Cytogenetics Study of *Vetiveria* Germplasm in Thailand

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Abstract

A cytological technique was developed to display chromosome morphology of 15 Thai and 5 introduced clones/ecotypes of vetiver from their root tip cells. They were classified as 13 *Vetiveria zizaniodes* and 7 *V. nemoralis* A. Camus. Humic acid solution was applied to obtain healthy root tips used for conventional slide preparation. The appropriate period for cutting the root tips was around 11 a.m. to 1 p.m. for further fixation. Enzyme mixture treatment appeared to be a promising way to separate groups of cells and to clear the cytoplasm. It was found that all clones/ecotypes studied had the same chromosome number, 2n = 2x = 20. Three B-chromosomes were observed only in one clone. The variation in satellite chromosomes in terms of number and morphology was evident in six clones/ecotypes. The total chromosome length varied from 1.2-8.4 μm. The karyotype consisted of metacentric and submetacentric chromosomes, all of which were nearly symetrical. Localization of the heterochromatic regions on the chromosome of vetiver was achieved by (a) modified C-band and (b) N-band. It was possible to characterize each pair of the vetiver chromosome. All ten pairs of chromosome were identified by these banding patterns, along with their length and arm ratios. They can be used as the basis for reproducible karyotype and for detecting chromosome relationship among clones/ecotypes. Each clone/ecotype showed at least one chromosome with a whole dark band, indicating a constitutive heterochromatin. The remainder indicated variation patterns from clone/ecotype to clone/ecotype of some faint and dark bands.
Keywords: Karyotype, banding, chromosome morphology, heterochromatin, vetiver

Introduction

Vetiver is known as a miracle plant because it possesses many beneficial characteristics and versatile uses especially in soil and water conservation and erosion control. Although vetiver is generally known to have originated in India, it is also found widely distributed naturally in all part of Thailand. From the result of recent botanical exploration, it was concluded that there are two species in Thailand, namely *Vetiveria zizanioides* Nash. and *V. nemoralis* A. Camus. The ecological difference between the two is so clear-cut that the former is commonly called the 'lowland' vetiver ('Faek Hom' in Thai) and the latter the 'upland' vetiver ('Faek Don' in Thai) (Chomchalow 1998).

Both species of Thai vetiver possess distinct germplasm variations which make them adapted to their different habitats. The term 'germplasm' is used to designate the variant originating in the province from which it was found e.g. 'Surat thani' 'Loei' 'Roi Et' ect. Due to small size chromosome hardly to obtain healthy and dividing root tip cells, its cytogenetics data is scarce. Only Lavania (1985) had reported nuclear DNA and karyomorphological studies in *V. zizanioides* from 20 germplasms in India. Since these chromosomes were small and frequently difficult to distinguish. The modified C-banding technique and the N-banding were applied to each germplasm of vetiver, to determine the banding patterns of the chromosome complement and to facilitate reproducible karyotypes. This study was conducted to obtain an appropriate cytogenetic technique and banding patterns as the basis for identification of all 10 chromosome pairs of the vetiver germplasm in Thailand.

Materials and Methods

Fifteen populations of vetiver germplasm collected from different geographical distributions in Thailand, maintained at Chalermphrakait Sakon Nakhon Province Campus and Ratchaburi Land Development Station were taken into account for the present study. Each population was put to root in a solution of humic acid 2-3 days. Root tips were collected when the roots were 1 to 1.5 cm. long and the appropriate time for root cutting was around 11-12 a.m. They were excised and pretreated in 0.2% colchicine at 12-14 ºC for 5 h., then fixed in a freshly prepared 95% ethanol- acetic acid (3:1 V/V) for 24 h. and stored in 70% ethanol. Chromosome preparations were made by enzymatic maceration and air-dried method (Fukui and Nakayama, 1996). Then root apices were rinsed with distilled water, stained and macerated in 2% aceto orcein for 5-7 min. For identification of the chromosome, specimens with well spread chromosomes were photographed. For chromosome lengths and arm ratios were measured from at least ten cells and averaged. Lengths of the chromosomes were measured from picture and converted into actual length according to magnifications. The number and size of satellites and B-chromosome were recorded.

For banding, the fixed root tips were stained in 1% acetocarmine for 1-2 h. at room temperature and squashed in 45% acetic acid. The cover glasses were removed by freezing method for further banding technique. (a) N-banding: the preparations were treated with 45% acetic acid at 50 ºC for 5-10 min and air-dried overnight. The slides were then incubated in hot phosphate buffer (1 M NaH_2PO_4) at 94 ºC for 2 min, washed thoroughly in running tap water, and air-dried. They were stained with 5% Giemsa solution for 10-15 min, rinsed briefly in tap water and air-dried. (b) Modified C-banding: the preparations were treated in a saturated barium hydroxide solution at 50 ºC for 2.5 min and rinsed in tap water. The slides were incubated in 2xSSC (0.3 M sodium chloride and 0.03 M trisodium citrate) at 50 ºC for 3-10 min and rinsed in tap water. The wet slides were stained with 5% Giemsa solution for 15-20 min, rinsed briefly in tap water and air-dried (Gill *et al*, 1991).

