DNA ENDOREPLICATION LEVEL IN ENDOSPERM DURING SEED DEVELOPMENT IN THREE MONOCOTYLEDONOUS SPECIES

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ABSTRACT

The DNA content after the Feulgen reaction in the endosperm of three monocotyledonous plant species (*Asparagus officinalis*, *Muscari comosum*, *Haemanthus katherinae*) differing in their 2C DNA content, was cytophotometrically measured. During endosperm development 1-6 endoreplication cycles take place, depending on the species. Differences in nuclear DNA endoreplication dynamics in the tested species are similar to those occurring in root parenchyma, but the endoreplication level in the endosperm is higher.

KEY: WORDS: DNA content, endosperm, monocotyledons, *Asparagus officinalis*, *Muscari comosum*, *Haemanthus katherinae*

INTRODUCTION

The term endocycle introduced by Nagl (1978) refers to the endoreplication cycle inside the nuclear envelope without formation of the mitotic spindle and without normal mitosis. This process increases the nuclear DNA content and the number of chromosome sets rises in geometrical sequence (Nagl 1978). Endoreplication causes full or partial multiplication of the genome which may – but does not have to – lead to intensified transcription and translation (Kononowicz and Janick 1988). Endoreplication processes become more intense during growth and differentiation of cells. Nuclear DNA endoreplication is common both in plants and animals.

According to Nagl (1978) 2C DNA content and DNA endoreplication dynamics are inversely correlated. Experiments performed by Olszewska and Osiecka (1982, 1983, 1984) did not confirm this relationship during differentiation of root parenchyma cells in angiosperms. These authors found that the dynamics of nuclear DNA endoreplication is a characteristic feature of a family among which annual species are characterized by a higher endoreplication level than herbaceous perennials, shrubs and trees. The relationship mentioned by Nagl (1976) can be observed only among species of the same family and with the same life cycle. A positive correlation between heterochromatin content and DNA endoreplication dynamics has been observed: annual species are characterized by a higher heterochromatin content than perennial ones, which explains the lower endoreplication dynamics of the latter (Olszewska et. al. 1988). The DNA endoreplication pattern seems to be a feature of the genome and is not modified either by generative or colchicine-induced polyploidization (Olszewska and Kononowicz 1981).

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).

In a species tested in this experiment, i.e., *Asparagus officinalis*, more than 90% of cells in the differentiated root zone contained 4-16 C DNA; in *Muscari comosum* only a small percentage of cells contained 4-8 C DNA, while in *Haemanthus katherinae* endoreplication did not occur (Olszewska and Osiecka 1982).

A particularly high level of endoreplication is observed in trophic tissues. The DNA content of the rat trophoblast is as high as 512-4096 C DNA (Zybina 1983).

In angiosperms the highest nuclear DNA contents were observed in the suspensor – 4096n, and in the endosperm – 192n in *Phaseolus coccineus* (Nagl 1978), 3076n in *Echinocystis lobata* (Turała-Szybowska 1966) and 1536C in *Vicia faba* subsp. *major* (Marciniak 1991).

The aim of the present study was to investigate the endoreplication level of nuclear DNA during nuclear and cellular endosperm development in three monocotyledonous plant species differing in respect to their 2C DNA contents, and to compare the possible similarities in dynamics of endoreplication in trophic and vegetative tissues.

MATERIAL AND METHODS

Endosperms of three monocotyledonous plant species: *Asparagus officinalis* (Liliaceae) 2C DNA 4.2pg, *Muscari comosum* (Liliaceae) 2C DNA 12.7pg, and *Haemanthus katherinae* (Amaryllidaceae) 2C DNA 117.7pg (Olszewska and Osiecka 1982) were used in the experiment.

