Seed Science Research (2009) **19**, 15–25 doi:10.1017/S0960258508186275

INVITED REVIEW

Nuclear DNA replication and seed quality

Elwira Sliwinska*

Department of Genetics and Plant Breeding, University of Technology and Life Sciences, al. Kaliskiego 7, 85-789 Bydgoszcz, Poland

Abstract

The quality of a seed (germination and vigour) is established during its development and maturation, but can be improved by post-harvest processing and pre-sowing treatments. During commercial seed production, maturity is usually estimated visually, relying on experience of the growers, but seed researchers are working to find molecular markers that can be applied easily to help in establishing optimal harvest time. One marker is cell cycle activity expressed as DNA replication in the seeds, analysed by flow cytometry. This fast and accurate method for the estimation of DNA content in plant nuclei allows detection of nuclei at different replication stages in different seed tissues and thus makes it possible to follow changes in the physiological state of a seed. DNA replication, as a late event during germination, can also be used to mark completion of germination and transition to early seedling growth. This information can be useful in the evaluation of seed quality and for following the advancement of priming. Flow cytometric analysis of ploidy can be also used as a basis for control of purity of some polyploid species seed lots.

Keywords: cell cycle, embryo, endoreduplication, endosperm, flow cytometry, nuclear DNA content

Introduction

The present-day seed market is very competitive; farmers require seed lots with a germination capacity close to 100% and high vigour. At the same time, one of the problems for seed producers is the lack of reliable, simple molecular markers that can predict after-sowing seed performance and which are faster than assessing germination and vigour using laboratory tests. Such markers could also be used for monitoring seed priming, a pre-sowing treatment commonly used for the enhancement and improvement of germination. Several germination/priming molecular markers, mainly proteins, such as β-tubulin (de Castro *et al.*, 1995), the β-subunit of 11-S globulin and LEA (late embryogenesis abundant) proteins (Job *et al.*, 1997; Capron *et al.*, 2000), the AUBE1 (a protein binding to the P1 promoter of the *RPL21* gene; Achard *et al.*, 2002) and others (Gallardo *et al.*, 2001) have been proposed as being of potential value. DNA replication also is an indicator of the transition of the seed to Phase II of germination (Bewley and Black, 1994), and therefore can be considered as an appropriate marker.

Flow cytometry is a fast and accurate method for estimating nuclear DNA content. It involves preparation of aqueous suspensions of intact nuclei, the DNA of which is stained using a DNA-specific fluorochrome. The result of the analysis is usually displayed in the form of a histogram of the relative fluorescence intensity, representing relative DNA content (see Fig. 1) (for a review see Doležel and Bartoš, 2005). The method can be used to study the cell cycle in seeds by isolating nuclei from the tissues of the embryo and/or endosperm (Bino *et al.*, 1993; Sliwinska, 2006). The information obtained can be used for determining the physiological state of a seed during its development, maturation, processing and germination, and therefore can be useful for monitoring the production of high-quality commercial seed lots. Different mitotic/endoreduplication activities occur in seeds at different developmental stages. Proliferating cells of the embryo pass through four stages in the mitotic cycle: G_1, the period of cell growth during which a nucleus possesses a 2C DNA content (where C = DNA content of a holoploid genome with chromosome number *n*, irrespective of the degree of plant ploidy); S, DNA replication phase which results

*Correspondence
Email: elwira@utp.edu.pl

https://doi.org/10.1017/S0960258508186275 Published online by Cambridge University Press

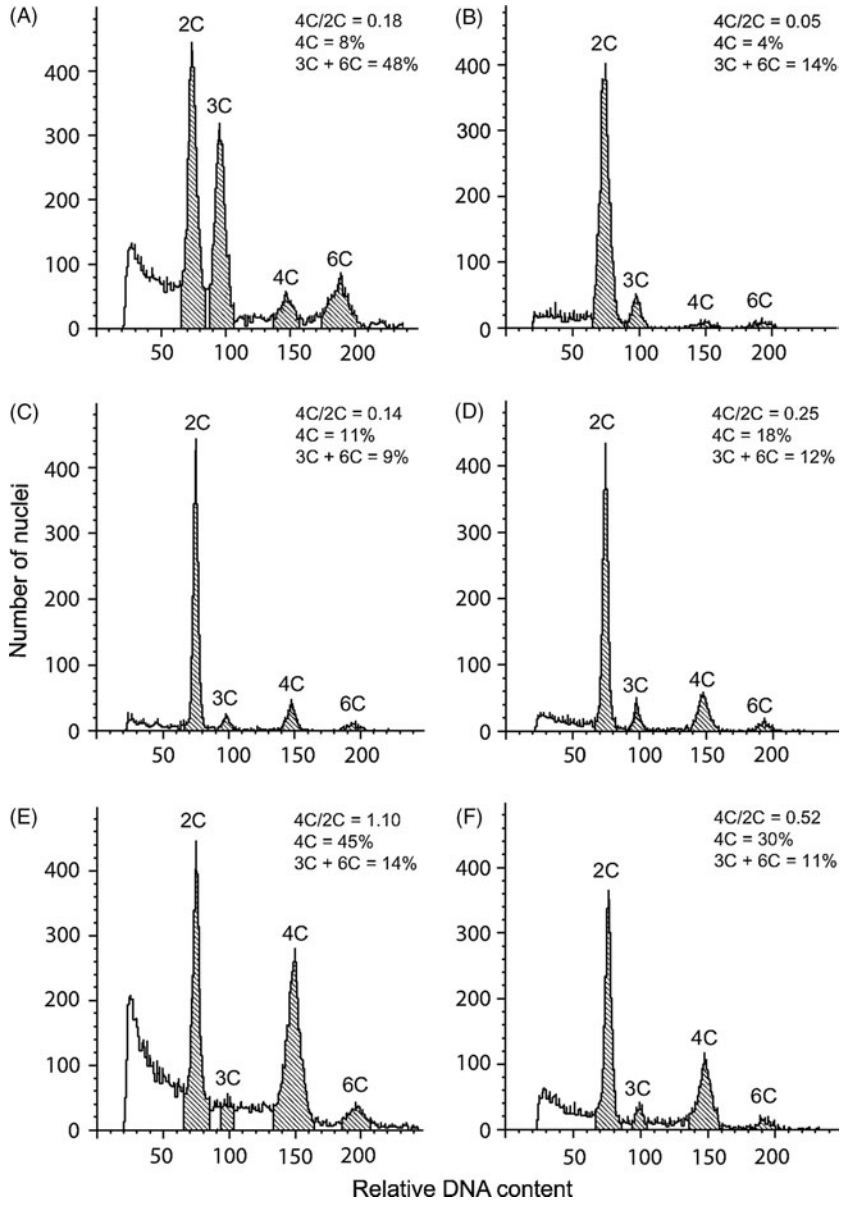


Figure 1. Flow cytometric DNA histograms of nuclear preparations from sugarbeet seeds. (A) and (B) true seed removed from the pericarp 3 weeks before full maturation, fresh and dried, respectively; (C) and (D) dry mature, whole true seed and radicle, respectively; (E) radicle of the germinated seed, at the transition from Phase II to Phase III (radicle protrusion); (F) radicle of the seed primed for 2 h in water and 2 h in 0.2% (w/v) NaOH. 2C and 4C, embryo nuclei; 3C and 6C, endosperm nuclei.

