# NUCLEAR DNA CONTENT VARIATIONS IN SUNFLOWER

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Summary

The variations in the amount of nuclear DNA which occur within the progeny of single plants of inbred lines of sunflower (Helianthus annuus L.) were studied. Cytophotometric analyses showed that, in the selfed line analyzed, the DNA content of seedlings obtained from seeds collected in the central portions of the heads (central seedlings; CS) is 14.7% lower than that of seedlings obtained from seeds collected in the peripheral portions (peripheral seedlings; PS). These analyses also showed that DNA sequences located in the heterochromatin are mainly involved in the variations. Reassociation kinetics indicated lower redundancies of highly and medium repetitive sequences in the DNA from CS than in that of PS, and this was confirmed by slot blot analyses performed with different DNA probes. Molecular hybridizations also indicated a lower amount of rDNA in the former than in the latter. Nuclear DNA content was found to be positively correlated to both cell volume, duration of mitotic cycle and flowering time. Thus, nuclear DNA content variations might play a role in determining a variability in development which may be of importance in buffering the effects of changing environmental conditions.

## INTRODUCTION

Genome size variation within Helianthus annuus has been reported by several authors (Nagl and Capesius, 1976; Olszewska and Osiecka, 1983; Cavallini et al., 1986). DNA amounts, as determined by Feulgen cytophotometry of root and shoot apical meristems, differ widely from one cultivar or line to another: a 55.9% variation exists between the most variant values.

Significant differences in the DNA content even occur within the progeny of a single homozygous plant according to the position of the seed in the head: the amount of DNA in seedlings raised from seeds developed at the periphery of the head is significantly greater than that in seedlings raised from seeds developed in its central part; these variations, never exceeding 25%, occur as a rule within every cultivar or line tested (Cavallini et al., 1986; 1989).

In this paper, we report the results of cytophotometric, biochemical and molecular analyses of nuclear DNA, showing that differences in DNA content within a selfed line are related to changes in given DNA fractions. Moreover, different phenotypic characters of plants were studied to establish possible correlations to DNA content variations.

## MATERIALS AND METHODS

A sunflower line selfed for 10 years was used. For cytological analyses, tissues were collected from plants grown in the garden or from seedlings obtained by germinating achenes in damp vermiculite. Fixation was in ethanol - acetic acid 3:1 (v/v). Squashes were made and Feulgen stained as already described (Cavallini et al.,1989). Feulgen/DNA absorption in individual cell nuclei were measured, at the wavelength of 550 nm, using a Barr & Stroud GN5-type integrating microdensitometer. Morphometric analyses were made on microphotographs of epidermal peels from mature leaves, which were stained with Delafield's haematoxylin. For other analyses, seedling roots were treated for 30 min with a 5 $\mu$Ci/ml solution of 3H-thymidine (The Radiochemical Centre) and recovered for different lengths of time in water; using these materials, the duration of mitotic cycle was calculated according to Quastler and Sherman (1959).

For biochemical analyses, DNA was extracted from the leaves of seedlings and reassociation kinetics were studied, as described in Cavallini et al. (1986). Slot DNA blots were performed according to Cecchini et al. (1992). Probes for hybridization were obtained by fractionating PS DNA at different Cot values to separate highly (HR: Cot < 0.22) and two fractions of medium (MR1: 0.22<Cot<2.1; MR2: 2.1<Cot<100) repeated sequences using the method described by Durante et al. (1989). An Eco RI 18+25S rDNA repeat of Phaseolus coccineus cloned into the vector pUC13 (Maggini et al., 1991) was also used as a probe. DNA probes were labeled with digoxigenin-11-dUTP by a DIG-DNA labeling kit (Boehringer) and hybridization was performed according to Maniatis et al. (1982). All hybridization experiments were repeated six times at least.

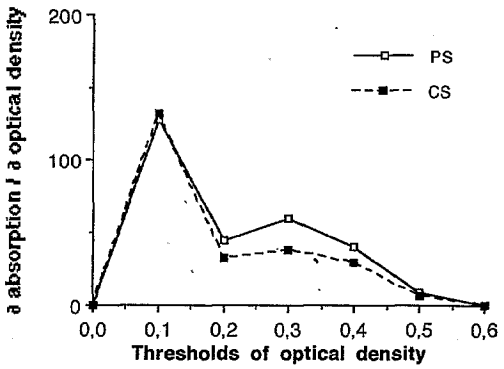


Fig. 1

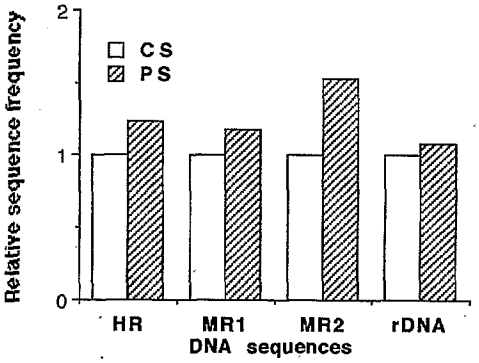


Fig. 2

Figure 1. First derivatives of Feulgen absorption curves at different thresholds of optical density of interphase (4C) nuclei in the shoot meristem of PS and CS of one and the same head of a selfed line. 20 nuclei for each of three PS and three CS were measured.

Figure 2. Normalized (CS=1) densitometrical values of slot blots loaded with DNA extracted from PS and CS and hybridized with different DNA probes. Each value is the mean of six experiments. Standard error never exceeding 1%.

