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Nuclear DNA synthesis during the induction of embryogenesis in cultured microspores and pollen of *Brassica napus* L.

P. Binarova [1, 2], K. Straatman [1], B. Hause [1], G. Hause [1], A. A. M. van Lammeren [1]

[1] Department of Plant Cytology and Morphology, Wageningen Agricultural University, Arboretumlaan 4, 6703 BD Wageningen, The Netherlands
[2] Institute of Experimental Botany, Czech Academy of Sciences, Sokolovska 8, 77200 Olomouc, Czech Republic

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Abstract. The dynamics of nuclear DNA synthesis were analysed in isolated microspores and pollen of *Brassica napus* that were induced to form embryos. DNA synthesis was visualized by the immunocytochemical labelling of incorporated Bromodeoxyuridine (BrdU), applied continuously or as a pulse during the first 24 h of culture under embryogenic (32 °C) and non-embryogenic (18 °C) conditions. Total DNA content of the nuclei was determined by microspectrophotometry. At the moment of isolation, microspore nuclei and nuclei of generative cells were at the G1, S or G2 phase. Vegetative nuclei of pollen were always in G1 at the onset of culture. When microspores were cultured at 18 °C, they followed the normal gametophytic development; when cultured at 32 °C, they divided symmetrically and became embryogenic or continued gametophytic development. Because the two nuclei of the symmetrically divided microspores were either both labelled with BrdU or not labelled at all, we concluded that microspores are inducible to form embryos from the G1 until the G2 phase. When bicellular pollen were cultured at 18 °C, they exhibited labelling exclusively in generative nuclei. This is comparable to the gametophytic development that occurs in vivo. Early bicellular pollen cultured at 32 °C, however, also exhibited replication in vegetative nuclei. The majority of vegetative nuclei re-entered the cell cycle after 12 h of culture. Replication in the vegetative cells preceded division of the vegetative cell, a prerequisite for pollen-derived embryogenesis.

Key words: *Brassica napus* – BrdU – Embryogenesis – Microspore and pollen culture – DNA synthesis

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Correspondence to: A. A. M. van Lammeren

Introduction

In higher plants, microspores undergo an ordered sequence of mitotic cell divisions which lead to the formation of pollen grains consisting of a vegetative cell and two sperm cells that are committed to specialized functions. The formation of embryos from microspores and pollen represents a fundamental switch in this development. Several studies on microspores and pollen at various developmental stages have shown that discrete developmental windows exist in which microspores and pollen become embryogenic in response to culture conditions. The re-entering of the cell cycles of the almost differentiated cells and the switch to embryogenic development were studied recently in tobacco pollen cultures. Using [^{3}H]-thymidine incorporation under embryogenic conditions Zarsky et al. (1992) showed that replication in the vegetative nucleus leads to embryo development.

Morphological studies in *Brassica napus* have indicated that embryogenesis can be induced in late microspores and early binucleate pollen (Fan et al. 1988; Pechan and Keller 1988; Telmer et al. 1992; Hause et al. 1993). The development of embryos from symmetrically divided microspores of *B. napus* has been studied in detail (Zaki and Dickinson 1990, 1991), but less attention has been paid to the development of embryos from early bicellular pollen. From immunocytochemical studies on the cytoskeleton and the behaviour of nuclei as visualized by 4,6-diamidino-2-phenylindole (DAPI), it is well-known that changes in the cytoskeletal patterning interact with deviating patterns of nuclear divisions in cultured late microspores and early bicellular pollen (Hause et al. 1992, 1993). In order to understand the early events of microspore- and pollen-derived embryogenesis in more detail we studied nuclear DNA synthesis in microspores and pollen during the first 24 h of culture using the im-

munolabelling of incorporated Bromodeoxyuridine (BrdU). This method has been successfully applied in plants for studying nuclear DNA synthesis in tissues, cultured cells and protoplasts (Levi et al. 1987; Pfosser 1989; Wang et al. 1989; Stroobants et al. 1990; Wang et al. 1991). The application of short pulses of BrdU allowed us to analyse the dynamics of replication in vegetative and generative nuclei.

Material and methods

Plant material

Plants of *Brassica napus* L. cv 'Topas' were first grown for 4 weeks under greenhouse conditions at 18 °–23 °C followed by a low temperature treatment at 10 °C in the light (300 µmol photons $m^{-2}s^{-1}$ for 16 h) and at 5 °C in the dark (8 h) until the onset of flowering. Flower buds, 3.2–3.8 mm long, were harvested from the terminal raceme.

