DNA endoreplication level in endosperm and in cotyledons during seed development in three dicotyledonous species

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Abstract

The DNA content of nuclear and cellular endosperm and in cotyledons in the course of seed development in *Helianthus annuus*, *Pisum sativum* and *Vicia faba* was cytophotometrically measured after Faulgen's reaction. *Helianthus annuus* is characterized by the lowest endoreplication dynamics attaining a maximum DNA content of 48C in nuclear endosperm, which corresponds to four endoreplication rounds, and 16C DNA (three rounds) in cotyledons. In *Pisum sativum* cellular endosperm in the studied stages of seed development was absent and the highest DNA content in cotyledons was 512C DNA; this corresponds to eight endoreplication rounds. In *Vicia faba*, the maximum DNA endoreplication level was 1536C in nuclear endosperm, which corresponds to nine endoreplication rounds; in cotyledons the maximum DNA content was 64C (five rounds). In the course of seed development, differences in the dynamics of DNA endoreplication in trophic tissues of the examined species seem to be similar to differences in the endoreplication level in root and leaf parenchyma, but the DNA endoreplication level is much higher in trophic tissues. Therefore, the endoreplication pattern might be species-specific and genetically conditioned.

Key words: dicotyledonous endosperm, cotyledons, DNA endoreplication

INTRODUCTION

Endoreplication, that is DNA synthesis out of the cell cycle, occurs commonly both in animals and plants. This phenomenon has been cytomorphologically studied since the late thirties. On the basis of enlargement of chromatin structures (e.g. heterochromatin) conclusions were drawn concerning the endoreplication level (ref. Nagl 1978). The increase in chromosome number estimated on that basis was presented as a multiple of number "n". Currently, two more accurate methods of measurement are used: 1) cyto-

photometric measurement after Feulgen's reaction, 2) cytofluorometric measurement after the use of fluorochroms selectively binding to DNA. The increase in DNA content in relation to the haploid genome is presented as a multiple of C value.

Growth and differentiation of cells and tissues are accompanied by DNA endoreplication. It has been shown that the percentage of nuclei with a DNA content higher than 4C increases during root development with increasing distance from the meristem (Olszewska and Osiecka 1982, 1983, 1984, Marciniak and Bilecka 1985, 1986). The dynamics of endoreplication are different in different species, e.g. DNA endoreplication does not occur in *Haemanthus katharinae*, *Tulipa kaufmannia*, *Levisticum officinale* during growth and differentiation of root parenchyma cells (Olszewska and Osiecka 1982, 1983, Marciniak and Bilecka 1985, 1986). In the differentiated zone of *Pisum sativum* and *Vicia faba* roots more than 90% of cells contain 4-8C DNA. Most cells of the differentiated zone of *Brassica oleracea* and *Armoracia lapatifolia* contain 8-16C DNA (Olszewska and Osiecka 1983).

A particularly high endoreplication level is observed in throphic tissues. In rodent throphoblasts (Zybina 1983) the DNA content is as high as 512-4096C.

In angiosperms the highest nuclear DNA contents were noted in suspensor — 4096n and in endosperm — 192n in *Phaseolus coccineus* (Nagl 1978), 3076n in *Echinocistis lobata* (Turała-Szybowska 1966). The highest ever described DNA content was 24576n in the endosperm haustorium of *Arum maculatum* (Nagl 1978).

The aims of the present study were to investigate the endoreplication level of nuclear DNA in endosperm and in cotyledon cells in three dicotyledonous plant species different in respect to 2C DNA content. Moreover, the DNA content in suspensor cells in *Pisum sativum* was analysed. Similarities in the endoreplication level of throphic tissues and other tissues of vegetative organs are discussed.

MATERIAL AND METHODS

Cells of cotyledons and endosperm of three dicotyledonous species: *Helianthus annuus* L. cv. Jadalny (2C DNA 5.0 pg, Olszewska and Osiecka 1983), *Pisum sativum* L. cv. Paloma (2C DNA 8.4 pg, Olszewska et al. 1990) and *Vicia faba* ssp. *major* cv. Hangdown (2C DNA 26.7 pg, Olszewska and Osiecka 1983) were used in the experiment. Tissues from seeds whose embryos were 1 mm in length (stage I), from seeds whose embryos were half their terminal size (stage II) and from mature seeds (stage III) were the subject of the study. Seeds at different stages of maturity were fixed in MAF mixture (96% methanol, 40% formalin, glacial acetic acid 80:15:5) for 24 h in room temperature. They were then washed in 70% methanol in which the seeds were stored. The material was hydrolized in 4N HCl (4M HCl/dm³ H₂O) in room

