Cytophotometric Determination of Nuclear DNA in Hevea Brasiliensis (willd. ex Adr. de Juss) Muell ARG.

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Among the angiosperms DNA content per nucleus varies over a 100 fold range (Rees & Jones, 1972). Polyploidy is responsible for some of these variations (Sparrow **et al**, 1972). The quantification of DNA has become a fascinating subject of research in recent years. In **Hevea**, an attempt has been made to study the characterization of the nuclear genome of **Hevea brasiliensis** (Low & Bonner, 1986). Analysis of variation in nuclear DNA content of chromosome manipulated plants in this crop has not yet been reported. This communication deals with the cytophotometric determination of nuclear DNA in different cytotypes.

Materials and Methods

The chromosome complement of the somatic cells of **Heava brasiliensis** (Willd. ex Adr. de Juss.) Muell. Arg.is 2n = 36. Tetraploidy (2n = 4x = 72) was induced, triploid (2n = 3x = 54) was synthesized (Saraswathy Amma **et al**, 1980, 1984, Saraswathy Amma, 1990) and spontaneous triploid (2n = 3x = 54) was identified (Nazeer & Saraswathy Amma 1987). Diploid, spontaneous triploid, synthesized triploid and tetraploid were incorporated in the study. Young shoot tips were collected from the different cytotypes of **Heava brasiliensis** and pretreated in saturated solution of PDB (Para dichlorobenzene) for 2.5 to 3.0 hours. The materials after pretreatment were washed thoroughly with water and fixed in acetic ethanol (1:3) for 24 hours and preserved in 70 per cent alcohol. The leaftips were hydrolized in INHCL for 20 minutes and washed thoroughly in water and kept in Feulgen solution (leuco-basic fuchsin 1% at PH 3.6) overnight at 5°C. The leaftips were washed with SO_2 water for 30 minutes with three changes of 10 minutes each. The root tips of **Allium cepa** were also treated in the same manner as the standard. Squash preparations were made in a [illegible] of glycerol. Four slides were prepared for each cytotype.

Photometric measurements were taken from twenty 4C nuclei from each slide on Vickers Scanning Cytophotometer M 85 at the Central Institute of Medicinal and Aromatic Plants (CIMAP), Lucknow. The 2C values were obtained from the 10 lowest readings among the 20 nuclei of each slide. This procedure minimised the risk of including values of 2C to 4C. Slides of each replication were prepared separately along with the control, **Allium cepa**. The absolute values were converted in picograms using **Allium cepa** as standard whose 4C nuclear DNA value is 67.08 pg. (Van't Hof, 1965). The data were subjected to statistical analysis.

Results and Discussion

The data on nuclear DNA amount in diploid, triploids, both spontaneous and synthesized and induced tetraploid are given in Table 1. There is significant difference in DNA content among the cytotypes. The 4C nuclar DNA content of the diploid is 44.29 pg. But the autoteraploid representing C_{20} generation showed an extract two fold increase. The triploids showed more or less thrice the value of haploid nucleus. The spontaneous triploid showed numerically less value to that of the synthesized triploid.

Table 1. 4C nuclear DNA content in three cytoypes of **Hevea brasiliensis.**

Cytoypes	2n	DNA (Pg)
Dipioid (RRII 105)	36	44.29
Triploid (Synthesized)	54	62.43
Triploid (Spontaneous)	54	60.19
Tetraploid (Induced)	72	89.37
SE		0.12
CD (5%)		0.32

Cytotypes with higher ploidy levels had corresponding higher DNA values. The same trend was reported in **Betula** (Schaefer & Miksche, 1977) Amaranthus (Ohri **et al**, 1981) and **Piper** (Rosabelle **et al**, 1986). There is a linear relationship between ploidy level and DNA content. The autotetraploid reprecenting C_{20} generation showed extract two fold increase in the DNA content. This shows that there has not been any loss of DNA after the induction of polyploidy. Triploids showed approximately 1: 1.5 with respect to diploid. A slight variation in nuclear DNA content between the two triploids can be correlated with reshuffling and rearrangements in chromosomes. The mean DNA content for annual species was significantly lower than for perennial species (Price, 1976; Bennett **et al**, 1977).

Acknowledgements

The authors are grateful to the Director, CIMAP, Lucknow, and Dr. B. R. Tyagi for access to Cytophotometer and to Dr. A. O. N. Panikkar, Joint Director for encouragements.

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