Cultivation of microspores and pollen

Microspores and pollen were isolated as described by Pechan and Keller (1988). They were cultured in the dark at a density of 2×10^4 ml^{-1} in NLN medium (Lichter 1982) with 13% sucrose and free of potato extract, pH 6.0, at 18 °C (non-embryogenic condition) and 32 °C (embryogenic conditions). Two days later, the cultures incubated at 32 °C were transferred to 25 °C. The numbers of embryos were counted after 3 weeks.

Two types of cultures were used to determine DNA synthesis during the first 24 h of culture under embryogenic and non-embryogenic conditions. The first culture was isolated from flower buds with sizes ranging from 3.2 to 3.4 mm and consisted of a mixture of microspores (Type A culture). The second culture (Type B culture) consisted mainly of late microspores, mitotic microspores and early bicellular pollen isolated from buds with sizes ranging from 3.6 to 3.8 mm.

BrdU labelling

Pulse labelling and continuous labelling with the thymidine substitute BrdU were applied to microspore and pollen cultures within the first 24 h of culture under embryogenic and non-embryogenic conditions. The BrdU labelling solution (supplied by Amersham) was added to the cultures at final concentrations of 1:500.

The viability of the cells was tested with fluorescein diacetate (FDA, Heslop-Harrison and Heslop-Harrison 1970) directly after the pulse labelling to determine the influence of increasing periods of BrdU incubation. The remaining cells were analysed for BrdU incorporation. Control cultures without BrdU were also analysed for viability.

The influence of BrdU on the embryogenicity of the culture was tested by adding BrdU to the cultures immediately after isolation, either for a period of 1 h or for 24 h. The numbers of embryos in the two cultures were counted 3 weeks after BrdU removal and compared with control cultures.

Two variants of BrdU labelling were used: (1) cells were continuously labelled for 4, 8, 12, 16 and 24 h of culture, and (2) cells were pulse-labelled in the last hour of culture period of 1, 4, 8, 12, 16 and 24 h. All of the experiments described were repeated at least twice.

Immunocytochemistry

Samples of microspores and pollen from BrdU-labelled and control cultures were fixed immediately after the labelling for 1.5 h in 3.5% paraformaldehyde in phosphate-buffered saline (PBS) supplemented with 0.1% Triton X-100. After fixation, the samples were rinsed, dehydrated, embedded in polyethylene glycol (PEG) and sectioned according to Van Lammeren et al. (1985). Sections were mounted on poly-L-lysine-coated slides, treated with 0.1 *M* NH_4Cl and washed twice with PBS. The last washing was done with 0.1% BSA in PBS. The sections were then incubated for 1 h with anti-BrdU monoclonal antibody (Amersham) containing nuclease. After rinsing in PBS, the secondary antibody goat anti-mouse IgG BODIPY (Molecular Probes) was applied at a dilution of 1:100, and the sections were incubated for 45 min. Both incubations were done in the dark at 26 °C. For DNA staining, the same slides were incubated in DAPI solution (0.01 mg/l) for 10 min, then washed in PBS and covered with Citifluor-glycerol (Citifluor Ltd., London). The fluorescence of BrdU-labelled and DAPI-stained nuclei was visualized with a Nikon Microphot epifluorescence microscope using the proper filters for DAPI and BODIPY. Black-and-white images were recorded on Kodak TMY 135-film.

The percentage of microspores and pollen grains having labelled nuclei was determined from a total number of 250–300 microspores and pollen grains for each sample in at least two independent experiments.

Microspectrophotometry

For the microspectrophotometric analysis control cells were collected immediately after isolation from the flower buds used for the Type A and Type B experiments. From the Type B culture, samples were also collected after 24 h of culture under embryogenic conditions. The cells were fixed in ethanol-acetic acid (3:1) and stored at −20 °C. Feulgen staining was performed according to Dolezel (1989), with hydrolysis in 5 mol l^{-1} HCl at 25 °C for 25 min. The slides were then stained for 60 min in Schiff reagent prepared according to Lilie (1951) using parafuchsin (Serva). Afterwards, the slides were washed by three changes of SO_2^- water, graded alcohols and xylene. Cover-slips were mounted with Depex (Serva). The amount of DNA was measured by mirror scanning cytophotometry with a Leitz MPV-3 microspectrophotometer interfaced to a microcomputer with a Nucleiscan programme (Dolezel 1989) taking erythrocytes as an internal standard. The reference 1C value was given by the early microspores obtained from the Type A and B culture.

