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Nuclear DNA changes within *Helianthus annuus* L.: origin and control mechanism

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Summary. Previous results suggested that the amount of nuclear DNA varies in one and the same progeny of *Helianthus annuus*, depending on the head portion in which seeds have developed. Accordingly, cytophotometric determinations were carried out in a selfed line, after Feulgen-staining, to obtain information on the developmental stages at which DNA changes are produced and on the mechanism controlling the variation. It was found that the Feulgen absorption values of mitotic prophases in immature anthers and pistils and of meiotic prophases I are the same in any flower of the head. In contrast, the Feulgen/DNA contents of early prophases in heart-shaped embryos differ significantly, increasing from those developing at the centre of the head to those developing at its periphery, and remaining unchanged in each embryo during further development and seed germination. Variations in the number of chromosomes do not account for the differences observed in nuclear DNA contents in which sequences comprised in heterochromatic nuclear regions are involved. The Feulgen absorption values of seedlings obtained from seeds developed in different portions of single heads increase or diminish starting from those found in the mother plant. This depends on whether these latter are relatively low or high and on the gradient of seed location in the head. The variation occurring within each single progeny covers the whole range existing within the line.

Key words: *Helianthus annuus* – DNA cytophotometry – Intraspecific DNA changes – Plant embryo development

Introduction

The results reported in a number of papers in the literature indicate that, particularly in plants, the nuclear genome might be organized into constant and fluid domains (Cullis 1983), so that both developmental and physiological stimuli and the response to changing environmental conditions may give rise to rapid changes (Walbot and Cullis 1985; Cionini 1988). As a general rule, these changes affect the amount of nuclear DNA and, apart from the cases of polyploidization or chromosome endoreduplication which multiply all the DNA sequences in the nucleus, the relative proportions of the fractions of the genome are modified. These programmed variations may be achieved through such processes as extra DNA synthesis (DNA amplification), nonreplication of certain sequences during a DNA synthetic phase (DNA underreplication), or the elimination of certain DNA sequences (DNA loss) (Bassi et al. 1984; Natali et al. 1986; Altamura et al. 1987; Cionini 1988).

These changes in nuclear DNA appear to be of great interest for both basic and applied research. They might constitute key mechanisms in the hierarchy of those controlling certain developmental processes as well as of those which govern the response of a plant to environmental stimuli. Moreover, quantitative flexibility of the genome deserves attention as a source of genetic variability which can be exploited in the improvement of species of agricultural interest.

An intriguing example of rapid changes in the amount and organization of the genome is seen in the results recently obtained with *Helianthus annuus*. By means of cytophotometric, karyological and biochemical analyses, it has been shown that large differences in nuclear DNA content diversify cultivated varieties or lines of sunflower. Variations in chromosome number or gross structure do not account for these changes in genome size, which, on the contrary, are paralleled by frequency variations in repeated DNA sequences. That the amount of nuclear DNA continuously varies with reproduction is

particularly interesting: even seedlings obtained from seeds collected from different portions of single heads of plants belonging to a selfed line have different DNA contents (Cavallini et al. 1986).

These results indicate that an unstable fraction exists in the genome of *H. annuus* and they raise many questions (Cavallini et al. 1986). In this paper, we report findings on the developmental stage at which the unstable DNA sequences vary and on the mechanism controlling the variation.

Materials and methods

A *Helianthus annuus* line selfed for 8 years was used. Tissues collected from plants grown in the garden or from seedlings obtained by germinating seeds (achenes) in damp vermiculite were fixed in ethanol-acetic acid 3:1 v/v or in 10% neutral formalin. In certain cases, fixation was preceded by treatment with an 0.05% aqueous solution of colchicine (Sigma) for 4 h at room temperature.

Squashes were made under a cover-slip in a drop of 45% acetic acid, after treatment with a 5% aqueous solution of pectinase (Sigma) for 1 h at 37 °C. The cover-slips were removed by the solid CO_2 method and the preparations were Feulgen-stained at different hydrolysis conditions in HCl: either 1N at 60 °C for 8 min or 5 N at 20 °C for 10–60 min for those made with materials fixed in ethanol-acetic acid, and 1 N at 60 °C for 20 min for those made with formalin-fixed materials. After staining, the slides were subjected to three 10-min washes in SO_2 water prior to dehydration and mounting in DPX (BDH). Preparations made to collect data which were to be directly compared were processed simultaneously whenever it was possible. When there were too many of them or when materials had to be collected at different times, squashes made with the root tips of a single plantlet of *Vicia faba* were concurrently stained for each group of slides and were used as standards. When it was necessary to use this method of comparison, certain differences in Feulgen/DNA absorptions observed between *H. annuus* preparations were further checked. This was done by analyzing preparations which were remade and processed simultaneously. Absorptions measured in *V. faba* (4C = 53.31 pg: Bennett and Smith 1976) were also used to convert relative Feulgen units into picograms of DNA. Feulgen/DNA absorptions in individual cell nuclei were measured at the wavelength of 550 nm using a Barr and Stroud GN5-type integrating microdensitometer.