Results and Discussion

The appropriate period of root tips cutting was around 11 am. – 1 pm. Humic acid solution was applied to increase rooting size, vigor root tips and cell division. Enzyme mixture treatment appeared to be a promising way to separate group of cells to clear the cytoplasm. Chromosome number obtained from mitotic divisions, number of satellite and karyotype formulae from various germplasms were presented in Table and also illustrated in Fig 1 and 2 respectively. The chromosome of all species studied of *Vetiveria* belong to the small category according to Lima de Faria (1980), because their length ranged between 1.8-8.4 µm. Karyotype morphology including chromosome length, centromere position and ratio, secondary constriction and staining properties was analyzed for 15 vetiver germplasm belonging to two species of the genus *Vetiveria*, *V. zizanioides* and *V. nemoralis* A. Camus. They had 20 chromosomes length in the 8 germplasms of *V. zizanioide* and the 7 germplasm of *V. nemoralis* were 1.8-7.8 µm. and 1.8-8.4 µm. respectively. The range of the chromatin length varied from 1.8-8.4 µm. The germplasm from Prachuab Khiri Khan had the longest chromosomes whereas Kamphaeng Phet 1 and Songkhla 3 were characterized with shorter chromosomal complement. Other characteristics such as variation in chromosome length within species, satellite number and size, constrictions and centromere placements were used to differentiate the germplasm.

B-chromosomes which are distinctly smaller than A-chromosome, arising by misdivision betray their origin by varying in number at mitosis in root: after misdivision their centromere are of insufficient strength for regular movement ever in somatic mitosis. (Darlington, 1973)

Karyotypes consisted of metacentric and submetacentric chromosome. The pattern of all germplasm studied was nearly symmetry (Table 1). Based on the number of satellite and B-chromosome, karyotypes were grouped into 3 categories for haploid complement: 1) with 1-2 satellite chromosomes e.g. Surat Thani, Chaing Mai, Chaiyaphum, Loei, Songkhla3, Mae Hong Son and Saraburi 2 germplasm. 2) with 3 B-chromosomes e.g. Roi Et germplasm and 3) without

satellite and B-chromosomes e.g. Chaing Rai, Trang 2, Kampang Phet 1, Prachub Kiri Khan, Songkhla 1, Songkhla 2 and Saraburi 1 germplasm.

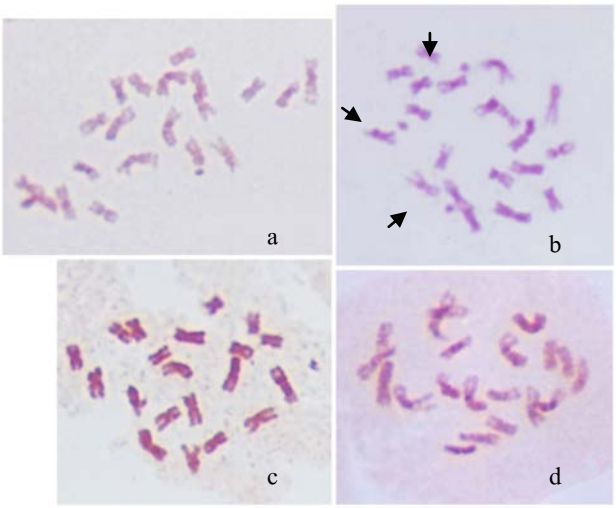

Fig 1. Metaphase cell of *Vetiveria* germplasm a) Surat Thani b) Roi Et c) Songkhla3 and d) Sara Buri2

Localization of the heterochromatic regions on the chromosomes of vetiver was achieved by a) modified C- banding and b) N-banding. It is possible to characterize each pair of the vetiver chromosomes. The absence of heterochromatin at the telomeric region and its presence in the centromeric region served as markers and facilitated easy identification of vetiver chromosome from those of haploid genome. Nevertheless, individual chromosome could be depicted and characterized by using a combination of chromosomes lengths, arm ratios, and their modified C-banding patterns.

According to their degree of asymmetry, the karyotypes of all germplasm studied were classified and fallen into category "1B".(Stebbins, 1971)