Tissues from seeds containing 1mm long embryos (stage I), from seeds with embryos at half of their final size (stage II), and from seeds grown to terminal size but not yet mature, that is, whose endosperm was still soft (stage III) were the subject of the study. Seeds at different stages of maturity were fixed in MAF (methanol, formalin, glacial acetic acid 80:15:5) then washed and stored in 70% methanol. The material was hydrolized in 4 N HCL at room temperature for 1 h, then placed in Schiff's reagent prepared from pararosaniline for 1 h at room tempera-

ture. After the Feulgen 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 a wave length of 550nm. Twenty telophase and ten prophase nuclei from young embryos of tested seeds were used to calculate 2C and 4C DNA values. In each experimental series, 100 nuclei from the endosperm obtained from different seeds were measured. The ploidy level of nuclei of endosperm cells was determined on the basis of the 2C and 4C 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 developing endosperm – On the initial phase of seed development of the three examined species cytokinesis was not observed in the endosperm; it was therefore in nuclear state. As the seeds matured, cytokineses appeared and the endosperm reached cellular state. A similar situation was described by Ryczkowski (1960) during endosperm development in *Haemanthus katharinae* and *Asparagus officinalis*.

In the nuclear state endosperm from stage I seeds, considerable mitotic activity was observed in nuclei with 6C and 12C DNA contents, i.e., where the DNA content was double or quadruple in comparison with the initial 3C (triploid value). In *Haemanthus katharinae* prophase were observed in nuclei with a 24C DNA content.

In the nuclear state endosperm from *Asparagus officinalis* in seeds at stage I, nuclei with 12C dominated (40%), nuclei with 6C and 24C DNA constituted together 49% (Fig. 1 A I). A similar situation was observed in the endosperm in cellular state, where group 12C DNA distinctly dominated (55%) and

Fig. 1. *Asparagus officinalis*. Nuclear DNA content in nuclear endosperm (A), cellular endosperm (B). I, II, III – successive stages of seed development. Ordinate – number of nuclei, abscissa – DNA content in C values.

groups 6C and 24C DNA together constituted 44% (Fig. 1 B I). In nuclear state endosperm of *Muscari comosum*, nuclei with 6C and 12C DNA dominated – 44% in each of the tested populations. Nuclei with a 24C DNA content constituted 11% and with 48C, 1% (Fig. 2 A I). In the cellular endosperm (from the same seeds), group 12C DNA dominated – 51%, group 6C DNA constituted 32% and 24C DNA 11% (Fig. 2 B I). In endosperm of *Haemanthus katharinae* (stage I), nuclei with a 12C DNA content constituted 75% and nuclei with 24C-DNA

Fig. 2. *Muscari comosum*. Nuclear DNA content in nuclear endosperm (A), cellular endosperm (B). I, II, III – successive stages of seed development. Ordinate – number of nuclei, abscissa – DNA content in C values.

18% (Fig. 3 A I). In cellular endosperm, group 12C DNA dominated – 58% and group 6C DNA constituted 34% (Fig. 3 B I).

In embryos which were half their terminal size (stage II) the C value was generally higher. In nuclear state endosperm of *Asparagus officinalis* there were no nuclei with a 3C DNA, nuclei with 24C DNA dominated, and constituted 37%, groups 12C and 48C DNA constituted 24% and 30% respectively, while 96C and 192C DNA – 5% and 1%. The highest observed poliploidy level in this species was 192C DNA (Fig. 1 A II). There were no nuclei with a 3C DNA content in the cellular endosperm of *Asparagus officinalis*. Nuclei with a 24C DNA content dominated – 40% nuclei with 48C DNA constituted 30% and 12C DNA 20% (Fig. 1 B II). In *Muscari comosum* endosperm, the 12C DNA group clearly dominated – 49%, groups 24C and 48C DNA constituted 29% and 4%. A 48C DNA content was the highest observed ploidy level in this species (Fig. 2 A II). In the cellular endosperm of *Asparagus officinalis*, the dominating group was 12C DNA – 62%. Groups 6C and 24C DNA constituted 14% and 21% (Fig. 2 B II). In *Haemanthus katharinae* apart from the dominating group of 12C DNA (54%), groups 3C – 11% and 6C 30% were observed. At this stage of nuclear endosperm de-

