MITOTIC ACTIVITY AND CHANGES IN DNA CONTENT DURING *ZEA MAYS* L. ENDOSPERM DEVELOPMENT

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ABSTRACT

Mitotic activity and nuclear DNA content in endosperm of *Zea mays* cv. Złota Karłowa were examined. DNA content was cytophotometrically measured on squashed preparations after Feulgen procedure. Mitotic activity in endosperm was determined till the stage when embryo sack reached 4.5 mm in length. Some of mitotic figures show multiplied DNA content. Endosperm nuclei have various DNA contents which increase throughout endosperm development. DNA content enhancement indicates endoreduplication in progress. Some nuclei with high DNA content display changes in chromatin structure, which are expressed by the presence of strands and aggregates of chromatin characterised by high staining intensity. A conclusion has been drawn that mitotic divisions and the endoreduplication phase of nuclear DNA may occur simultaneously and dominate one over another at different phases of endosperm development.

KEY WORDS: endosperm, mitotic activity, endoreduplication, DNA content, *Zea mays*.

INTRODUCTION

The development of endosperm, which is formed as a result of double fertilization in the embryo sac, significantly surpasses the development of the embryo. For example in *Triticum aestivum* the first divisions of a fertilized secondary nucleus take place as early as 6 h after pollination, while the first division of a zygote – only 22 h after pollination (Raghavan 1997). Regardless of the fact whether a forming embryo uses up the whole of endosperm or remains in a seed as a storage material for a developing seedling, the endosperm development may be accompanied by cellularization, or this process is a little delayed. In *Arabidopsis*, during cellularization the embryo reaches an early heart-shaped stadium (Berger 1999), while in *Cucumis* cellularization was observed in endosperm already at the globular stage of embryo (Faris and Niemirowicz-Szczyt 1999). In cereals, where endosperm seeds are common, endosperm is of syncytial nature till the moment when the number of nuclei reaches several hundred or even a few thousand and then the fast cellularization occurs. In *Triticum aestivum* it starts already on the second day after pollination (Oryol and Shmarayev 1987) and after 5 days the endosperm consists of five thousand fully formed cells (Raghavan and ref. 1997). In rice and maize central vacuole is fully closed 4 days after pollination (Olsen et al. 1999).

The early stages of endosperm development are accompanied by intensive synchronous mitotic divisions. Along with its development – in nuclear phase – the number of

dividing nuclei diminishes (Orłowa 1991). The mitotic activity level depends not only on endosperm developmental phase (Kowles and Phillips 1985), but on its region as well (Phillips et al. 1983). In *Arabidopsis*, when the embryo is in heart-shaped stage, a small part of endosperm in the chalazal region is still a multinucleate mass, while the rest of it is cellularized (Berger 1999). In cereal grains the division activity of nuclei in the outer part of starch endosperm, described as a subaleurone layer, persists till the medium developmental stage (Oryol and Shmarayev 1987; Olsen et al. 1999).

The period of endosperm development characterized by numerous mitotic divisions is followed by an increase in the nuclear DNA content resulting from endoreduplication, which is a common and basic process during this tissue development (Kowles et al. 1992; Marciniak 1991, 1993). Borisjuk et al. (1995) determined the beginning of endoreduplication in *Vicia faba* for the 8th day after pollination. Similarly, in maize endoreduplication starts on the 9th day after pollination and the increase in nuclear DNA content is accompanied by an increase in volume of nuclei. 14 days after pollination the DNA content reaches 48C (Cavallini et al. 1995), which indicates four endoreduplication rounds. The size of nuclei generally coincides with their DNA content (Kowles and Phillips 1985, Melaragno et al. 1993), but in maize endosperm such a correlation was insignificant (Kowles et al. 1990). However, in soybean a very distinct correlation between DNA content and seed size was observed (Chung et al. 1998).

In order to determine whether mitotic activity in endosperm is limited only to early developmental stages, and whether nuclei with multiplied genome may undergo mitosis, mitotic activity and changes in nuclear DNA content during *Zea mays* endosperm development were examined.