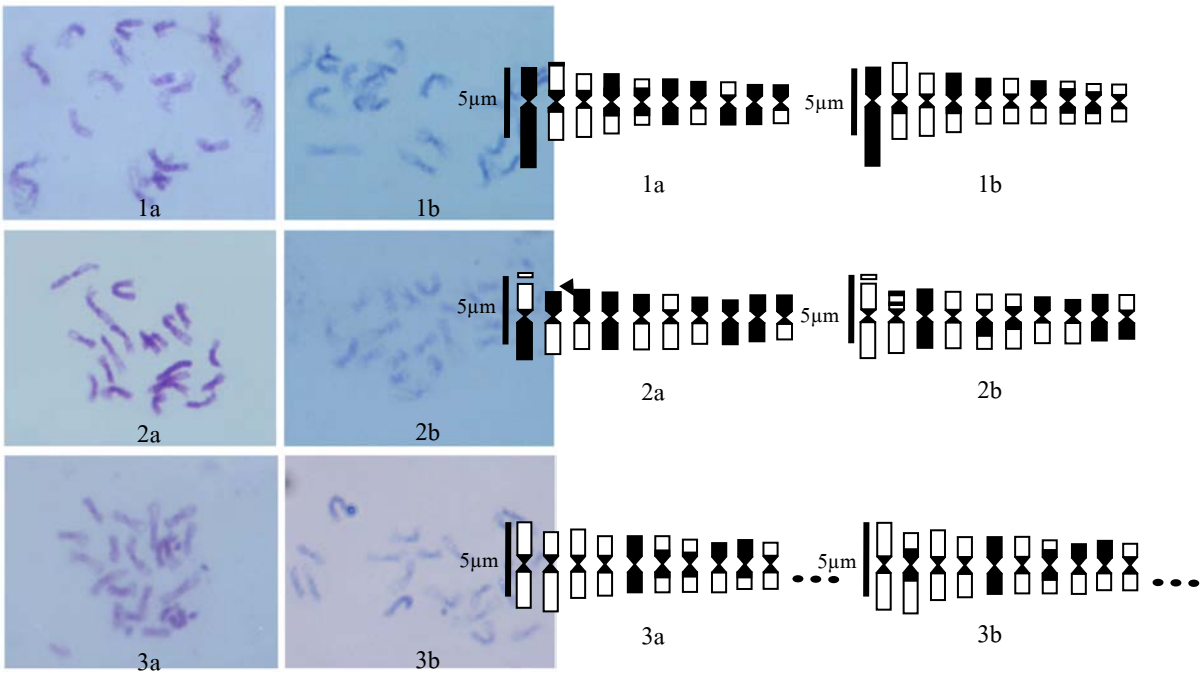

Fig 2. Chromosome banding
a) mC-band: 1. Chaiyaphum, 2. Sara Buri2 and 3. Roi Et
b) N-band: 1. Chaiyaphum, 2. Sara Buri2 and 3. Roi Et

They can be used as the basis for making reproducible karyotypes and detecting chromosome relationship among germplasms. Haploid complement of each germplasm showed at least one chromosome with whole dark band indicating a constitutive heterochromatin. B-chromosome showed whole dark band that indicating a constitutive heterochromatin. The remainder indicated variation patterns of some faint and dark bands from germplasm to germplasm.

Table 1. Location of collection, 2*n* chromosome, number of satellites and karyotype formulae for populations of *Vetiveria* germplasm in Thailand.

Germplasm	2*n*	Number of Satellite	Karyotype formulae
Surat Thani^{Z}	20	2	1MstB+3MC+1MstD+3MD+1SmC+1SmC
Chiang Rai^{Z}	20	0	4MC+4MD+2SmD
Chiang Mai^{Z}	20	2	1MB+1MstC+2MC+1SmC+1SmstC+4MD+1 SMD
Chaiyaphum^{N}	20	1	1MB+5MC1MstC+1SmC+2MD
Trang2^{N}	20	0	2MB+4MC+4MD
Kamphaeng Phet1^{N}	20	0	2MC+7MD+1ME
Roi Et^{N}	20+3b	0	1MB+2SmC+1MC+5MD+1SmD+3b
Prachuap Khiri Khan^{N}	20	0	1MA+3MB+1SmC+5MC
Loei^{N}	20	1	1MB+3MC+1MstC+2SmC+3MD
Songkhla1^{Z}	20	0	1SmB+1MB+2MC+5MD+1SmD
Songkhla2^{Z}	20	0	2MC+1SmD+6MD
Songkhla3^{Z}	20	1	2MC+5MD+1SmD+1SmstD+1ME
Mae Hong Son^{N}	20	0	3MC+1SmD+6MD
Sara Buri1^{N}	20	0	1SmB+1MB+8MC
Sara Buri2^{N}	20	2	1MstB+2MC+3SmC+1SmD+1MstD+2MD

^{Z} = lowland vetiver, ^{N} = upland vetiver

M = metacentric chromosome, Sm = submetacentric chromosome, st = satellite chromosome

b = B-chromosome

A = chromosome size ≤ 8 μm, B = chromosome size 6-8 μm, C = chromosome size 4-6 μm

D = chromosome size 2-4 μm, E = chromosome size ≥ 2 μm

References

Chomchalow, N. 1998. Amazing Thai Vetiver**.** FAO/RAP Publication 1998/40 / Royal Development Projects Board, Bangkok.

Darlington, C.D. 1973. Chromosome Botany and the origins of Cultivated Plants. George Allen & Unwin, London.

Fukui, K. and S. Nakayama. 1996. Plant chromosome: Laboratory Methods. CRC press, Inc. Florida.

Gill, B.S., B. Friebe and T.R. Endo. 1991. Standard karyotype and nomenclature system for description of chromosome bands and structural aberrations in wheat (*Triticum aestivum*). Genome 34: 830-839.

Lavania, U. C. 1985. Nuclear DNA and karyomorphological studies in vetiver (*Vetiveria zizanoides* L.) Nash. Cytologia 50: 177-185.
Stebbins, G.L. 1971. Chromosome Evolution in Higher Plants. Edward Arnold Publishers, London.