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Meiosis of vetiver germplasm in Thailand

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Abstract

The use of vetiver (Vetiveria zizanoides) anther for basic scientific research has not been well documented. This study was undertaken to identify cytologically the different developmental stages of vetiver anther to correlate them with previous karyomorphological study of vetiver germplasm in Thailand. Meiotic division in vetiver studied seemed to be normal only a few showed abnormality.

Keywords: *Chromosome configuration, cytogenetics, anther, microsporogenesis*

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).

Many aspects of maize genetics and cytogenetics can be properly understood only when view with full knowledge of the development of the gametophyte (Rhoades 1958; Lin 1987; Simcox *et al.* 1987) Maize microsporogenesis was studied by Chang and Neuffer (1989)

Microsporogenesis and megasporogenesis comprises three stages that culminate in the production of gametes. Pre-meiosis, meiosis and post-meiosis stages are controlled and coordinated by several diverse genes (Baker *et al.* 1976, Koduru and Roa 1981, Kaul and Murthy 1985). Meiosis, in addition to being the stage of longest duration, is also the stage that consumes the most cellular energy and is controlled by a large number of genes than the other stages (Golubovskaya 1979, 1989). Meiocyte is a highly special size cell capable of producing four haploid cell, mutations, hybridizations, environmental stress, endogamy, among other factors, may alter the constitution or the expression of genes that act during meiosis, resulting in abnormal microspores. Meiotic abnormality can cause pollen sterility, there was no correlation between these abnormalities and sterility. Many failures in genetics and mutation breeding experiments have occurred because of a lack of understanding of the mechanism and gamete formation. The purpose of the present study was to observe chromosome association in various stages of meiotic division and to serve as a background of chromosome behavior in vetiver.

Material and Methods

Five populations (DLD 009, Trang 2, Mae Hongson, Chiang Rai and Songkhla 3) of vetiver ecotypes collected from different geographical distributions in Thailand, maintained at Chalermphrakiat Sakon Nakhon Province Campus of Kasetsart University was taken into account for the resent study. For

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meiotic analysis, the appropriate stage of young inflorescences were collected between 9.00 to 10.00 a.m. and fixed in Carnoy solution (ethanol: acetic acid, 3: 1 v/v) for 24 hr. after which they were transferred to 70% alcohol and stored at 4˚C or -20˚C. Pollen mother cells (PMCs) were prepared by the squash technique followed by staining with 2% propionic orcein. The number of cells analyzed per flower ranged from 100 to 200. All meiotic abnormalities were carefully examined. For observation chromosome behavior, specimens with well spread chromosomes were photographed.

Result and Discussion

Meiotic studied of vetiver was attempted and reported for first time. A total of approximate 1,000 cells undergoing meiosis were analyzed. The vetiver defined stages, leptotene, zygotene, pachytene, diplotene, diakinesis, first metaphase, first anaphase, first telophase, second prophase, second metaphase, second anaphase, and second telophase (quartet) were presented sequentially in Figs. 1-12. All these stages take place inside the spore mother cell wall. The protoplasm is very dense and has no vacuoles and the nucleus is located in the center. Significant nuclear events are observed during this period. Homologous chromosome pair and chiasmata are formed along chromosome arms, accompanied by crossing over that exchanges genetics materials (Rhodes 1950, 1955). Genetic shifting of the genome occurs by random distribution of the homologous chromosome, the reduction division reduces the chromosome number to half, and the haploid cells divide again to form four microspores (Stantley and Linskens 1974).

From this study, it revealed mostly bivalent configurations. A few types of meiotic abnormalities were observed (Table. 1). Different type of meiotic abnormalities were observed and mainly related to chromosome migrating precociously to 1 pole and laggards (Fig. 13a) may result from late chiasma terminalization. Bivalents with interstitial chiasma may delay the terminalization process, while chromosomes do not accompany those with solely terminal chiasma. Univalent chromosomes, laggards and non-oriented bivalents may produce micronuclei (Fig. 13a) if they fail to reach the poles in time to be included in the main telophase nucleus. The behavior of micronuclei among cells was varied. Another meiotic abnormality observed at low frequency was quadrivalent (IV) chromosome (Fig. 13c). Ten bivalents were observed in normal meiocyte. (Fig. 13d).

