INTRODUCTION

Over the past years, estimation of nuclear DNA content or C-value is gaining importance in molecular biology. Attempts are being made to understand the importance of C-value in a wide range of research areas such as taxonomy, evolution, biodiversity, plant breeding, genomics, development and physiology. The concept of “nucleotype” initially provided the impetus for using C-value as a parameter to understand phylogeny and evolution. C-value has also become a focus of molecular biology because of the recent discoveries showing that repetitive, noncoding DNA sequences constitute a major part of the eukaryotic genomes including *Arabidopsis* and humans, and that retrotransposon activity could lead to huge differences in C-values of even closely related organisms. Like the transposons comprise most of the intergenic noncoding, the introns constituting the intragenic, nonsense DNA sequences are also in the limelight for their role in evolution of new genes. While the intergenic, transposon activity could shuffle the genomic DNA and result in several types of chromosomal rearrangements, such as deletions, inversions, duplications, formation of telocentric and dicentric chromosomes, the intragenic activity of introns – intron homing – may cause rearrangement of genes and even production of new proteins. These aspects known from animal examples offer exciting and challenging opportunities to examine the situation in plants. The focus here is on the relevance of C-values in angiosperms in the context of both fundamental and evolutionary significance.

Swift (1950), working on angiosperms, defined the DNA content of an unreplicated haploid nuclear genome as its C-value. DNA content is often loosely referred to as genome size but strictly speaking, genome size in eukaryotes is the DNA amount in the basic (monoploid) chromosome set (x). Thus, DNA C-value equals the genome size for diploid species whereas for polyploids, it is the sum total of two or more genomes involved (Leitch et al., 1998).

Nuclear genome size in eukaryotes is measured in picograms (pg) of DNA (1pg = 10^{-12} g). The DNA content per genome is usually constant, both between cells of an individual and between individuals of the same species (Dounce, 1955). For rapid changes in genome size that occur during developmental events, the term ‘plastic
genome" has become popular. On the other hand, there are differences between genome size between individuals of one population or between populations of the same species that are so large that neither taxonomic artifacts nor chromosomal heteromorphism can apply and that are unexplainable in the context of the current taxonomy or the breeding system of the organism (Greilhuber, 1998). Variation in DNA amount has been shown to correlate with a wide range of important characters in angiosperms including chromosome volume or length (Bennett et al., 1983; Anderson et al., 1985). It indicates that: 1) genome size has adaptive significance and 2) nuclear DNA can influence the phenotype, independent of its encoded informational content (Bennett, 1971, 1972, 1973).

Both prokaryotic and eukaryotic species show a wide range of variation in nuclear DNA amount (Sparrow et al., 1972). Mirsky and Ris (1951) were the first to demonstrate huge interspecific variation in C-values that bore no correlation to the genes coded by the species. Comparisons of phenotypic differences of closely related species with differing genome sizes have been made in several groups of plants and animals (Rees and Jones, 1972, Bachmann and Rheinsmith, 1973; Price et al., 1974). Eukaryotes may have same chromosome number, yet differ in their genome size and vice versa (Jones et al., 1998). The changes in genome size may not be restricted to species divergence but also associated with various environmental conditions (Jassiensi and Bazzaz, 1995; Reeves et al., 1998; Grime, 1998; Poggio et al., 1998).

In eukaryotes, C-value is known to vary from around 0.009 pg (= 9 Mbp) in yeast Saccharomyces cerevisiae to approximately 700 pg (= 675 Mbp) in Amoeba dubia (see Cavalier-Smith, 1985). Yet, there is little correlation between the genome size and the organismic complexity, a phenomenon referred to as C-value paradox (Thomas, 1971). In flowering plants, C-values range from 0.3 pg in Arabidopsis thaliana (= 100 Mbp) to over 127.4 pg (= 120, 000 Mbp) in Fritillaria assyriaca. This is the largest range known for any eukaryotic group (Bennett and Leitch, 1997; Hanson et al., 2001). Gymnosperms seem to have relatively larger C-values than the angiosperms. Smaller C-values in fact may have helped angiosperms to acquire evolutionary flexibility, quicker cell cycle etc. to compete with the gymnosperms (Leitch et al., 1998). Although, C-value is a key biodiversity parameter, the number of angiosperms examined so far is very low. Bennett
and colleagues have provided six lists of C-value of 3493 angiosperms, representing only 1% of the global angiosperm flora (see Bennett et al., 2000). Even within these limited information, a wide range of variation seems to exist within families and many genera. A range of variation (2.9 to 5.2 pg, 80%) at a constant chromosome number has been found among 42 collections of seed progeny derives from established plants of Poa annua (Mowforth and Grime, 1989). In certain groups, karyotypic studies along with genome size may reveal diversity which is not otherwise apparent from morphological studies, as in the Australian genus Bulbine (Ohri, 1998).

