

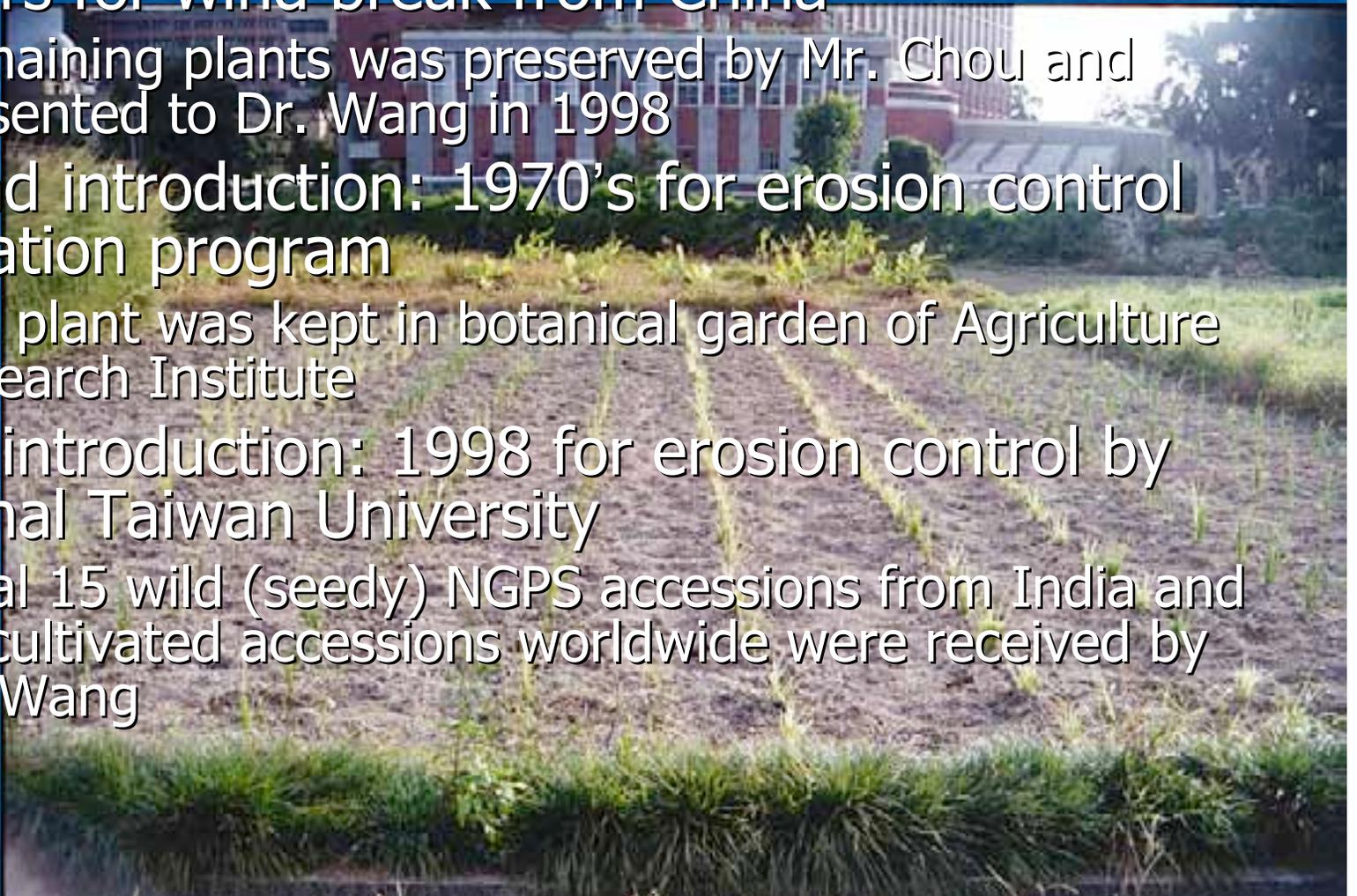
THE VETIVER AGRONOMIC RESEARCH PROGRESS IN TAIWAN FOR THE PAST DECADE

I-Chen Tsai, Wen-Hui Yang, Ya-Szu Yang, Jien-Hu Liu,
and Yue-Wen Wang

Department of Agronomy, National Taiwan University

The introductions of vetiver into Taiwan

- First introduction: Around 1900 by melon farmers for wind break from China
 - Remaining plants was preserved by Mr. Chou and presented to Dr. Wang in 1998
- Second introduction: 1970's for erosion control evaluation program
 - The plant was kept in botanical garden of Agriculture Research Institute
- Third introduction: 1998 for erosion control by National Taiwan University
 - Total 15 wild (seedy) NGPS accessions from India and 27 cultivated accessions worldwide were received by Dr. Wang



The List of Vetiver Germplasm in Taiwan

- Wild type vetiver (Nouthern India type, Seedy vetiver)
 - 15 accessions from USDA NGPS collected in India
- Cultivated vetiver (Southern India type, non-seedy vetiver)
 - USA(5), Japan(1), Malaysia(5), Australia(2), Malawi(2), Costa Rica(1), Sri Lanka (1), Panama(1), China(1), Taiwan(5), Fiji(1), Germany(1), total 26 lines
- Breeding stocks
 - Five half-sib populations created in the year 2000.



The primary concerns

- The weedy potential of vetiver as invasive plant when applied as large scale erosion control measure.
 - Will vetiver adapt to new environment?
 - Will vetiver becomes weed?
- The vegetative propagation of cultivated vetiver produce homogeneous population with potential of epidemic outbreak of pest.
 - Genetic variation of vetiver
 - Method of reproduction of vetiver
 - Breeding potential of vetiver

Weedy potential in Taiwan

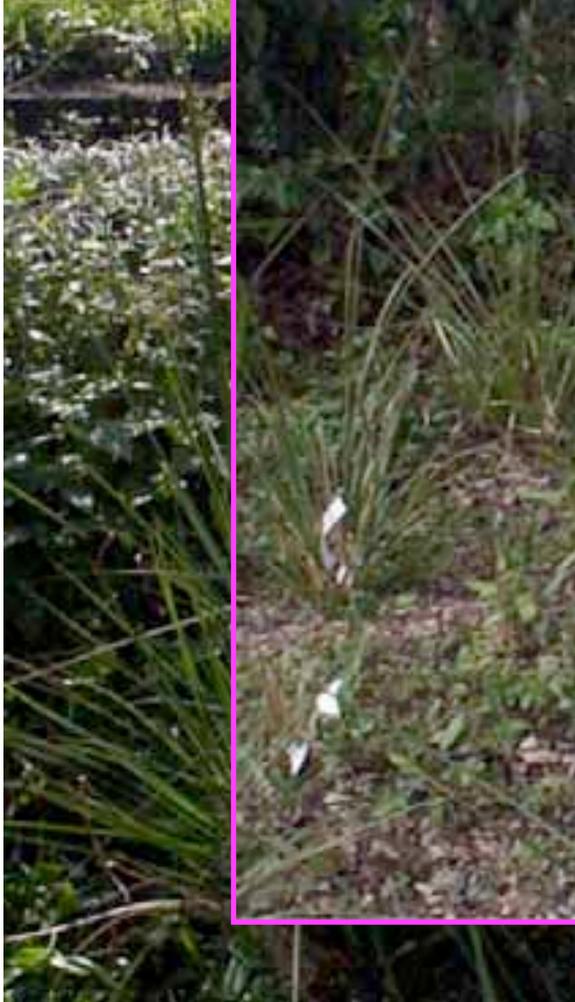
- Studied in isolated nurseries in University experiment farms located in different altitudes (sea-level, 1000, and 2100 meters)
 - Southern India vetiver (Cultivated)
 - Ohito (VVZ009) and Taiwan I (VVZ010)
 - Northern India vetiver (Seedy)
 - 253 plants from the 14 seedy Northern India vetiver accessions were used for evaluation
- The traits for evaluation
 - Survival
 - reproductive organs: seed and vegetative spreading organs (Stolon and rhizome)