With the same instrument and at the same wavelength, the Feulgen/DNA absorptions of chromatin fractions with differing condensation were determined by measurements on one and the same nucleus, after selecting different thresholds of optical density in the instrument. The instrument, which is capable of measuring a range of densities from less than 0.1–2.5, does not read all parts of the nucleus where optical density is greater than the preselected limit, regarding them rather as a clear field.

Results

Most analyses were replicated using preparations that had been Feulgen-stained at different fixations and hydrolysis conditions. The same results were obtained within the same fixation and hydrolysis condition, regardless

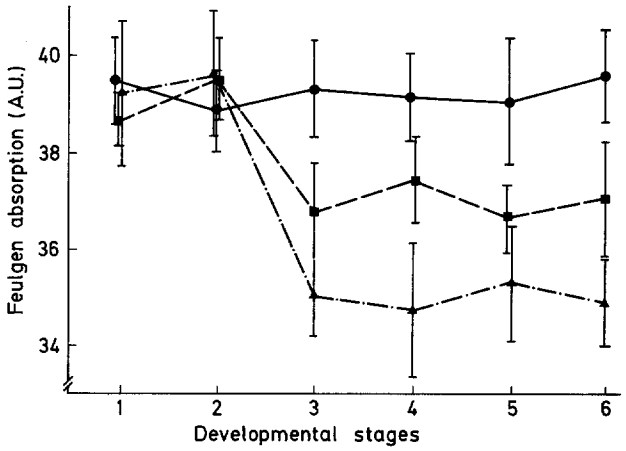

Fig. 1. Feulgen absorptions of early mitotic prophases in immature anthers and pistils (1), of meiotic prophases I (2), of early prophases in embryos at the heart (3), early cotyledonary (4) and late cotyledonary (5) stages, and in the meristems of seedlings (6). All materials, as well as the seeds from which the seedlings were obtained, were collected from one and the same head and the data obtained from those located in its peripheral (●——●), intermediate (■– – –■) and central (▲–·–·–▲) portions were kept separate. Each point is the mean of 60 determinations: 20 for each of three flowers, embryos or seedlings. Confidence limits at 0.01 level of probability

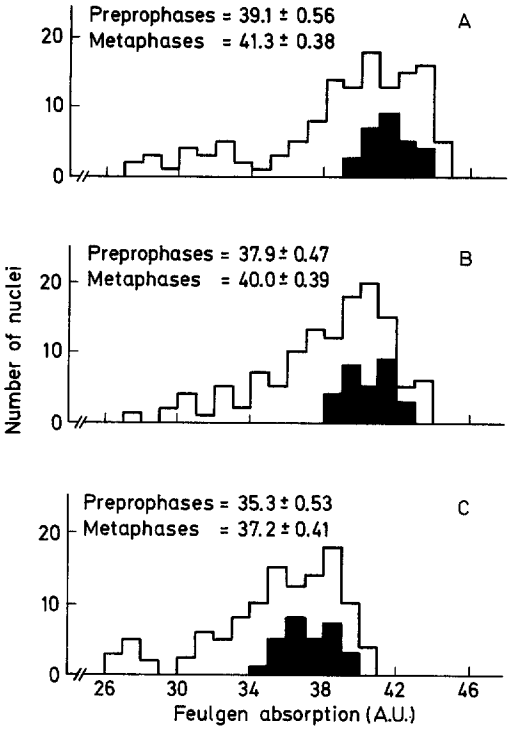

Fig. 2A–C. Feulgen absorptions of early prophases (open bars) and euploid C-metaphases (solid bars) in heart-shaped embryos developing in the peripheral (**A**), intermediate (**B**) and central (**C**) portions of one and the same head. Three embryos were analyzed for each head portion. The mean absorption values (±SE) of prophases and euploid metaphases are also shown

