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. Zlota Karlowa 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 characterized 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 Niemirowicki-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 (Orlowa 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 starchy 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, Melaaragno 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. Zlota Karlowa) 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 1 mm 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 multiplication 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).

Cytophometric 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

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<th>TABLE 1. DNA content in endosperm telophase and prophase nuclei of Zea mays.</th>
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<td>Endosperm sack diameter (mm)</td>
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Fig. 1. Mitotic activity in developing embryo and endosperm of Zea mays. Ordinate: number of mitotic nuclei (%), abscissa: length of embryo sack (mm).
with 24C DNA was found (Table 1). In older endosperm, prophaes and telophases 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 (Oryol 1991). An embryo sack grows quickly and in *Triticum aestivum* in endosperm 250 µm long the larger part of the central cell is already cellularized (Oryol and Shmarayev 1987). Our study shows that in mai-
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 (μm²), abscissa: DNA content (AU).

In older maize endosperm, starting from embryo sac 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 sacs 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 much more numerous. The presence of many aggregates of chromatin and its distinctive strands most probably result from polytenization of endosperm nucleus (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 Echinoctis lobata endosperm (Turaša 1966). Chromosome-like structures in the central part of barley endosperm were described even 20 days after anthesis (Giese 1992). The presence of polyplid mitoses in the examined maize endosperm in embryo sac of 1.8 mm in length and mostly in sacs 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).
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.
LITERATURE CITED


AKTYWNOŚĆ MITOTYCZNA I ZMIANY ZAWAROŚCI JĄDROWEGO DNA PODCZAS ROZWOJU BIELMA *ZEA MAYS*  

STRENCZCZENIE  

Badano aktywność mitotyczną oraz zawartość jądrowego DNA w rozwijającym się bielmie *Zea mays*, cz. *Zo- 
hta Karkowa. Zawartość DNA mierzono cytofotometrycznie na preparatach gniecionych po zastosowaniu procedu- 
ry Feulgena. Stwierdzono, że aktywność mitotyczną w bielmiu występuje aż do czasu, kiedy woreczek zalążkowy osiąga 4,5 mm długości. Część figur mitotycznych wykazuje zwięciochronioną zawartość DNA. W jądrach biel- 
mowych zawartość jądrowego DNA jest różna i wzrasta w miarę rozwoju tkanki. Wzrost poziomu DNA wskazu- 
je na postępującą endoreduplikację. Część jąder o wysokim poziomie DNA wykazuje zmiany struktury chromaty- 
ny wyrażające się obecnością silniej barwiających się pasmi i grudek chromatynowych. Wyciągnięto wniosek, że 
okres występowania podziałów mitotycznych i faza endoreduplikacji jądrowego DNA mogą zachodzić jednocze-
śnie i dominować w różnych okresach rozwoju bielma.

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