Twenty weeks after transplanting

Sea-level

1000 meter altitude

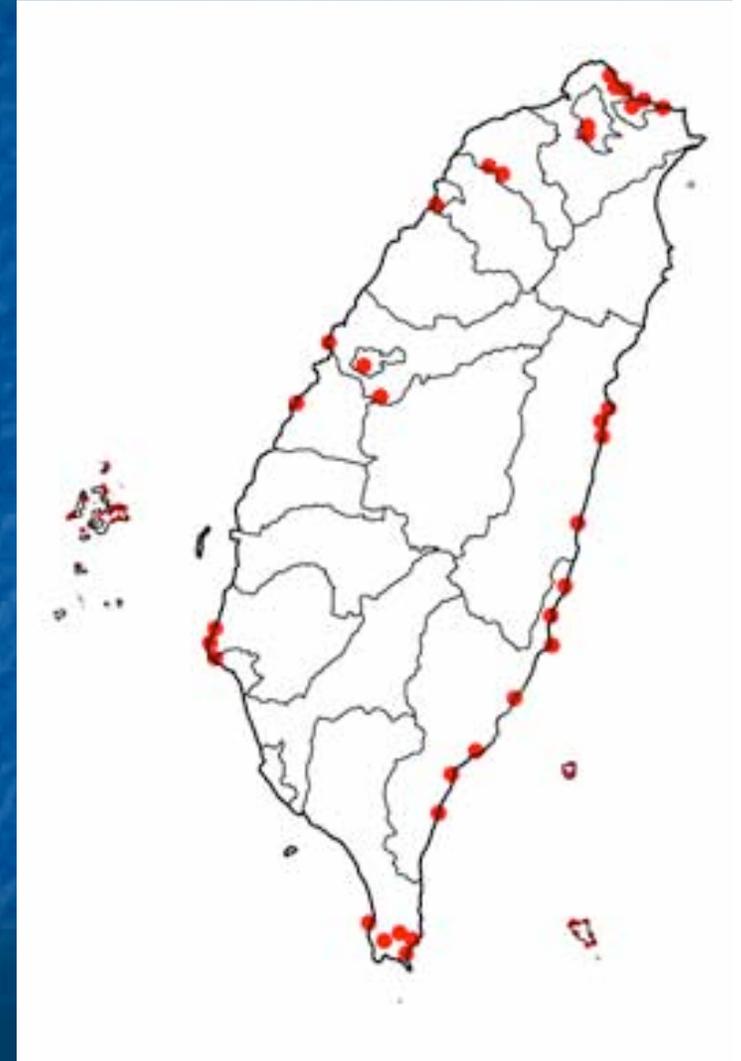
2100 meter altitude



	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
Stolon or rhizome growth			
Northern India type	None	None	None
Southern India type	None	None	None
Fertility (Seed set)%			
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

Adaptation in Taiwan

- The regional differences
- Precipitation
 - amount and season distribution
- Light
 - Day length and light intensity
- Temperature
 - Tropical, sub-tropical, mountain (temperate)
- Soil
 - pH, texture
- Harsh environments
 - Beach, mudstone, rocky



Latitude adaptation

- Northern Taiwan
- Central Taiwan
- Southern Taiwan



Altitude adaptation

- Northern Taiwan (Tai-Ping Mountain)

- 800m
- 1900
- 2100

- Central Taiwan (Sun-Moon Lake)

- 800m



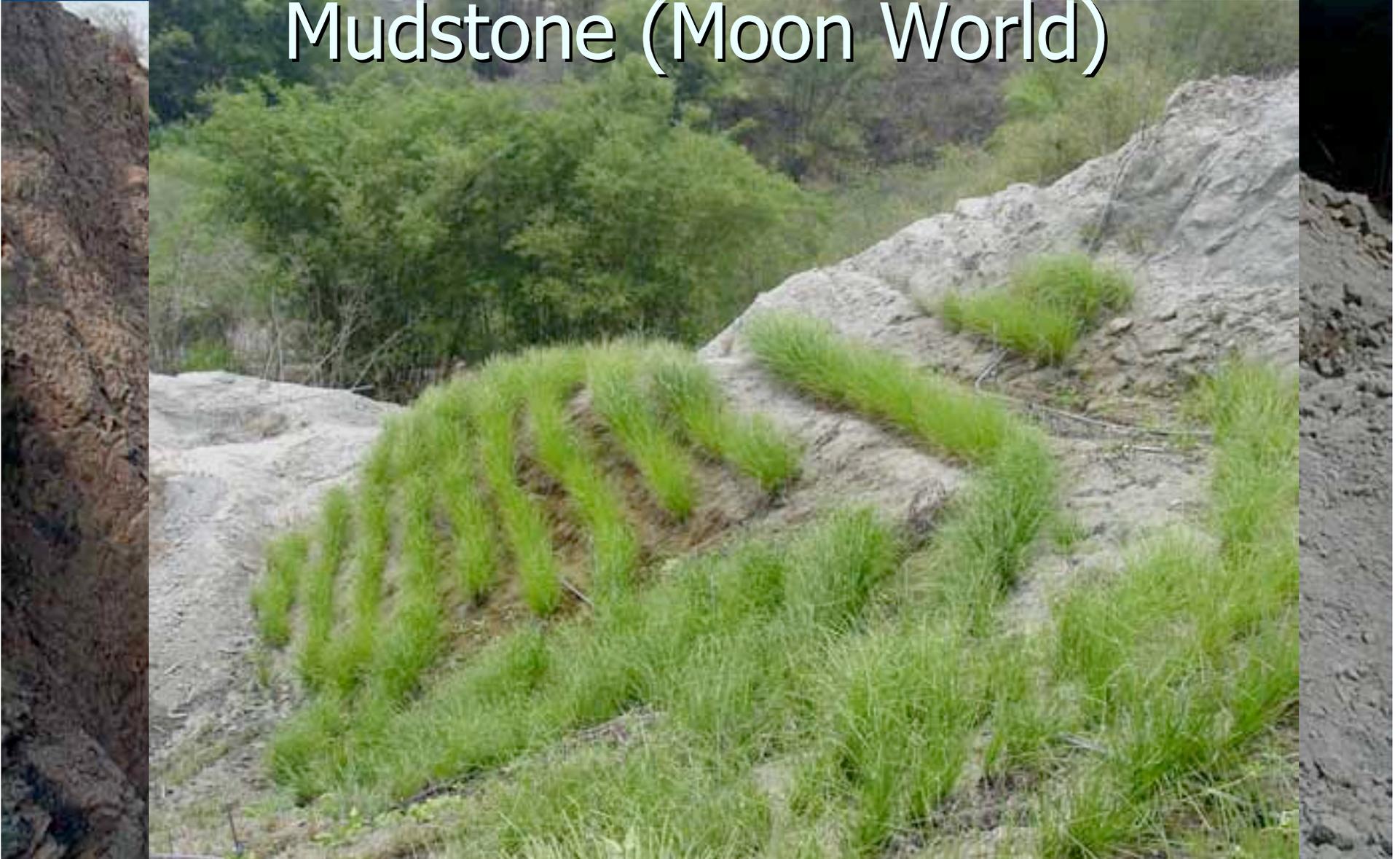
Harsh Environment Adaptation

- Saline soil
 - Salt condensation field of Taiwan Salt Co.
- Beach
 - Chayi Budai Port
- Mudstone



