ELSEVIER

Journal of Cereal Science 43 (2006) 209–215

Journal of CEREAL SCIENCE

www.elsevier.com/locate/jnlabr/yjcrs

Development of the endosperm of *Sorghum bicolor* during the endoreduplication-associated growth phase

Aleš Kladnik [a], Prem S. Chourey [b,c], Daryl R. Pring [b,c], Marina Dermastia [a,*]

[a] *Department of Biology, Biotechnical Faculty, University of Ljubljana, Vecna pot 111, SI-1000 Ljubljana, Slovenia*
[b] *Department of Plant Pathology and Agronomy, University of Florida, Gainesville, FL 32611-0680, USA*
[c] *United States Department of Agriculture, Agricultural Research Service, Gainesville, FL 32611-0680, USA*

Received 13 July 2005; received in revised form 5 September 2005; accepted 8 September 2005

Abstract

Spatial occurrence of endoreduplication, a variant of the cell cycle resulting in endopolyploidy, was investigated in the developing sorghum caryopsis between 5 and 16 DAP (days after pollination). This was a period of intense mitotic and endoreduplication-associated endosperm growth. Endopolyploidy was quantitatively analyzed on median caryopsis sections using image densitometry that provided in situ cytometrical data. In the endosperm, the first endopolyploid nuclei with a nuclear DNA content of 12C (where 1C represents the nuclear DNA content of a non-replicated haploid genome) were detected at 5 DAP. In subsequent days progressively higher levels of endopolyploidy occurred, and nuclei with the highest amount of DNA (96C) were first observed at 10 DAP. The highly endopolyploid nuclei were located only in the central region of the endosperm and their occurrence coincided with the onset of starch deposition in the endosperm. Cells with non-endopolyploid nuclei (3C and 6C) were found exclusively in the peripheral layers of the endosperm. No starch was observed in the basal part of the endosperm where the highest level of endopolyploidy was 24C. The volume of endosperm nucleai and cells showed a positive correlation with the level of endopolyploidy. Endoreduplication was also prominent in the pericarp, where the highest level of endopolyploidy was 16C.

Keywords: Endoreduplication; Endosperm; Grain; Pericarp; Sorghum bicolor

1. Introduction

Sorghum (*Sorghum bicolor* (L.) Moench), is a major cereal crop plant and is evolutionarily closely related to maize (Swigoňová et al., 2004). As in all cereal species, the caryopsis is composed of three major parts: the pericarp (seed coat), the endosperm and the embryo.

The pericarp is derived from the ovary wall and adheres strongly to the seed coat of the ovule (Evers and Millar, 2002). In sorghum and rice, the pericarp is the major site of starch deposition during first days after pollination (DAP) and before starch deposition in the endosperm (Earp et al., 2004; Sato, 1984). By maturity all starch disappears and the cells in which it was present are squashed or broken down. Exceptions to this are some varieties of sorghum where cells and starch granules persist until maturity (Evers et al., 1999).

In flowering plants, the result of double fertilization is a diploid zygote and a triploid primary endosperm cell. The latter subsequently develops into a storage tissue, the endosperm, which is structurally adapted to ensure efficient translocation of nutrients from the sporophyte to the developing embryo. At maturity, the cereal endosperm is composed of five specialized cell types: the central starchy endosperm, the subaleurone layer, the aleurone layer, the basal endosperm transfer layer and the embryo-surrounding region (Olsen et al., 1999). Endosperm development is determinate and includes syncytial and cellular phases (Berger, 2003; Costa et al., 2004; Olsen et al., 1999). In sorghum in the syncytial phase, as many as 28 nuclei were observed 8 h after pollination (Paulson, 1969). About 48 h after pollination, the syncytial endosperm is cellularised, and this is followed by a period of intense mitotic activity (Berger, 2003; Costa et al., 2004; Olsen et al., 1999; Paulson, 1969). The outer cells of developing cereal endosperm give rise to the aleurone layer (Olsen et al., 1999). In sorghum, the aleurone layer consists of roughly rectangular cells that are much smaller than in the rest of the

Abbreviations: DAP, days after pollination; IOD, integrated optical density.

* Corresponding author. Tel.: +386 1 423 33 88; fax: +386 1 257 33 90.
E-mail address: marina.dermastia@bf.uni-lj.si (M. Dermastia).

0733-5210/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jcs.2005.09.004

endosperm. Small, elongated cells also occur at the base of the sorghum endosperm (Artschwager and McGuire, 1949).

