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IN VITRO REGENERATION OF *ADHATODA VASICA* NEES THROUGH ADVENTITIOUS ORGANOGENESIS AT DIPLOID LEVEL OF GENOME

YOGEETA ANAND and Y.K.BANSAL

Tissue Culture Laboratory, Department of Bioscience, R.D. University, Jabalpur 482 001 (M.P.). India.

The chromosomal analysis of *in vitro* regenerated plants of *Adhatoda vasica* Nees revealed the predominance of normal chromosome complement (2n=34) in the roots. Only a low level of chromosomal variation was recorded in the cells of calli. The normal appearance of the regenerated phenotype suggests that plant regeneration occurs through adventitious organogenesis at diploid level of genome.

Keywords: *Adhatoda vasica;* Chromosomal analysis; Diploid; Genome; Organogenesis.

Introduction

The technique of plant cell, tissue and organ culture has led to the development of several tools, viz. micropropagation, anther culture, *in vitro* selection, embryo rescue, somaclonal variation, somatic hybridization and transformation to assist the plant breeder. Out of these, the somaclonal variation occupies a unique position, because it may be occurring both as an advantage and a disadvantage of the tissue system[1].Somaclonal variation has been associated with changes in chromosome number and structure[2,3] point mutation, DNA methylation[4,5] and activation of transposons.

Several factors such as genotype[6], ploidy of the explant [7] tissue culture procedures, tissue source [8] and choice and concentration of plant growth regulators [9, 10] have been presumed to affect the nature and frequency of this cytogenetic variability. However, the interaction of one or more of these factors in creating variability or stability of chromosomes and DNA *in vitro* cultures is only little understood[11].

The present investigation deals with the evaluation of chromosome complement of cultured tissue of *Adhatoda vasica* Nees, a medicinal plant of high value[12].

Materials and Methods

Fresh calli produced over leaf explants inoculated on B5 medium[13] supplemented with 2, 4-D (1mg/l) and root tips of plants regenerated on Kin (o.1 mg/l) were excised and subjected to the usual cytological treatments[14]. The accurate count of chromosome number in cultured tissue in this species was found difficult due probably to the presence of dense oil contents and small size of the chromosomes. Hence in all cases only an approximate number of chromosomes, sufficient enough to indicate the ploidy status of the cultured tissue under observation have been determined.

Results and Discussion

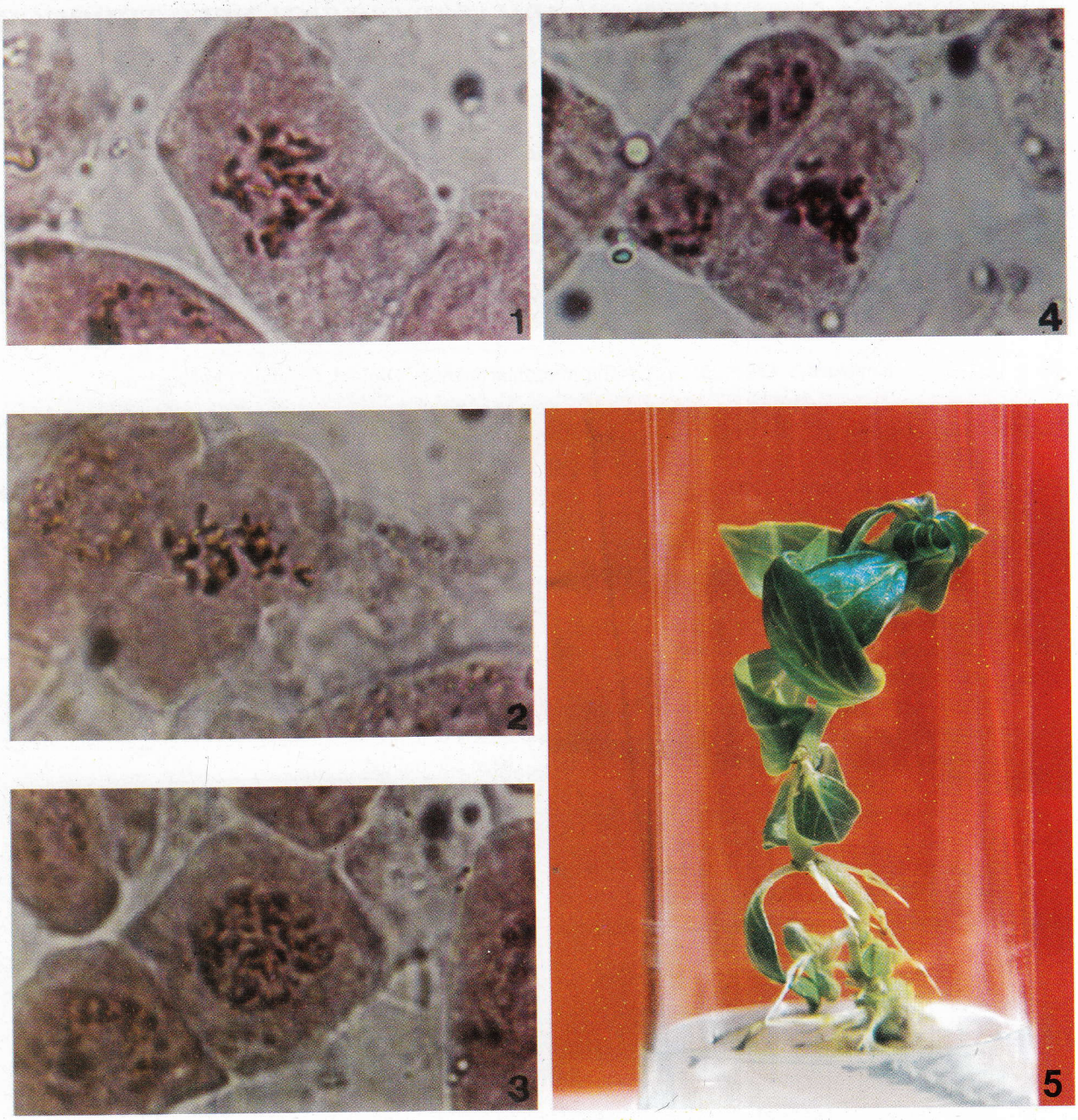
Cytological investigation of the calli as well as regenerated roots revealed a MI of 5.99 in the former while that of 7.44 in the latter (Table 1). The most abundant stages among the dividing calli in the present work were metaphases and anaphases.