temperature for 1 h, then placed in Schiff's reagent prepared from pararosaniline. After Feulgen's reaction the tissues were isolated and squashed preparations were embedded in Euparal. Measurements were taken in a Zeiss histophotometer (Jena). The DNA content was determined at 550 nm. Twenty telophase and ten prophase nuclei from young embryos of the tested seeds were used to calculate 2C and 4C DNA content; the ploidy levels of endosperm and cotyledons were determined on the basis of the above data. The DNA content was expressed in arbitrary units (AU) which were obtained by multiplying the appropriate structure surface by extinction.

RESULTS

DNA CONTENT IN NUCLEI OF NUCLEAR ENDOSPERM

In endosperm from the youngest seeds (stage I) considerable mitotic activity was observed in nuclei with a 6C and 12C DNA content, that is, where the DNA content was double or quadruple compared with the initial 3C (triploid) value. These two groups were the major nuclear population in *Helianthus annuus* (Fig 1, 3AI) and *Pisum sativum* (Fig. 1d, 4AI) while in *Vicia faba* most nuclei contained 24C and 48C DNA (Fig. 2a, 5AI).

Helianthus annuus nuclear endosperm with 6C DNA (58%) and 12C DNA (25%) contents dominated in seeds with medium sized embryos (Fig. 3 AII). In *Pisum sativum*, 12C and 24C DNA contents were dominant but 48C DNA contents also appeared, which were the highest polyploidy level in nuclear endosperm in this species (Fig. 1e, f, 4AII). In *Vicia faba* stage II seeds the following groups of nuclei were found 6C, 12C, dominating 24C (33.5%), 48C, 96C and single 384C and 1536C DNA contents. The highest polyploidy level could be observed in *Vicia faba* ssp. *major* (Fig. 5AII).

In *Helianthus annuus* stage III seeds, nuclei with 12C DNA content constituted 62%, with 24C DNA 16% and, a new group, 48C DNA (1.5% nuclei appeared). These nuclei had the highest ploidy level in *Helianthus annuus* (Fig. 1b, 3AIII). Nuclear endosperm in *Vicia faba* contained DNA in amounts from 6C to 384C (Fig. 2b-e, 3AIII). Single nuclei with a 6C DNA content could be observed. There was no clearly dominating group. In *Vicia faba* some nuclei with a higher ploidy level of 96-192C DNA showed some abnormalities, that is, nuclei with constrictions suggesting amitosis or nuclear fusion appeared (Fig. 2b, c-e). Nuclei with one or more constrictions were observed. This type of nucleus were found very frequently on the chalazal pole. The DNA content in nuclei with numerous constrictions was not measured. Such pictures were often found in *Vicia faba*, less frequently in *Pisum sativum* and were not observed in *Helianthus annuus*.

PLATE I

Fig. 1. *Helianthus annuus*: a and b — nuclear endosperm, x 500; c — cellular endosperm, x 250. *Pisum sativum*: d, e and f — nuclear endosperm, x 250

PLATE II

Fig. 2. *Vicia faba*: a and b — nuclear endosperm, x 250; c, d and e — nuclear endosperm, x 500

Fig. 3. *Helianthus annuus*. Nuclear DNA content in nuclear endosperm (A), cellular endosperm (B) and in cotyledons (C). I, II, III — successive stages of seed development. Ordinate — number of nuclei, abscissa — DNA content in C values

DNA CONTENT IN NUCLEI OF CELLULAR ENDOSPERM

Cellular endosperm was not found in *Pisum sativum* seeds. In young *Helianthus annuus* and *Vicia faba* seeds (stage I) cells with 6C and 12C DNA nuclei dominated (Fig. 3 and 5 BI).

In the endosperm of stage II *Helianthus annuus* seeds, nuclei with 6C DNA content dominated (Fig. 3 BII). In *Vicia faba* from the same stage of development, the dominating groups were 12 and 24 C DNA and single nuclei with 192C DNA content were observed; this was the highest ploidy level in *Vicia faba* cellular endosperm (Fig. 5 BII).

In *Helianthus annuus* stage III seeds nuclei with 6 and 12C DNA were the dominating groups. The maximum ploidy level in the cellular endosperm in this species was 24C DNA (Fig. 3 BIII). In *Vicia faba* 12 and 24C DNA were the dominating groups (Fig. 5 BIII).

