Effect of harvest time and soaking treatment on cell cycle activity in sugarbeet seeds

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Abstract

Cell cycle activity in dry and germinating untreated and treated (soaked in water and subsequently in fungicide) seeds of two sugarbeet cultivars, collected at commercial harvest time (late mature seeds) and about 2 weeks before this (immature seeds), was investigated by flow cytometry, and by immuno-detection of β-tubulin and the B-subunit of the 11 S globulin. Germination capacity and field emergence were tested. With dry seeds of both cultivars, higher G_2/G_1 ratios were observed in the radicle tips of late mature seeds, as compared with those from immature seeds. The late mature seeds contained more partly degraded (soluble) B-subunit of 11 S globulin, typical of germinating or primed sugarbeet seeds. Thus events associated with the onset of germination had occurred in the seed lots collected at commercial harvest time. The cytoskeleton protein β-tubulin was not detectable in dry seeds from either harvest. Western blotting revealed an accumulation of β-tubulin during germination and this was faster in the late mature harvested seeds which was correlated with the onset of DNA replication. Soaking enhanced the rate of cell cycle activation during germination as well as vigour, germination capacity, and field emergence. There was positive correlation between the G_2/G_1 ratio and the traits examined in laboratory and field tests. It is concluded that a combined analysis of proteins and cell-cycle-related events can be used in understanding and predicting sugarbeet seed quality.

Keywords: 11 S globulin, *Beta vulgaris* L., β-tubulin, DNA replication, flow cytometry, germination markers, seed treatment

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Introduction

The quality of sugar-beet (*Beta vulgaris* L.) seed is a measure of the extent to which the genetic potential is achieved through seed production. This is modified by processing and treatment with fungicides, insecticides and pelleting (Longden, 1986). To obtain seeds with high vigour and germination capacity, as much as 75–90% of the harvested seed crop has to be rejected (Longden, 1986; Durrant and Loads, 1990). Crucial aspects of seed production are environmental requirements such as temperature, relative humidity and rainfall, which are especially important during seed ripening (Lexander, 1969; Wood *et al.*, 1980; Longden, 1986). Low temperatures during seed development, as well as early harvest, can cause low germination capacity (Lexander, 1981, and references therein). Grimwade *et al.* (1987) observed a linear relationship between the maturity of a seed lot and the subsequent germination capacity. They suggested that this phenomenon is caused by the frequent occurrence of underdeveloped seeds in less mature fruits. The other factor affecting germination capacity is the presence of endogenous inhibitors. Battle and Whittington (1969a) and Inoue and Yamamoto (1977) claimed that the amount of inhibitors in sugarbeet fruit wall decreases with time to harvest. Long periods of overhead irrigation or heavy rainfalls may remove inhibitors by leaching (Snyder, 1963; Battle and Whittington, 1969b), or may directly affect the embryos (Longden, 1971) due to the repeated soaking and drying of fruits.

Recent studies have focused on the identification of germination markers that can be used to dissect the key processes involved in germination, and monitor the effect of seed treatments such as priming that are aimed at improving the germination quality of seed lots. These treatments, which are based upon

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controlled hydration of the seeds, promote early germinative metabolic processes, thus accounting for a rapid and uniform emergence in the field (Bradford, 1986).

Flow cytometry, allowing fast and exact analysis of the DNA content of nuclei isolated from plant tissues, has opened possibilities for studying cell cycle activity in developing and germinating sugarbeet seeds as well as for estimating seed vigour (Śliwińska, 1996, 1997, 1998). The G_2 / G_1 ratio from radicle tip nuclei is related to particular cell cycle stages and it is suggested that this gives information about the physiological state of the embryo (Bino *et al.*, 1995, 1996). With seeds from several species, an increase in this ratio occurs during imbibition and before radicle protrusion. Consequently, the G_2 / G_1 ratio can be considered as an early marker of the progression of seed germination.

One of the proteins involved in the cell cycle is β-tubulin, a component of the microtubular cytoskeleton (Alberts *et al.*, 1994). Accumulation of β-tubulin is a cell-cycle-related marker for following the initial processes that are activated during the early stages of seed germination (de Castro *et al.*, 1995). Another pre-germination marker is the B-subunit of a major sugarbeet storage protein, the 11 S globulin, which becomes more soluble to extraction by low-ionic-strength buffers during early germination and priming (Job *et al.*, 1997). The solubilization is a result of an endoproteolytic attack on the A-chain of the 11 S globulin, which liberates a complex composed of the B-subunit of the 11 S globulin storage protein and a peptide of the A-chain. This corresponds to an initial event of the mobilization of the storage protein that occurs before radicle emergence.