From the previous karyomorphological study of vetiver germplasm, the ecotypes with 1-2 satellite chromosomes seem to have many micronuclei in meiosis, e.g. Mae Hong Son and Songhkla 3 but ecotype without satellite chromosome appear to be none micronuclei e.g. Trang 2 and Chiang Rai (Kongprakhon *et al.* 2003). From this study, the meiotic division was considered along with karyomorphological abnormalities, numerous micronuclei appear to be related to the number of satellite chromosomes. Mae Hong Son and Songkhla 3 ecotypes which represented karyotype with 1-2 chromosomes had numerous micronuclei in meiotic cells. Trang 2 and Chiang Rai ecotypes which represented not only karyotype without satellite chromosome but also invisible micronuclei.

From the experiment, we suggest to collect and analyze a large number of cells including *V. nemoralis* before made conclusion about meiotic abnormalities of vertiver germplasm studied.

Legend

Figs. 1-12 Vetiver microsporogenesis (3,300X). Stages are transitory and represent a momentary expression, which may not be a good representation of the complete events. They are demonstrated in sequence as follow, with an arrow indicating the event. N = nucleolus.

Fig. 1 Leptotene. Cell becomes round with dense protoplasm. The chromatin treads are greatly extended and coiled around nucleolus and chromosome become visible.

Fig. 2 Zygotene. Synapsis is initiated. Single-and double-strand configuration is evident, the chromosomes (arrow) are visible. The pairing of the homologous chromosome is complete. The condensed of chromosome show detail of heterochromatin and knobs (arrow). The nucleolus (N) and chromosome pattern of chromosome are visible.