In maize the endosperm cells undergo endoreduplication, resulting in highly endopolyploid nuclei in the starchy endosperm cells (Kowles and Phillips, 1985; Kowles et al., 1990; 1997; Larkins et al., 2001; Vilhar et al., 2002) and aleurone cells in barley (Keown et al., 1977). Endoreduplication is a variant of the cell cycle, in which chromosomal DNA is replicated without intervening mitotic division, leading to endopolyploid nuclei with polytene chromosomes (Joubès and Chevalier, 2000). The resulting larger, endopolyploid nuclei are often, but not always, associated with an increase in cell size (Leiva-Neto et al., 2004; Sugimoto-Shirasu and Roberts, 2003; Vilhar et al., 2002). Nevertheless, in maize, cells with highly endopolyploid nuclei occupy a major part of the volume of the starchy endosperm (Vilhar et al., 2002). Central endosperm cells in sorghum caryopsis have very large nuclei with diameters often measuring 15 μm (Artschwager and McGuire, 1949).

Understanding the development of cereal grains and particularly, the distribution of functionally- and nutritionally-important components, is important if the utilization of the grain is to be optimized. In this study we demonstrate a technique which allows analyses to be performed on tissue sections, combining information on spatial distribution of various cytological parameters at the light microscope level. Using image cytometry we demonstrate that caryopsis development in sorghum is similar to that of maize and other cereals. We show the spatial and temporal occurrence of endoreduplication in different parts of sorghum caryopsis between 5 and 16 DAP, the period of intense mitotic and endoreduplication-associated growth of the endosperm. Additionally, we demonstrate a relationship between starch accumulation and endoreduplication.

2. Experimental

2.1. Preparation of tissue sections

Sorghum (*Sorghum bicolor* (L.) Moench) samples were prepared using the male-sterile line A3T×7000 and pollination with B3T×7000. Flowering proceeds basipetally in sorghum and about four days are required for style emergence to the base of the panicle (Pring and Tang, 2004). The line A3T×7000 was pollinated when flowering was complete at the base of the panicle. The pollination results in 100% fertilization at the fourth day and thus is in synchrony when samples were collected over time. Developing caryopses were collected at different days after pollination (DAP) and immediately fixed in cold FAA fixative (3.7% formaldehyde, 5% acetic acid, 50% ethanol) for 24 h, followed by dehydration in series of ethanol and tertiary butyl alcohol and embedding in Paraplast Plus (Sherwood Medical Co., St Louis, MO, USA). Paraffin embedded caryopses were sectioned to 12–20 μm thick sections (depending on the development stage) on a rotary microtome (Microm 325, Carl Zeiss, Germany).

2.2. Staining for starch with I_2/KI

Tissue sections were dewaxed in xylene and rehydrated in an ethanol series to water, stained in aqueous solution of 2% iodine and 3% potassium iodide (I_2/KI) for 1 min, washed in water, dehydrated quickly through the ethanol series to xylene and mounted with DPX (Fisons Scientific Equipment, Loughborough, England).

2.3. Image analysis

The image analysis system consisted of the Axioskop 2 MOT microscope (Carl Zeiss, Jena, Germany) with the KS400 software package (Carl Zeiss Vision, Hallbergmoos, Germany) and either a video CCD camera (DXC-950P, Sony, Tokyo, Japan) for DNA measurements or a digital color camera (AxioCam MRc, Carl Zeiss Vision, Hallbergmoos, Germany) for measurements of cell size and pericarp thickness.

2.4. Measurement of pericarp thickness and number of cell layers

Pericarp thickness and numbers of cell layers were measured on median longitudinal tissue sections of sorghum caryopses imaged using UV excitation. Both parameters were measured on the dorsal side of the caryopsis, away from the embryo, in three different caryopses for each developmental stage. Pericarp thickness was measured perpendicularly to the pericarp surface at its thickest part and the number of cell layers was counted in the same region.

2.5. Measurement of nuclear DNA and nuclear volume

The nuclear DNA was measured by image densitometry using the interphase-peak method (Vilhar et al., 2001; Vilhar and Dermastia, 2002) adapted for use with tissue sections (Kladnik et al., 2004; Vilhar et al., 2002). Longitudinal sections of caryopses were dewaxed in xylene, rehydrated through an ethanol series to water, hydrolysed in 5 M HCl for 75 min at 20 °C, stained with Schiff's reagent for 120 min at 20 °C, washed for 45 min in six changes of 0.5% potassium metabisulphite in 50 mM HCl, dehydrated and mounted in DPX (Fisons Scientific Equipment, Loughborough, England). Integrated optical density (IOD) and coordinates of the nuclei were measured on a series of grayscale images of caryopses recorded with a 40× objective. IOD is linearly related to the amount of DNA and was used to estimate the relative amount of DNA in individual nuclei. The nuclear amount of DNA was expressed in C-value units, where 1C represented the nuclear DNA content of a non-replicated haploid genome. The volume of a nucleus was estimated as the volume of a sphere based on the area of projection of a nucleus in the *z*-axis of the tissue section.