Fig. 4. *Pisum sativum*. Nuclear DNA content in nuclear endosperm (A), cotyledons (B) and suspensor (C). I, II, III — successive stages of seed development. Ordinate — number of nuclei, abscissa — DNA content in C values

DNA CONTENT IN NUCLEI OF COTYLEDON CELLS

The nuclei of the youngest cotyledon (stage I) were found to have a 2, 2-4 and 4C DNA content. Mitotic activity was observed in this group; apart from these, cells with an 8C DNA content were present. In *Pisum sativum* cotyledons, nuclei with 16C and 32C DNA were also present (Fig. 4BI). No nuclei with an intermediate, 4C-8C DNA level were observed. In *Helianthus annuus* in cotyledons of seeds with medium sized embryos (stage II) groups with 2C and 2-4C DNA content dominated (Fig. 1c; 3CII). In *Pisum sativum* cotyledons, groups with 2-8C DNA content were not to be found, the dominating group was 32C DNA and a 128C group appeared (Fig. 4BII). In *Vicia faba*, the number of nuclei with a 2C and 2-4C DNA content decreased while nuclei with 8C DNA became more numerous (Fig. 5CII). Only in *Helianthus annuus* in cotyledons of the oldest seeds (stage III) groups with 2, 2-4, 4 and 8C DNA could be observed, the last one dominating (62%). The highest found poliploidy level was 16C DNA (Fig. 3CIII). In the oldest *Pisum sativum* cotyledons (stage III) cells with nuclei

Fig. 5. *Vicia faba*. Nuclear DNA content in nuclear endosperm (A), cellular endosperm (B) and cotyledons (C). I, II, III — successive stages of seed development. Ordinate — number of nuclei, abscissa — DNA content in C values

with 16-512C DNA appeared. Groups with 64 and 128C DNA dominated (Fig. 4BIII). In mature *Vicia faba* cotyledons, nuclei with a 8-12C DNA content could be found. Group 32C DNA dominated (Fig. 5CIII).

In all of the examined species at this stage of development seeds contained mature embryos in which no mitotic activity was observed and the cytoplasm was rich in storage substances.

DNA CONTENT IN SUSPENSOR CELLS NUCLEI IN *PISUM SATIVUM*

It was possible to take measurements of the DNA content in suspensor cell nuclei only in *Pisum sativum*. The data presented in the histogram (Fig. 4CIII) come from all of the examined developmental stages of pea seeds. Suspensor cells were characterized by a high ploidy level. Nuclei with DNA content of 128C dominated (56%). A few nuclei with 512C DNA content were also present (Fig. 4 CIII).

DISCUSSION

The increase in DNA content through endoreplication seems to be proved as far as root parenchyma and cotyledon parenchyma cells are concerned. Also the increase in DNA content in differentiated mesophyll tissues can be attributed to endoreplication (Damsz and Łuchniak 1988). However, the increase in DNA content in nuclear endosperm may be under doubt because of the syncytial character of this tissue, though it is difficult to exclude nuclear fusion. In the course of transformation from nuclear endosperm to cellular endosperm, a binucleate cell may be formed, thus giving rise to a cell with a higher ploidy level. Pictures suggesting either amitosis or fusion were found in *Pisum sativum* and *Vicia faba*. They were the most numerous on the chalazal pole of medium sized seed embryos, while on the micropylar pole, mitotic activity was still observed. Similar pictures were described in the course of *Iris pseudoacorus* endosperm development (Olszewska and Gabora 1966). The occurrence of large layers of nuclear endosperm displaying the same level of DNA content seems to prove endoreplication.

According to Nagl (1976) one can expect higher endoreplication levels in species with a low 2C DNA content than in species with a high 2C DNA amount. The results of Olszewska's experiments (Olszewska and Osiecka 1982, 1983, 1984) indicate that in angiosperms, the dynamics of nuclear DNA endoreplication is a taxonomic feature and is dependent on the 2C DNA content only within the same family and is correlated with the life cycle type. Within the same family when the 2C DNA content is similar, annual species exhibit higher dynamics of endoreplication than perennials, shrubs and trees. The species which are the subject of the present study differ in 2C DNA content and in respect to this feature they can be arranged as follows *Helianthus annuus*, *Pisum sativum* and *Vicia faba*; they can be ordered similarly taking into account the maximum endoreplication level, while according to Nagl's hypothesis (1978) an opposite arrangement should be found. Annual species contain a higher percentage of heterochromatin in nuclei than perennial ones (Olszewska et al. 1988). This may be attributed to a positive correlation between the heterochromatin content and dynamics of nuclear DNA endoreplication. Endoreplication of nuclear DNA compensate for high heterochromatin content in the plant genome and enable a cell to increase its transcriptional potential in the course of differentiation (Olszewska et al. 1988).