In the present work, the effect of harvest time and soaking treatment on the nuclear replication activity, the accumulation of β-tubulin and the B-subunit of 11 S globulin in sugar-beet seeds was examined. The aim of this study is to find a marker for estimation of seed maturity and initiation of germination, which could help predict optimal harvest time and suggest appropriate seed treatments to obtain high vigour.

Materials and methods

Seeds

Seed samples (in fact fruits) of two triploid sugarbeet cultivars were obtained in 1996 from commercial production fields in southern France and central Poland. The seeds produced in France, cv. A, were a cultivar from the German seed company Strube GmbH & Co KG. The seeds produced in Poland, cv. B, were of the cultivar Kawajana from the Polish seed company Kutnowska Hodowla Buraka Cukrowego.

Seeds from each cultivar were collected at two times: about 2 weeks before the commercial harvest time and at the late mature state, the latter corresponding to the commercial harvest time. The harvest dates were 10 and 27 June for cv. A, and 14 and 28 August for cv. B. Secondary floral axes were collected and dried at room temperature until they reached about 12% water content, and then the seeds were hand-threshed, cleaned, rubbed and sized (3.50–4.75 mm). Part of the seeds from each lot was soaked for 3 h in water (water:seed ratio as 5:1, on a weight basis) and 3 h in 0.2% solution of Funaben T (45% thiram + 20% carbendazim, in a ratio of Funaben T:seeds as 3:1, on a weight basis) and subsequently dried for 24 h at room temperature. This treatment, comparable as performed in some commercial practice, is aimed at removal of soluble inhibitors from the pericarp and for control of damping off. Seeds were stored at room temperature until analysis.

Germination test and vigour index

Seed samples were tested at about 5 months after the harvest. A laboratory germination test was performed according to ISTA rules (ISTA, 1985), with some minor modifications (pleated filter paper at 65% relative substrate moisture content, in darkness, at 15°C and 20°C). The germination capacity after 4 and 14 d as well as the seedling length vigour index (the percentage of seeds with radicle or radicle / hypocotyl axis over 5 mm long at 15°C or over 15 mm long at 20°C, after 96 h), was determined. No abnormal seedlings were encountered. At the end of the test, non-germinated fruits were inspected by removing the lid and scored as full (containing true seeds) or empty. Three replicates of 100 seeds were tested for each seed sample.

Field test

The seeds (four replicates of 100 seeds) were sown on 23 April 1997, in Polanowice, central Poland. Field emergence was determined by counting the number of plants established 3 d (first count) and 21 d (final count) after the first seedlings had appeared. When the seedlings reached the two- to four-leaf stage, they were collected from the field, cleaned and the fresh weight of 100 entire seedlings determined.

Flow cytometry

The distal part of the radicle, about 1–2 mm long, was cut off and used for flow cytometric analysis. Previously, it had been demonstrated that upon germination, cell cycle activation mainly takes place in this tissue (Bino *et al.*, 1992; Śliwińska, 1997). Samples of individual radicle tips were prepared according to

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Galbraith *et al.* (1983), with some minor modifications, from dry and from germinating seeds (pleated filter paper, 65% relative substrate moisture content, 15°C, darkness), after 24, 48 and 72 h. The radicle tip was chopped with a sharp razor blade in a plastic Petri dish with 750 µl nucleus-isolation buffer (12.1 g TRIS, 0.5 g $MgCl_2.6H_2O$, 5.0 g NaCl, 1.0 ml Triton X-100 and deionized water to 1 l, pH 7.0). After chopping, the suspension was passed through an 88 µm mesh nylon filter and 20 µl propidium iodide (PI) was added. For each sample 5000 nuclei were analysed at about half an hour after preparation, using a Coulter Epics XL-MCL (Coulter Electronics, USA) flow cytometer. For each seed lot and germination time 20 replications were analysed and the average G_2 / G_1 ratio was calculated from the flow cytometric profiles.

Analysis of β-tubulin accumulation

Seeds were subjected to germination under the same conditions as for flow cytometry. Fifty radicle tips for each seed sample were collected from dry seeds and germinating seeds after 24 and 48 h. The procedures for protein extraction, electrophoretic separation and immuno-detection were basically as previously described by de Castro *et al.* (1995, 1998). The radicle tips were ground, using liquid nitrogen, and the powder was extracted with MODIL buffer (80 mM Tris-HCl, 2% (w/v) sodium dodecyl sulphate (SDS), 15 mg ml^{-1} dithiothreitol (DTT) and 12.5% (v/v) glycerol, pH 9) at 95°C for 10 min. The samples were centrifuged at 17 000 *g* for 7 min and the supernatant was used for analysis of total proteins. For each sample, 60 µg total protein were loaded on an SDS gradient gel (Pharmacia, Sweden) and run at 15°C for 80 min. Subsequently the proteins were transferred by electroblotting to a PVDF membrane (Amersham) for Western blotting analysis. The membranes were probed with anti-β-tubulin monoclonal antibodies (Boehringer-Mannheim, clone KMX-1). After washing, the membranes were probed with a peroxidase conjugated goat-anti-mouse IgG secondary antibody (Boehringer-Mannheim). Immuno-chemoluminescence detection was performed using chemiluminescence blotting substrate (Boehringer-Mannheim) and photo - graphic film according to de Castro *et al.* (1998).