2.6. Measurement of cell volume

The size of cells was measured on the median longitudinal sections of caryopses, according to Vilhar et al. (2002); Kladnik et al. (2004) using a 10× objective. Cell walls were imaged using their auto-fluorescence with UV excitation and interactively outlined. The area of the cell's transect and the coordinates of cell centers in the tissue section. The volume of a cell was estimated as the volume of a sphere based on the area of the cell transect. Endopolyploidy data were linked with the volume of cells using the nearest neighbor method developed for maize endosperm (Vilhar et al., 2002).

2.7. Estimation of total number and volume of endosperm cells

The total number and volume of cells in the different endopolyploidy classes were estimated using the 3D model of the endosperm developed for maize (Vilhar et al., 2002).

3. Results

3.1. Endopolyploidy and number of endosperm cells

Between the second DAP, when the endosperm was already cellular (data not shown), and the fifth DAP the caryopsis was primarily filled with nucellar tissue and the endosperm enlarged much more rapidly than the embryo (Fig. 1). There was a steep increase in the number of endosperm cells between 8 and 10 DAP (Table 1, Fig. 2A), indicating an exponential growth curve of the endosperm. Afterwards growth slowed to a stationary phase between 12 and 16 DAP.

The first endopolyploid nuclei with a 12C DNA content were observed at 5 DAP, and they were scattered in the basal and apical part of the endosperm (Fig. 1). Two more endoreduplication cycles were completed by 8 DAP, giving rise to 24C and 48C cells. The highest level of endopolyploidy in the endosperm nuclei was 96C and occurred for the first time at 10 DAP (Figs. 1, 3 and 4). A constant number of cells between 12 and 16 DAP indicated cell division had ceased by 12 DAP (Table 1, Fig. 2A). Thus by 12 DAP the pool of nuclei that participated in endoreduplication was effectively fixed in size. Therefore, the progression of nuclear DNA to higher C-values resulted in a decreased number of cells with 3C and 6C nuclear DNA (Fig. 2A).

Because endopolyploid cells are not known to undergo mitosis (Barlow, 1978; Larkins et al., 2001; Schweizer et al., 1995), we divided the endosperm into a putative mitotic cell population (i.e. 3C and 6C cells) and a population that had entered the endoreduplication cycle (i.e. cells with *C*-values higher than 6C), (Fig. 3). At 5 DAP, most cells in the endosperm were 3C and 6C cells, but at 8 DAP, such cells were restricted to the outermost layers. Nevertheless, the number of presumptively dividing cells increased until 12 DAP and slightly decreased thereafter, but at 16 DAP still represented 54% of all endosperm cells. The peak of mitotic activity during 8 and 12 DAP coincided with four completed endoreduplication cycles and the number of endopolyploid cells subsequently increased in the central part of the endosperm (Figs. 3, 2A).

The endoreduplication started in the nuclei of the central starchy endosperm cells and proceeded basally and outward (Figs. 1 and 3). Endoreduplication was not detected in the aleurone layer. In the endosperm transfer layer close to the placental sac (Maness and McBee, 1986) no more than one endoreduplication cycle (i.e. 12C) was observed (Figs. 1 and 3). In the basal part of the endosperm, cells had lower levels of endopolyploidy as compared to the central starchy endosperm (Fig. 3).