in a doubling of DNA content (2C → 4C); G_2, a second growth period during which a nucleus retains a 4C DNA content; and M, mitosis, when genetic material is divided into two daughter nuclei (4C → 2C; Bewley and Black, 1994). Non-proliferative cells usually enter the quiescent G_0 state from G_1 phase of the cell cycle. The endosperm in angiosperms is formed by the fusion of two nuclei from the female gametophyte and one nucleus from the male gametophyte; thus its nuclei in the G_1 phase of the cell cycle possess a 3C DNA content and in G_2 it is 6C. In addition, usually some of the cells of the endosperm undergo endoreduplication, during which the nuclei go through repeated rounds of DNA replication that are not followed by mitosis (endocycles), resulting in endopolyploid cells (4C → 8C → 16C → 32C, etc.) (Nagl, 1976; Dilkes *et al.*, 2002). Because of the presence of somatic cells with different DNA contents, the endosperm is called a polysomatic tissue. In some species, e.g. bean (*Phasoleus vulgaris*) and cucumber (*Cucumis sativus*), the embryo also exhibits polysomaty (Bino *et al.*, 1993; Jing *et al.*, 2000; Sadowski *et al.*, 2008).

https://doi.org/10.1017/S0960258508186275 Published online by Cambridge University Press

Seed maturation and cell cycle activity

Seed development involves embryo and permanent or temporary extra-embryonic storage tissue formation, and seed maturation (Bewley and Black, 1994). During early embryo and endosperm development high cell cycle activity occurs, while later during maturation it usually becomes gradually arrested (especially in orthodox seeds). Cell cycle activity in the embryo can be identified by the proportion of nuclei with a 4C DNA content. Early in development this proportion can reach 45% in sugarbeet (*Beta vulgaris*), 30% in pepper (*Capsicum annuum*) and about 15–20% in soybean (*Glycine max*) and neem (*Azadirachta indica*) (Chamberlin *et al.*, 1993; Sacandé *et al.*, 1997; Sliwinska, 1998; Portis *et al.*, 1999). Differential cessation of DNA replication and mitosis during maturation usually leads to an increased proportion of nuclei containing 2C DNA (G_0/G_1); in some species only 2C cells are present in the mature dry seeds while in the others a small number of 4C, and sometimes also 8C, cells occur (Deltour, 1985; Bino *et al.*, 1993) (Table 1). In sugarbeet seeds, the 4C/2C ratio is 0.8 at the beginning of embryo development, decreases up to 24–28 days after pollination (DAP) as the embryo becomes fully morphologically developed, and then is stabilized at about 0.1 in the mature state (containing about 10% 4C nuclei; Sliwinska, 2000). In pepper, nuclear DNA replication in embryonic tissues stops 1–2 weeks after the embryo reaches its final curled morphology, while arrest of nuclear DNA synthesis coincides with the onset of desiccation tolerance; at this stage 4C nuclei are no longer present (Portis *et al.*, 1999). Similarly, in neem embryos, after initial DNA replication, the cell cycle comes to a standstill at maturation, after arresting only 3% of the nuclei at the G_2 phase (4C; Sacandé *et al.*, 1997). During *Arabidopsis thaliana* embryo growth, cell cycle activity increases up to the torpedo/walking-stick stage and becomes gradually arrested at maturation (Raz *et al.*, 2001). Also, in tomato (*Lycopersicon esculentum*) the cessation of nuclear DNA replication marks the completion of embryo histodifferentiation (de Castro and Hilhorst, 2006).

In contrast, recalcitrant seeds of sycamore (*Acer pseudoplatanus*) and silver maple (*Acer saccharinum*) contain a high proportion of 4C nuclei, over 30% during development and after maturation is completed (Finch-Savage *et al.*, 1998; Kazeko and Troyan, 2000). In seeds of another recalcitrant tree, that of the tropical legume guaba (*Inga vera* ssp. *afinis*), the proportion of 4C nuclei remains constant (about 20%) during development and maturation (Faria *et al.*, 2004). These and other observations lend support to the hypothesis of Deltour (1985) that a decline in water content in orthodox seeds during maturation drying causes the inhibition of DNA replication, resulting in the accumulation of nuclei at the pre-synthetic G_1 and G_0 phases.

There are only a few reports on DNA replication during seed development in species having polysomatic seeds in the mature state, but DNA replication appears to show a different profile and the proportion of the 4C nuclei does not always reflect the progress of maturation. In polysomatic seeds only some of the 4C nuclei are in the G_2 stage of the mitotic cell cycle, and other nuclei have just entered first endocycle (having, however, the same DNA content they cannot be distinguished by flow cytometry). Jing *et al.* (2000)

Table 1. The highest C-values in the nuclei of developing and mature seed of selected species

	Highest C-value				
	Embryo		Endosperm		
Species	During development	In mature seed	During development	In mature seed	Reference
Arabidopsis	8C	2C	6C	6C	Raz *et al.*, 2001
Cucumber	8C	8C	–	–	Jing *et al.*, 2000
	16C	16C	96C	6C	Sadowski *et al.*, 2008
Guaba	4C	4C	–	–	Faria *et al.*, 2004
Maize	–	–	192C	No nuclei	Dilkes *et al.*, 2002
	–	–	768C	No nuclei	Kowles *et al.*, 1992
Neem	4C	4C	–	–	Sacandé *et al.*, 1997
Norway maple	8C	8C	–	–	Finch-Savage *et al.*, 1998
Pepper	4C	2C	6C	6C	Portis *et al.*, 1999
Rice	–	–	74C	>30C	Ramachandran and Raghavan, 1989
Soybean	4C	4C	9.7C	6C	Chamberlin *et al.*, 1993
Sugarbeet	4C	4C	24C	6C	Sliwinska, 1998
Sycamore	8C	4C	–	–	Finch-Savage *et al.*, 1998
Tomato	8C	4C	–	–	Liu *et al.*, 1997

https://doi.org/10.1017/S0960258508186275 Published online by Cambridge University Press

found 2C, 4C and 8C DNA in cucumber radicle tip nuclei, but in cotyledons and the nodal region of the embryo only 2C and 4C nuclei were present; no alterations in DNA profiles were detected from 28 to 56 DAP. However, according to recent studies of Sadowski *et al.* (2008), until 35 DAP the cucumber embryo possesses over 1% 16C nuclei, before declining to none at 56 DAP. By this time the germination capacity of the mature dry seeds has increased from about 40% to over 90%. Thus, it seems that, in this species, the replication of DNA (or lack thereof) during the second endocycle (8C → 16C), and not during the mitotic cycle, is indicative of the physiological state of the seeds. The significance of nuclei in the dry seed being at different stages of the cell cycle is still not clear; studies on seeds containing endoreduplicated nuclei are desirable to find an explanation for this phenomenon (Larkins *et al.*, 2001).