Results

BrdU incorporation and effects on viability after short pulse labelling

The minimal pulse length needed to detect DNA synthesis was determined by adding BrdU to cultured microspores for 10, 20, 30, 45 and 60 min at the end of a 12-h culture period at 32 °C. The shortest pulse of BrdU which enabled the detection of the S-phase in microspores and pollen was 30 min (Fig. 1 a,a'). A 1-h pulse labelling was sufficient to show replication in generative, vegetative and microspore nuclei. It was exceptional that progression through the cell cycle from S phase to mitosis was observed in microspores within a 1-h pulse of BrdU labelling (Fig. 1 b,b'). Bromodeoxyuridine pulses of 1 h had no influence on the viability of the culture, but a continuous BrdU labelling during 24 h caused a 10–20% decrease in viability. Embryogenicity was not changed

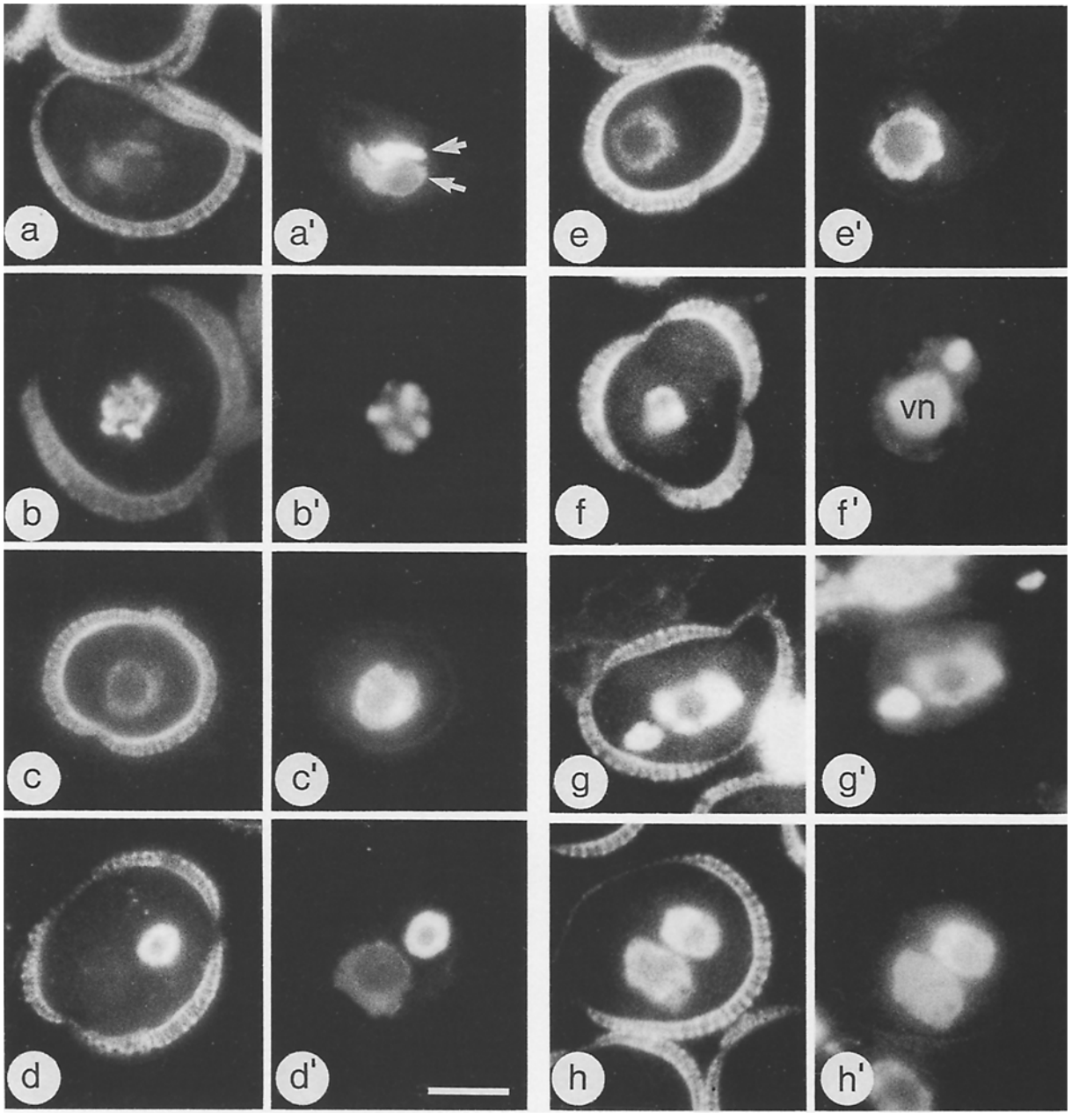
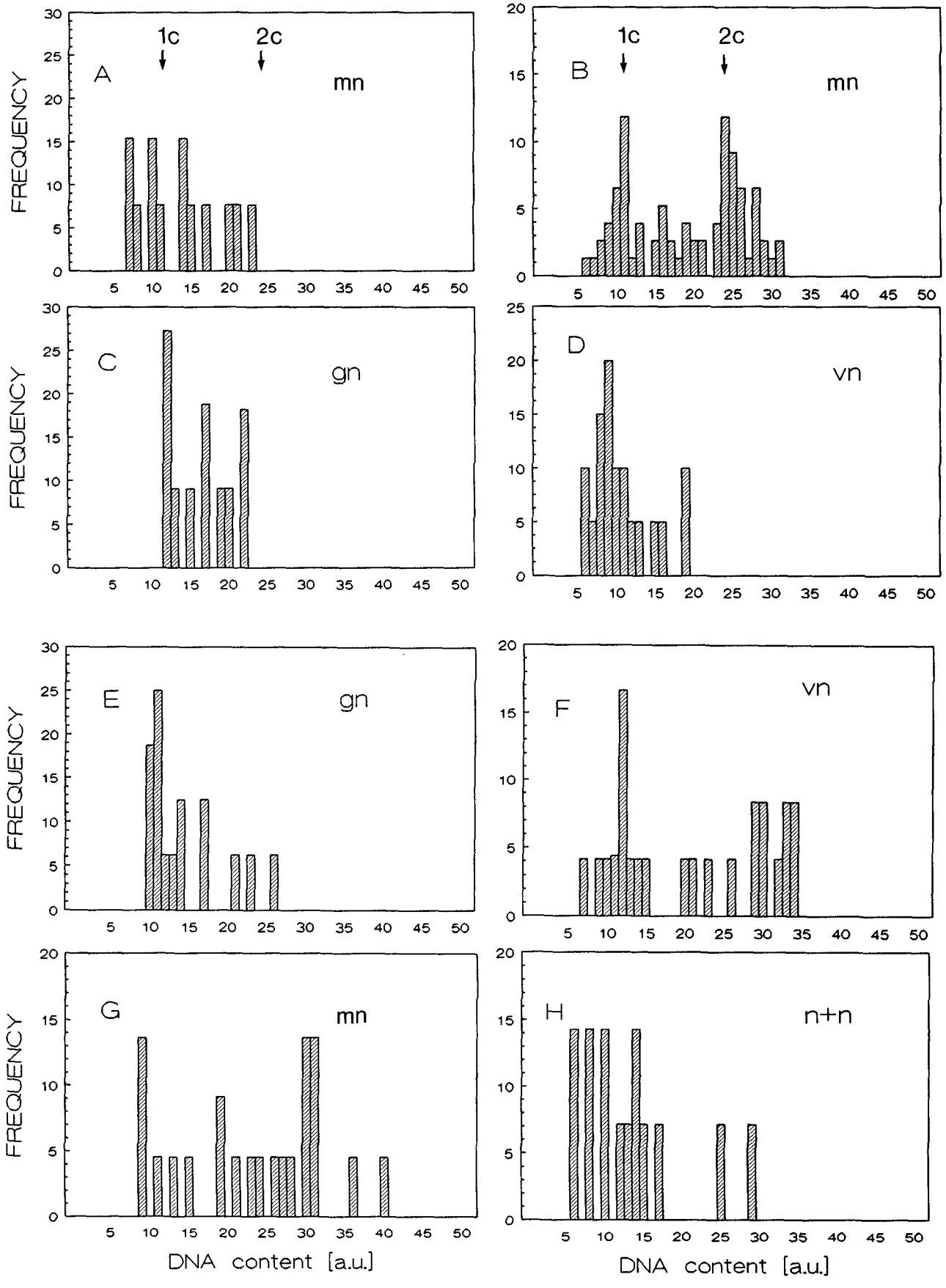