Harsh Environment

Mudstone (Moon World)



Conclusions for adaptation and weedy potential

- Vetiver can adapt to Taiwan in any conditions below 1500 meter altitude
- Weedy potential
 - The Northern India type vetiver produce abundant seeds should be restricted for application.
 - The Southern India type even though did produce seed but the amount of seed is not enough to make impact to the environment. But the seed production is contradictory to the previous finding and require further study

Root Extension Rate of Vetiver

- Three vetiver accessions for the study
 - Ohito VVZ009
 - Taiwan I VVZ010
 - VVZ008-18 a selected reduced fertility plant from PI196257



Temperature treatment

- 15/13, 20/15, 25/20, 30/25, 35/30 °C in phytotron for 12/12hours day/night



Vetiver

Temperature treatments

Genotype	15/13°C	20/15°C	25/20°C	30/25°C	35/30°C
Ohito	10.6? 7.5	54.7? 4.4	79.4? 20.9	73.3? 1.5	81.7? 15.0
Taiwan	16.0? 5.5	51.2? 16.5	80.3? 10.9	82.3? 7.5	86.0? 9.6
VVZ008-18	11.2? 1.5	38.4? 1.9	47.0? 7.9	50.2? 8.4	53.0? 23.1

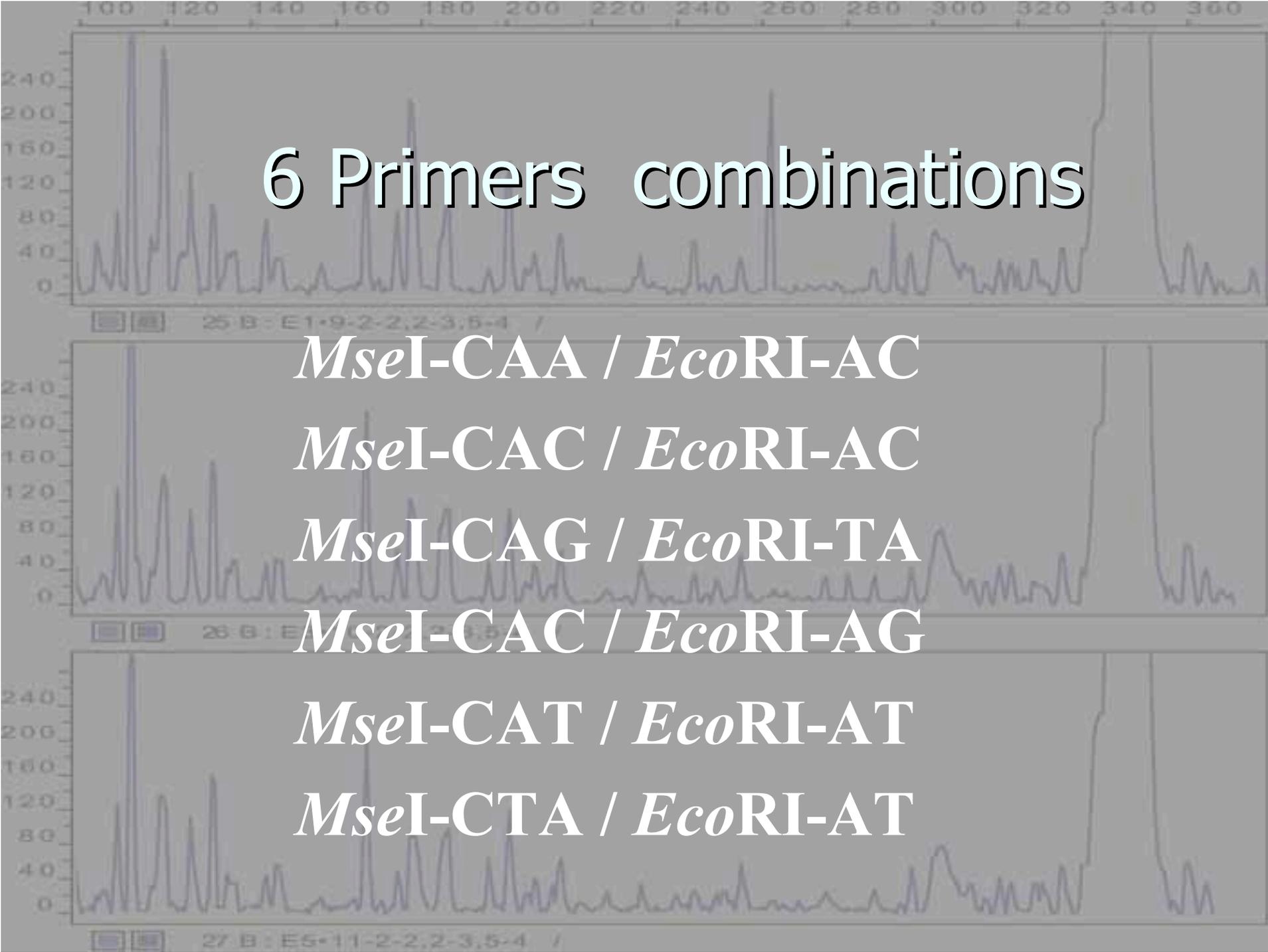


Conclusions

- The growth of vetiver root corresponding to the temperature was studied in this research with the following conclusions.
- Approximately three centimeters per day of root extension was observed when the soil temperature reached 25°C.
- The maximum growth of vetiver was achieved when the temperature rise up to 25°C.
- The root extension rate was higher in 35/30°C treatment, the difference was not significant statistically then the ones of 25/20 and 30/25 °C.
- The vetiver still had detectable underground growth in the 15/13 °C temperature treatment in this study, which suggested the vetiver is not dormant at this temperature. It is still possible to establish vetiver hedgerow under this temperature, although the time will be longer.