Endosperm development is characterized by three developmental stages: syncytial karyokinesis, and mitosis and endoreduplication (Kowles *et al.*, 1992; Dilkes *et al.*, 2002). After initial high mitotic activity, the number of endosperm nuclei undergoing endoreduplication greatly increases, such that the mitotic index falls to nearly zero. Subsequently, in some species, especially in grains of cereals, the starchy endosperm cells undergo programmed cell death. Due to endoreduplication, endosperm nuclei in rice (*Oryza sativa*) can reach 74C (determined using Feulgen microspectrophotometry and therefore probably underestimated; it is likely to be 96C) and in maize (*Zea mays*) even 768C, although in other species the highest DNA content is much lower, and at maturity usually only 3C and 6C nuclei are present (Ramachandran and Raghavan, 1989; Kowles *et al.*, 1992) (Table 1). In gymnosperms, the endosperm is a haploid tissue (generated by proliferation of one meiotic daughter cell), but in addition to 1C nuclei it can also contain endopolyploid ones, e.g. in the endosperms of seeds of some *Cupressaceae* 1C–6C nuclei are present (Pichot and El Maataoui, 1997).

In some seeds, the endosperm is completely absorbed at maturity [e.g. pea (*Pisum sativum*), bean, sugarbeet and other non-endospermic seeds], while in others, it is present after maturation is completed [e.g. maize, wheat (*Triticum aestivum*), rice, carrot (*Daucus carota*); endospermic seeds]. Thus, the ratio of endosperm to embryo nuclei or extent of endoreduplication in the endosperm provides information on seed maturity (Sliwinska, 2006). In sugarbeet, the ratio between the embryo and endosperm nuclei is about 3:2 in 21-day-old developing seeds, and about 9:1 in 36-day-old (mature) seeds (Sliwinska, 1998). In *Arabidopsis*, at the end of the growth phase of the embryo its volume increases about tenfold compared to during early development, while the endosperm is degraded and is reduced to a one-cell-thick layer (Raz *et al.*, 2001). Similarly, in cucumber seeds, the proportion of endosperm nuclei is highest at 21 DAP (45%), decreases to 13% at harvest and to 8% after seed processing (Sadowski *et al.*, 2008). In this species, the decrease in endoreduplication in endosperm cells from three endocycles to one (the presence of nuclei with a DNA content not higher than 12C) coincides with a decline in water content from an initial value of almost 90% to about 40%, and with the establishment of a germination capacity of over 90%. However, the decrease in endoreduplication intensity does not mark seed maturity in all cases. In sugarbeet seeds, already by about 3 weeks after pollination (2 weeks before maturity) the endopolyploid (12C and 24C) nuclei are no longer present (Sliwinska, 1998), and in the endosperms of pepper and *Arabidopsis* no endoreduplication at all occurs during seed development (Portis *et al.*, 1999; Raz *et al.*, 2001).

Based on the above-mentioned reports it can be concluded that in species producing orthodox seeds the ratio between the embryo/endosperm cell number, together with the proportions of the nuclei containing different DNA contents in the embryo, provide information on seed maturity. For example, if in a dry sugarbeet seed the proportion of 4C nuclei is over 10% and that of the endosperm nuclei is below 10%, it can be assumed that the seed was harvested after completing maturation (Fig. 1C). However, in some species there are no, or only small, changes in cell cycle activity in a fresh seed during maturation drying, which is often an essential stage for the establishment of high germination and vigour. But since seeds of different developmental stages invariably exist in one and the same plant/production field, flow cytometry can help seed producers to fix the most economic harvest date, when the proportion of fully developed seeds is the highest.

Not often, however, do seed growers have access to flow cytometers; they mostly rely on their experience to visually estimate seed maturity. Hence, the seed industry receives partly processed (dried and cleaned) seed lots of different quality from different growers. Can the quality of these lots be determined by flow cytometry? Studies on sugarbeet seeds show that it can be. Sliwinska (2003) analysed fresh and dried seeds collected at five different times during maturation drying, starting from the stage when the embryo was already fully developed. She found that after drying there are significant differences in the proportions of the 4C embryo nuclei, and both 3C and 6C endosperm nuclei, between seeds of different maturities. In seeds dried at an early stage of maturation, the proportion of the 2C embryo nuclei is higher than in those harvested after completing maturation drying (Fig. 1A–C). Probably because of lower tolerance of the nuclei with a higher DNA content to rapid water loss, in early-harvested seeds these nuclei were not able to

https://doi.org/10.1017/S0960258508186275 Published online by Cambridge University Press

withstand desiccation (and therefore were not detected during flow cytometric analysis), whereas slow drying on the mother plant (to a water content below 30%) prevented damage during after-harvest drying. Consequently, seeds with a higher 4C/2C ratio (fully mature) exhibited a higher vigour and germination capacity. The same relationship was observed by Sliwinska and Pedersen (1999) and Sliwinska *et al.* (1999) when studying sugarbeet seed lots of the same cultivar but of different quality, or harvested at different maturation stages.

DNA replication during germination

Cell cycle arrest in a quiescent seed is reversed during germination (reviewed by Vázquez-Ramos and de la Paz Sánchez, 2003). The completion of germination is characterized by cell elongation and, during subsequent seedling growth, cell division resumes (Raz *et al.*, 2001). Replication of DNA is a relatively late event in germination; it does not usually occur until some hours after a seed imbibes water, when DNA damage sustained during developmental drying, or imbibition or both has been repaired and considerable protein synthesis has occurred (Chen and Osborne, 1970; Osborne, 1977). The time of commencement of DNA replication can be the indication of seed vigour, since low-quality seeds need longer for DNA repair, and even further degradation of DNA may take place upon imbibition (Osborne, 1977). Therefore, the 4C/2C ratio has been proposed as a marker for the advancement of seed germination and can be used to estimate seed quality. Since the radicle is considered to be the part of the embryo where the increase in 4C nuclei is predominantly located, the ratio is usually established using this tissue (Bino *et al.*, 1992, 1996) (Fig. 1D–F). However, in some species, e.g. *Arabidopsis*, the hypocotyl is the organ in which there is intensive DNA replication during germination, accompanying its elongation (Sliwinska, Bassel and Bewley, unpublished). Since the boundary between the radicle and the hypocotyl is hard to discern in the embryo, most probably in some studies the basal part of the hypocotyl has been included along with the radicle during its isolation by dissection, and consequently the increase in 4C nuclei was incorrectly attributed as originating from the radicle only.