The predominant chromosome complement observed in calli and root squashes in this species was diploid (2n=34) (Figs. 1, 2) as determined under *ex vitro*

Table 1. Mitotic analysis of 4 week old callus and regenerated roots of *Adhatoda vasica Nees*

No.	Hormone (mg/l)	Source	Tcs	Tdc	MI (Mean+SE)	Diploma Complement No %	Hypodiploid No %	Hyperdiploid No %	Euploid No %
1.	Control	*Callus*	2500	75	3.00±0.82	75 100±0.20	- -	- -	- -
		Root	2450	70	2.85±0.66	65 92.8±3.04	- -	- -	5 7.1±0.68
2.	2, 4D (1)	*Callus*	3904	234	5.99±0.54	208 88.89±2.12	9 3.85±0.20	17 7.26±0.84	- -
		Root	3208	188	5.86±1.02				
3.	KIN (0.1)	*Callus*	3840	284	7.39±0.92	268 94.3±3.62	8 2.8±0.16	8 2.8±0.20	- -
		Regenera	4464	332	7.47±0.82	324 97.29±4.02	4 1.20±0.8	4 1.20±0.04	- -
		Root							

MI - Mitotic Index, Tcs - Total cells scanned, Tdc- Total dividing cells.



Figs. 1,2. Chromosome complement of cultured tissue of *Adhatoda vasica* Nees . Normal diploid complement (2n = 34) of *A. vasica* Nees (x1000)

Fig.3. Hyperdiploid chromosome number in callus tissue. (x1000)

Fig. 4. Hypodiploid chromosome number in callus tissue.(x1000)

Fig. 5. Regenerated plantlet of *A.vasica.*

conditions [15-17]. The occurrence of a few variable chromosomal complements of hyperdiploid (Fig.3) and hypodiploid (Fig.4) number in calli and roots of the cultured cells was also noticed. In the present study the roots of the regenerated plants (Fig.5) showed much less variations of the normal (diploid) chromosome complement than observed in calli.

Tissue squashes in *A.vasica* showed cellular composition made-up of a variety of cell types, sizes and wall thickenings. Cytodifferentiation in the form of tracheids and sieve elements was also visible during growth and maturation of cells.

Plants propagated with diploid genomes are phenotypically homogenous exhibiting genetic stability necessarily required for the maintenance of genetic stocks by means of *in vitro* culture[18-19].

Thus it is inferred that despite the occurrence of some variations in the callus cultures, culture of *A..vasica* Nees may provide a stable genome leading to its selection during regeneration of whole plants from cell cultures and filtering out the most radically variant cell lineage[20].

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