Detection of the B-subunit of 11 S globulin

The untreated dry seeds were analysed for their content of liberated B-subunit from the 11 S globulin as described previously (Job *et al.*, 1997). For this, 100 seeds were ground with two stainless steel balls in a stainless steel cylinder, using a grinder (Retsch) operating at full speed for 1 min. Eight millilitres of extraction buffer E, consisting of 50 mM HEPES, pH 8.0, and 1 mM EDTA, were added to the powder. This suspension was stirred at 5°C for 15 min, and then centrifuged (Sigma type 3K30 centrifuge) at 9000 *g* for 10 min at 5°C. Four millilitres of the supernatant were withdrawn and subjected to a second, clarifying centrifugation at 35 000 *g* for 10 min at 5°C. This fraction was the soluble protein extract. For the extraction of total proteins, SDS was added to the remaining suspension in buffer E to 2% (v/v). Following 15 min incubation at 5°C, this suspension was centrifuged as described above. Aliquots were kept at −20°C until use. Protein concentrations in the various extracts were measured according to Bradford (1976) with bovine serum albumin as standard.

Proteins were transferred from the polyacrylamide gel on to nitrocellulose (Gelman Sciences) using a semi-dry electroblotter. Blots were incubated for 1 h at 25°C in PBS containing 3% (w/v) BSA, then for 1 h with primary antibodies against the B-subunit of sugarbeet 11 S globulin (dilution 1:5000) in PBS containing 1% BSA, followed by three washes with PBS. Following incubation with peroxidase-conjugated secondary antibodies (goat anti-rabbit IgGs) in 1% PBS-BSA, antigens were revealed by adding peroxidase substrate solution (1 µl of 30% H_2O_2 ml^{-1} and 0.5 mg of 4-chloro-1-naphthol ml^{-1}. Samples from mature and primed seeds from a commercial seed lot, provided by KWS Klein-wanzlebener Saatzucht AG (Einbeck, Germany), served as standards.

Statistical analysis

A single factor analysis of variance and a Duncan's test were performed on the G_2 / G_1 ratios, and, after angular transformation of the results, on the laboratory and field tests parameters. An analysis of correlation between studied traits was performed.

Results

Germination and field emergence

Final germination capacity (after 14 d) varied between 64% and 88% for cv. A and between 62% and 75% for cv. B (Table 1). For seeds harvested at the commercial harvest time, the vigour was higher for cv. A than cv. B. However, for the earlier harvested seeds this trait was close to zero for both cultivars. The relatively immature seeds also had reduced germination capacity and field emergence (Tables 1 and 2). The seedlings developed from these seeds achieved a lower fresh weight in the stage two- to four-leaf seedling as well (Table 2). The beneficial effect of soaking was evident for the early harvested seeds of both cultivars; it improved vigour, rate of germination,

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Table 1. Vigour and germination capacity of two triploid sugarbeet cultivars estimated in laboratory germination test at 15°C and 20°C

Cultivar	Temperature	Harvest time	Seed treatment	Seedling length vigour index (%)	Germination capacity (%) after		Ungerminated seeds (%)	
					4 d	14 d	full	empty
A	15°C	early	untreated	0 d*	7 d	64 c	19 a	17 a
			soaked	7 c	59 c	78 b	10 b	12 b
		commercial	untreated	16 b	66 b	81 b	8 b	11 b
			soaked	49 a	83 a	88 a	2 c	10 b
	20°C	early	untreated	1 d	61 c	70 c	15 a	15 a
			soaked	21 c	71 b	77 b	13 a	10 b
		commercial	untreated	43 b	81 a	84 a	3 b	13 ab
			soaked	62 a	82 a	83 a	4 b	13 ab
B	15°C	early	untreated	0 c*	11 d	67[NS]	13[NS]	20[NS]
			soaked	0 c	30 c	68	11	21
		commercial	untreated	9 b	48 b	63	14	23
			soaked	26 a	67 a	70	11	19
	20°C	early	untreated	2 c	49 c	62 b	10[NS]	28 a
			soaked	15 b	54 c	63 b	9	28 a
		commercial	untreated	17 b	69 b	72 a	10	18 b
			soaked	45 a	74 a	75 a	8	17 b