An increase in the proportion of nuclei in the G_2 phase of the cell cycle (possessing a 4C DNA content) has been detected in germinating seeds of many species, including several reports on changes in nuclear replication activity in tomato (Bino *et al.*, 1992, 1996; Liu *et al.*, 1994, 1997; de Castro *et al.*, 1995, 2000). In the radicle of the dry seeds of this species only about 5% of the nuclei are arrested in the G_2 phase of the cell cycle and their proportion increases during imbibition, reaching as high as 40% after 48 h, before radicle protrusion, which occurs first after 3 d. Also, in the pepper radicle tip, in which only 2C nuclei are present in the mature dry state, 4C nuclei appear 1–2 d after imbibition in water while radicle emergence starts 2 d later; at this time the proportion of the 4C nuclei is over 50% (Lanteri *et al.*, 1992; Saracco *et al.*, 1995). Thus, DNA replication precedes pepper seedling growth. In maize seeds, the transition from early to late stages of germination is accompanied by a decrease in 2C nuclei and a sizeable increase in 4C nuclei – from 10% in dry seeds to over 30% at 72 h of imbibition, with two periods of substantial increase, between 18 and 30 h, and between 60 and 72 h (Georgieva *et al.*, 1994). The 4C/2C ratio remains relatively constant (about 0.1) until 12 h, slightly increasing up to 60 h and then increasing fourfold to exceed 1 at 72 h of germination. An increase in 4C nuclei also marks an advancement in germination in jack pine (*Pinus banksiana*), sugarbeet, coffee (*Coffea arabica*) and barley (*Hordeum vulgare*) (Wyman *et al.*, 1996; Sliwinska *et al.*, 1999; da Silva *et al.*, 2008; Gendreau *et al.*, 2008). In contrast, in the nuclei of the radicle and young seedlings (up to an axis length of 2 mm) of barrel clover (*Medicago truncatula*) there is no nuclear DNA replication but the proportion of 4C nuclei is as high as 45% (Faria *et al.*, 2005).

Upon imbibition of some species, beside or instead of an increase in 4C nuclei, the presence of endopolyploid nuclei with higher DNA content has been observed. In the well-known polysomatic species *Arabidopsis*, between 30 and 48 h of germination (shortly before and at radicle protrusion) a population of 8C nuclei arises (Barrôco *et al.*, 2005; Masubelele *et al.*, 2005). The percentage of the embryo nuclei in the whole dry seeds of this species exhibiting DNA replication is negligible (0.7%), while following imbibition it increases up to 5–7% (4C + 8C nuclei). In germinating seeds of sugarbeet, in contrast, 8C nuclei were observed as early as about 2 d before radicle emergence (after 24 h of imbibition) and, additionally, by 48 h of imbibition a small population (1.5%) of 16C nuclei was present (Sliwinska, 1996, 2000). At that time the 4C/2C ratio exceeded 0.5 (being 0.1 in the dry seeds); it reached 1, similar to that in maize and barley (Georgieva *et al.*, 1994; Gendreau *et al.*, 2008), when the radicle became visible, marking the end of germination (Fig. 1E). Another pattern of DNA synthesis has been observed during germination of cabbage (*Brassica oleracea*) seeds; here the onset of DNA replication precedes visible germination, but 8C and 16C nuclei appear first during protrusion of the radicle tip through the seed coat, or very shortly thereafter (Górnik *et al.*, 1997).

Although seeds of different species show different patterns of nuclear DNA synthesis, by observing them it is usually possible to follow the advancement of

https://doi.org/10.1017/S0960258508186275 Published online by Cambridge University Press

germination by comparing time necessary to complete Phase II of this process, and thus identify the quality of a certain seed lot. Observations on cell cycle activity also allow determination of the time of transition from germination to seedling growth, which is when seed desiccation tolerance starts to be lost (Côme and Thévenot, 1982; de Castro *et al.*, 2000). This information is useful for establishing an appropriate seed harvest time, especially when there is rain during the maturation/ripening period that can induce germination of seeds still attached to the mother plant (pre-harvest sprouting) (Sliwinska, 2000).

Flow cytometric analysis can also provide information on seed dormancy. In imbibed dormant tomato seeds, nuclear DNA replication is blocked (the proportion of the 4C nuclei does not increase above that observed in dry seeds) (Groot *et al.*, 1997; de Castro *et al.*, 2001). Breakage of dormancy induces an increase in the number of 4C nuclei in the radicle tip, which precedes its protrusion. Similarly, the removal of dormancy from cherry (*Prunus avium*) and Norway maple (*Acer platanoides*) seeds by stratification coincides with a change in nuclear DNA content (Finch-Savage *et al.*, 1998; Pawłowski *et al.*, 2004).

Monitoring seed priming by flow cytometry

Seed quality can be improved by processing and pre-sowing treatments such as priming. These treatments, which are based upon controlled hydration of the seeds (using water or non-osmotic and osmotic solutions), promote germinative metabolism; since this promotion is retained after subsequent dehydration, it leads to rapid and uniform seedling emergence in the field (Bradford, 1986). One of the problems that producers face when applying priming to seeds is that it has to be finished before completion of germination, otherwise there is a decrease in germinability caused by a loss of desiccation tolerance following radicle protrusion. Additionally, there is variation among seed lots, even of the same species, in their response to priming, and thus conditions of the treatment have to be optimized individually for each lot. Depending on the species, laboratory germination tests, which are routinely applied to assess seed quality control, take several to a dozen or more days and therefore slow down the final processing of the seeds, exposing the seed industry to economic losses. Molecular markers of germination that allow an immediate check of priming advancement, including DNA replication, could reduce these losses.

Changes in nuclear replication stages upon priming have been studied by flow cytometry, mostly on pepper, tomato and sugarbeet, species producing orthodox seeds. The effect of osmopriming, which consists of incubating seeds in an osmotic solution, has been followed in pepper and tomato (Bino *et al.*, 1992; Lanteri *et al.*, 1992, 1993, 1994, 1995, 1996, 1997, 1998, 2000; Saracco *et al.*, 1995; Liu *et al.*, 1996; Van Pijlen *et al.*, 1996; Gurusinghe *et al.*, 1999; Özbingöl *et al.*, 1999; de Castro *et al.*, 2000). Osmopriming, even when applied for a prolonged period of time, prevents germination by restraining processes that are involved in radicle elongation and protrusion, but not DNA replication. The rate of DNA replication during priming depends on the seed moisture content as well as on temperature and oxygen availability. In most osmoprimed pepper seed lots there is advancement of embryonic root tip nuclei into S and G_2 phases of the cell cycle, as measured by an increase in the percentage of nuclei with 4C DNA content, although DNA replication is retarded compared to that of seeds imbibed in water. The appearance of 4C nuclei in pepper radicle tips is usually first noticeable after about a week of incubation in osmotic solution, and after 2 weeks they exceed 40%. Moreover, the lag period necessary for DNA repair before its replication during subsequent introduction of primed seeds to germination conditions, was reduced to as little as 12 h (from 36 h for untreated seeds similarly imbibed in water), when the osmopriming was applied for 14 or 21 d. Significant correlations were found between the frequency of priming-induced nuclear replication and the improvement of pepper seed vigour, as measured by the reduction in mean germination time. Thus, the 4C/2C ratio can be used to predict seedling performance of this species.