MATERIAL AND METHODS

Young seeds of field-grown maize (*Zea mays* cv. Żłota Karłowa) were examined. Embryo sacks of 1-7 mm in size together with embryos were isolated from young seeds, fixed in MAF (methanol:formalin:glacial acetic acid, 80:15:5 v/v) for 24 h at room temperature. After 1 h hydrolysis in 4 N HCl the material was stained with Schiff's reagent and squashed preparations were made according to the method described earlier (Marciniak 1991). Nuclear DNA content was assayed cytophotometrically with the use of computer analysis of microscopic pictures IMAL 1024 at a wave length of 550 nm. In order to define 2C and 4C DNA content 10 prophase and 20 telophases from a young embryo developing in endosperm of 1 mm in diameter were measured. These data were used to determine the polyploidy level of endosperm nuclei. Values are presented in AU (arbitrary units). The mitotic index of endosperm and embryo were calculated using the same preparations from which DNA content was measured.

RESULTS

Mitotic activity of endosperm and embryo

The mitotic index was calculated from squashed preparations, thus it was impossible to establish the examined regions of endosperm precisely. However, the method of endosperm isolation aimed at preparing central regions of endosperm for observation.

The mitotic index established during consecutive periods of *Zea mays* endosperm development is the highest in embryo sack of 1 mm in length where it reaches 6.3%, then along with its growth, the mitotic activity decreases (Fig. 1). In embryo sacks of 2 mm in length mitotic divisions in endosperm were observed only in one out of five preparations and the mitotic index was 1.1%. At the following stages of endosperm development the nuclear divisions were not so numerous as in earlier phases and in embryo sacks of 4.0 and 4.5 mm in length in endosperm the mitotic indices were 0.3% and 0.26%, respectively (Fig. 1) when an embryo was 1mm long. In embryo sacks of 7 mm in length no mitotic divisions were found in endosperm.

Mitotic divisions in embryos at chosen stages of seed development were observed both in the youngest and oldest stages of development. The highest mitotic index – 14.4% (Fig. 1) was observed in the embryo at globular stage located in a seed with endosperm of 1 mm in length and then mitotic activity of the embryo decreased gradually, reaching 1,76% in embryos with a differentiated cotyledon situated in endosperm of 7 mm in length (Fig. 1).

Changes in nuclear DNA content in endosperm

In embryo sacks of 1 and 1.4 mm in length the maximum ploidy of nuclei in endosperm is 6C DNA and the number of 3C and 6C nuclei is similar (Fig. 2). The multi-

Fig. 1. Mitotic activity in developing embryo and endosperm of *Zea mays*. Ordinate: number of mitotic nuclei (%), abscissa: length of embryo sack (mm).

plication of DNA content occurs only in nuclei from embryo sacks of 1.8 and 2.0 mm in length, where DNA content in endosperm reaches even 24C, but 3C and 6C nuclei dominate (Fig. 2). At the next examined stages the nuclear DNA content in endosperm increases gradually reaching 48C level in embryo sacks of 2.5 and 3.0 mm in length and 96C level – corresponding to six endoreplication rounds – in sacks of 3.5 and 4 mm in length (Fig. 2). In older endosperm with sacks of 4.5 mm in length, a considerable percentage (7.1%) of nuclei is typical of 96-192C classes, while 3C and 3-6C DNA nuclei were not observed (Fig. 2). At the oldest developmental stage of endosperm no nuclei with DNA content lower than 48C were found, 96C, 96-192C and 192C classes dominated and single nuclei reached 384C DNA level (Fig. 2).

Cytophotometric measurements of DNA contents in telophases and prophase of endosperm in embryo sacks of 1 and 1.4 mm in length showed 3C and 6C DNA levels in telophases and prophase, respectively (Table 1). Starting from developmental stage of embryo sack of 1.8 mm in length, prophase with 12C DNA and telophases with 6C DNA contents appeared in endosperm (Table 1), while telophases from an embryo reached 2C DNA content (Fig. 5D). At this stage of endosperm development one prophase

Endosperm sac diameter (mm)	Telophase	Prophase
1	3C	6C
1.4	3C	6C
1.8	3C and 6C	6C and 12C and 24C
2	without divisions	
2.5	3C and 6C	6C and 12C and 24C
3	3C and 6C	12C and 24C
4	3C	6C and 12C

Fig. 2. Nuclear DNA content in developing endosperm of *Zea mays*. Ordinate: number of nuclei (%), abscissa: DNA content in C values.

with 24C DNA was found (Table 1). In older endosperm, prophase and telophase with 6C, 12C and 24C DNA contents were common. Mitotic figures with multiplied DNA content were observed at all the examined stages of endosperm development (Table 1, Fig. 5A, B, C).