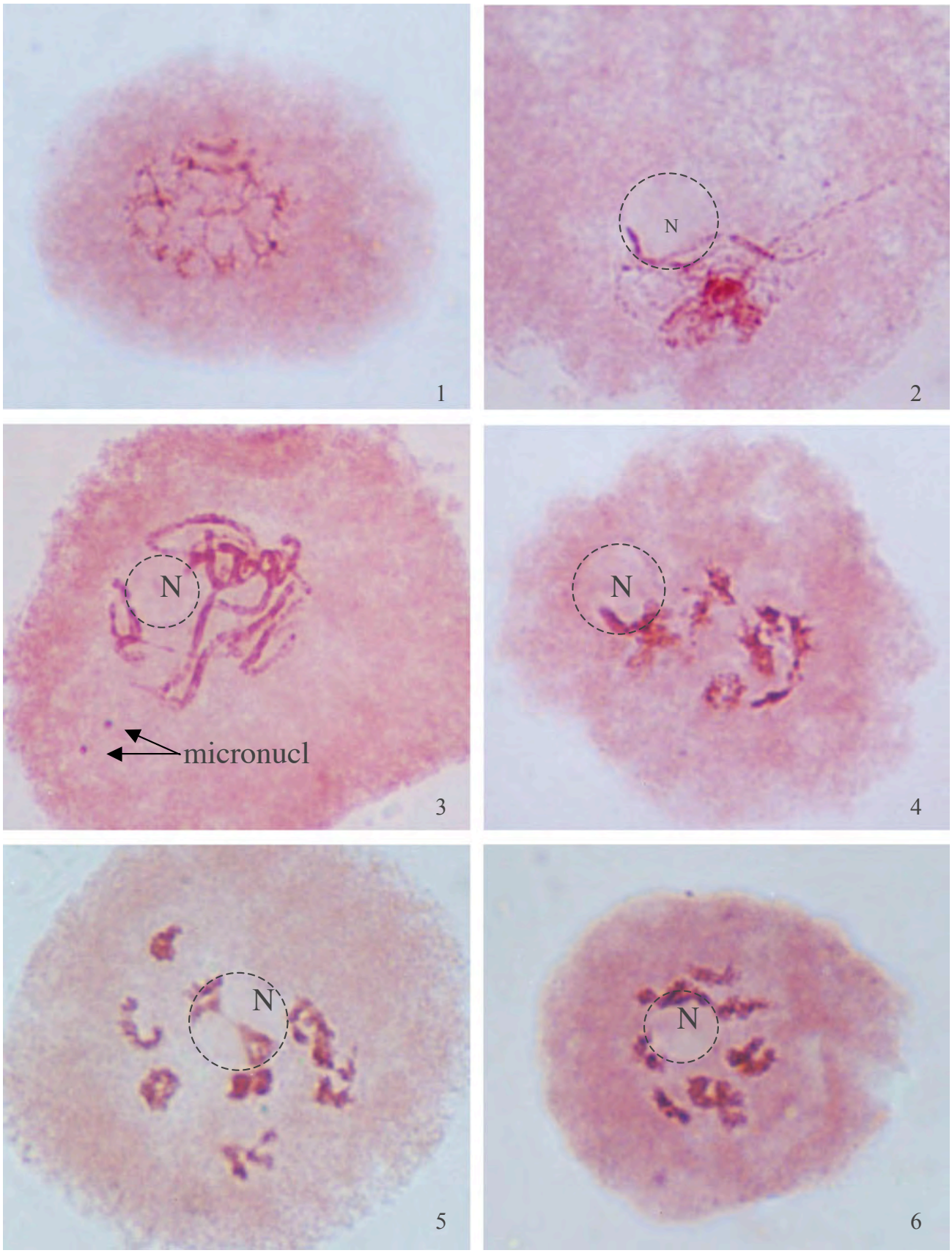

Fig. 3. Pachytene. The paired chromosomes are further condensing to become very thick threads. Individual chromosomes can be identified by their relative lengths, distinctive chromomere patterns, position of knobs and other recognizable characteristics.