Table 1. Feulgen absorptions (arbitrary units) at different thresholds of optical density of interphase 4C (G_2) nuclei in the shoot meristems of late cotyledonary embryos developed in different portions of one and the same head. For each threshold of optical density, the additional absorption and the percentage increase in relation to the values obtained at the former threshold are given. Twenty nuclei for each of three embryos for each portion of the head were measured

Threshold of optical density	Head portion								
	Peripheral			Intermediate			Central		
	Absorption (mean ± SE)	Additional absorption	Increase (%)	Absorption (mean ± SE)	Additional absorption	Increase (%)	Absorption (mean ± SE)	Additional absorption	Increase (%)
0.1	21.9 ± 0.39	–	–	21.8 ± 0.34	–	–	22.3 ± 0.26	–	–
0.2	25.2 ± 0.26	3.3	15.1	25.6 ± 0.49	3.8	17.4	25.9 ± 0.51	3.6	16.1
0.3	30.7 ± 0.45	5.5	21.8	29.5 ± 0.58	3.9	15.2	29.1 ± 0.48	3.2	12.4
0.4	37.3 ± 0.56	6.6	21.5	34.7 ± 0.74	5.2	17.6	33.5 ± 0.61	4.4	15.1
0.5	38.7 ± 0.37	1.4	3.8	36.2 ± 0.41	1.5	4.3	34.8 ± 0.31	1.3	3.9

of which were used. The data given below were obtained after fixation in ethanol-acetic acid and hydrolysis in hot HCl.

The Feulgen absorptions of 4C nuclei in materials collected at different developmental stages and in different portions of one and the same head are given in Fig. 1. The absorption values of mitotic prophases in immature anthers and pistils and of meiotic prophases I are seen to be the same in any flower of the head. The results obtained in the male and female lines did not differ and have been pooled. Measurements were also found in microsporocytes (not shown) and their results did not differ either. However, the Feulgen/DNA contents of early prophases in heart-shaped embryos developing in different portions of the head differ significantly, increasing from embryos collected at the centre of the head to those collected at its periphery. On average, there are 2.4 more picograms of DNA per 4C (G_2) nucleus in the heart-shaped embryos developing at the periphery of the head than in those developing at its centre (22.0 versus 19.6 pg, respectively). Neither during subsequent embryo development nor during seed germination do further DNA changes occur, since Feulgen absorptions remain unchanged in the embryos located in one and the same head portion and in the seedlings obtained from seeds collected in it. No significant difference was ever found between shoot and root meristems and the results obtained in both have been pooled.

Since extensive aneusomaty has been shown to occur in *H. annuus* and to be present in embryos (Cremonini and Cavallini 1986), the possibility had to be considered that a differential occurrence of this phenomenon in embryos developing in different head portions might account for the variations observed in nuclear DNA content. The Feulgen/DNA absorptions of early prophases and euploid C-metaphases in the meristems of heart-shaped embryos collected in different portions of one and the same head are given in Fig. 2. Aneusomaty actually occurs in the embryos, since large ranges of absorption values are obtained by measuring prophases; however, the frequency of aneuploid cells appears to be similar in embryos collected from different portions of the head, while the Feulgen/DNA contents of euploid metaphases differ significantly.

The results of measurements taken on interphase nuclei at different thresholds of optical density, listed in Table 1, show that these differences in nuclear DNA content are mainly due to DNA fractions contained in relatively more spiralized, optically dense chromatin (heterochromatin). Indeed, at low thresholds of optical density when nuclear parts with dense chromatin are not read, comparable absorption values are obtained in embryos collected from different head portions. By increasing the threshold of optical density when heterochromatin is also read, the nearer the location of the embryo on the head is to its periphery, the more the Feulgen absorptions increase, so that differing total absorption values are obtained.

The data in Table 2 show the correlation between the variation in genome size in a progeny and the genome size of the mother plant. The Feulgen absorptions in the meristem of the primary root, collected at the seedling stage, of six plants with different DNA contents (from 19.3 to 21.9 pg per 4C [G_2] nucleus) are compared with the Feulgen absorptions in the primary root meristems of seedlings obtained from their seeds. The Table shows that the DNA content increases progressively, starting from the values measured in the mother plant, from seedlings obtained from seeds developed in the central portion of the head to those obtained from seeds developed at its periphery, when the genome size of the mother plant is relatively small. The opposite trend is detectable when the genome size of the mother plant is relatively large: the DNA content of seedlings obtained from seeds developed at the periphery of the head is comparable to that of the mother plant, while it decreases progressively in seedlings obtained from seeds developed towards the centre of the head. When the genome of the mother plant