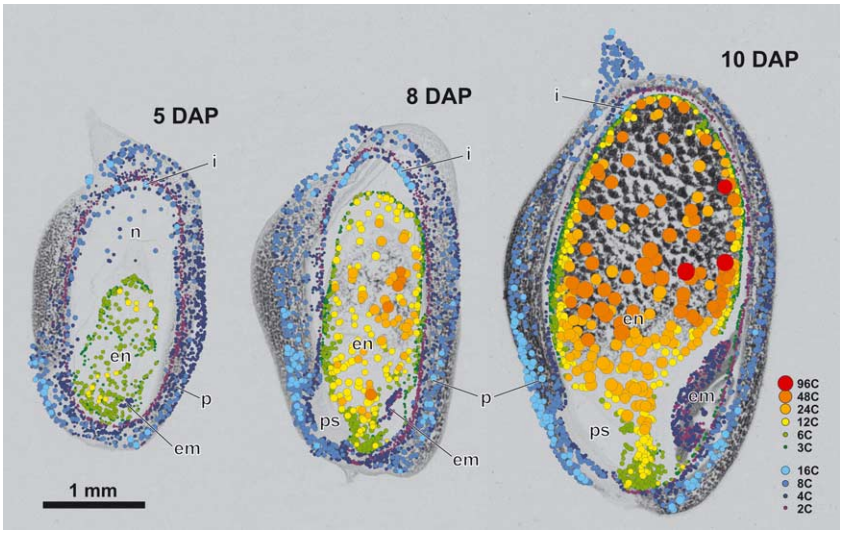

Fig. 1. Endopolyploidy and starch content in the sorghum caryopsis. The bubble graph, representing the nuclei of different *C*-value classes, is superimposed on the images of iodine-potassium iodide stained longitudinal sections of caryopses. Measurements of the DNA content and staining for starch were performed on the different sections of the same caryopsis, therefore the graphs and images are not completely aligned. The bubble diameters are linearly related to the diameters of the nuclei in the tissue sections. em, embryo; en, endosperm; i, inner integument; n, nucellus; p, pericarp; ps, placental sac.

Table 1
Total number of cells in the endosperm and endosperm volume at different stages of development of *Sorghum bicolor* caryopsis

Days after pollination	Total number of cells[a]	Endosperm volume (mm^3)[a]
5	59,228±3749	0.371±0.007
8	89,932±13,288	1.329±0.177
10	207,482±28,891	4.295±0.983
12	263,164±8490	5.339±0.497
16	279,820±3487	7.603±0.839

[a] Data are mean±standard error of three caryopses for each developmental stage, calculated from median longitudinal sections of caryopses with the 3D model of the maize endosperm (Vilhar et al., 2002).

3.2. Endopolyploidy and endosperm volume

The total endosperm volume, estimated using a 3D model (Vilhar et al., 2002), increased from 0.4 mm^3 at 5 DAP to almost 7.6 mm^3 at 16 DAP. In contrast, between 8 DAP and 16 DAP, the total volume of the peripheral layers with 3C and 6C cells increased by only 1.5-fold. In the same period, the volume of the central endosperm with 12C and higher endopolyploid cells increased 8.5-fold (Figs. 3, 2B). Although 48C and 96C cells represented only 8% of all endosperm cells (Fig. 2A), they occupied more than 44% of the endosperm volume at 12 and 16 DAP (Fig. 2B). Moreover, by 16 DAP, 90% of the total endosperm volume consisted of 12C and higher endopolyploid cells (Fig. 2B).

Both nuclear and cell volumes increased with an increasing level of endopolyploidy (Fig. 4) but remained relatively constant at the same endopolyploidy level, regardless of developmental stage. Similar constancy was found in both the diploid cells of the embryo as well as the initially triploid cells of the endosperm. At 5 DAP, the volume of 2C and 4C embryo nuclei equaled the volume of 3C and 6C nuclei in the endosperm (Fig. 4A). However, at the same time, the volume of both 2C and 4C cells in the embryo was smaller than that of 3C cells in the endosperm (Fig. 4B). The analysis of variance (ANOVA) of cell and nuclear volumes in different endopolyploidy classes revealed that endopolyploidy explains about 65% of the overall variance in endosperm cell volume and even 95% in nuclear volume at the later stages of development (Table 2).

3.3. Correlation between the endopolyploidy and starch deposition

The first detectable amount of endosperm starch was observed at 8 DAP and after pollination and its content increased until 10 DAP (Fig. 1) and further (data not shown). The location of starch accumulation correlated with highly endopolyploid cells. Notably, starch deposition was observed

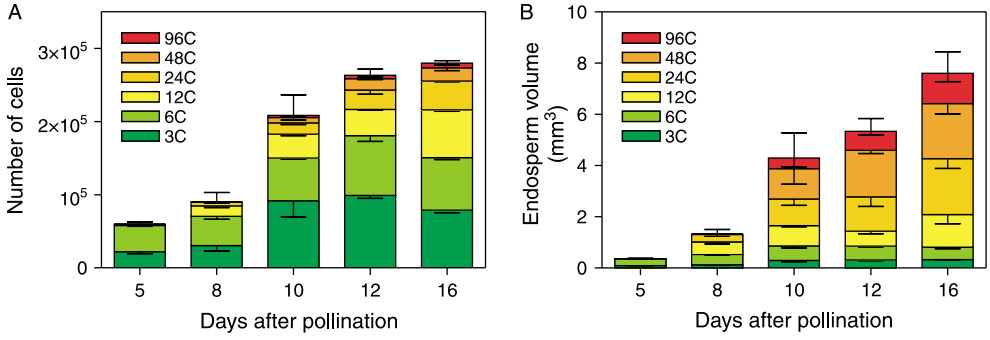

Fig. 2. The total number of endosperm cells belonging to different *C*-value classes (A) and the volume of the endosperm comprised of the cells of different *C*-value classes (B). The downward pointing error bars are standard errors for the mean number of cells/endosperm volume in each *C*-value class and the upward pointing error bars are the standard errors for the mean number of all endosperm cells/total endosperm volume in three caryopses at each developmental stage. Data were calculated from the 3D model of the endosperm (Vilhar et al., 2002).