Estimation of the genetic variation compared to related species

- AFLP marker system



6 Primers combinations

MseI-CAA / *EcoRI*-AC

MseI-CAC / *EcoRI*-AC

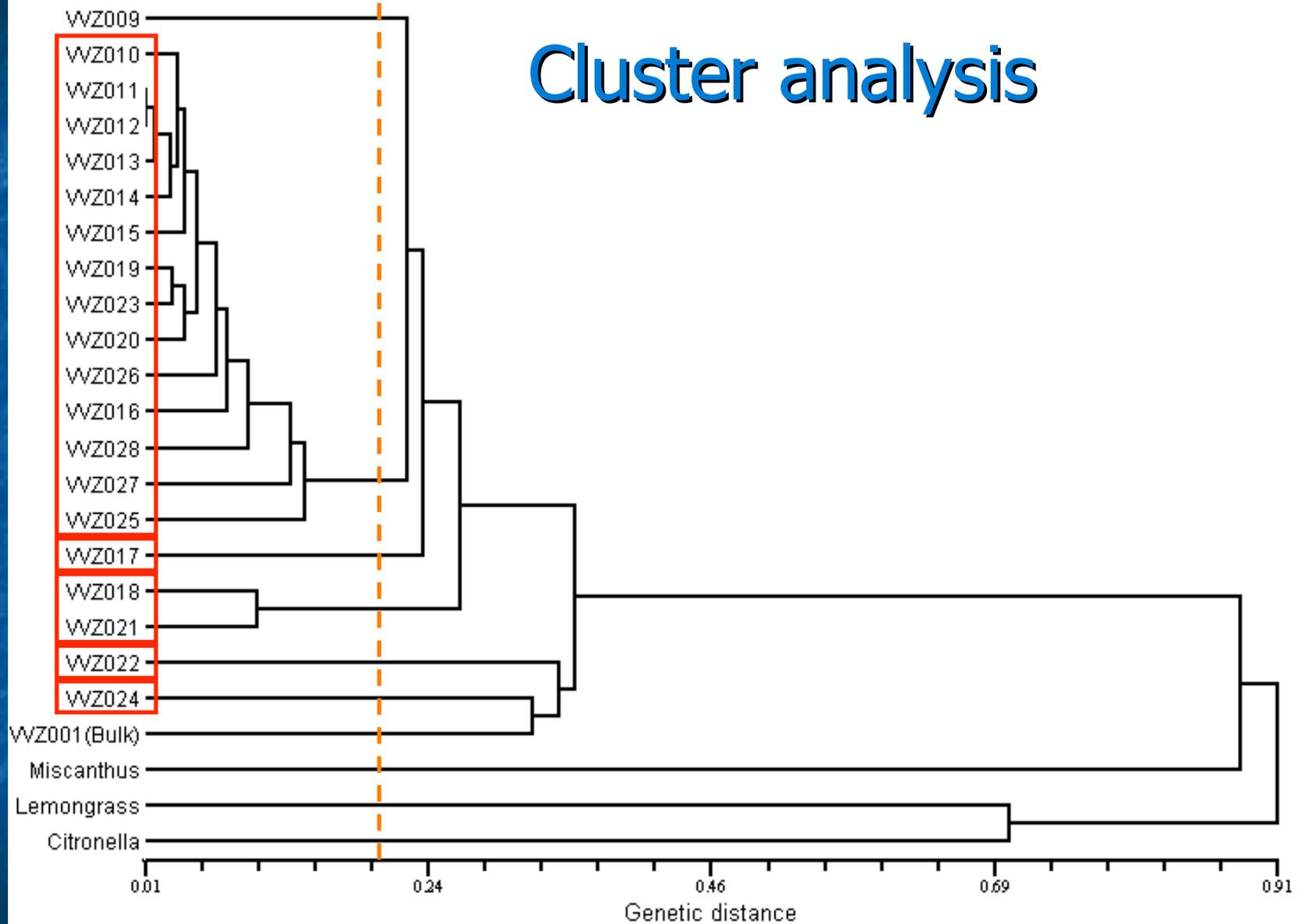
MseI-CAG / *EcoRI*-TA

MseI-CAC / *EcoRI*-AG

MseI-CAT / *EcoRI*-AT

MseI-CTA / *EcoRI*-AT

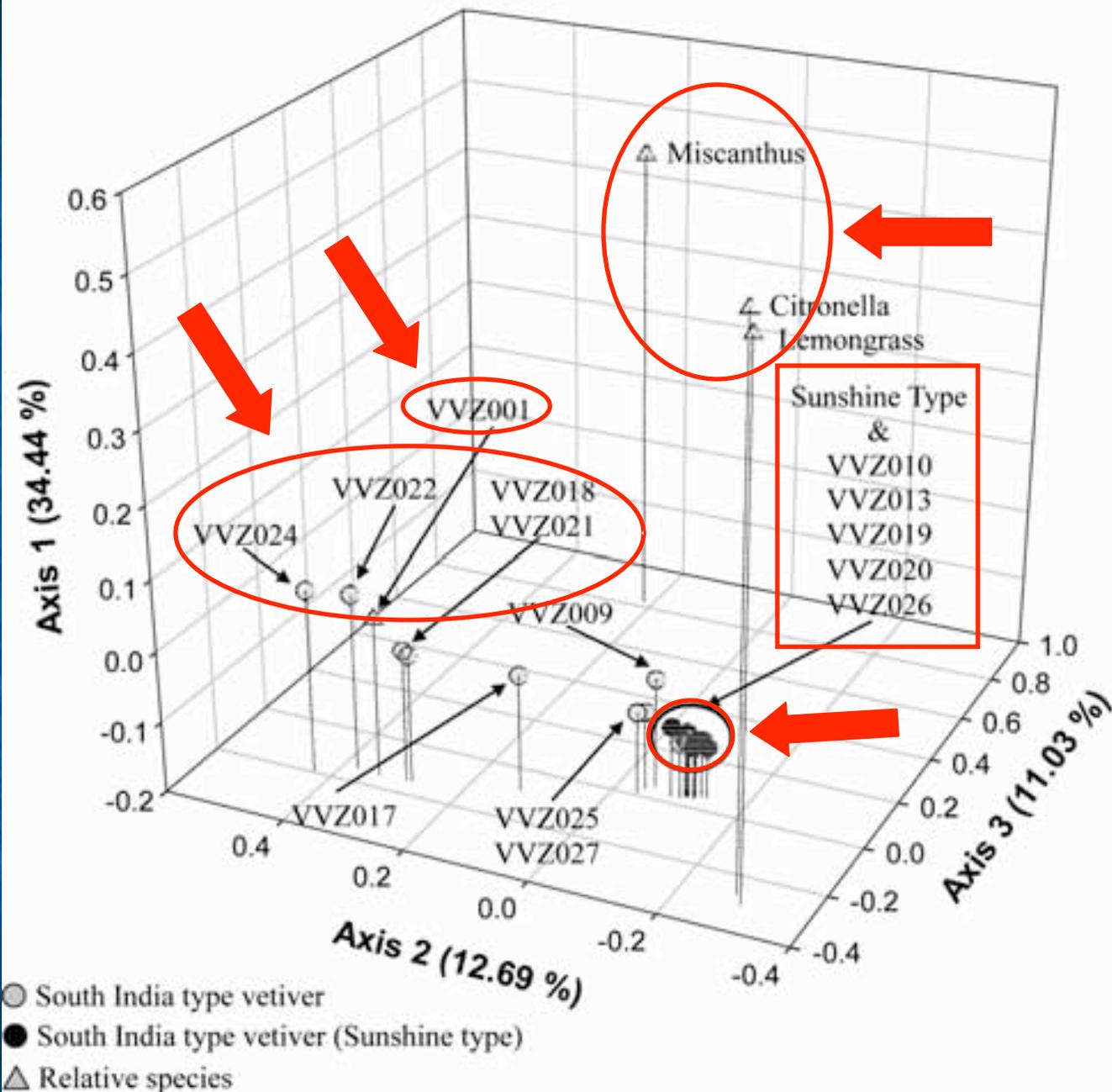
Cluster analysis



PCO analysis

All samples

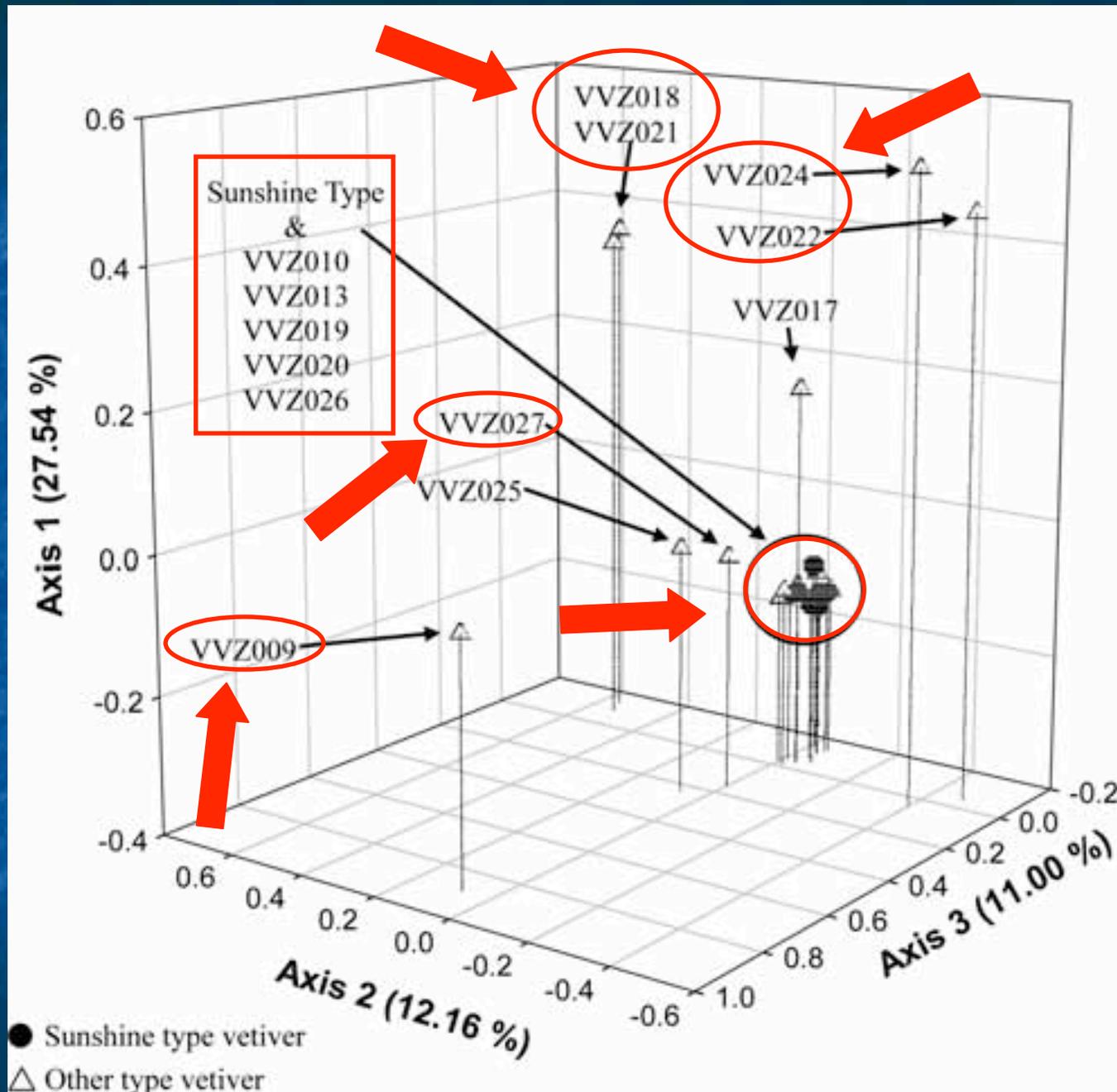