Fig. 1 a–h. Fluorescence micrographs of semi-thin sectioned microspores and pollen of *Brassica napus* cultured under embryogenic (32 °C) and non-embryogenic (18 °C) conditions. **a–h** show the incorporation of BrdU, labelled with BODIPY; **a′–h′** depict the position of the nuclei in the same cells stained with DAPI. *Bar* represents 10 μm for all micrographs. **a–a′** Two-cellular structure from Type A culture, grown at 32 °C for 12 h, exhibits fluorescence in the two nuclei after a 30-min BrdU pulse; **b–b′** microspore grown at 32 °C for 8 h shows labelling in the prophase nucleus after a 1-h BrdU pulse; **c–c′** late microspore from Type A culture, grown at 18 °C for 4 h with BrdU, shows labelling in its nucleus; **d–d′** pollen from Type B culture, grown at 18 °C for 4 h with BrdU, exhibits labelling in the generative nucleus only; **e–e′** microspore from Type B culture, grown at 32 °C for 4 h with BrdU, shows labelling in the nucleus; **f–f′** pollen from Type B culture, grown at 32 °C for 1 h in the presence of BrdU, shows labelling in the vegetative nucleus (*vn*); **g–g′** pollen from Type B culture, grown at 32 °C for 12 h with a 1-h BrdU pulse, exhibits labelling in both the vegetative and the generative nucleus; **h–h′** symmetrical division in Type B culture, grown at 32 °C for 12 h, exhibits labelling in the two nuclei after a 1-h BrdU pulse


A
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after a 1-h pulse labelling, but 24 h of continuous labelling reduced the number of embryos by 8% to 17%.

Quantification of nuclear DNA by microspectrophotometry

Analysis of the Type A culture showed that predominantly microspores were present (Table 1). This mixture of microspores had DNA contents corresponding to values from 1C up to 2C at the onset of culture. Microspores with C values between 1 and 2 were in the S-phase (Fig. 2A). Analysis of the Type B culture revealed that it contained 30% microspores and 70% bicellular pollen (Table 2). The microspores had 1C to 2C DNA contents at the time of isolation (Fig. 2B). Late microspores were in G2. Bicellular pollen contained vegetative nuclei with DNA contents of about 1C at the time of isolation (Fig. 2D). Generative nuclei showed 1C levels and higher DNA contents, indicating further progress through the cell cycle up to G2 (Fig. 2C).

After 24 h of culture under embryogenic conditions, a portion of the vegetative nuclei of the bicellular pollen reached the G2 phase of the cell cycle (Fig. 2F). Some nuclei showed DNA contents higher than 2C at that time. The generative nuclei were predominantly in G1, but some did reach G2 within 24 h of culture (Fig. 2E). Microspores which had been cultured for 24 h exhibited a range of C values from about 1C up to about 3C (Fig. 2G). Daughter cells formed by the symmetrical division of microspores were often at the G1 phase. The DNA contents indicated that some daughter nuclei progressed through the S phase and reached the G2 phase (Fig. 2H), and others exhibited progression through the cell cycle simultaneously.

Differences in nuclear DNA synthesis in Type A and B cultures

Type A culture

At the onset of culture the Type A culture consisted of a mixture of microspores at early and late developmental stages. Table 1 gives an overview of both, the developmental fate of these microspores from the onset of culture up to a period of 24 h and the incorporation of BrdU as a signal for replicative DNA synthesis during the first 24 h under embryogenic and non-embryogenic conditions. DAPI-stained nuclei exhibited a shift from the late microspore stage to the early bicellular pollen stage (up to 18% of the cells) within 24 h when cultured at 32 °C. Embryo formation in Type A cultures was less than 0.5%.

Under non-embryogenic conditions 0.8% of the microspores replicated DNA during an 8-h labelling period. Figure 1c,c′ is an example of such labelling after 4 h of incubation. After 24 h of continuous labelling up to 1.8% of the microspores and newly formed pollen were labelled (Table 1). Incorporation only occurred in nuclei of late microspores and in generative nuclei at the bicellular pollen stage.

