INTERSPECIFIC VARIATION IN THE AMOUNT OF DNA IN *OCIMUM* L

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SIGNIFICANT variations in the amount of DNA at an interspecific level have been reported earlier[1–6]. The existence of widespread variation in DNA content per nucleus is indicative of the role of quantitative variation in the evolution of several species in different genera[7–9].

In the present investigation, the DNA contents of the four diploid *Ocimum* species have been determined and the results have been shown to be correlated to chromosome number. The genus *Ocimum* provides with an ideal material for such an investigation, as it contains not only a large number of species but also widely different chromosome numbers[10] ranging from $2n = 16$ to $2n = 128$. It was thought worthwhile to ascertain the extent to which the change in diploid number is correlated with variation in DNA content.

The present work deals with four different species of the genus *Ocimum* belonging to the family Lamiaceae (table 1). These plants were grown in the experimental plot of our department, under identical conditions to eliminate the ecological variations, if any.

For cytophotometric measurements of DNA content, young leaf tips were fixed in acetic acid-ethanol mixture (1:2) for 24 hr and then transferred to 45% acetic acid for 5 min. The leaf tips. were then hydrolyzed for 12 min in 1(N) HCl at 60° C, rinsed with distilled water and stained for 2 hr with Schiff's reagent. They were finally squashed in 45% acetic acid. Fixation of the leaf tips in neutral formalin (40%) under the same experimental set-up was also carried out for confirmation.

The 4C nuclear DNA content was determined with a Reichert Zetopan microspectrophotometer and a single wavelength (550 nm) method. Thirty nuclei at metaphase were scored by using four leaf tips smears from each of the two plants per species. As the magenta colour diffuses out into the cytoplasm, the transmittance readings were taken in the microspectrophotometer immediately after squashing. The amount of 4C nuclear DNA was measured on the basis of the optical density in terms of arbitrary units of the relative absorbances.

The diploid chromosome number and the 4C nuclear DNA content of the four different species of *Ocimum* are shown in table 1, from which, it may be noted that the diploid chromosome number varies from 24 to 48 while 4C DNA content per nucleus varies approximately 3.5 fold from 0.068 in *O. sanctum* ($2n = 32$) to 0.242 in *O. canum* ($2n = 24$). In general, there is no linear relationship between 4C DNA content per nucleus and the diploid chromosome number of different species (figure 1).

Table 1 *4C nuclear DNA content of four different species of Ocimum L (in arbitrary units of absorbance)*

Name of the species	Chromosome number ($2n$)	4C DNA content ± S.E. (amount/Cell
Ocimum canum Sims	24	0.242 ± 0.0029
Ocimum sanctum L	32	0.068 ± 0 0035
Ocimum viride Willd	38	0.117 ± 0.0029
Ocimum basilicum L	48	0.118 ± 0.0028