Table 2. Feulgen absorptions (arbitrary units) of early prophases in the primary root meristems of seedlings obtained from seeds developed in different portions of the heads of six plants with differing genome size as compared with the Feulgen absorptions of early prophases in the primary root meristem of each mother plant. Twenty prophases were measured for each mother plant and for each of three seedlings for each head portion

Mother plant (mean ± SE)	Seedlings from seeds in different head portions					
	Peripheral		Intermediate		Central	
	Mean ± SE	Variation (%)	Mean ± SE	Variation (%)	Mean ± SE	Variation (%)
34.4±0.57	37.5±0.46	+ 9.0	36.3±0.43	+5.5	35.0±0.38	+ 1.7
35.2±0.47	39.4±0.63	+11.9	36.8±0.25	+4.5	34.7±0.25	− 1.4
36.1±0.62	37.9±0.22	+ 5.0	37.1±0.61	+2.8	36.2±0.66	+ 0.3
37.6±0.43	38.1±0.26	+ 1.3	34.9±0.36	−7.2	34.8±0.30	− 7.4
38.5±0.39	38.2±0.37	− 0.8	36.2±0.33	−6.0	34.9±0.33	− 9.4
39.1±0.58	39.3±0.59	+ 0.5	37.2±0.52	−4.9	34.7±0.30	−11.2

is medium-sized, the DNA content tends both to increase and to decrease in the progeny, according to the gradient of seed location in the head. It is worth noting that there is no significant difference either in the highest or in the lowest Feulgen absorption values obtained by analyzing each progeny.

Discussion

Confirming the results already obtained by other means, including biochemical analyses (Cavallini et al. 1986), our cytophotometric data point to the presence within *H. annuus* of an unstable fraction of DNA located in the heterochromatic nuclear regions (Table 1), and hence mainly made up of repeated sequences.

We are aware that the determination of DNA content by the scanning cytophotometry of Feulgen-stained nuclei may be subject to certain reservations. Indeed, certain factors may interfere with DNA stainability and/or the measurement of the amount of the stain: the chemical nature and spatial configuration of chromatin due to its proteic component and functional state, the presence of particular substances in the cells, especially in differentiated tissues, or the size of the nuclei measured Frölich and Nagl 1979; Mello and Vidal 1980; Greilhuber 1986). However, none of these conditions differed in the cells we analyzed, since Feulgen/DNA absorptions were measured on early prophases, and each time meristematic tissues were compared at the same developmental stages.

Therefore, we feel justified in stating that the differences in the Feulgen absorption values we obtained do actually reflect real differences in nuclear DNA content and our results seem to allow us to draw two conclusions: (i) the variations in genome size which have already been shown to exist within one and the same progeny even in selfed lines of *H. annuus* (Cavallini et al. 1986) occur during early embryogenesis (Fig. 1); (ii) the DNA content of the mother plant affects the direction in which that of its progeny may vary. By this mechanism, the changes in genome size are maintained within definite limits. Indeed, the variations in the amount of DNA occurring in each single progeny seem to cover the whole range occurring within the line (Table 2). Any malfunction of this control mechanism may be thought to produce the differences in the amount and organization of nuclear DNA which are established in different cultivated varieties or lines of sunflower (Nagl and Capesius 1976; Olszewska and Osiecka 1983; Michaelson and Price 1985; Cavallini and Cionini 1986; Cavallini et al. 1986).

The reason(s) an embryo developing at the periphery of the head has more DNA than one developing at the centre must remain a subject for speculation for the time being. These DNA changes might perhaps occur in response to diversification in the microenvironment in which embryogenesis takes place, due to possible differences in the availability of water and nutrients in different portions of the head, and/or to differences in the times at which embryos develop there, since reproduction events occur on the head in centripetal succession. Without entirely excluding the remote possibility that any microenvironmental variations which might occur simply allow or inhibit the synthesis of DNA sequences to be considered as selfish DNA (Doolittle and Sapienza 1980; Orgel and Crick 1980), the hypothesis might be put forward that nuclear DNA changes play some sort of role in buffering the effects of changing environmental conditions, so as to avoid or to limit their influence on the growth of the embryos.

Work is in progress to identify and to isolate unstable DNA sequences in order to obtain information on their structure and organization at the chromosomal level and to establish if correlations exist between genome sizes and the phenotype of embryos and plants.

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