Under embryogenic conditions the percentage of microspores and pollen with BrdU-labelled DNA increased from 0.6% after 4 h of continuous labelling to 4.4% after 24 h of continuous BrdU labelling. Replicating nuclei were mostly observed in late microspores; only a few pollen with labelled vegetative nuclei were found (Table 1).

Type B culture

The Type B culture consisted initially of a mixture of late microspores, mitotic microspores and early bicellular pollen (Table 2). Relative to the Type A culture, embryo formation in the Type B culture was much higher, up to 6.5%. Table 2 gives an overview of both the developmental fate of the microspores and pollen from the onset of culture up to a period of 24 h and the incorporation of BrdU as a signal for nuclear DNA synthesis during the first 24 h under embryogenic and non-embryogenic conditions.

Under non-embryogenic conditions (18 °C), BrdU incorporation was always observed in the late microspore stage, but the majority of the replicating nuclei were the generative nuclei in middle or late binucleate pollen (Fig. 1d,d′).

DNA synthesis under embryogenic conditions (32 °C) was much higher. The total number of labelled nuclei increased from 4% after continuous labelling for 4 h to about 15% after 24 h of BrdU labelling. An example of a labelled microspore nucleus is given in Fig. 1e,e′. The rate of entrance of microspores into the S phase was constant during the 24-h period.

The beginning of DNA synthesis in the vegetative nucleus of bicellular pollen was observed as early as 1 h

Fig. 2A–H. Frequency histograms of Feulgen-stained nuclei of freshly isolated (**A–D**) and cultured (**E–H**) microspores and pollen of *Brassica napus*. DNA contents are measured by cytophotometry and expressed in arbitrary units (*a.u.*) Culture was in the embryogenic condition for 24 h. n = number of analysed nuclei. In A and B the reference 1C and 2C values of DNA are indicated by *arrows* in the microspore populations from Type A and B cultures. **A** Microspore nuclei (*mn*) from Type A isolation ($n=13$); **B** microspore nuclei (*mn*) from Type B isolation ($n=76$); **C** generative nuclei (*gn*) of pollen from Type B isolation ($n=11$); **D** vegetative nuclei (*vn*) of pollen from Type B isolation ($n=20$); **E** generative nuclei (*gn*) of pollen from Type B culture ($n=16$); **F** vegetative nuclei (*vn*) of pollen from Type B culture ($n=24$); **G** microspore nuclei (*mn*) from Type B culture ($n=22$); **H** nuclei ($n+n$) after symmetrical division ($n=14$)

Table 1. Nuclear DNA synthesis in isolated microspores of *B. napus* during the first 24 h of culture under non-embryogenic and embryogenic conditions. Cells of Type A culture were labelled with BrdU continuously (C) or for 1 h at the end of the cultivation period

Developmental stage of microspores and pollen (%)						
	EMs	MMs +LMs	M!	EB	MB +LB	Σ
After isolation	24	74	–	2	–	100
24 h in culture at 32 °C	4.3	77.7	–	18	–	100

Culture under non-embryogenic conditions (18 °C)								
Period of labelling	% labelled cells							T
	LMs	M!	BC				Σ	
			g	g+v	v	n+n		
4 h C	–	–	–	–	–	–	–	250
4 h 1 h	–	–	–	–	–	–	–	235
8 h C	0.8	–	–	–	–	–	0.8	285
8 h 1 h	–	–	–	–	–	–	–	218
16 h C	1.7	–	0.4	–	–	–	2.1	295
16 h 1 h	0.8	–	–	–	–	–	0.8	235
24 h C	1.4	–	0.4	–	–	–	1.8	284
24 h 1 h	0.4	–	–	–	–	–	0.4	211

Culture under embryogenic conditions (32 °C)								
Period of labelling	% labelled cells							T
	LMs	M!	BC				Σ	
			g	g+v	v	n+n		
4 h C	0.6	–	–	–	–	–	0.6	305
4 h 1 h	0.4	–	–	–	–	–	0.4	251
8 h C	1.7	–	–	–	–	–	1.7	235
8 h 1 h	0.4	–	–	–	–	–	0.4	261
16 h C	2.0	–	–	–	0.6	–	2.6	291
16 h 1 h	0.4	–	–	–	–	–	0.4	245
24 h C	3.2	–	–	0.4	0.8	–	4.4	254
24 h 1 h	0.8	–	–	–	0.4	–	1.2	250