413 polymorphic bands



PCO
analysis

South India
type

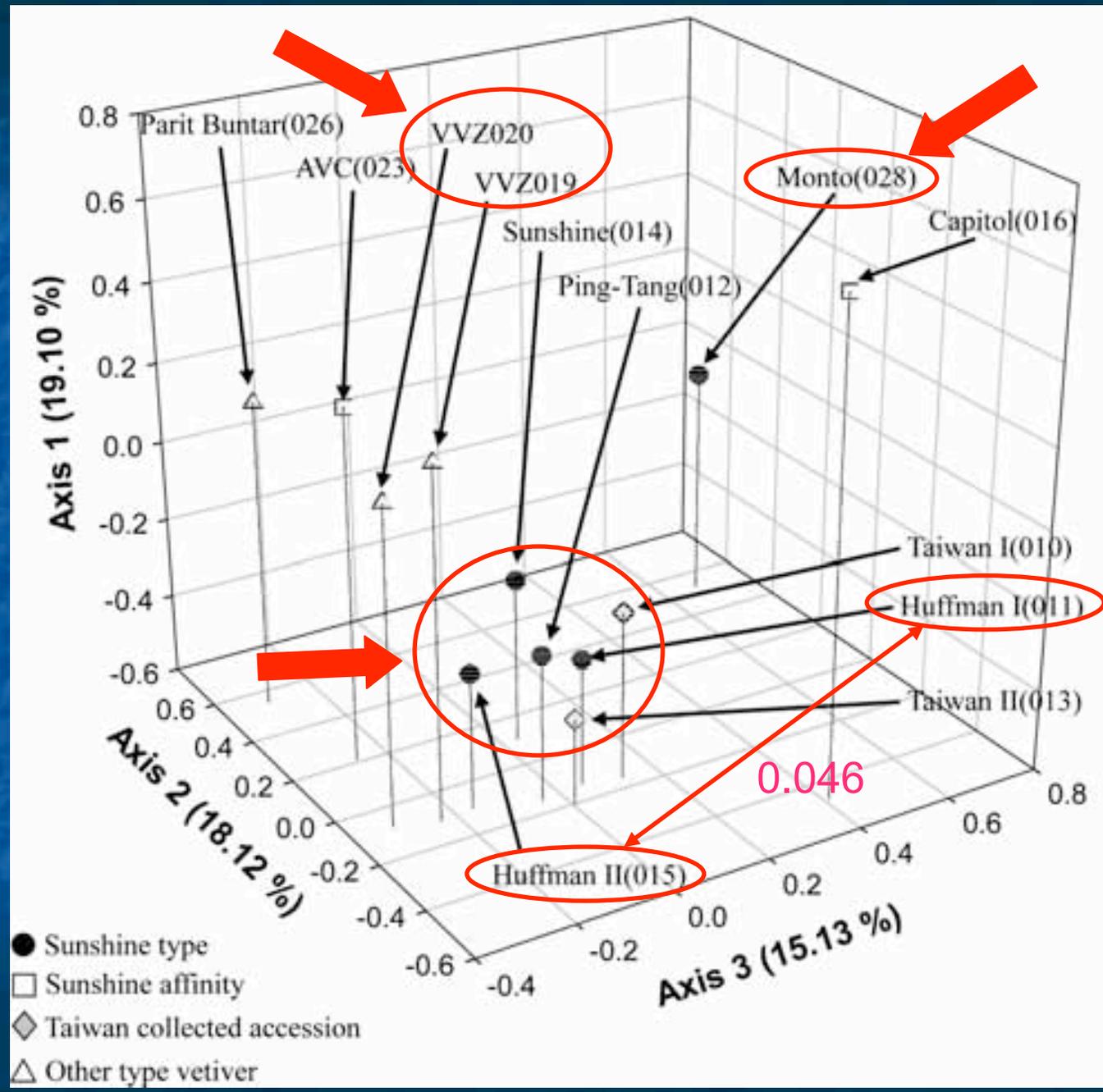
128
polymorphic
bands



PCO analysis

Sunshine type

34 polymorphic bands



Estimation of the genetic relationship between Northern & Southern India type vetiver

- RAPD markers

7 Primers

- Operon Technologies, Alameda, Calif

OPT-09 5'-CACCCCTGAG-3'

OPT-17 5'-CCAACGTCGT-3'

OPV-01 5'-TGACGCATGG-3'

