Aneuploid Variation of Chromosome Number in the Somatic Cells of Piper magnificum Trel

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Aneuploid Variation of Chromosome Number in the Somatic Cells of *Piper magnificum* Trel.[†]

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Summary Aneuploid variation of chromosome number in somatic cells at growing root tips of *Piper magnificum* Trel., a South American *Piper* species of ornamental value is reported. Of the total 142 mitotic metaphase plates examined, about 62% of the cells were having normal chromosome number of $2n=26$. The variations observed were $2n=22$, 24, 25, 27, and 28 among which $2n=24$ was the most frequent (27.46%) and others were in less than 5.0%. Higher frequency of the cells with $2n=24$ is attributed as a reason for the dual chromosome numbers ($2n=24$ and $2n=26$) reported for the species. The role of precocious segregation of chromosomes and lagging during anaphase in generating aneuploid variation of chromosomes is discussed. This is the first report of such variation in *P. magnificum*.

Key words Chromosomal variation, Ornamental *Piper*, South American species of *Piper*.

Piper magnificum is a South American species of *Piper* which is important for its showy glabrous leaves with prominent veins (Fig. 1). It is well known for having the largest chromosomes among the *Piper* species (Dasgupta and Dutta 1976, Nair 1994). Two chromosome numbers have been reported for *P. magnificum* such as $2n=24$ (Dasgupta and Dutta 1976) and $2n=26$ (Smith 1966, Joseph *et al.* 1998, Mathew *et al.* 1998, 1999). Karyomorphology of *P. magnificum* was reported by Dasgupta and Dutta (1976) and Joseph *et al.* (1998). Meiotic studies were conducted by Mathew *et al.* (1998, 1999). The present study reports the existence of aneuploid variation of chromosomes in the root tip cells of *P. magnificum*.

Materials and methods

Actively growing root tips were collected from the fresh rooted cuttings of *Piper magnificum* maintained at the garden of Indian Institute of Spices Research, Calicut and cytological analysis was conducted following the method standardized by Nair *et al.* (1993) for black pepper (*Piper nigrum* L.). Root tips were collected between 11.00 and 11.30 AM and pretreated with a saturated solution of α-bromonaphthalene at 4–5°C for 4 h. Subsequently, the samples were washed thoroughly in double distilled water and fixed in a 3 : 1 : 1 mixture of ethyl alcohol, acetic acid and chloroform for 24 h.

The fixed root tips were hydrolysed with 1 N HCl at 60°C for 4–6 min and stained in 2% lacto-propionic orcein for 4 h and squashed in 45% propionic acid. One root tip each was squashed per slide. Observations and photomicrographs were taken from temporary slides using Olympus Vanox research microscope fitted with PM-10 camera. A total of 142 mitotic metaphase plates with reasonably good chromosome spread from 6 slides were counted to record chromosome numbers. Frequency of cells having different chromosome numbers was recorded separately and percentage was calculated. Fifty anaphase plates were observed at random from all the 6 slides for recoding chromosomal abnormalities during disjunction.

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Table 1. Numerical variation of chromosomes in root tip cells of *Piper magnificum* Trel.

Slide No	Number of cells observed	Frequency of chromosome numbers observed					
		$2n=22$	$2n=24$	$2n=25$	$2n=26$	$2n=27$	$2n=28$
1	27	1	6	—	17	1	2
2	15	1	3	1	9	—	1
3	25	2	8	1	12	1	1
4	27	2	8	—	17	—	—
5	21	—	5	—	15	—	1
6	27	—	9	—	18	—	—
Total	142	6 (4.23)	39 (27.46)	2 (1.41)	88 (61.97)	2 (1.41)	5 (3.52)*

* Values in parentheses indicate Percentage.

Fig. 1. A plant of *Piper magnificum* raised in garden pot.

Results

Considerable variation in chromosome number was observed among the mitotic metaphase cells counted. Of the 142 cells counted 88 were having $2n=26$, 39 were having $2n=24$, 6 were having, $2n=22$, 5 were having $2n=28$ and 2 each were having $2n=25$ and 27 (Table 1). Mitotic metaphase plates showing different chromosome numbers are presented in Fig. 2A–G. A few chromosomes in certain cells showed indications of precocious division and separation (Fig. 2F, G→).