Abbreviations: BC, Bicellular structure; EB, early bicellular structure; EMs, early microspores; g, generative nucleus; LB, late bicellular structure; LMs, late microspores; M!, mitosis; MB, mid-bicellular structure; MMs, mid-microspores; *n*, nucleus of symmetrically divided microspore; T, total number of analysed cells; Σ, sum of percentages; *v*, vegetative nucleus

after culture (Fig. 1 f,f'). In this case no labelling was found in the generative nucleus. Pollen in which the generative and the vegetative nuclei were labelled were also observed (Fig. 1 g,g' for 8 h of culture). Their number increased only twice after 24 h of culture. The highest

Table 2. Nuclear DNA synthesis in isolated microspores and bicellular pollen of *B. napus* during the first 24 h of culture under non-embryogenic and embryogenic conditions. Cells of Type B culture were labelled with BrdU continuously (C) or for 1 h at the end of the cultivation period. For abbreviations see Table 1

Developmental stage of microspores and pollen (%)						
	EMs	MMs +LMs	M!	EB	MB +LB	Σ
After isolation	3.5	26	7.5	53	10	100
24 h in culture at 32 °C	1	17	0	24	58	100

Culture under non-embryogenic conditions (18 °C)								
Period of labelling	% labelled cells							T
	LMs	M!	BC				Σ	
			g	g+v	v	n+n		
4 h C	0.4	–	0.8	–	–	–	1.2	250
4 h 1 h	0.4	–	–	–	–	–	0.4	231
8 h C	1.3	–	2.7	–	–	–	4.1	294
8 h 1 h	–	–	0.7	–	–	–	0.7	280
16 h C	1.4	–	5.9	–	–	–	7.3	287
16 h 1 h	–	–	0.7	–	–	–	0.7	290
24 h C	1.3	–	10.3	–	–	–	11.6	224
24 h 1 h	–	–	1.04	–	–	–	1.04	287

Culture under embryogenic conditions (32 °C)								
Period of labelling	% labelled cells							T
	LMs	M!	BC				Σ	
			g	g+v	v	n+n		
4 h C	1.6	–	1.2	0.8	0.4	–	4.0	250
4 h 1 h	0.4	–	0.8	0.4	1.3	–	2.9	239
8 h C	1.9	–	0.4	2.9	1.9	–	7.9	208
8 h 1 h	0.4	0.4	0.8	0.4	0.4	–	2.4	237
16 h C	2.2	0.3	1.6	1.2	3.5	2.2	11.0	318
16 h 1 h	0.4	–	0.4	0.4	1.2	0.4	2.9	237
24 h C	1.3	–	2.3	1.7	5.0	4.9	15.2	297
24 h 1 h	0.8	–	0.3	–	1.2	–	1.8	287

number of labelled vegetative cells was found after 24 h of continuous BrdU labelling. Pollen with replicating vegetative nuclei often showed a configuration with the generative cell arrested near the intine and clearly separated from the vegetative cell. The incorporation patterns observed after 1 h of BrdU pulse often showed a high heterogeneity in labelling in vegetative nuclei as compared to the homogenous labelling found in generative nuclei. Symmetrically or almost symmetrically divided

microspores with labelled DNA in both nuclei were often observed from 12 h of culture onwards (Fig. 1 h,h'; Table 2). Both nuclei were labelled after continuous incubation with BrdU and incidentally after 1 h of pulse labelling. On the other hand, we also found symmetrically divided microspores in which the daughter nuclei were not labelled after a continuous BrdU treatment for 8 or 16 h.

Discussion

The application of BrdU pulses enables the visualization of nuclear DNA replication (Lacy et al. 1991). Pulse labelling clearly provides information on the dynamics of DNA synthesis, whereas continuous labelling visualizes total DNA synthesis.

Type A and Type B cultures were analysed because they consisted of two different populations of microspores and pollen. In the Type A culture, which contained mainly microspores, replication appeared only at low percentages and no symmetrical divisions were found although a portion of the microspores were in a late stage at the onset of the culture. This corresponds to results of Telmer et al. (1992). The Type B culture consisted of a mixture of late microspores, mitotic cells and up to 53% young bicellular pollen, and was most appropriate to obtain high yields of embryos.