A similar pattern of DNA replication occurs in primed tomato seeds: the proportion of 4C nuclei increases up to fivefold after incubation in −1.1 MPa polyethylene glycol (PEG) (Bino *et al.*, 1992; Lanteri *et al.*, 1994; Liu *et al.*, 1996; Van Pijlen *et al.*, 1996; Özbingöl *et al.*, 1999; de Castro *et al.*, 2000). However, Gurusinghe *et al.* (1999) observed no increase in nuclear DNA content in one seed lot upon priming, although its germination rate response to PEG treatment was similar to that of other similarly treated seed lots that showed an augmented proportion of 4C nuclei. They concluded that the presence of, or increase in, 4C nuclei is not essential for germination advancement, and hypothesized that the greater initial percentage of 4C nuclei in the one seed lot that behaved differently reduced its capacity for additional cell cycle activity. Increases in germination rate without the commencement of DNA replication were observed earlier for some seed lots of pepper; this was explained as being due to a longer lag period being necessary for them to complete any physiological processes that normally precede DNA replication (Lanteri *et al.*, 1993; Saracco *et al.*, 1995). These lots, usually of lower vigour, would need longer priming or a higher osmotic potential of the solution used for the treatment to achieve a more advanced stage of germination.

https://doi.org/10.1017/S0960258508186275 Published online by Cambridge University Press

A similar phenomenon was observed in primed seeds of cauliflower (*Brassica oleracea* var. *botrytis*) differing in vigour (Powell *et al.*, 2000). Priming in PEG led to a clear increase in 4C nuclei in seeds of both high and low vigour; but when priming using aerated hydration was applied, no change in the proportion of the nuclei with 4C DNA occurred in low-vigour seeds. Nevertheless, priming increased the rate of germination of both high- and low-vigour lots of cauliflower (although not to the same extent), even if it was not always accompanied by the accumulation of nuclei in the G_2 phase of the cell cycle.

For commercial purposes, sugarbeet seeds are exposed to priming in various non-osmotic solutions that wash out germination inhibitors from the pericarp, control damping off and/or initiate germination (Sliwinska and Babinska, 1999). Not all of these solutions induce progress in the cell cycle. Redfearn and Osborne (1997) observed that tetramethyl thiuram-steeping for 6 h did not significantly affect seed nucleic acid content in comparison with untreated seeds; however, following an advancement treatment, involving an additional 88 h of incubation of humid seeds at 25°C, the percentage of root-tip nuclei with 4C DNA and above rose from 2% to 24%. Similarly, a 6-h soaking of sugarbeet seeds in water and a fungicide solution did not influence the 4C/2C ratio, although in treated seeds the rate of DNA replication upon imbibition was faster (Sliwinska *et al.*, 1999). As shown in other reports, the activation of the cell cycle and the commencement of DNA synthesis in sugarbeet seeds by priming depends mainly on the conditions used, especially time of the treatment, but the initial quality of the seed lot and cultivar can also be of importance (Sliwinska and Babinska, 1999; Sliwinska and Jendrzejczak, 2002; Sliwinska and Sadowski, 2004). After applying a 2-h soaking in tap water to seed lots of three sugarbeet cultivars, followed by 2 h in 0.2% (w/v) NaOH solution, a significant increase in the 4C/2C ratio was detected in the radicles of seeds of two cultivars (Fig. 1D, F) but not in those of a third one, which initially possessed a higher proportion of 4C nuclei and was of a slightly higher quality (Sliwinska and Babinska, 1999). Sliwinska and Jendrzejczak (2002) applied up to 14 cycles of 2-, 4- and 8-h hydration–dehydration to seed lots of different vigour and found that the 4C/2C ratio increased faster when longer periods of hydration were applied. The treatments were more beneficial to low-quality seeds, but the pattern of DNA replication was similar for both seed lots. However, an increase of germination of the low-vigour seeds almost to the level of the high-vigour seeds, immediately after the first hydration, suggests that the initial difference in quality between the lots was due to the presence of germination inhibitors in the pericarp, rather than to the status of DNA in the embryo. When some bioactive compounds (algae) were added during hydropriming of the sugarbeet seed lots of different vigour it appeared that their advantageous effects were similar to those obtained after hydration in water. But in three out of four seed lots they negatively influenced the increase of the proportion of the 4C nuclei during priming; and one of the two bioactive compounds used induced a lower than water increase in germination capacity, of seed lots of lower vigour at suboptimal temperature (Sliwinska and Sadowski, 2004). The commercial use of these compounds would appear both to be uneconomical and to have a negative effect on early seedling growth.

Large-seeded legume species are susceptible to imbibition injury, and hydropriming is not suitable for the improvement of their seeds; an alternative method of priming, matriconditioning, can be applied. During this treatment seeds are mixed with a solid matrix of particles and water, and allowed to imbibe slowly to reach an equilibrium hydration level just below that required for radicle protrusion (Khan *et al.*, 1990). Flow cytometry has been used to study the cell cycle progression during matriconditioning of lentil (*Lens culinaris*) seeds (Sadowski and Sliwinska, 2007). An increase in the 4C/2C ratio in the radicle tips upon matriconditioning suggests that this method of priming induces germination. The usefulness of flow cytometry to establish the effectiveness of matriconditioning has also been confirmed for melon (*Cucumis melo*) seeds (Sadowski, 2007). Commencement of DNA replication was earlier in treated melon seeds of higher quality, and the percentage of nuclei with higher than 2C DNA content increased more when a higher volume of water was applied to a wet vermiculite matrix during matriconditioning. Since melon seeds already show polysomaty in the dry state and the cells undergo up to two endocycles during germination, the 4C/2C ratio does not reflect the onset of DNA replication, whereas the 8C/4C and (8C + 16C)/(2C/4C) ratios, as well as the mean C-value, increase during treatment and are therefore the recommended parameters by which to follow the advancement of priming in this species.

Flow cytometry can also be used for establishing germination advancement when, after priming, the seeds are to be stored. In this case, the treatment that is preferable is one that does not affect the nuclear replication stages, but does improve seed performance (Lanteri *et al.*, 1993; Saracco *et al.*, 1995). This is because the activation of nuclear DNA replication, together with other processes taking place in cells during Phase II of germination, causes alterations in the mechanisms responsible for resistance to seed deterioration imposed during storage.

https://doi.org/10.1017/S0960258508186275 Published online by Cambridge University Press

Estimation of seed lot purity

Most of the sugarbeet seeds obtainable from European companies are produced in the south of Europe. In these regions seed plants are often exposed to the risk of pollination by their diploid wild relative, *Beta vulgaris* ssp. *maritima*. As a consequence, seed lots can be contaminated by so-called weed beets, persistent annual weeds of a sugarbeet crop (Ewans and Weir, 1981). Since most of the European cultivars are triploid hybrids between a diploid mother plant and a tetraploid pollinator, and weed beets are diploids, they can be easily distinguished by estimating their ploidy. During hybrid seed production in the field there is also the danger of including some seeds with the tetraploid male component. The presence of seeds of other than triploid ploidy can be easily detected by flow cytometry at any developmental stage, also directly in commercial lots of seeds partly processed by growers, using any part of the true seed removed from the pericarp (Sliwinska, 1997, 2000). This kind of analysis is already routinely used by seed producers, who control contamination with undesirable ploidies in all seed lots obtained from different growers by eliminating those with a considerable proportion of diploid and/or tetraploid seeds.