Among the fifty anaphase plates observed, four cells showed lagging chromosomes and one showed sticky bridge. Counting the number of anaphase chromosomes segregating to the poles was not possible due to the heavy stickiness of chromosomes.

Discussion

The present results on aneupoid variation in somatic chromosomes of *Piper magnificum* explains the earlier reports of 2 different chromosome numbers by different researchers. Smith (1966) reported the chromosome number of *Piper magnificum* as $2n=26$ while Dasgupta and Datta (1976) reported it as $2n=24$. Later reports (Joseph *et al.* 1998, Mathew *et al.* 1998, 1999) supported the report of Smith (1996). From the present results it is clear that somatic cells in the root tips of *Piper magnificum* shows variation in chromosome number. However, the majority of cells (62%) possess $2n=26$ and next higher frequency (27.46%) was observed for $2n=24$. Frequency of cells having other chromosome numbers such as $2n=22$, 25, 27 and 28 were less than 5%. The reason for the report of chromosome number of *Piper magnificum* as $2n=24$ by Dasgupta and Dutta (1976) may be due to the few number of mitotic metaphase plates (about 5) used by them for analysis. Conversely, it is also be possible that they used a variant clone of *Piper magnificum* for cytological analysis, similar to the studies by Jose and Sharma (1983) in *Piper betle*. In view of the present observations, the chromosome number of *P. magnificum* may be accepted as $2n=26$.