Fig. 3. *Haemanthus katharinae*. Nuclear DNA content in nuclear endosperm (A), cellular endosperm (B). I, II, III – successive stages of seed development. Ordinate – number of nuclei, abscissa – DNA content in C values.

velopment, 2% of the examined population reached the maximum polyploidy level – 48C DNA (Fig. 3A II). In the cellular endosperm of *Haemanthus katharinae* the dominating groups were 6C and 12C DNA, 41% and 45%, respectively. Nuclei with 3C DNA constituted 12%, with 24C DNA, 2% (Fig. 3 D II). In *Haemanthus katharinae* seeds grown to terminal size (stage III), nuclear state endosperm was not observed. In the remaining two species in the central part of the endosperm small patches of nuclear state endosperm were observed. In the nuclear state endosperm of *Asparagus officinalis*, group 24C DNA dominated and constituted 53%. Groups 6C and 12C DNA constituted 3% and 27% while groups 48C and 96C DNA constituted 19% and 4% respectively (Fig. 1 A III). In cellular endosperm in *Asparagus officinalis* the group with 6C DNA constituted 4%, the group with 12C DNA dominated, 49%, the group with 24C DNA constituted 38%

with 48C DNA 9%, and with 96C DNA, 1% each (Fig. 1 B III). In the endosperm in nuclear state in *Muscari comosum*, groups 12C and 24C DNA dominated, making up 43% and 38% of the examined population. The maximum polyploidy level, 48C, occurred in 15% of the examined nuclei (Fig. 2 A III). In the cellular endosperm in *Muscari comosum* there were no nuclei with a 3C and 6C DNA content. The group with 24C DNA dominated, 58%, nuclei with 12C DNA constituted 33% and with 48C DNA 9% (Fig. 2 B II). In the cellular endosperm of *Haemanthus katharinae*, nuclei with a 3C DNA content constituted 12% with 6C DNA dominating (61%). Nuclei with 12C DNA constituted 25% and 24C DNA – 2% (Fig. 3 B III). It can be stated that during endosperm development in all the three examined species an increase in DNA content above 3C (triploid) took place. In *Muscari comosum* and *Asparagus officinalis* group 3C DNA disappeared completely in more advanced stages of endosperm development, so all nuclei in the endosperm underwent 1 to 6 endoreplication cycles. The maximum polyploidy level – 192C DNA which corresponds to 6 endoreplication cycles – was observed in the nuclei of *Asparagus officinalis* endosperm.

DISCUSSION

A direct proof of endoreplications is the incorporation of ³H thymidine into the nuclei of over-meristematic cells during growth and differentiation of roots (Olszewska and Osiecka 1982, 1983). Numerous attempts of incorporating ³H thymidine into developing endosperm have not brought positive results, so it seems probable that the endosperm contains a huge amount of endogenous DNA precursors and is a tissue to some degree inaccessible to external substances, i.e., supplied during incubation of isolated young seeds. However, a convincing proof of endoreplication in nuclear and cellular endosperm is the decrease in the number of nuclei with lower and an increase in nuclei with higher ploidy levels. This process is especially evident in *Asparagus officinalis* and *Muscari comosum* (Figs. 1 and 2). A similar relationship was described during endosperm development in rice (Ramachandran and Raghavan 1989) and maize (Kowles et al. 1990). The plants tested in this experiment are perennials so they represent the same life-cycle that is characterized by a lower endoreplication level (Olszewska and Osiecka 1984). These species differ markedly in their 2C DNA content (Table 1). In respect to this feature they can be arranged as follows: *Asparagus officinalis*, *Muscari comosum* and *Haemanthus katharinae*. Data presented in Table 1 show that during growth and development of roots in *Haemanthus*, endoreplication of nuclear DNA does not take place, while during endosperm