Thus, as shown here, the pattern of DNA replication during seed development, maturation and germination is different for different species. Nevertheless, once established, it can be used to estimate the physiological state of the seeds. For example, in sugarbeet it is possible to recognize seed maturity by the ratio between the endosperm and embryo nuclei of fresh and dried seeds, and the proportion of 4C nuclei in a dry seed; this proportion also provides information on the advancement of germination and allows the progress of priming treatments to be followed (Fig. 1). Thus, for this species, in addition to estimating the purity of seed lots, flow cytometry can be used for establishing an optimal harvest time and predicting seed performance in the field. Presently, since the cost of flow cytometers and of sample preparation is relatively low, and even portable cytometers are available, the routine use of this instrument by seed producers/companies to monitor seed quality of different species is worthy of consideration.

Acknowledgements

The author thanks Professor J.D. Bewley (University of Guelph, Canada) for his inspiration and critical comments on the manuscript, and Dr S. P. C. Groot (Plant Research International, Wageningen, The Netherlands) for his thoughtful comments.

References

Achard, P., Job, D. and Mache, R. (2002) A nuclear transcription factor related to plastid ribosome biogenesis is synthesised early during germination and priming. *FEBS Letters* **518**, 48–52.

Barrôco, R.M., van Poucke, K., Bergervoet, J.H.W., de Veylder, L., Groot, S.P.C., Inzé, D. and Engler, G. (2005) The role of the cell cycle machinery in resumption of postembryonic development. *Plant Physiology* **137**, 127–140.

Bewley, J.D. and Black, M. (1994) *Seeds. Physiology of development and germination* (2nd edition). New York, Plenum Press.

Bino, R.J., de Vries, J.N., Kraak, H.L. and van Pijlen, J.G. (1992) Flow cytometric determination of nuclear replication stages in tomato seeds during priming and germination. *Annals of Botany* **69**, 231–236.

Bino, R.J., Lanteri, S., Verhoeven, H.A. and Kraak, H.L. (1993) Flow cytometric determination of nuclear replication stages in seed tissues. *Annals of Botany* **72**, 181–187.

Bino, R.J., Bergervoet, J.H.W., De Vos, C.H.R., Kraak, H.L., Lanteri, S., Van Der Burg, W.J. and Zheng, X.Y. (1996) Comparison of nuclear replication activity and protein expression patterns during tomato seed germination. *Field Crops Research* **45**, 71–77.

Bradford, K.J. (1986) Manipulation of seed water relations *via* osmotic priming to improve germination under stress conditions. *HortScience* **21**, 1105–1112.

Capron, I., Corbineau, F., Dacher, F., Job, C., Côme, D. and Job, D. (2000) Sugarbeet seed priming: effects of priming conditions on germination, solubilization of 11-S globulin and accumulation of LEA proteins. *Seed Science Research* **10**, 243–254.

Chamberlin, M.A., Horner, H.T. and Palmer, R.G. (1993) Nuclear size and DNA content of the embryo and endosperm during their initial stages of development in *Glycine max* (Fabaceae). *American Journal of Botany* **80**, 1209–1215.

Chen, D. and Osborne, D.J. (1970) Hormones in the translational control of early germination in wheat embryos. *Nature* **226**, 1157–1160.

Côme, D. and Thévenot, C. (1982) Environmental control of embryo dormancy and germination. pp. 271–298 *in* Khan, A.A. (Ed.) *The physiology and biochemistry of seed development, dormancy and germination*. Amsterdam, Elsevier Biomedical Press.

da Silva, E.A.A., Toorop, P.E., van Lammeren, A.A.M. and Hilhorst, H.W.M. (2008) ABA inhibits embryo cell division events during coffee (*Coffea arabica* 'Rubi') seed germination. *Annals of Botany* **102**, 425–433.

de Castro, R.D. and Hilhorst, H.W.M. (2006) Hormonal control of seed development in GA- and ABA-deficient tomato (*Lycopersicon esculentum* Mill. cv. Moneymaker) mutants. *Plant Science* **170**, 462–470.

de Castro, R.D., Zheng, X.Y., Bergervoet, J.H.W., De Vos, C.H.R. and Bino, R.J. (1995) β-Tubulin accumulation and DNA replication in imbibing tomato seeds. *Plant Physiology* **109**, 499–504.

de Castro, R.D., van Lammeren, A.A.M., Groot, S.P.C., Bino, R.J. and Hilhorst, H.W.M. (2000) Cell division and subsequent radicle protrusion in tomato seeds are inhibited by osmotic stress but DNA synthesis and

https://doi.org/10.1017/S0960258508186275 Published online by Cambridge University Press

formation of microtubular cytoskeleton are not. *Plant Physiology* **122**, 327–335.

de Castro, R.D., Bino, R.J., Jing, H.-C., Kieft, H. and Hilhorst, H.W.M. (2001) Depth of dormancy in tomato (*Lycopersicon esculentum* Mill.) seeds is related to the progress of the cell cycle prior to the induction of dormancy. *Seed Science Research* **11**, 45–54.

Deltour, R. (1985) Nuclear activation during early germination of the higher plant embryo. *Journal of Cell Science* **75**, 43–83.

Dilkes, B.P., Dante, R.A., Coelho, C. and Larkins, B.A. (2002) Genetic analyses of endoreduplication in *Zea mays* endosperm: evidence of sporophytic and zygotic maternal control. *Genetics* **160**, 1163–1177.

Doležel, J. and Bartoš, J. (2005) Plant DNA flow cytometry and estimation of nuclear genome size. *Annals of Botany* **95**, 99–110.

Ewans, A. and Weir, J. (1981) The evolution of weed beet in sugar beet crop. *Genetic Resources and Crop Evolution* **29**, 301–310.

Faria, J.M.R., van Lammern, A.A.M. and Hilhorst, H.W.M. (2004) Desiccation sensitivity and cell cycle aspects in seeds of *Inga vera* subsp. *affinis*. *Seed Science Research* **14**, 165–178.

Faria, J.M.R., Buitink, J., van Lammern, A.A.M. and Hilhorst, H.W.M. (2005) Changes in DNA and microtubules during loss and re-establishment of desiccation tolerance in germinating *Medicago truncatula* seeds. *Journal of Experimental Botany* **56**, 2119–2130.

Finch-Savage, W.E., Bergervoet, J.H.W., Bino, R.J., Clay, H.A. and Groot, S.P.C. (1998) Nuclear replication activity during seed development, dormancy breakage and germination in three tree species: Norway maple (*Acer platanoides* L.), sycamore (*Acer pseudoplatanus* L.) and cherry (*Prunus avium* L.). *Annals of Botany* **81**, 519–526.

Gallardo, K., Job, C., Groot, S.P.C., Puype, M., Demol, H., Vandekerckhove, J. and Job, D. (2001) Proteomic analysis of *Arabidopsis* seed germination and priming. *Plant Physiology* **126**, 835–848.