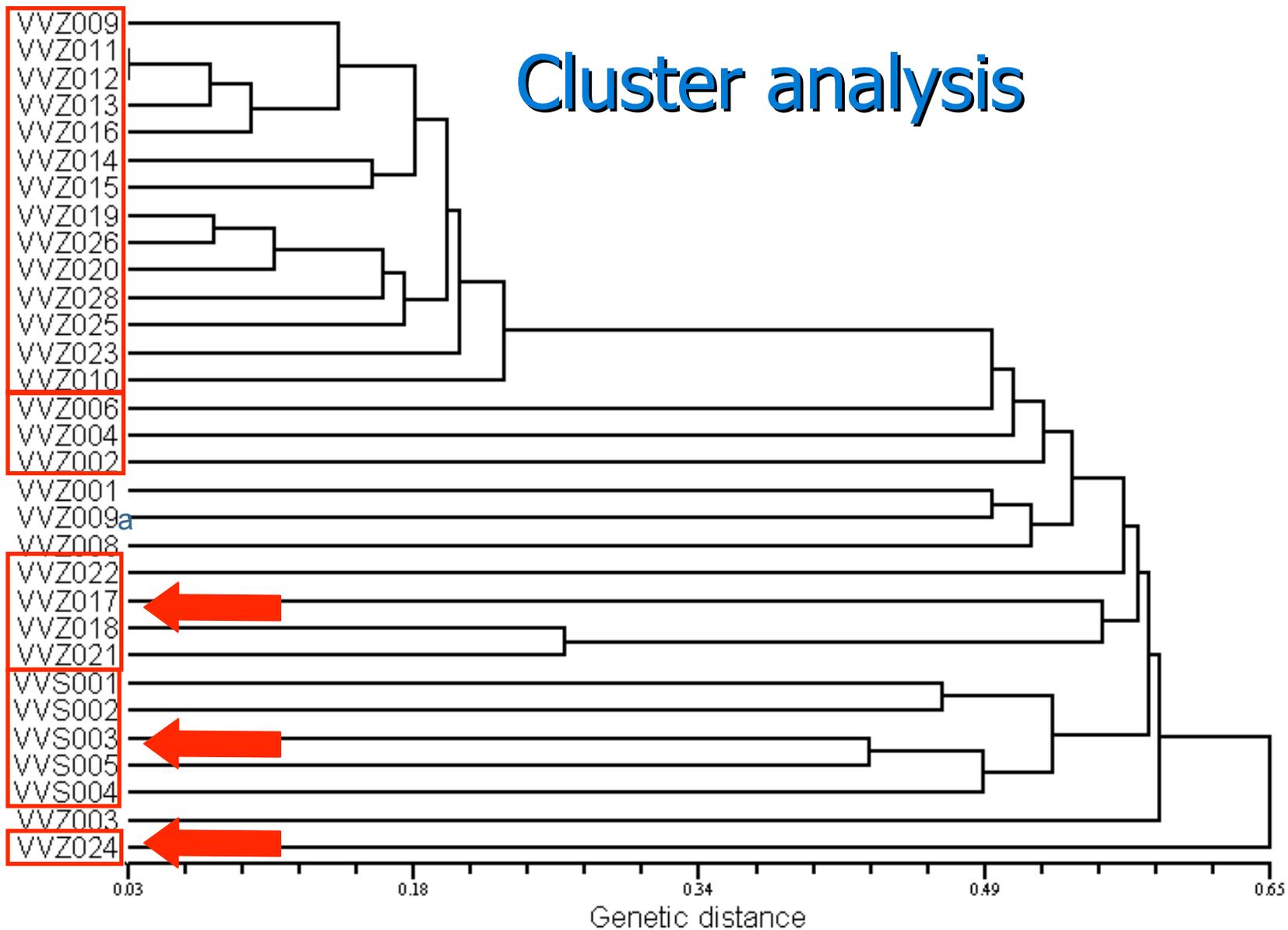
OPV-04 5'-CCCCTCACGA-3'

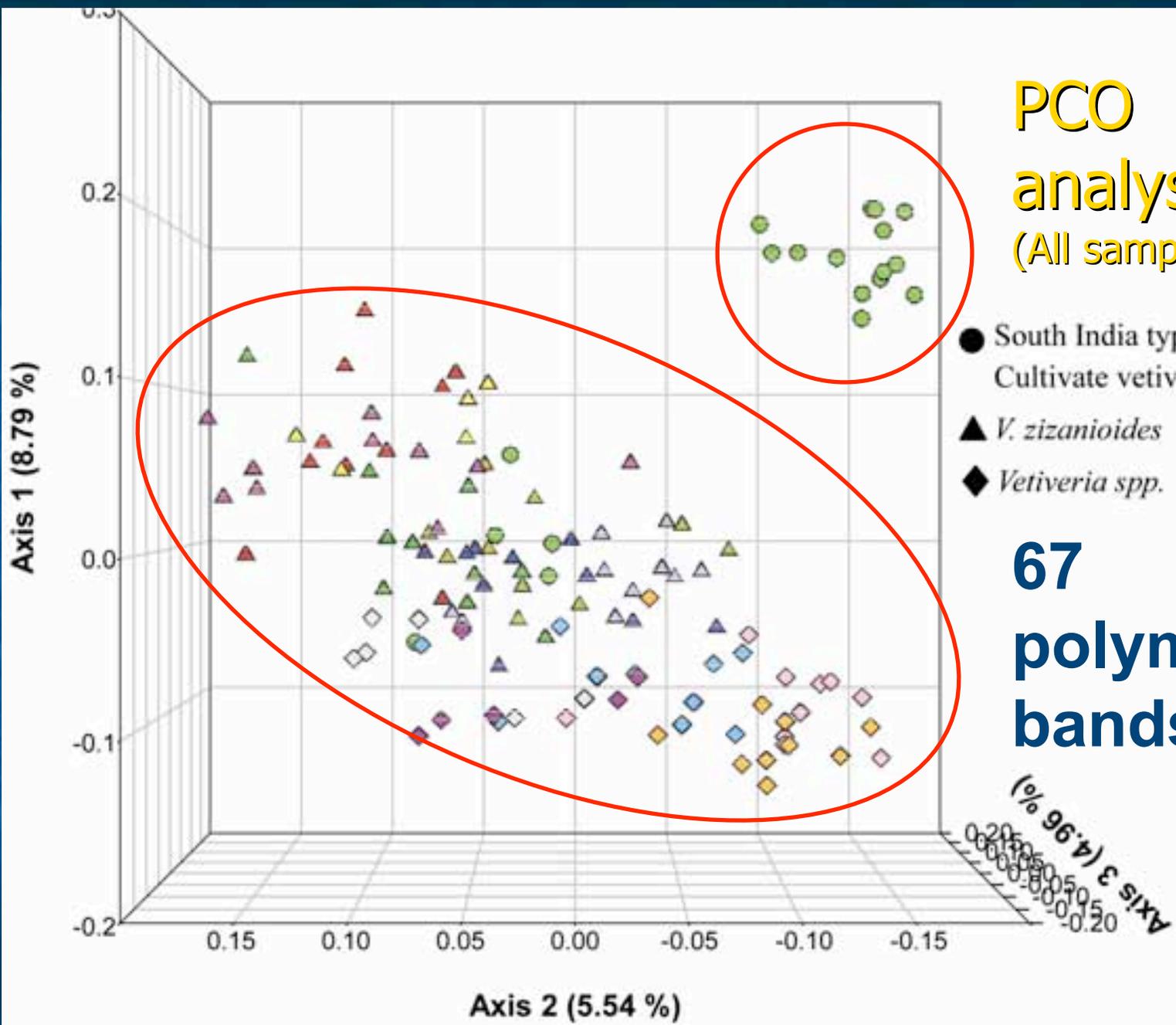
OPV-07 5'-GAAGCCAGCC-3'

OPX-03 5'-TGGCGCAGTG-3'

OPX-06 5'-ACGCCAGAGG-3'