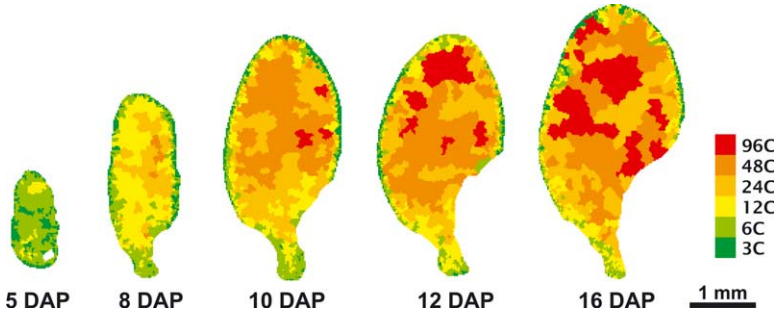

Fig. 3. Distribution of cells of different ploidy classes in sorghum endosperm from 5 to 16 DAP. Because the nuclear DNA amount was not measured in all endosperm cells, the *C*-value classes were assigned to all endosperm cells using the nearest neighbor method from the 3D model of the endosperm (Vilhar et al., 2002).

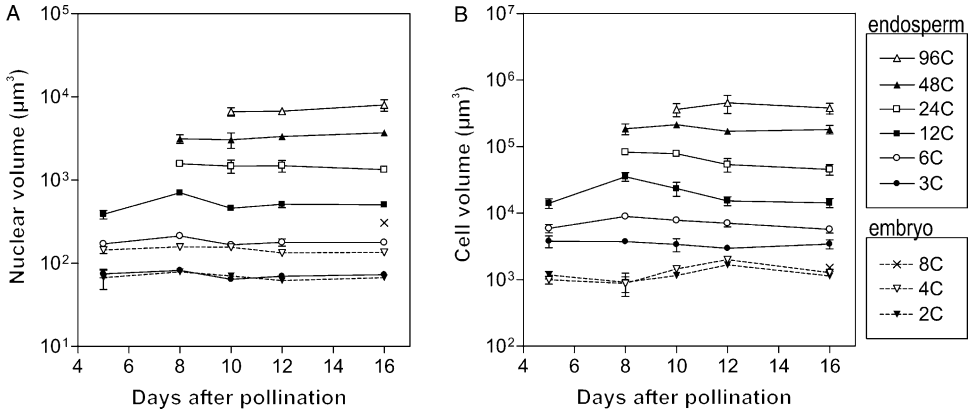

Fig. 4. Volume of nuclei and cells in the endosperm and embryo of *Sorghum bicolor*. Mean volume of nuclei of different ploidy levels ± standard error ($N=3$) at different developmental stages, shown on a logarithmical scale (A). Mean volume of cells associated with nuclei of different ploidy levels ± standard error ($N=3$) at different developmental stages, shown on a logarithmical scale (B).

in parts of the endosperm where 48C and 96C nuclei were prevalent. No starch was detected at the base of the endosperm, which contained cells with an endopolyploidy level 24C and lower (Fig. 1).

3.4. Structure of the maternal tissues of the caryopsis

At 5 DAP pericarp cells were mainly isodiametric and arranged in about 20 cell layers (Fig. 5). The number of cell layers remained relatively constant throughout the period of caryopsis development examined (Fig. 5). The thickness of the pericarp at the dorsal side of the caryopsis at 5 DAP was about 385 µm and increased to about 500 µm at 8 DAP due to cell enlargement. After 10 DAP (Fig. 5) the pericarp cells started to elongate in the apical-basal direction of the caryopsis and compress in the transverse direction, resulting in a decreased thickness of the pericarp to about 315 µm at 16 DAP. The pericarp already contained a significant amount of starch at 5 DAP and the density of staining for starch increased as the caryopsis developed (Fig. 1).

The developing endosperm completely displaced the nucellus between 8 and 10 DAP, as reported by Paulson (1969), (Fig. 1). Between the endosperm and inner integumental cells, a prominent cuticular layer known as the hyaline layer (Evers and Millar, 2002; Paulson, 1969) was visible (Fig. 6). Whether the cuticular layer derives from the nucellar epidermis or from the integument (Evers and Millar, 2002; Paulson, 1969) remains to be clarified.