TABLE 1. Comparison of DNA content and endoreplication level in different tissues of studied species

Species	2C DNA pg.	Endosperm nuclear state		Endosperm cellular state		Root parenchyma	
		C(max)	No. of endoreplication rounds	C(max)	No. of endoreplication rounds	C(max)	No. of endoreplication rounds
<i>Asparagus officinalis</i>	4,2 ± 0,1 ⁽¹⁾	192	6	192	6	16 ⁽¹⁾	3
<i>Muscari comosum</i>	12,7 ± 0,33 ⁽¹⁾	48	4	48	4	8 ⁽¹⁾	2
<i>Haemanthus katharinae</i>	117,7 ± 2,4 ⁽¹⁾	48	4	24	3	2 ⁽¹⁾	No endoreplication

⁽¹⁾ Olszewska and Osiecka, 1982

Fig. 4.

Nuclei from nuclear endosperm:

- a – *Asparagus officinalis*, small nuclei – 2C DNA, big nucleus – 12C DNA,
 b – *Muscari comosum* – 12C DNA,
 c – *Haemanthus katherinae* – 12C DNA; x 1000.

development it appears at a low level of 48C which corresponds to 4 rounds of DNA replication. In *Muscari*, the nuclei of root parenchyma in differentiated zones reach an 8C DNA level which corresponds to two replication rounds. During endosperm development nuclei attain a ploidy level of 48C DNA which corresponds to 4 rounds of replication. In *Asparagus officinalis*, the species with the lowest 2C DNA content, nuclei from differentiated zones of roots contain 16C DNA, which corresponds to 3 rounds of DNA endoreplication, while during endosperm development the maximum ploidy level is 192C DNA, which corresponds to 6 rounds of DNA endoreplication. It is worth noticing that *Haemanthus* contains only 0.5% heterochromatin (ref. in Olszewska et al. 1988). The heterochromatin contents in *Muscari* and *Asparagus* were not examined, but the nuclei structure in these species suggests the highest heterochromatin amount in *Asparagus officinalis* (Fig. 4). These results are in agreement with the hypothesis according to which a low amount of DNA (Nagl 1976) and high heterochromatin content are related to the dynamics of DNA endoreplication; it has been shown that a positive correlation exists between heterochromatin content and endoreplication dynamics (Olszewska et al. 1988). The data in Table 1 suggest that during endosperm development, differences in DNA endoreplication dynamics in each species are similar to species-specific differences in endoreplication in root parenchyma, but the level is higher in the endosperm (*Asparagus*, *Muscari*) or endoreplication occurs only on a low level in a species in which this process is not observed during root cell differentiation (*Haemanthus*).

In conclusion, during endosperm development, 1-6 endoreplication cycles occur depending on species and during cellular endosperm development 3-6 cycles; differences in nuclear DNA endoreplication dynamics in the studied species are similar to those occurring in root parenchyma but the endoreplication level in endosperm is higher.

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POZIOM ENDOREPLIKACJI DNA W BIELMIE PODCZAS ROZWOJU NASION U TRZECH GATUNKÓW ROŚLIN JEDNOLIŚCIENNYCH

STRESZCZENIE

Metodą cytofotometryczną po reakcji Feulgena badano zawartość DNA w bielmie trzech gatunków roślin jednoliściennych różniących się zawartością 2C DNA (*Asparagus officinalis*, *Muscari comosum*, *Haemanthus katharinae*). Podczas rozwoju bielma następuje, w zależności od gatunku, 1-6 cykli endoreplikacyjnych. Różnice dynamiki endoreplikacji jądrowej DNA u badanych gatunków są zbliżone do występujących w miękiszu korzeni, z tym, że poziom endoreplikacji w bielmie jest wyższy.

SŁOWA KLUCZOWE: zawartość DNA, bielmo, jednoliścienne, *Asparagus officinalis*, *Muscari comosum*, *Haemanthus katharinae*