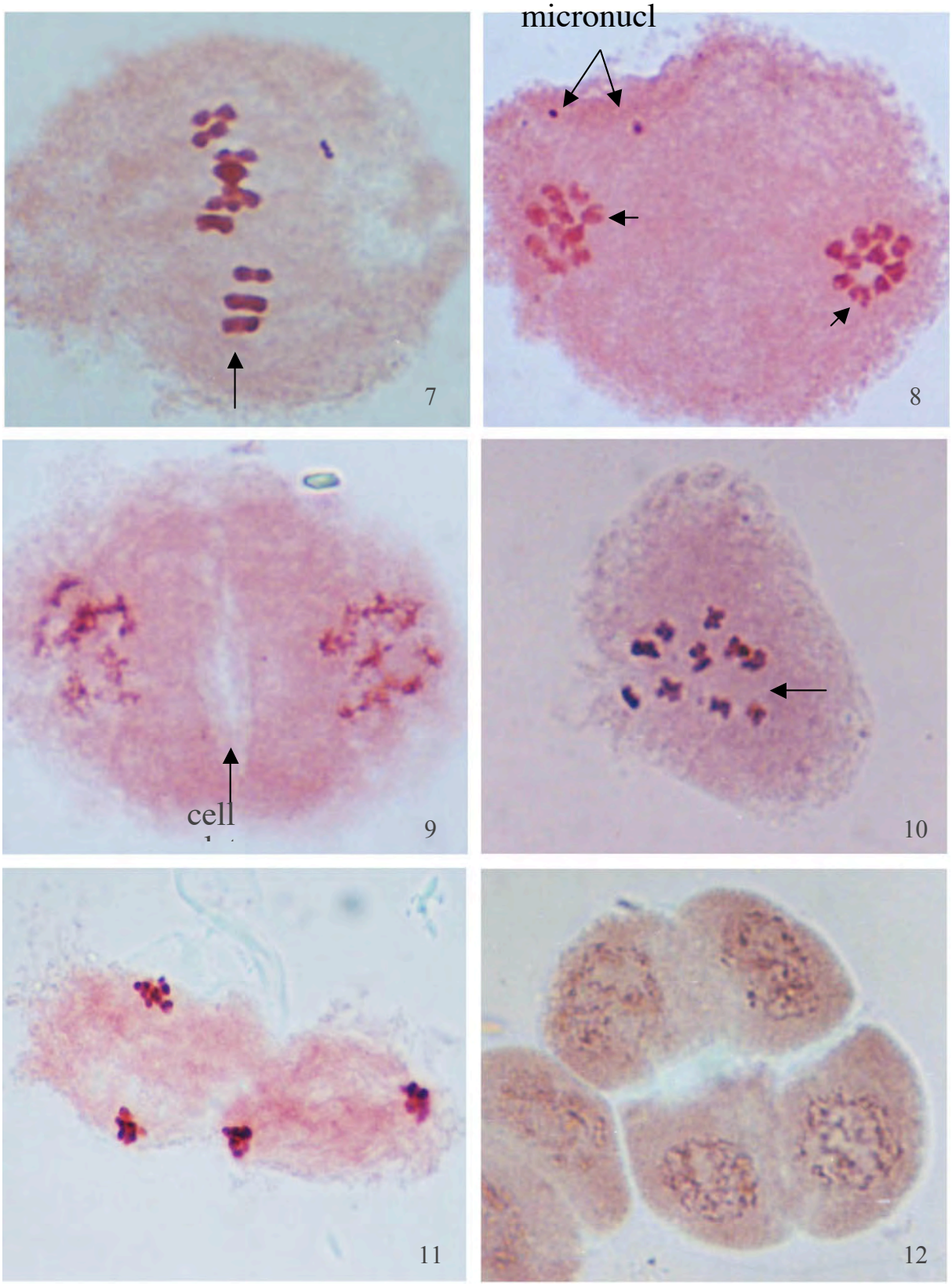

Fig. 4. Diplotene. The chromosomes continue to condense into short, thick threads. The paired chromosomes appear to be repulsing one another, except regions where an actual crossover took place. The chiasmata are frequently seen as X-shaped (arrow) and looped chromosome configurations.
Fig. 5 Late diplotene. The chiasmata are terminalized and very short condensed chromosome pairs are separated from each other. The X-shaped and looped chromosome configurations are still observed.
Fig. 6 Diakinesis. The condensed chromosome pairs are separated from each other and become thick staining bodies. The chiasmata and the X-shaped and looped configurations are still seen. The nucleolus starts to disappear.

Fig. 7 Metaphase I (side view). The nucleolus has disappeared. The paired chromosome (bivalent) lie at the equatorial plate (arrow) of the spindle structure. The chiasmata have moved to the end of the paired chromosomes. Both ring and rod bivalents are observed.
Fig. 8 Anaphase I. The paired chromosomes separate and move toward the opposite poles. The V-shaped configuration (arrow) of the chromosome is due to movement of the centromere ahead of the arms. The number of the chromosomes at each pole now is reduced to half (10-10).
Fig. 9 Telophase I. The chromosomes at each pole are now extended. The nucleolus reappears and the cytoplasm divides (arrow) (cytokinesis) to form two half-mooned cells.
Fig. 10 Metaphase II. The chromosomes (each has two sister chromatids) lie at the equatorial plate (arrow) of the spindle structure. Nucleoli have again disappeared.
Fig. 11 Anaphase II. The two sister chromatids seen collectively as a dark staining mass are now separated and have moved towards the opposite poles.
Fig. 12 Telophase II. The chromosomes at each pole are extended, the nucleoli reappear, and the cytoplasm divides to form four cone-shaped cells.

