profile of the vetiver lines revealed by the selected Operon primers combinations in RAPD analysis.

	VVZ 017	VVZ 018	VVZ 021	VVZ 022	VVZ 024	VVZ 032	VVZ 009	VVZ 010	VVZ 011	VVZ 012	VVZ 013	VVZ 014	VVZ 015	VVZ 016	VVZ 019	VVZ 020	VVZ 023	VVZ 025	VVZ 026	VVZ 027	VVZ 028	VVZ 029	VVZ 030	VVZ 031	VVZ 033
OPN01 (5'CTCACGTTGG 3')																									
950bp							◆	◆	◆	◆	◆	◆	◆	◆	◆	◆	◆	◆	◆	◆	◆	◆	◆	◆	◆
320bp							◆							◆		◆	◆	◆	◆	◆	◆	◆	◆	◆	◆
OPV04 (5'CCCCTCACGA 3')																									
2100bp	◆			◆										◆				◆	◆	◆	◆	◆	◆		
1400bp	◆								◆		◆			◆	◆	◆	◆	◆	◆	◆	◆	◆	◆	◆	◆
1150bp	◆	◆	◆		◆			◆	◆	◆	◆			◆	◆	◆	◆			◆	◆				◆
OPK18 (5'CCTAGTCGAG 3')																									
1250bp								◆	◆	◆	◆		◆	◆	◆	◆			◆	◆	◆	◆	◆		◆
900bp				◆	◆				◆		◆			◆			◆	◆		◆		◆	◆		◆
830bp	◆			◆				◆					◆					◆	◆				◆		
480bp	◆	◆					◆	◆		◆	◆	◆	◆		◆		◆	◆	◆	◆	◆	◆	◆	◆	◆

The breeding potential of vetiver (Yang, 2002)

The lack of seed production is one of the important characteristics of cultivated vetiver for the application in erosion control. It also poses a challenge to breed new variety other than introduction and selection from existing germplasm. The method of reproduction of vetiver was proved to be self-incompatible in previous study and total 251 seedlings germinated from five open-pollinated out-crossing half-sib populations with pollen donors from both cultivated and wild seedy vetiver lines were planted in the nursery in September, 2001. After one year of growth, twelve seedlings were dead and 18 remained in vegetative stage and the rest 220 seedlings advanced to reproductive stage. The height of all the remaining 238 plants was ranging from 100 to 310 cm. The number of tiller and inflorescence was ranging from 6 to 249 and 0 to 130, respectively. The fresh weight of all the tillers of each plant was ranging from 77 to 8300 gm. The establishment of the five half-sib populations of the cultivated vetiver lines proved the breeding of vetiver through hybridization of cultivated vetiver lines is possible. The enormous variation existed in the investigated agronomic traits indicated a fair chance for breeding.

Conclusion

The vetiver has been applied in erosion control and phytoremediation extensively worldwide in the past decades. We are delighted to be able to gather some germplasm for the conduction of the basic agronomic studies as well as breeding works. The growing demand of energy in recent years and the development of energy conversion technology has made the cellulose fiber a source of biofuel feedstock. The production of bioenergy crops would demand a lot of acreage in order to supply enough quantity of biomass and thus would compete the land resource with food and forage crops. The adaptability of vetiver will make it a best candidate for exploiting the land in the margin area and relief the competition with food/forage crops. The integration of vetiver into the bio-energy production system shall be the bright future of vetiver.

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Contribution of the paper

It reported the agronomic research conducted by the author in Taiwan during 1997-2003. The genetic relationship between the cultivated and wild vetiver was identified and DNA fingerprinting of the major cultivated vetiver lines was proposed as well. The fertility of vetiver has been addressed and the self-incompatibility was proposed. The breeding potential of vetiver for biomass production was studied and positive conclusion was found.