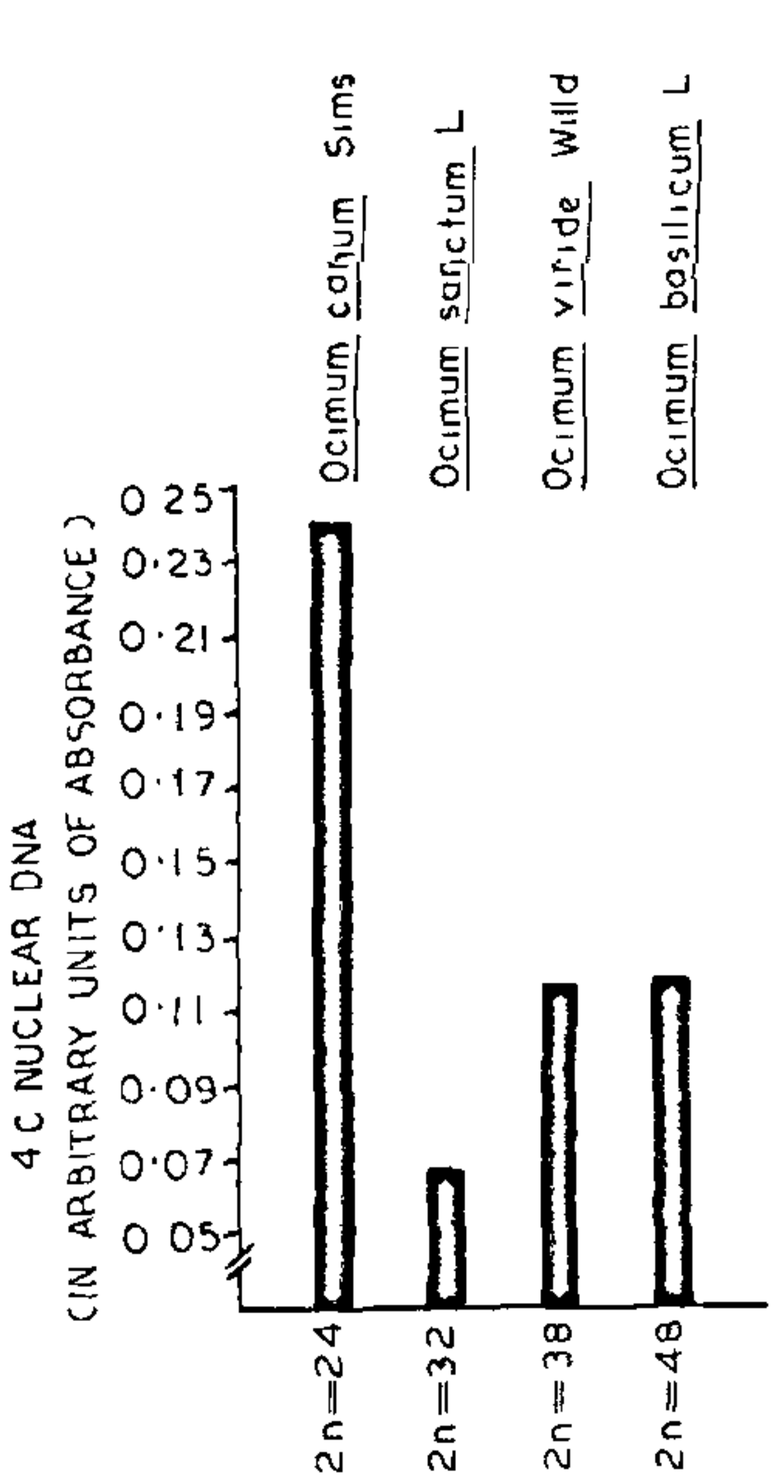

Figure 1. Bar diagram representing total nuclear DNA amount in different species of *Ocimum*.

The analysis of correlation coefficient reveals a negative correlation ($r = -0.556$) between the diploid chromosome number and the amount of 4C nuclear DNA. It therefore appears that at least in the four species of *Ocimum* studied, the variation in the amount of DNA is not necessarily correlated with the change in chromosome number. The significant differences in the amount of DNA ($P < 0.01$) may suggest that there has been widespread changes in nuclear DNA content with the evolutionary divergence of the different species of *Ocimum*. It is not likely that a major portion of the DNA present in the different species is repetitive in nature. It is to be ascertained to what extent this variation in the amount of nuclear DNA can be attributed to the repetitive sequences.

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SUPEROXIDE DISMUTASE: AN INDICATOR OF SO_2-TOLERANCE IN PLANTS

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SULPHUR DIOXIDE, a major gaseous air pollutant, can induce foliar injury and cause reduction in growth and yield in many plant species. Laboratory studies have revealed variation in SO_2 sensitivity among plants. These can be attributed to differences in SO_2 uptake rates, biochemical properties and genetic expression[1,2]. SO_2-toxicity involves the formation of either sulphite or free radicals during photo-oxidation of sulphite to sulphate[3]. Evidence regarding free radicals toxicity is based on the reports that SO_2 injury increases in plants treated with diethyldithiocarbamate, an inhibitor of superoxide dismutase (SOD), which decomposes free radicals and that the use of chemicals that scavenge free radicals reduce SO_2 or bisulphite-induced chlorophyll destruction and leaf injury[3-5]. In the present study, the differential sensitivity of two crop plants to SO_2 based on changes in their SOD activity and chlorophyll content was assessed.

Gram (*Cicer arietinum* L cv T-3) and broad bean (*Vicia faba* L cv Local) plants were grown in pots (2 plants/pot) under greenhouse conditions for 40 days at day and night temperatures of 25 ± 0.5 and 20 ± 0.5°C, respectively and relative humidity of $70 \pm 5\%$. After 40 days, 10 plants of each species were transferred to environmentally controlled fumigation chambers and exposed under illuminated conditions to 0.5 ± 0.01 ppm SO_2 for 2 hr[6]. During fumigation SO_2 concentration within the chamber was measured continuously with a CEA SO_2 analyzer. Control plants were placed in identical chambers flushed with filtered air system.

Leaf samples of three age groups i.e. young, mature and old, were collected 24 hr after fumigation from control and SO_2-exposed plants for the determination of chlorophyll content[6] and SOD activity[7]. Five replicates were taken for each analysis. The total chlorophyll content of SO_2-exposed plants was expressed as percentage of the control. SOD activity was assayed by the method of McCord and Fridovich[7], which is based on SOD inhibition of superoxide-mediated ferricytochrome reduction. One unit of SOD activity was defined as that which inhibited 50% of the reaction rate per cm^2 leaf area.

The present findings showed that young leaves of the gram had higher SOD activity than those of broad bean. With increasing age, leaves of both these plants exhibited simultaneous decrease in SOD activity and chlorophyll content. However, with respect to control such decreases in SO_2-exposed plants were significantly ($P < 0.01$) higher.

Recent reports have indicated that accumulation of superoxide radicals (O_2^-) in the chloroplasts of illuminated SO_2-exposed plants causes destruction of chlorophyll pigments[3]. The present results show that leaves gradually lose SOD, a scavenger of O_2^-,