Cluster analysis



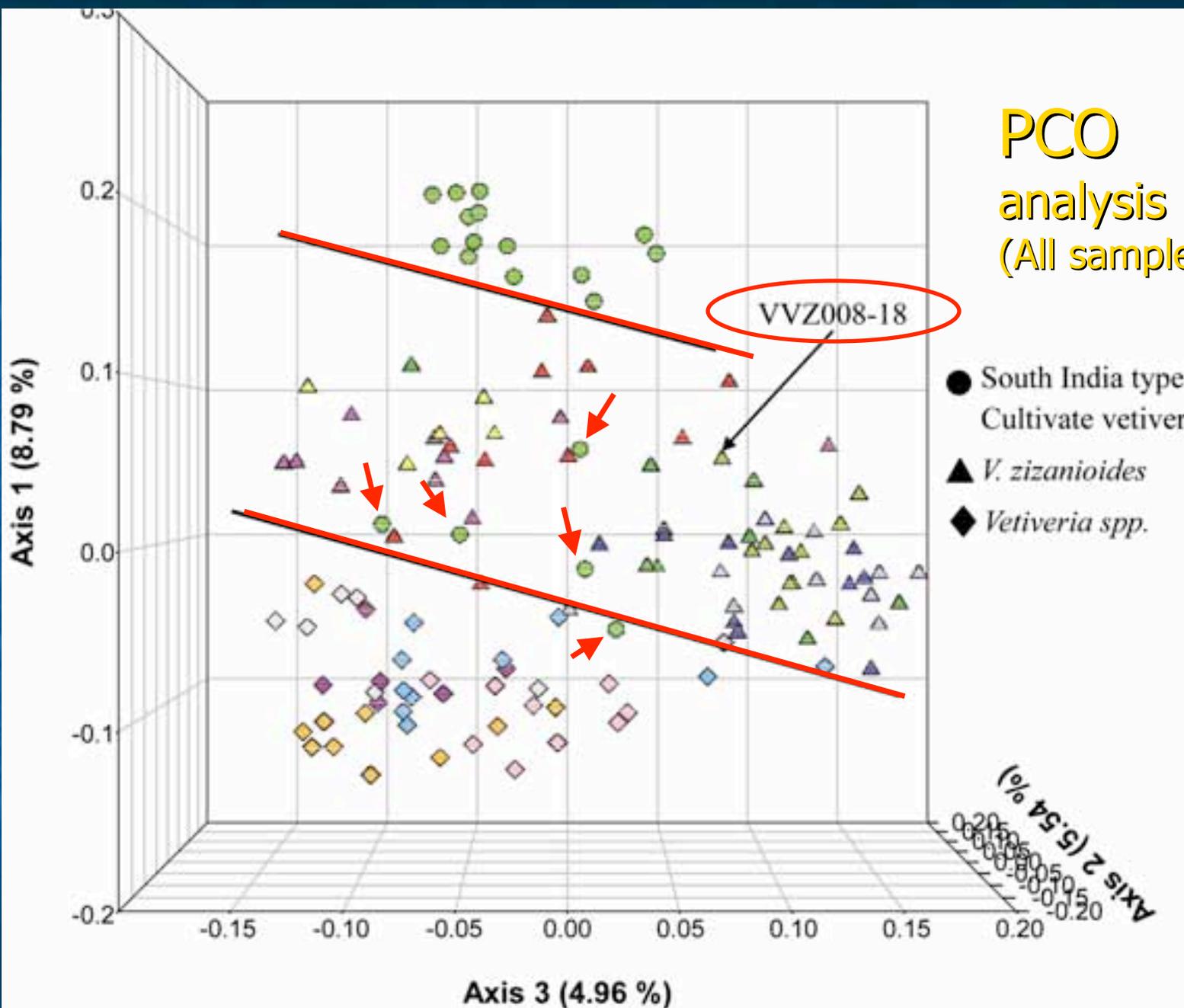


**PCO
analysis
(All samples)**

- South India type Cultivate vetiver
- ▲ *V. zizanioides*
- ◆ *Vetiveria spp.*

**67
polymorphic
bands**

PCO analysis (All samples)



The fingerprinting of vetiver (AFLP)

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		◆	◆								◆	◆				◆		◆			◆		◆			

The method of reproduction

- The seedy vetiver was used in the controlled pollination experiment for open-pollination in the field as well as phytotron under 5 different temperature treatments for self-pollination
- The open-pollination resulted in almost 100% seed set, while the self-pollination resulted in less than 1% with no difference to zero statistically
- The self-incompatibility was concluded for vetiver

Confirmation of the self-incompatibility

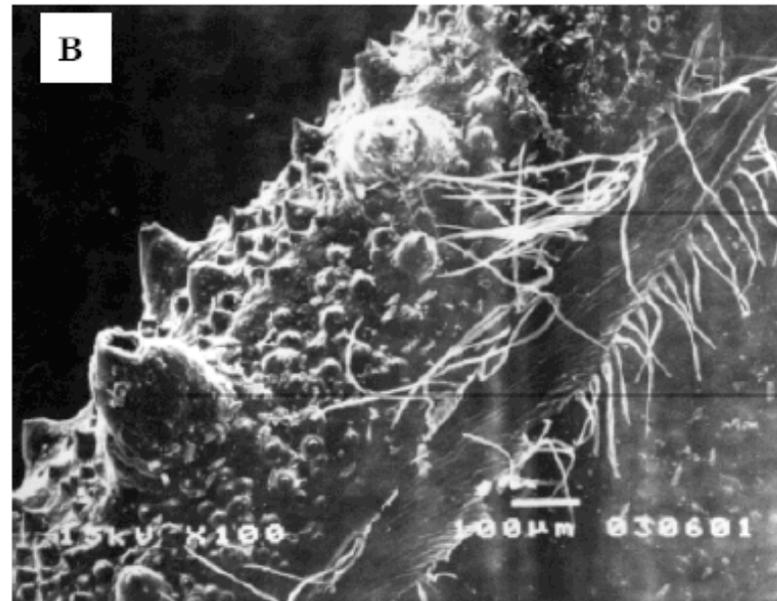
- The five cultivated vetiver accessions were open-pollinated and half-sib populations were created. Each of the seedling was tested using the SCAR marker to identify the pollen donor and was confirmed to be hybrid.