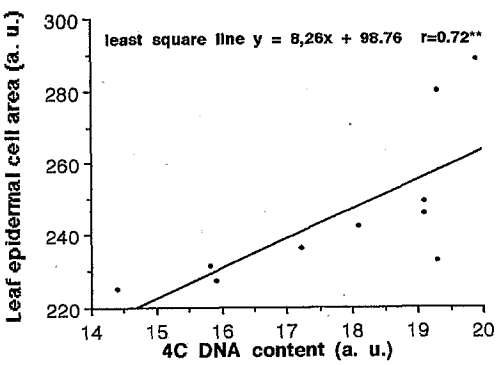


Fig. 3

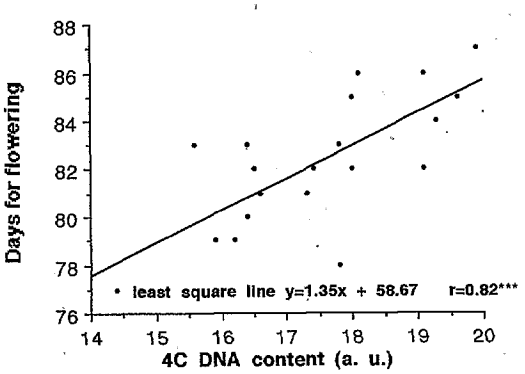


Fig. 4

Figure 3. Relationship between 4C nuclear DNA content and the areas of leaf epidermal cells in 10 plants of a single progeny of a selfed line.

Figure 4. Relationship between 4C nuclear DNA content and flowering time in 20 plants of a single progeny of a selfed line.

## RESULTS

Nuclear DNA contents in seedlings obtained from seeds collected in the central portion (central seedlings; CS) or from the peripheral portion (peripheral seedlings; PS) of one and the same head were analyzed by means of Feulgen cytophotometry of early prophases: on average, 4C DNA contents of 17.12 pg and 19.64 pg, respectively, were found (14.7% difference). Interphase nuclei were also measured at different thresholds of optical density; this method allows to estimate DNA contained in fractions of chromatin with different degrees of condensation. The first derivative curves of the values obtained clearly showed that the differences in DNA content were mainly due to DNA fractions contained in optically dense chromatin (heterochromatin: 0.3-0.4 thresholds of optical density; Fig. 1).

The DNAs extracted from leaves of PS or CS were studied by means of reassociation kinetics. The analysis of the reassociation curves, that is detailed in Table 1, revealed differences in the redundancy of HR, MR1 and MR2 sequences (see Materials and Methods): all these sequences were more represented in PS than in CS (+39.83%, +35.12%, and +113.82%, respectively).

The results of the reassociation kinetics were confirmed by slot blot analyses performed with HR, MR1 and MR2 DNA probes. Slot blots also indicated that the rDNA too was underrepresented in CS (Fig. 2).

Nuclear DNA content was found to be positively correlated to the area of leaf epidermal cells (fig. 3) and to mitotic cycle time (8h45min in PS and 7h15min in CS). Nuclear DNA content was also shown to be positively correlated to flowering time (Fig. 4).

## DISCUSSION AND CONCLUSIONS

Our cytological, biochemical and molecular analyses suggest that the changes in the content of nuclear DNA that occur within the progeny of single plants of sunflower are mainly due to variations in the redundancy of repeated sequences (Table 1, Fig. 2) which are, at least in part, localized in heterochromatin (Fig. 1).

It is worth noting that variations in nuclear DNA content occur at the same extent in every line tested and continuously take place, at each generation, during embryo development (Cavallini et al., 1989). According to Bennett's (1972) nucleotype hypothesis, changes of DNA content may affect development and adaptation through their effects on cell parameters as cell volume and mitotic cycle time. In multicellular plants, nucleotypic effects would be additive, so that they

Table 1. Scatchard type analysis of the reassociation curves of the DNAs extracted from PS and CS from a single head of a selfed line.

| DNA sample | DNA sequences | C$_o$t range | Frequency | C$_o$t $\frac{1}{2}$ pure (moles xsec/l) | Reassociation constant (1/ molesxsec) | Kinetic complexity(bp) | No. of copies per 1C-genome* |
|---|---|---|---|---|---|---|---|
| PS | HR | < 0.22 | 0.140 | $3.22 \times 10^{-3}$ | 43.5 | $3.67 \times 10^{3}$ | $1.72 \times 10^{5}$ |
| CS | | < 0.23 | 0.126 | $3.53 \times 10^{-3}$ | 35.7 | $4.02 \times 10^{3}$ | $1.23 \times 10^{5}$ |
| PS | MR1 | 0.22-2.1 | 0.142 | $9.65 \times 10^{-2}$ | 1.47 | $1.10 \times 10^{5}$ | $5.81 \times 10^{3}$ |
| CS | | 0.23-2.4 | 0.150 | $1.20 \times 10^{-1}$ | 1.25 | $1.37 \times 10^{5}$ | $4.30 \times 10^{3}$ |
| PS | MR2 | 2.1-100 | 0.352 | $2.64 \times 10^{0}$ | 0.133 | $3.01 \times 10^{6}$ | $5.26 \times 10^{2}$ |
| CS | | 2.4-100 | 0.184 | $2.58 \times 10^{0}$ | 0.714 | $2.94 \times 10^{6}$ | $2.46 \times 10^{2}$ |

* calculated on a cytophotometrically determined 1C value of 4.91 pg and 4.28 pg for PS and CS, respectively.

would influence characters as growth rate and minimum generation time (Bennett, 1987). Our results showing that flowering time is positively correlated to DNA content (Fig. 4) fully match this view.

The creation of new variability at each generation may be of importance in buffering the effects of changing environmental conditions; this variability could be useful in plant breeding programs.

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