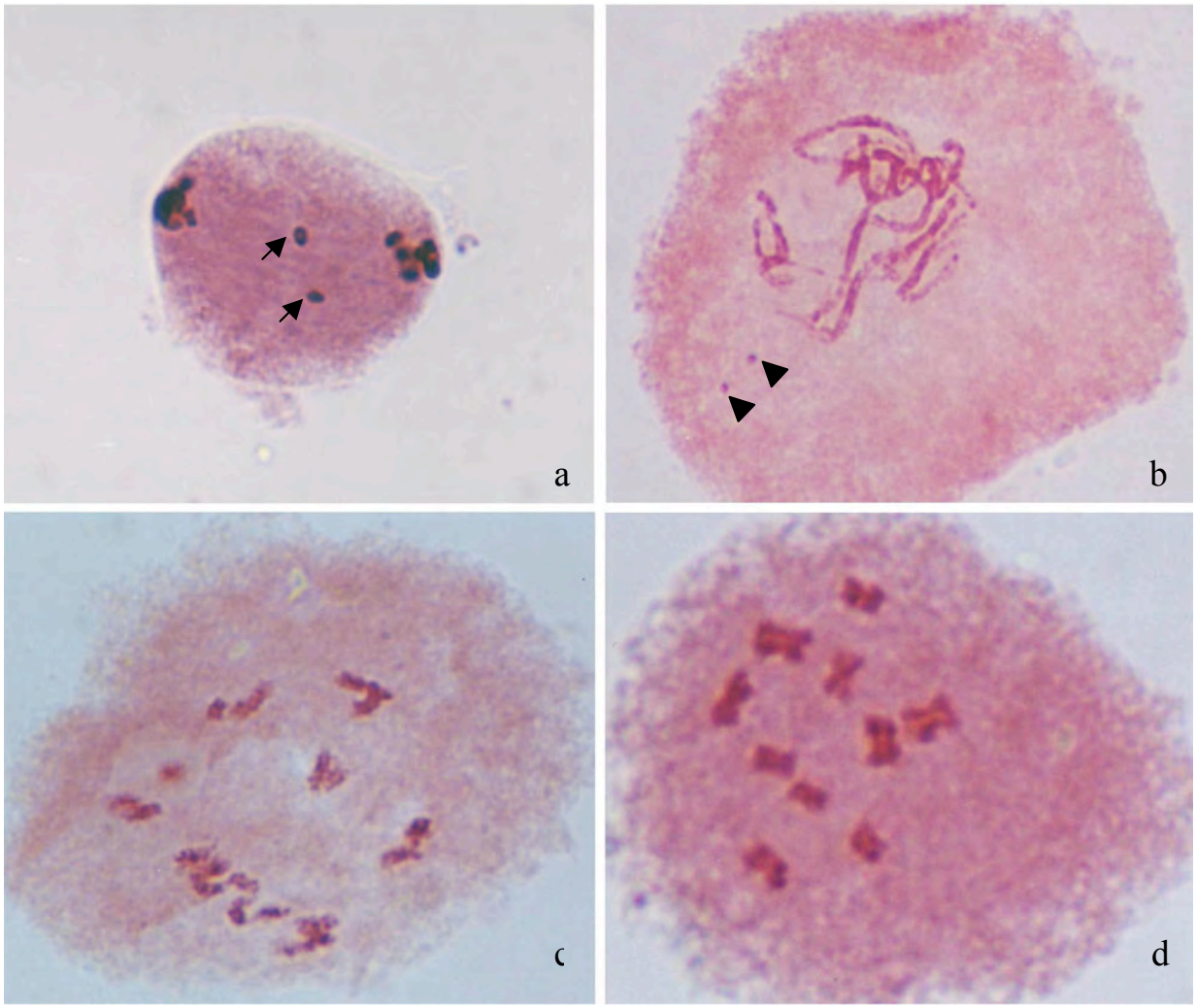

Fig. 13. Normal and abnormality chromosome behavior of vetiver meiotic cells.
a) laggard b) micronuclei c) quadrivalent (IV) chromosome and d) 10 bivalent (II) chromosome. (3,300X)

Table 1. The frequency of minor meiotic abnormality recorded among vetiver cells analyzed

Vetiver germplasm *(V. zizanioides)*	Frequency and number of meiotic abnormality cells					Total
	bivalent	univalent	quadrivalent	micronuclei	laggard	
DLD009 (z)	(0.96) 178 (1.0)	-	(0.04) 6	-	-	184
Trang 2 (z)	200 (0.68)	-	-	- (0.23)	- (0.09)	200
Mae Hong Son (z)	137 (0.91)	-	- (0.09)	47	16	200
Chiang Rai (z)	182 (0.76)	-	18	- (0.24)	-	200
Songkhla 3 (z)	152	-	-	48	-	200

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