3.5. Endopolyploidy in the maternal tissues of the caryopsis

At 5 DAP, the pericarp cells contained nuclei with 2C to 16C DNA. Throughout the pericarp most of the nuclei were 4C. Two C nuclei were located in the cell layers close to the endosperm, while 8C and a few 16C nuclei were located primarily in the apical and basal part of the pericarp. The endoreduplication cycles continued until 8 DAP, resulting in a higher proportion of 8C and 16C nuclei. After 8 DAP, 2C cells were disposed in a ring in the innermost pericarp layer, 4C cells were scattered throughout the pericarp tissue, 8C cells dominated in the outer pericarp layers and 16C cells were relatively frequent in the basal part of the pericarp (Fig. 1).

The cells of the inner integument that are elongated in the direction perpendicular to the ovule surface (Fig. 6), completed at least one endoreduplication cycle before 5 DAP, resulting in predominantly 8C cells. At 8 DAP the inner integument in the apical part of the caryopsis was composed mainly of cells with 8C and 16C nuclear DNA. After 10 DAP the number of inner integument cells decreased (Fig. 1).

A large proportion of the nucellus was already degraded at 5 DAP, and the remaining cells contained nuclei with 4C and 8C DNA. Further degradation continued until 8 DAP when no nuclei were observed (Fig. 1).

4. Discussion

Although detailed morphological studies of the sorghum caryopsis can be traced to the first part of 20th century (Artschwager and McGuirre, 1949 and references therein), little is known about the role of endoreduplication during sorghum caryopsis development. Considering the close evolutionary links between sorghum and maize (Swigoňová et al., 2004), the absence of endoreduplication investigation in sorghum is noteworthy, as maize endosperm is generally used as a model for the mechanism of plant endoreduplication and the study of physiological consequences in a seed storage tissue (Larkins et al., 2001).

Table 2
The portion of variation in the volume of cells and nuclei in the endosperm of *Sorghum bicolor* explained by endoreduplication, expressed as the R^2 value from one-way analysis of variance of cell and nuclei volume of different ploidy classes

Developmental stage	5 DAP[a] (%)	8 DAP[b] (%)	10 DAP[c] (%)	12 DAP[c] (%)	16 DAP[c] (%)
R^2 cell volume	8	43	62	67	64
R^2 nuclei volume	55	83	90	92	94

[a] The number of ploidy classes was: three at 5 DAP (3C–12C).
[b] The number of ploidy classes was: five at 8 DAP (3C–48C).
[c] The number of ploidy classes was: six at 10–16 DAP (3C–96C).

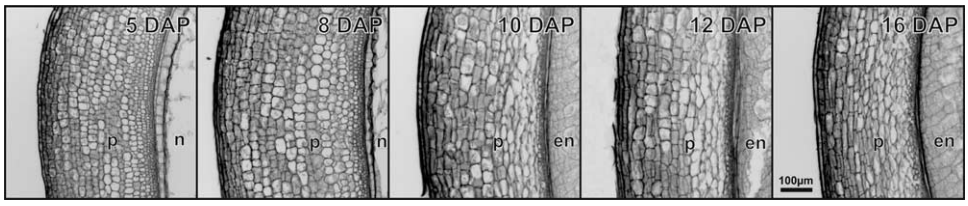

Fig. 5. Pericarp in the caryopsis of *Sorghum bicolor* from 5 to 16 days after pollination (DAP). Shown is the dorsal part of the caryopsis, away from embryo, where pericarp is the thickest. Images of the median longitudinal sections of caryopses were taken using UV excitation and presented as grayscale negatives. en, endosperm; n, nucellus; p, pericarp.

We show here the first evidence of endoreduplication in developing sorghum caryopsis. The profile of endoreduplication in sorghum endosperm is similar to the maize endosperm described previously by cytometry (Vilhar et al., 2002) and by flow cytometry (Kowles and Phillips, 1985; Kowles et al., 1990). Endosperm enlargement relies upon two cellular processes, cell division and cell growth; the latter may be related to the endoreduplication of nuclear DNA (Sugimoto-Shirasu and Roberts, 2003). A positive correlation between the amount of nuclear DNA and cell volume was very clear at all stages of development. Both nuclear and cell volumes were constant amongst all *C*-value classes (Fig. 4). The only exceptions were the volumes of 12C nuclei and cells at 8 DAP, which were higher than other volumes in this *C*-value class (Fig. 4). The higher volumes of 12C nuclei and cells at 8 DAP coincided with the first occurrence of higher endopolyploidy classes (Fig. 4) and the beginning of the cell division peak (Fig. 2A). It is noteworthy that this is also the stage at which starch deposition was initiated (Fig. 1). After 8 DAP, starch accumulated only in that part of the endosperm where cells with 48C DNA and higher were observed. The nuclei of these cells had proportionally very large volumes (Fig. 4A), as reported previously (Artschwager and McGuire, 1949). Endoreduplication and the associated increased cellular volume may thus provide better efficiency of storage in starchy endosperm cells, as less volume is occupied by cell walls and membranes. Additionally, the tissue expands without mitoses that temporarily disrupt cell structure and gene expression (Barlow, 1978). In both sorghum and maize endoreduplication precedes starch accumulation but in maize endoreduplication begins at 10 DAP (Kowles and Phillips, 1985; Schweizer et al., 1995; Tsai et al., 1970) while in sorghum it begins at 5 DAP. However, in contrast with the suggestion that endoreduplication is a mechanism for increasing cell size and gene expression (Larkins et al., 2001), the lower level of endopolyploidy in a transgenic maize with reduced endoreduplication did not affect cell size and only slightly reduced starch accumulation (Leiva-Neto et al., 2004).