Structure of endosperm nuclei

In embryo sacks of at least 3.5 and 4 mm in length, in endosperm there occur nuclei containing chromatin in the form of strongly staining strands and aggregates (Fig. 5E, F, G, H) in addition to nuclei with dispersed chromatin.

Along with endosperm growth this kind of chromatin organisation is observed in longer nuclei and in sack of 7 mm in length such nuclei reach the maximum size (Fig. 5H). Comparative analysis of DNA content of the above mentioned nuclei and those with dispersed chromatin, carried out in endosperm of sacks of 4 mm in length, showed a higher level of ploidy in nuclei containing chromatin strands and aggregates (Fig. 3), as well as a much higher DNA content of a similar area (Fig. 4). DNA content in nuclei containing chromatin strands and aggregates ranges from 12C to 96C DNA. Nuclei with 24C DNA and 48C DNA dominate and those with 96C constitute 10% of this group (Fig. 3). In nuclei with dispersed chromatin the DNA content falls between 3C and 96C DNA, but 48-96C and 96C nuclei constitute only 2.8% each.

DISCUSSION

The period of intensive synchronous mitotic divisions of fertilized central nucleus occurs in cereals during the first 3 days and then the wavy character of mitosis loses synchronisation (Orłowska 1991). An embryo sack grows quickly and in *Triticum aestivum* in endosperm 250 μ m long the larger part of the central cell is already cellularized (Oryol and Shmarayev 1987). Our study shows that in mai-

Fig. 3. C DNA content in nuclei with dispersed, granular and stranded chromatin in embryo sacks of 4 mm in length. Ordinate: number of nuclei (%), abscissa: DNA content (C). White – nuclei with dispersed chromatin, black – nuclei with granular and stranded chromatin.

Fig. 4. Relationship between C DNA content (AU) and nucleus area in endosperm of 4 mm embryo sac length. A – nuclei with dispersed chromatin; B – nuclei with granular and stranded chromatin. Ordinate: size of nuclei (μm^2), abscissa: DNA content (AU).

ze embryo sacks of 1 mm in length mitotic index in endosperm is 6.3%. Along with growth of embryo sacks the mitotic activity in endosperm decreases significantly. Thus, it seems that in this species that stage is one of the final of intensive proliferation, which occurs on 8th day after pollination at the latest (Kowles and Phillips 1988), and until 10th day after pollination the majority of nuclei belong 3C and 6C DNA class (Sun et al. 1999). At this phase the embryo is globular and characterized by proliferation activity. Mitotic activity in the central part of maize endosperm disappears between the 12th and the 14th day after pollination (Kowles and Phillips 1988), but on peripheries of this tissue it persists till the 20th day after pollination (Phillips et al. 1983). Thus, mitotic activity ranging between 1% and 1.7% observed in older endosperm most probably originates from peripheral regions of endosperm where the number of observed anaphases and telophases is three times higher than in the central regions (Kowles and Phillips 1988). In embryo sacks of 2 mm in length in all preparations except one the mitotic index in endosperm was zero (Fig. 1, Table 1). In barley after the phase of intensive mitotic activity there are an inhibition of this activity and a 2-day hiatus preceding cellularization of endosperm (Brown et al. 1994). During that time there occur changes in the organization of nucleus and cytoplasm leading to the formation of nuclear-cytoplasmic domains, defined by pattern of microtubules distribution (Brown et al. 1994), accompanied by changes in nucleus shape from spherical to elliptical (Brown et al. 1996).

The phase characterized by lack of mitotic divisions directly precedes an increase in DNA content caused by en-