Microspores and pollen cultured for 24 h under non-embryogenic conditions exhibited DNA replication in the nuclei of late microspores and in the nuclei of generative cells of middle and late bicellular pollen. Vegetative nuclei did not show DNA replication. These results are comparable with those expected during gametophytic development and are in agreement with the data of Aruga et al. (1982) and Zarski et al. (1992). Thus, 18 °C is an acceptable control state at which embryogenesis does not occur.

Microspore-derived embryogenesis

Microspore-derived embryogenesis starts with symmetrical divisions induced in late microspores (Zaki and Dickinson 1991; Hause et al. 1993). In the present investigation the daughter nuclei were sometimes labelled and sometimes unlabelled. When they were labelled, either the BrdU was incorporated in the DNA during the S phase of the microspore or the daughter nuclei progressed through the cell cycle simultaneously. The former explanation implies that microspores were at S phase or still in G1. The latter explanation is possible because it was often observed that the two daughter nuclei were labelled after a 1-h BrdU pulse; 1 h is too short for the progression of the cell cycle from the microspore S phase, via mitosis, to G1 of the bicellular structure. Some symmetrically divided microspores had unlabelled nuclei in cultures even though BrdU was present continuously. This indicates that microspores which are isolated in G2 can also give rise to embryogenesis. So it can be concluded that microspores can be induced to enter the embryogenic pathway from G1, during S phase, up to G2. We sometimes observed microspores with C values larger than 2 (Fig. 2 G). This is most likely caused by endoreduplication within the microspore nucleus, which has also been demonstrated in microspore cultures of *Zea mays* (Pretova et al. 1993).

Pollen-derived embryogenesis

When isolated bicellular pollen were cultured at 32 °C, vegetative nuclei could enter the S phase within 1 h. This observation shows that the re-entering of the cell cycle is an immediate response to the high temperature and might be of great importance to our understanding the initial changes that occur during development. Many vegetative nuclei were replicating DNA after 12 h of culture at 32 °C. These data together with the quantitative determinations of DNA contents at the onset of culture and after incubation at 32 °C indicate that the vegetative nucleus in *B. napus* is arrested in the G1 phase in vivo. It re-enters the cell cycle within the induction period at 32 °C. Similarly, Aruga et al. (1982) and Zarsky et al. (1992) confirmed that the vegetative nuclei of pollen of *Nicotiana tabacum* are arrested in G1. Contrary to these results, De Paepe et al. (1990) found that the DNA content of the vegetative nuclei of pollen from *Nicotiana sylvestris* corresponds to the G2 phase of the cell cycle.

Bicellular pollen with labelled generative nuclei appeared early in culture at 32 °C. They were probably isolated in the middle or late bicellular stage of pollen development and are not competent to switch to the developmental pathway (Telmer et al. 1992).

Replication in the vegetative nucleus was sometimes preceded by DNA replication in the generative nucleus. Similarly, Zarsky et al. (1992) observed in tobacco that replication in the generative nucleus was first completed, and only then followed by DNA replication in the vegetative cell when induced to embryogenesis by starvation. As compared to tobacco pollen embryogenesis, the replicating vegetative nuclei of *B. napus* were more often found together with non-labelled nuclei of generative cells. Generative cells were often attached to the intine, a characteristic of embryogenic development in bicellular pollen (Hause et al. 1993). Vegetative nuclei exhibiting DNA contents up to 3C most likely represent examples of endoreduplication. This pathway is not expected to give rise to embryogenesis because cell division is absent (see also Pretova et al. 1993).

High temperature treatment in Type B cultures resulted in up to 6.5% embryos after 3 weeks of culture, whereas up to 15.2% of the microspore and pollen population

(Table 2) exhibited BrdU labelling within 24 h. It should be realized that the labelling of late microspores (1.3%) and generative cells (2.3%) not necessarily leads to embryo formation. So at least 11.6% of the cells changed DNA replication in the embryogenic direction. As it was observed that multicellular structures stopped further development regularly (B. Hause, unpublished), probably because of concurrence or disturbed endogenic regulation, it is understandable that the eventual percentage of embryos is lower than 11.6.

It can be concluded that our qualitative and quantitative analysis of nuclear DNA synthesis revealed the dynamics of the replication with respect to microspore- and pollen-derived embryogenesis. Embryogenic cultures can be started with microspores from late G1 to G2 phase. Vegetative cells of pollen always have to re-enter the cell cycle before embryogenesis can occur.

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