Bacterial genome size is probably directly related to the number of genes and is a good measure of genetic and organismic complexity. But in eukaryotes, the existence of vast amounts of apparently non-genic DNA has long been a major puzzle. This additional non-genic DNA may be an incidental result of mutational and intragenomic selective forces. Whether all this additional DNA, sometimes a much as 50,000 fold in excess of protein coding requirements, has a function or is it merely selfish or junk/parasitic DNA (Crick, 1979; Orgel and Crick, 1980) are the questions that need to be addressed for understanding chromosome organization. Similarly, the significance of C-values in chromosomal evolution has not received due attention. These aspects are discussed here in the context of extant information provided in the review of literature section.

A database for angiosperm C-values: http://www.Rbgkew.org.uk/cval/database1 provides updated information on C-values known in angiosperms. A general account of C-values is also available from special issues of Annals of Botany (vol. 82. Suppl. A, 1999) and Current Opinion in Genetics and Development (vol.14 : 599-705 (2004)).
MATERIAL AND METHODS

Taxa listed in Table 1 have been used in present study.

The nuclear DNA measurements were carried out with Leica DMW Feulgen microspectrophotometer set at 541 nm wavelength. For the purpose of internal standard against which the measurements were made, *Allium cepa* var. Nasik-red root tips were selected and seeds were germinated on a moist filter paper. The root tips of both onion and experimental material for present study were excised, washed and fixed for 2 hours in 4% formaldehyde prepared in ice-cold, 0.067 M phosphate buffer (pH 7). The root tips were then washed under running water for 24 hours to remove any traces of formaldehyde. They were then fixed in freshly prepared, pre-cooled 1:3 aceto-alcohol and subsequently stored in 70% alcohol.

The root tips were then washed briefly in distilled water and were hydrolysed in 5 N HCl for about 50 min at 20°C. After a wash in distilled water for 30 min the root tips were stained for 1 hour in Feulgen in total darkness at 20°C. They were then washed 3 times, each of 10 min duration, in 0.5% sodium metabisulphite solution prepared in 0.15N HCl to remove nonspecifically bound feulgen in the cytoplasm.

Squash preparations were made from these root tips in 50% glycerol. The stained root tips were macerated in a drop of 50% glycerol on a slide as per standard cytological preparatory protocols.

All the above steps were uniformly maintained for both experimental materials as well as the internal standard. In each case, three replicates were prepared. For measurements, telophase nuclei were selected under 100 X oil immersion objective.

DNA measurements were made by using the telophase nuclei of the internal standard as reference by focussing the pilot lamp aperture to the size of the nucleus. Then the aperture was focussed on the cytoplasm by adjusting voltage to the value of 900. The aperture then was adjusted to overlap the telophase nucleus without changing the aperture size. The aperture was closed so that its size was directed to the photomultiplier. On the computer screen the actual absorbance was seen. In this manner measurements for 30 nuclei from different preparations were taken for each material for the purpose of
average. Simultaneously, statistical details like number of entries, mode, mean, standard error, histogram etc. were displayed on the screen and were recorded.

DNA content was estimated by using the following formula (Labani and Elkington 1987).

\[ 2C \text{ DNA amount} = \frac{A}{B \times C \text{ Pg}}. \]

Where, 

- \( A = 2C \text{ DNA of the internal standard} \)
- \( B = \text{mean absorption value in arbitrary unit for the internal standard} \)
- \( C = \text{mean absorption value for the experimental material relative to that of the internal standard used} \).