Table 1 presents the level of DNA content in different tissues of the tested plants. Taking into account the triploidy of endosperm tissue, the results concerning the increase in DNA content through endoreplication were presented by a number of endoreplication rounds i.e. the C value. Data presented in Table 1 show that there is some similarity between the DNA content in root parenchyma and mesophyll of mature leaves. The examined trophic tissues — endosperm and cotyledons — attain much higher DNA contents still

maintaining the tendency to endoreplication dynamics characteristic of other previously tested tissues; if in the given species endoreplication in parenchyma was not observed it appeared in the endosperm and cotyledons on a low level, while if the differentiation of parenchyma cells was accompanied by endoreplication, it appeared in trophic cells on a high level. In *Helianthus annuus* there is no endoreplication in the course of differentiation of root parenchyma (Cavallini and Cionini 1986) or it is on a very low level (Olszewska and Osiecka 1983). In the course of cotyledon development the level of endoreplication is 16C DNA and in endosperm few nuclei contain 48C DNA. A similar DNA content was found in *Helianthus annuus* endosperm (24C) by Cavallini and Cionini (1986) without stating the stage of endosperm development.

Table 1

DNA content and endoreplication level in different tissues of the studied species

Tissue	DNA content, C	Species		
		<i>Helianthus annuus</i> cv. Jadalny	<i>Pisum sativum</i> cv. Paloma	<i>Vicia faba</i> subs. <i>major</i> cv. Hang-dawn
Root parenchyma	2C DNA, pg Author	5.0 ¹	8.4 ²	26.7 ¹
	Maximum Dominating	4 ¹ 2 ¹	16 ¹ 8 ¹	8 ¹ 4-8 ¹
	No. of endoreplication rounds	1	3	2
Mesophyll	Maximum Dominating	4 ⁴ 2 ⁴	8 ³ 4 ³	4 ³ 2 ³
	No. of endoreplication rounds	1	2	1
Cotyledon	Maximum Dominating	16 8	512 128	64 32
	No. of endoreplication rounds	3	8	5
Nuclear endosperm	Maximum Dominating	48 12	48 24	1536 24
	No. of endoreplication rounds	4	4	9
Cellular endosperm	Maximum Dominating	24 6	— —	192 24
	No. of endoreplication rounds	3	—	6

¹ Olszewska and Osiecka 1983

² Olszewska et al. 1989

³ Damsz and Łuchniak 1988

⁴ Cavallini and Cionini 1986

From the above data it might be assumed that the differences in the dynamics of DNA endoreplication in trophic tissues in three tested species seem to be similar to the differences in the endoreplication level in nuclei of root parenchyma and mesophyll. However in trophic tissues the endoreplication level is many times higher.

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*Poziom replikacji DNA w bielmie i liścieniach podczas rozwoju nasion
u trzech gatunków roślin dwuliściennych*

Streszczenie

Metodą cytofotometryczną po reakcji Feulgena badano zawartość DNA w bielmie jądrowym, komórkowym i liścieniach podczas rozwoju nasion *Helianthus annuus*, *Pisum sativum* i *Vicia faba*. Najmniejszą dynamiką endoreplikacji charakteryzuje się *Helianthus annuus*, osiągając w bielmie jądrowym maksymalną zawartość 48C DNA, co odpowiada 4 rundom endoreplikacji, zaś w liścieniach 16C DNA (3 rundy). U *Pisum sativum* największa zawartość DNA występuje w komórkach liścieni — 512C DNA. Odpowiada to 8 rundom endoreplikacji. Natomiast u *Vicia faba* maksymalny poziom endoreplikacji DNA — 1536C — występuje w bielmie jądrowym, co świadczy, że jądra te przeszły 9 rund endoreplikacji; w liścieniach maksymalna zawartość wynosi 64C (5 rund). Podczas rozwoju nasion różnice dynamiki endoreplikacji DNA u poszczególnych badanych gatunków są podobne do różnic w poziomie endoreplikacji w komórkach miękiszowych korzeni i mezofilu, z tym że w tkankach troficznych poziom endoreplikacji DNA jest znacznie wyższy. Wzór endoreplikacji jest więc cechą gatunkową, uwarunkowaną genetycznie.