Gendreau, E., Romaniello, S., Barad, S., Leymarie, J., Benech-Arnold, R. and Corbineau, F. (2008) Regulation of cell cycle activity in the embryo of barley seeds during germination as related to grain hydration. *Journal of Experimental Botany* **59**, 203–212.

Georgieva, E.I., López-Rodas, G., Hittmair, A., Feichtinger, H., Brosch, G. and Loidl, P. (1994) Maize embryo germination. I. cell cycle analysis. *Planta* **192**, 118–124.

Górnik, K., de Castro, R.D., Liu, Y., Bino, R.J. and Groot, S.P.C. (1997) Inhibition of cell division during cabbage (*Brassica oleracea* L.) seed germination. *Seed Science Research* **7**, 333–340.

Groot, S.P.C., de Castro, R.D., Liu, Y. and Bino, R.J. (1997) Cell cycle analysis in dormant and germinating tomato seeds. pp. 395–402 *in* Ellis, R.H.; Black, M.; Hong, T.D. (Eds) *Basic and applied aspects of seed biology*. Dordrecht, Kluwer Academic Publishers.

Gurusinghe, S.H., Cheng, Z. and Bradford, K.J. (1999) Cell cycle activity during seed priming is not essential for germination advancement in tomato. *Journal of Experimental Botany* **50**, 101–106.

Jing, H.-C., Bergervoet, J.H.W., Jalink, H., Klooster, M., Du, S.-L., Bino, R.J., Hilhorst, H.W.M. and Groot, S.P.C. (2000) Cucumber (*Cucumis sativus* L.) seed performance as influenced by ovary and ovule position. *Seed Science Research* **10**, 435–445.

Job, C., Kersulec, A., Ravasio, L., Chareyre, S., Pepin, R. and Job, D. (1997) The solubilization of the basic subunit of sugarbeet seed 11-S globulin during priming. *Seed Science Research* **7**, 225–243.

Kazeko, L.E. and Troyan, V.M. (2000) The relationship between the mitotic activity and moisture content of recalcitrant seeds of *Acer saccharinum* (L.) during maturation, post maturation drying and germination. *Seed Science Research* **10**, 225–232.

Khan, A.A., Miura, H., Prusinski, J. and Ilyas, S. (1990) Matriconditioning of seeds to improve emergence. *Proceedings of national symposium on stand establishment for horticultural crops*. Minneapolis, USA, pp. 19–40.

Kowles, R.V., Yerk, G.L., Srinc, F. and Phillips, R.L. (1992) Maize endosperm tissue as an endoreduplication system. pp. 65–88 *in* Setlow, J. (Ed.) *Genetic engineering*. New York, Plenum Press.

Lanteri, S., Bino, R.J. and Kraak, H.L. (1992) Flow cytometric determination of nuclear replication stages in pepper seeds during germination and after priming treatments. *Capsicum Newsletter* **4**, 249–253.

Lanteri, S., Kraak, H.L., De Vos, C.H.R. and Bino, R.J. (1993) Effects of osmotic preconditioning on nuclear replication activity in seeds of pepper (*Capsicum annuum*). *Physiologia Plantarum* **89**, 433–440.

Lanteri, S., Saracco, F., Kraak, H.L. and Bino, R.J. (1994) The effects of priming on nuclear replication activity and germination of pepper (*Capsicum annuum*) and tomato (*Lycopersicon esculentum*) seeds. *Seed Science Research* **4**, 81–87.

Lanteri, S., Belletti, P., Nada, E. and Quagliotti, L. (1995) Flow cytometric determination of nuclear replication stages in pepper seeds during priming and germination. *Capsicum and Eggplant Newsletter* **14**, 72–75.

Lanteri, S., Nada, E., Belletti, P., Quagliotti, L. and Bino, R.J. (1996) Effects of controlled deterioration and osmoconditioning on germination and nuclear replication in seeds of pepper (*Capsicum annuum* L.). *Annals of Botany* **77**, 591–597.

Lanteri, S., Belletti, P., Marzach, C., Nada, E., Quagliotti, L. and Bino, R.J. (1997) Priming-induced nuclear replication activity in pepper (*Capsicum annuum* L.) seeds. Effect on germination and storability. pp. 451–459 *in* Ellis, R.H.; Black, M.; Hong, T.D. (Eds) *Basic and applied aspects of seed biology*. Dordrecht, Kluwer Academic Publishers.

Lanteri, S., Quagliotti, L., Belletti, P., Scordino, P., Triglia, A. and Musumec, F. (1998) Delayed luminescence and priming-induced nuclear replication of unaged and controlled deteriorated pepper seeds (*Capsicum annuum* L.). *Seed Science and Technology* **26**, 413–424.

Lanteri, S., Portis, E., Bergervoet, J.H.W. and Groot, S.P.C. (2000) Molecular markers for the priming of pepper seeds (*Capsicum annuum* L.). *Journal of Horticultural Science and Biotechnology* **75**, 607–611.

Larkins, B.A., Dilkes, B.P., Dante, R.A. and Coelho, C.M. (2001) Investigating the hows and whys of DNA endoreduplication. *Journal of Experimental Botany* **52**, 183–192.

Liu, Y., Bergervoet, J.H.W., De Vos, C.H.R., Hilhorst, W.M., Kraak, H.L., Karssen, C.M. and Bino, R.J. (1994) Nuclear replication activities during imbibition of

https://doi.org/10.1017/S0960258508186275 Published online by Cambridge University Press

abscisic acid- and gibberellin-deficient tomato (*Lycopersicon esculentum*) seeds. *Planta* **194**, 368–373.

Liu, Y., Bino, R.J., van der Burg, W.J., Groot, S.P.C. and Hilhorst, H.W.M. (1996) Effect of osmotic priming on dormancy and storability of tomato (*Lycopersicon esculentum* Mill.) seeds. *Seed Science Research* **6**, 49–55.

Liu, Y., Hilhorst, H.W.M., Groot, S.P.C. and Bino, R.J. (1997) Amounts of nuclear DNA and internal morphology of gibberellin- and abscisic acid-deficient tomato *(Lycopersicon esculentum* Mill.) seeds during maturation, imbibition and germination. *Annals of Botany* **79**, 161–168.

Masubelele, N.H., Dewitte, M., Maugham, S., Collins, C., Huntley, R., Nieuwland, J., Scofield, S. and Murray, J.A.H. (2005) D-Type cyclins activate division in the root apex to promote seed germination in *Arabidopsis*. *Proceedings of the National Academy of Sciences, USA* **102**, 15694–15699.

Nagl, W. (1976) DNA endoreduplication and polyteny understood as evolutionary strategies. *Nature* **261**, 614–615.

Osborne, D.J. (1977) Nucleic acids and seed germination. pp. 319–333 *in* Khan, A.A. (Ed.) *The physiology and biochemistry of seed dormancy and germination*. Amsterdam, North Holland Biomedical Press.

Özbingöl, N., Corbineau, F., Groot, S.P.C., Bino, R.J. and Côme, D. (1999) Activation of the cell cycle in tomato (*Lycopersicon esculentum* Mill.) seeds during osmoconditioning as related to temperature and oxygen. *Annals of Botany* **84**, 245–251.