The SEM morphology of vetiver seed



bristle

cilia



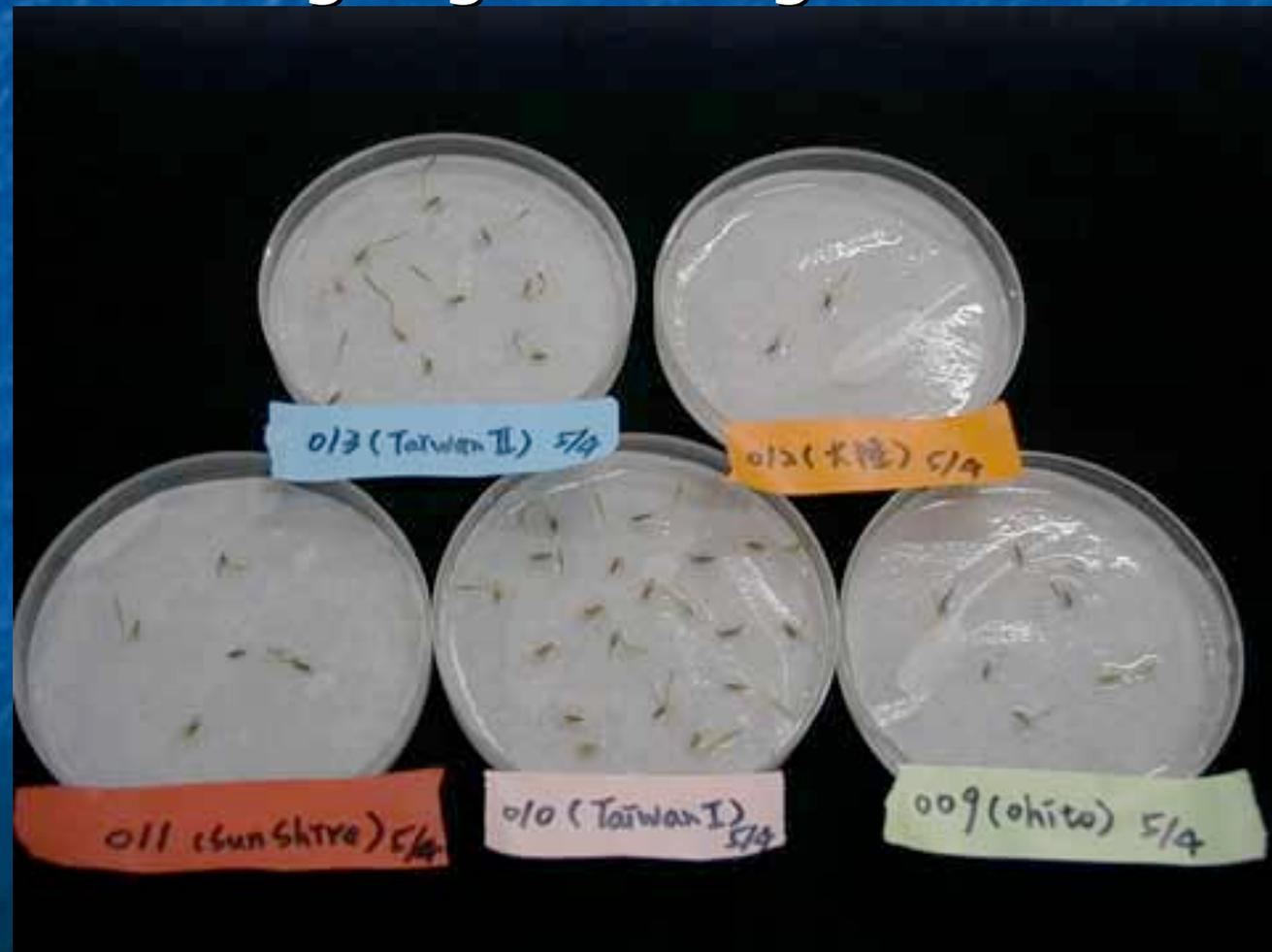
cilia

bristle

Scanning electron micrographs of caryopsis of cultivated and wild vetiver
(A° Cultivated vetiver VVZ009° B° Wild vetiver VVZ004 Rougher).

The optimum germination condition for vetiver

- 23°C-8hr Light/13°C-16hr dark was identified using a gradient germination table



The breeding potential of vetiver

- After one year of field growth, 220 out of the 251 seedling from the 5 half-sib families advanced to reproductive growth, 18 remained in vegetative growth and 12 died.
- 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 8,300 gm.

CO₂ sequestration : semi-open air

- A preliminary test conducted in Tai-Power Company



CO₂ sequestration : box in greenhouse



CO₂ sequestration preliminary result

- Five accessions put into test
- The amount of fresh weight increase was different between accessions
- The partition of the fresh weight between the shoot and root among accessions was also different

Energy and vetiver

- Shade
- CO₂ concentration
- Photosynthesis
- partition of photosynthates

Phytotron No. 3 Light room
Day 30°C/14 Hour
Night 25°C/10 Hour

260-370 $\mu\text{mol m}^{-2} \text{s}^{-1}$
0% Shade

120-157 $\mu\text{mol m}^{-2} \text{s}^{-1}$
57% Shade

8-18 $\mu\text{mol m}^{-2} \text{s}^{-1}$
97% Shade

0% Shade



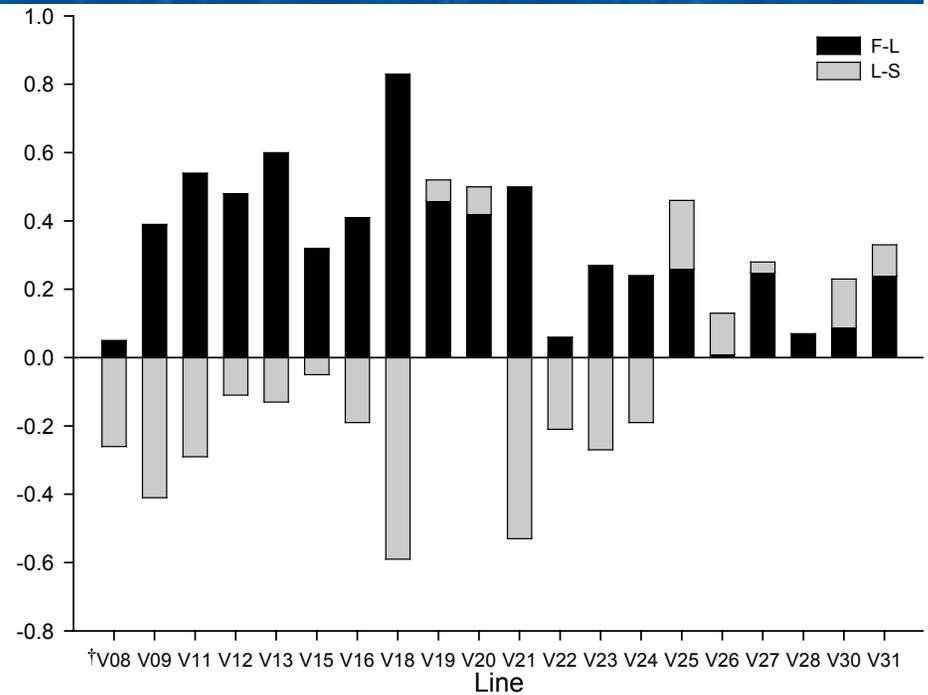
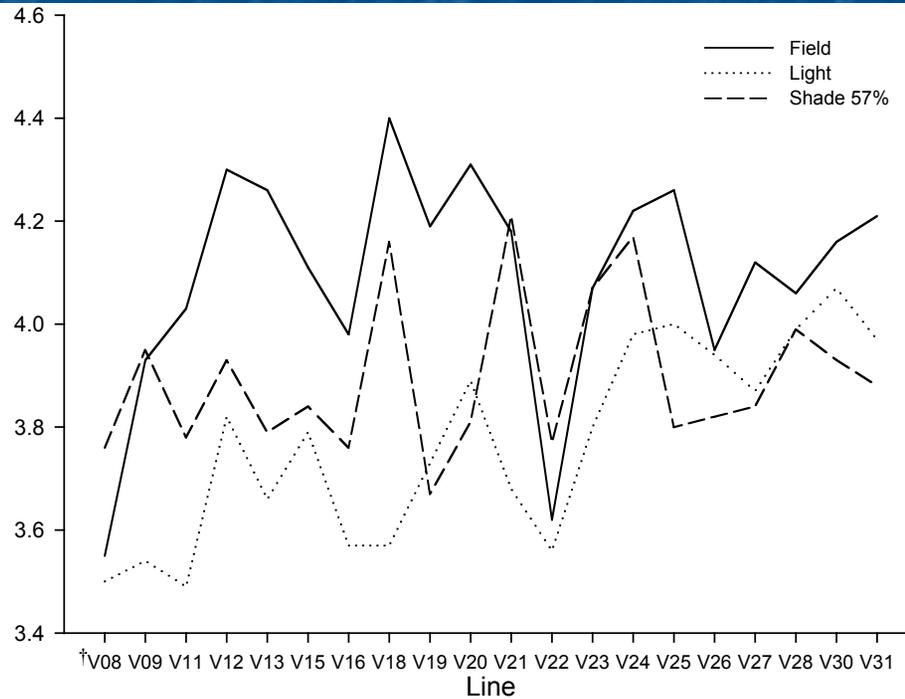
57% Shade



97% Shade



Chlorophyll ab ratio respond differently to treatments between lines



Source of Variation	CHL a/b	
	F value	Pr > P
Line	8	<.0001
Treatment	267.16	<.0001
Line×Treatment	2.86	0.2466

Grouping by Duncan's Multiple Range Test is not consistent with the dendrograph

008-18 (野生型培地茅之選系)