doreduplication (Brown et al. 1994). In embryo sack of 2 mm in length, in endosperm characterized by lack of mitoses and significant domination of 3C and 6C DNA nuclei, 12C and even 24C DNA nuclei (5.2%) were observed, which indicates the endoreduplication. An increase in number of 12C and 24C DNA nuclei, as well as the appearance of 48C nuclei on 2nd day after pollination indicate transition from mitosis to endoreduplication cycle (Sun et al. 1999). Moreover, the size of endoreduplicated nuclei was at least twice as big as that of the dominating ones, which is related to multiplication of genome (Kowles and Phillips 1985). At the successive stages of maize endosperm development, nuclear DNA content increases reaching 96C and even 192C DNA in the oldest examined phase (7 mm) with simultaneous disappearance of nuclei with low DNA content. This phenomenon was described by several authors (Kowles and Phillips 1985; Ramachandran and Raghavan 1989; Marciniak 1991, 1993). In maize, 16 days after pollination 56% of cells contain poliploid nuclei (Kowles et al. 1997). Endoreduplication and increase in size of nuclei following high mitotic activity were also observed in dicotyledonous species (Borisjuk et al. 1995), and progression of DNA content changes accompanies consecutive endosperm development phases both in dicotyledonous species (Marciniak 1991) and the monocotyledonous ones (Kowles et al. 1990; Marciniak 1993; Cavallini et al. 1995). Data obtained by flow cytometry significantly correlate with those of Feulgen microspectrophotometry (Johnston et al. 1999).

In older maize endosperm, starting from embryo sack of 3.5 mm in length, strands and aggregates of chromatin stain strongly in many nuclei different in size and shape, however, these structures are much more common in embryo sacks of 7 mm in length. Among interphase endosperm nuclei some of them contain condensed chromatin (Kowles and Phillips 1988) while others dispersed chromatin, and the latter being more numerous. The presence of many aggregates of chromatin and its distinctive strands most probably result from polytenization of endosperm nuclei (Kowles and Phillips 1985; Phillips et al. 1985) and the presence of numerous mitotic figures with DNA content reaching even 24C DNA clearly indicates the possibility of mitotic divisions in nuclei with multiplied DNA level. The presence of metaphases with multiplied number of distinct chromosomes was documented and described in *Echinocistis lobata* endosperm (Turała 1966). Chromosome-like structures in the central part of barley endosperm were described even 20 days after anthesis (Giese 1992). The presence of polyploid mitoses in the examined maize endosperm in embryo sack of 1.8 mm in length and mostly in sacks of 2.5 and 3 mm in length points out to the fact that proliferation and endoreduplication processes do not always occur in a definite order. The examination of mitotic index and DNA content in maize endosperm on successive post-pollination days, indicates that a rapid decrease in mitotic divisions occurs concomitantly with the increase in DNA content (i.e. about 10-12 days after pollination) (Kowles and Phillips 1988).

In conclusion, cytological and cytochemical analyses reported in this paper indicate that mitotic divisions and multiplied DNA content may occur simultaneously and dominate one over another at different phases of endosperm development.

Fig. 5A-C: mitotic figures in developing endosperm, A – 3 mm length embryo sac endosperm; B – 3.5 mm length embryo sac endosperm; C – 4 mm length embryo sac endosperm; D – mitotic figure in embryo; E-H – nuclei with granular and stranded chromatin organisation in endosperm tissue; E and F – 4 mm length embryo sac endosperm; G – 4.5 mm length embryo sac endosperm; H – 7 mm length embryo sac endosperm.

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AKTYWNOŚĆ MITOTYCZNA I ZMIANY ZAWARTOŚCI JĄDROWEGO DNA PODCZAS ROZWOJU BIELMA *ZEa MAYS*

STRESZCZENIE

Badano aktywność mitotyczną oraz zawartość jądrowego DNA w rozwijającym się bielmie *Zea mays*, cv. Złota Karłowa. Zawartość DNA mierzono cytofotometrycznie na preparatach gniecionych po zastosowaniu procedury Feulgena. Stwierdzono, że aktywność mitotyczna w bielmie występuje aż do czasu, kiedy woreczek zalążkowy osiąga 4,5 mm długości. Część figur mitotycznych wykazuje zwielokrotnioną zawartość DNA. W jądrach bielmowych zawartość jądrowego DNA jest różna i wzrasta w miarę rozwoju tkanki. Wzrost poziomu DNA wskazuje na postępującą endoreduplikację. Część jąder o wysokim poziomie DNA wykazuje zmiany struktury chromatyny wyrażające się obecnością silnie barwiących się pasm i grudek chromatynowych. Wyciągnięto wniosek, że okres występowania podziałów mitotycznych i faza endoreduplikacji jądrowego DNA mogą zachodzić jednocześnie i dominować w różnych okresach rozwoju bielma.

SŁOWA KLUCZOWE: bielmo, aktywność mitotyczna, endoreduplikacja, zawartość DNA, *Zea mays*.