Pawłowski, T.A., Bergervoet, J.H.W., Bino, R.J., Hilhorst, H.W.M. and Groot, S.P.C. (2004) Cell cycle activity and β-tubulin accumulation during dormancy breaking of *Acer platanoides* L. seeds. *Biologia Plantarum* **48**, 211–218.

Pichot, C. and El Maataoui, M. (1997) Flow cytometric evidence for multiple ploidy levels in the endosperm of some gymnosperm species. *Theoretical and Applied Genetics* **94**, 865–870.

Portis, E., Marzachi, C., Quagliotti, L. and Lanteri, S. (1999) Molecular and physiological markers during seed development of peppers (*Capsicum annuum* L.): DNA replication and β-tubulin synthesis. *Seed Science Research* **9**, 85–90.

Powell, A.A., Yule, L.J., Jing, H.C., Groot, S.P.C., Bino, R.J. and Pritchard, H.W. (2000) The influence of aerated hydration seed treatment on seed longevity as assessed by the viability equations. *Journal of Experimental Botany* **51**, 2031–2043.

Ramachandran, C. and Raghavan, V. (1989) Changes in nuclear DNA content of endosperm cells during grain development in rice (*Oryza sativa*). *Annals of Botany* **64**, 459–468.

Raz, V., Bergervoet, J.H.V. and Koornneef, M. (2001) Sequential steps for developmental arrest in *Arabidopsis* seeds. *Development* **128**, 243–252.

Redfearn, M. and Osborne, D.J. (1997) Effect of advancement on nucleic acids in sugarbeet (*Beta vulgaris*) seeds. *Seed Science Research* **7**, 261–267.

Sacandé, M., Groot, S.P.C., Hoekstra, F.A., de Castro, R.D. and Bino, R.J. (1997) Cell cycle events in developing neem (*Azadirachta indica*) seeds: are they related to intermediate storage behavior? *Seed Science Research* **7**, 161–168.

Sadowski, J. (2007) Effect of conditioning on DNA synthesis, catalase activity and germination of melon (*Cucumis melo* L.) seeds. PhD thesis, University of Technology and Life Sciences in Bydgoszcz, Poland (in Polish).

Sadowski, J. and Sliwinska, E. (2007) Cell cycle, membrane integrity and germination of matriconditioned lentil (*Lens culinaris* Medik.) seeds. pp. 179–186 *in* Navie, S.; Adkins, S.; Ashmore, S. (Eds) *Seeds: biology, development and ecology*. Wallingford, CAB International.

Sadowski, J., Bartunek, M. and Sliwinska, E. (2008) Germination and cell cycle activity changes during development and storage of cucumber (*Cucumis sativus* L.) seeds. *Polish Journal of Natural Sciences Suppl.* **5**, 139.

Saracco, F., Bino, R.J., Bergervoet, J.H.V. and Lanteri, S. (1995) Influence of priming-induced nuclear replication activity on storability of pepper (*Capsicum annuum* L.) seeds. *Seed Science Research* **5**, 25–29.

Sliwinska, E. (1996) Flow cytometric analysis of the cell cycle of sugar-beet seed during germination. *Journal of Applied Genetics* **37A**, 254–257.

Sliwinska, E. (1997) Flow cytometric analysis of sugar-beet seeds different in vigour. pp. 577–584 *in* Ellis, R.H.; Black, M.; Hong, T.D. (Eds) *Basic and applied aspects of seed biology*. Dordrecht, Kluwer Academic Publishers.

Sliwinska, E. (1998) Cell cycle activity during development of sugar beet seed. pp. 51–59 *in* Małuszyńska, J. (Ed.) *Plant cytogenetics*. Katowice, Silesian University Publishers (Prace Naukowe Uniwersytetu Śląskiego 1696).

Sliwinska, E. (2000) Analysis of the cell cycle in sugarbeet seed during development, maturation and germination. pp. 133–139 *in* Black, M.; Bradford, K.J.; Vázquez-Ramos, J. (Eds) *Seed biology: advances and applications*. Wallingford, CAB International.

Sliwinska, E. (2003) Cell cycle and germination of fresh, dried and deteriorated sugar-beet seeds as indicators of optimal harvest time. *Seed Science Research* **13**, 131–138.

Sliwinska, E. (2006) Nuclear DNA content analysis of plant seeds by flow cytometry. pp. 7.29.1–7.29.13 *in* Robinson, J.P.; Darzynkiewicz, Z.; Dean, P.N.; Orfao, A.; Rabinovitch, P.S.; Stewart, C.C.; Tanke, H.J.; Wheeless, L.L. (Eds) *Current protocols in cytometry*. New York, John Wiley & Sons.

Sliwinska, E. and Babinska, L. (1999) Effect of presowing hydration treatment on DNA replication activity in the embryo of sugar beet (*Beta vulgaris* L.). *Electronic Journal of Polish Agricultural Universities, Agronomy* **2**, Available at www.ejpau.media.pl/series/volume2/issue2/agronomy/art.-04.html (accessed December 2008).

Sliwinska, E. and Jendrzejczak, E. (2002) Sugar-beet seed quality and DNA synthesis in the embryo in relation to hydration-dehydration cycles. *Seed Science and Technology* **30**, 597–608.

Sliwinska, E. and Pedersen, H.C. (1999) Determination of nuclear replication stages during germination of sugar-beet seeds differing in vigour. *Electronic Journal of Polish Agricultural Universities, Agronomy* **2**, Available at http://www.ejpau.media.pl/series/volume2/issue1/agronomy/art.-01.html (accessed December 2008).

Sliwinska, E. and Sadowski, H. (2004) Comparison of the effects of bioactive compounds and hydration treatments on germination and cell cycle activity of sugar-beet seeds. *Seed Science and Technology* **32**, 917–920.

Sliwinska, E., Jing, H.C., Job, C., Job, D., Bergervoet, J.H.W., Bino, R.J. and Groot, S.P.C. (1999) Effect of harvest time

https://doi.org/10.1017/S0960258508186275 Published online by Cambridge University Press

and soaking treatment on cell cycle activity in sugar-beet seeds. *Seed Science Research* **9**, 91–99.

Van Pijlen, J.G., Groot, S.P.C., Kraak, H.L., Bergervoet, J.H.W. and Bino, R.J. (1996) Effects of pre-storage hydration treatments on germination performance, moisture content, DNA synthesis and controlled deterioration tolerance of tomato (*Lycopersicon esculentum* Mill.) seeds. *Seed Science Research* **6**, 57–63.

Vázquez-Ramos, J.M. and de la Paz Sánchez, M. (2003) The cell cycle and seed germination. *Seed Science Research* **13**, 113–130.

Wyman, J., Tremblay, M.-F. and Laliberté, S. (1996) Cell cycle activation during imbibition and visible germination in embryos and megagametophytes of jack pine (*Pinus banksina* Lamb.). *Annals of Botany* **78**, 245–253.

Received 25 August 2008
accepted after revision 26 November 2008
© 2009 Cambridge University Press

https://doi.org/10.1017/S0960258508186275 Published online by Cambridge University Press