Endoreduplication was also demonstrated in other parts of the caryopsis, particularly in the pericarp. To our knowledge this is the first report of endoreduplication in this tissue. However, in contrast to the highly endopolyploid endosperm cells, at most two endoreduplication cycles were completed in pericarp cells, resulting in a nuclear DNA content of 16C. In sorghum, the pericarp is the major site of starch deposition during the first days after pollination and before starch deposition in the endosperm (Earp et al., 2004). It is evident from this study that starch accumulated in parts of the pericarp where cells with nuclear DNA content 4C and higher prevailed.

To conclude, advances in microscopical techniques and data reconstruction presented here revealed details of the endosperm development which cannot be determined using bulk analytical methodology. The results of our study proved the similarities in maize and sorghum development and they show that cell size and endoreduplication are correlated in sorghum endosperm. Moreover, endoreduplication correlated with starch deposition in endosperm and pericarp, but it is currently not known if the underlying control mechanisms are the same in both tissues.

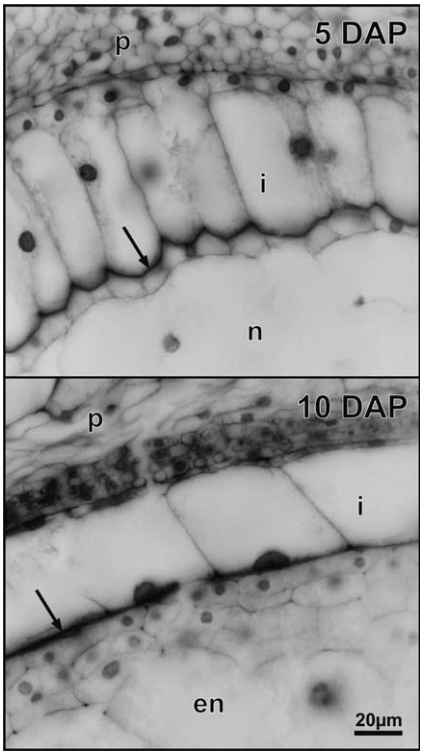

Fig. 6. Inner integument and the hyaline layer in caryopsis of *Sorghum bicolor* 5 and 10 days after pollination (DAP). Shown is the apical part of the caryopsis. Images of the median longitudinal sections of caryopses were taken using UV excitation and presented as grayscale negatives. Arrows point to the hyaline layer between the inner integument and nucellus epidermis. At 10 DAP the nucellus is completely replaced by the endosperm. en, endosperm; i, inner integument; n, nucellus; p, pericarp.

Acknowledgements

The authors thank Ms Shayna Sutherland and Dr Hoang Tang for technical assistance. They also thank Dr Carol Ritzen Kem for critical reading of the manuscript. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the US department of Agriculture. This was a cooperative investigation of the US Department of Agriculture—Agricultural Research Service, Institute of Food and Agricultural Science, University of Florida and the University of Ljubljana, Slovenia (Slovenian Research Agency, grant No. P1-0212). This paper is Florida Agricultural Experiment Station Journal Series Number R-10995.

References

Artschwager, E.R., McGuire, C., 1949. Cytology of reproduction in *Sorghum vulgare*. Journal of Agricultural Research 78, 659–673.

Barlow, P.W., 1978. Endopolyploidy—towards an understanding of its biological significance. Acta Biotheoretica 27, 1–18.

Berger, F., 2003. Endosperm, the crossroad of seed development. Current Opinion in Plant Biology 6, 42–50.

Costa, L.M., Gutierrez-Marcos, J.F., Dickinson, H.G., 2004. More than a yolk: the short life and complex times of the plant endosperm. Trends in Plant Science 9, 507–514.