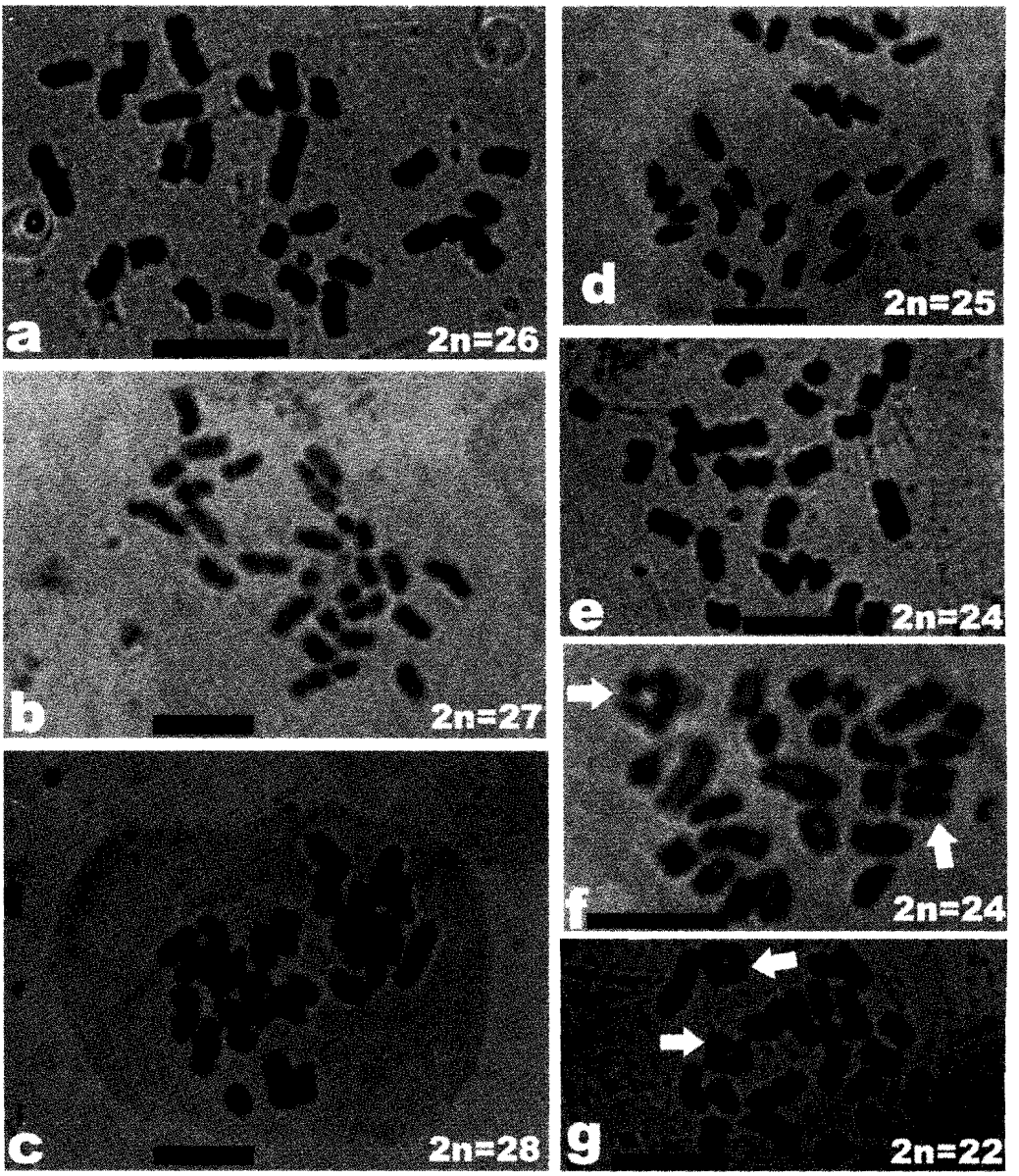

Fig. 2. Somatic cells at mitotic metaphase stage from root tips of *Piper magnificum* showing variation in chromosome number. a. $2n=26$ (Normal), b. $2n=27$, c. $2n=28$, d. $2n=25$, e. $2n=24$, f. $2n=24$ showing two precociously separating chromosomes after replication (→), g. $2n=22$ showing two precociously separating chromosomes after replication (→), Bars represent 5 μm.

Instances of variation in somatic chromosome number were reported previously by few authors (Hegwood and Hough 1958, Mix *et al.* 1978). A mosaic pattern of chromosome numbers was observed by Hegwood and Hough (1958) in somatic tissues in the White Winter Pearmain apple and 6 of its seedlings. They observed the basic euploid chromosome number in higher frequency and variable numbers, both higher and lower than the euploid mode at random in cells of meristematic layers of shoot buds. According to D'Amato (1977) a great variation in chromosome number may occur in somatic cells of microspore derived plants in various species. In root tips of microspore regenerated plants of barley, Mix *et al.* (1978) observed haploid, diploid, tetraploid as well

as aneuploid cells. Similar to the case of White Winter Pearmain apple (Hegwood and Hough 1958), in the present study on *Piper magnificum* also cells with normal euploid chromosome number were in higher frequency and variants were in lower frequency (Table 1).

Numerical variation of chromosomes in somatic cells may usually arise due to abnormal mitosis (Hegwood and Hough 1958, Nair and Ravindran 1994). Hegwood and Hough (1958) attributed the reason for mosaic pattern of chromosome numbers in somatic tissues in the White Winter Pearmain apple and its seedlings to abnormalities in the mitotic process. They indicated the heritable nature of such variation which further indicates the genetic control of the phenomenon. While discussing the somatic association of chromosomes and other mitotic abnormalities in *Vanilla planifolia*, Nair and Ravindran (1994) indicated abnormal segregation of chromosomes during anaphase and chromosome lagging as the reasons for the numerical variation of chromosomes in the root tip cells of the same plant. In the present study chromosome lagging was observed in 8% of the cells and indications of precocious division and separation of a few chromosomes were evident in the metaphase plates itself. Such lagging of chromosomes and inclusion of precociously divided chromosomes selectively to one pole may result in aneuploid variation of chromosomes in daughter cells. *Piper magnificum* being a vegetatively propagated ornamental, such variations might have perpetuated through clonal generations. Sticky bridge was observed in an anaphase cell and it may not create any numerical changes. But, it is possible that such abnormalities may induce structural changes in chromosomes which can be ascertained by a detailed karyotype analysis of cells with different chromosome numbers.

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