Earp, C.F., McDonough, C.M., Rooney, L.W., 2004. Microscopy of pericarp development in the caryopsis of *Sorghum bicolor* (L.) Moench. Journal of Cereal Science 39, 21–27.

Evers, T., Millar, S., 2002. Cereal grain structure and development: some implications for quality. Journal of Cereal Science 36, 261–284.

Evers, A.D., Blakeney, A.B., O'Brien, L., 1999. Cereal structure and composition. Australian Journal of Agricultural Research 50, 629–650.

Joubès, J., Chevalier, C., 2000. Endoreduplication in higher plants. Plant Molecular Biology 43, 735–745.

Keown, A.C., Taiz, L., Jones, R.L., 1977. The nuclear content of developing barley aleurone cells. American Journal of Botany 64, 1248–1253.

Kladnik, A., Vilhar, B., Chourey, P.S., Dermastia, M., 2004. Sucrose synthase isozyme SUS1 in the maize root cap is preferentially localized in the endopolyploid outer cells. Canadian Journal of Botany 82, 96–103.

Kowles, R.V., Phillips, R.L., 1985. DNA amplification patterns in maize endosperm nuclei during kernel development. Proceedings of the National Academy of Sciences of the United States of America 82, 7010–7014.

Kowles, R.V., Srienc, F., Phillips, R.L., 1990. Endoreduplication of nuclear DNA in the developing maize endosperm. Developmental Genetics 11, 125–132.

Kowles, R.V., Yerk, G.L., Haas, K.M., Phillips, R.L., 1997. Maternal effects influencing DNA endoreduplication in developing endosperm of *Zea mays*. Genome 40, 798–805.

Larkins, B.A., Dilkes, B.P., Dante, R.A., Coelho, C.M., Woo, Y., Liu, Y., 2001. Investigating the hows and whys of DNA endoreduplication. Journal of Experimental Botany 355, 183–192.

Leiva-Neto, J.T., Grafi, G., Sabelli, P.A., Dante, R.A., Woo, Y., Maddock, S., Gordon-Kamm, W.J., Larkins, B.A., 2004. A dominant negative mutant of cyclin-dependent kinase A reduces endoreduplication but not cell size or gene expression in maize endosperm. The Plant Cell 16, 1854–1869.

Maness, N.O., McBee, G.G., 1986. Role of placental sac in endosperm carbohydrate import in sorghum caryopses. Crop Science 26, 1201–1207.

Olsen, O.-A., Linnestad, C., Nichols, S.E., 1999. Developmental biology of the cereal endosperm. Trends in Plant Science 4, 253–257.

Paulson, I.W., 1969. Embryogeny and caryopsis development of *Sorghum bicolor* (L.) Moench. Crop Science 9, 97–102.

Pring, D.R., Tang, H.V., 2004. Transcript profiling of male-fertile and male-sterile sorghum indicates extensive alterations in gene expression during microgametogenesis. Sexual Plant Reproduction 16, 289–297.

Sato, K., 1984. Starch granules in tissues of rice plants and their changes in relation to plant growth. Japan Agricultural Research Quarterly 18, 79–86.

Schweizer, L., Yerk-Davis, G.L., Phillips, R.L., Srienc, F., Jones, R.J., 1995. Dynamics of maize endosperm development and DNA endoreduplication. Proceedings of the National Academy of Sciences of the United States of America 92, 7070–7074.

Sugimoto-Shirasu, K., Roberts, K., 2003. Big it up: endoreduplication and cell-size control in plants. Current Opinion in Plant Biology 6, 544–553.

Swigoňová, Z., Lai, J., Ma, J., Ramakrishna, W., Llaca, V., Bennetzen, J.L., Messing, J., 2004. Close split of sorghum and maize genome progenitors. Genome Research 14, 1916–1923.

Tsai, C.Y., Salamini, F., Nelson, O.E., 1970. Enzymes of carbohydrate metabolism in the developing endosperm of maize. Plant Physiology 46, 299–306.

Vilhar, B., Dermastia, M., 2002. Standardisation of instrumentation in plant DNA image cytometry. Acta Botanica Croatica 61, 11–26.

Vilhar, B., Greilhuber, J., Dolenc Koce, J., Temsch, E.M., Dermastia, M., 2001. Plant genome size measurement with DNA image cytometry. Annals of Botany 87, 719–728.

Vilhar, B., Kladnik, A., Blejec, A., Chourey, P.S., Dermastia, M., 2002. Cytometrical evidence that the loss of seed weight in the *miniature1* seed mutant of maize is associated with reduced mitotic activity in the developing endosperm. Plant Physiology 129, 23–30.