* Values for a particular parameter and cultivar at determined germination temperature followed by the same letter are not significantly different at $P = 0.05$ (Duncan's test)
[NS] No significant difference

Table 2. Field emergence and fresh weight of the seedlings of two triploid sugarbeet cultivars

Cultivar	Harvest time	Seed treatment	Field emergence – first count (%)	Field emergence – final count (%)	Fresh weight of 100 seedlings in two- to four-leaf stage (g)
A	early	untreated	10 c*	19 c	111 c
		soaked	23 b	39 b	191 bc
	commercial	untreated	54 a	60 a	258 ab
		soaked	51 a	65 a	281 a
B	early	untreated	3 c	10 c	99 b
		soaked	16 b	31 ab	172 a
	commercial	untreated	20 b	29 b	174 a
		soaked	30 a	37 a	214 a

* Values for a particular parameter and cultivar followed by the same letter are not significantly different at $P = 0.05$ (Duncan's test)

final laboratory germination and field emergence (Tables 1 and 2). For the late mature harvested seeds of both cultivars, the soaking treatment was also beneficial, but to a lesser extent. This treatment improved neither the germination capacity at 20°C nor the field emergence of cv. A mature seeds (commercial harvest time), nor the final germination capacity of the cv. B seeds.

Nuclear DNA replication

For the dry seeds from both harvest dates, most of the cells in the radicle tip were arrested in the G_1/G_0 phase of the cell cycle, with a 3C DNA content in accordance with their (triploid) ploidy level. However, there was a considerable number of radicle tip cells containing nuclei with the double amount of DNA

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Table 3. G_2 / G_1 ratio in radicles of dry and germinating seeds of two triploid sugarbeet cultivars

Cultivar	Harvest time	Seed treatment	G_2 / G_1 ratio at time after start of imbibition (h) 0	24	48	72
A	early	untreated	0.063 **b***B**	0.069 **b** B	0.269 **b** A	0.355 **c** A
		soaked	0.071 **b** C	0.060 **b** C	0.283 **b** B	0.826 **b** A
	commercial	untreated	0.179 **a** C	0.176 **a** C	0.491 **a** B	0.837 **b** A
		soaked	0.206 **a** C	0.170 **a** C	0.591 **a** B	1.101 **a** A
B	early	untreated	0.066 **b***B**	0.064 **b** B	0.153 **c** B	0.758 NSA
		soaked	0.066 **b** C	0.100 **b** C	0.490 **b** B	1.007 A
	commercial	untreated	0.148 **a** C	0.161 **a** C	0.398 **b** B	0.831 A
		soaked	0.139 **a** C	0.158 **a** C	0.794 **a** B	1.157 A

* Values for particular cultivars and germination time (in columns) followed by the same small letter are not significantly different at $P = 0.05$ (Duncan's test)
** Values for particular seed lot (in lines) followed by the same capital letter are not significantly different at $P = 0.05$ (Duncan's test)
NS No significant difference

(6C), which were apparently arrested in the G_2 phase of the cell cycle. For both cultivars, the frequency of G_2 phase nuclei was significantly higher with seed harvested at the late mature stage, compared to the immature seeds (Table 3). The 6 h soaking did not influence the G_2 / G_1 ratio in dry seeds.

The G_2 / G_1 ratio increased during imbibition and prior to radicle protrusion (Table 3), which was in almost all cases later than 72 h after the start of imbibition. For seed lots, other than the early harvested and untreated seeds of cultivar B, a significant increase in G_2 occurred between 24 and 48 h after the start of imbibition and the DNA replication further progressed during the third day. The soaking treatment resulted in a faster rate of DNA replication for both cultivars at both harvest dates.

Positive correlations between the G_2 / G_1 ratio and traits characterizing quality of the seeds were found (Table 4).

Table 4. Selected coefficients of correlation between the different traits

Trait	G_2 / G_1 ratio In dry seeds	After 24 h germination	After 48 h germination	After 72 h germination
1	0.890**	0.768**	0.712**	0.667**
2	0.810**	0.740**	0.707**	0.748**
3	0.641**	0.407	0.328	0.447
4	0.852**	0.800**	0.796**	0.798**
5	0.862**	0.749**	0.599*	0.399
6	0.804**	0.634**	0.450	0.279
7	0.857**	0.788**	0.661**	0.507*
8	0.804**	0.708**	0.610**	0.527*
9	0.856**	0.786**	0.715**	0.670**

1: seedling (radicle or radicle / hypocotyl axis) length vigour index at 15°C
2: germination capacity after 4 d at 15°C
3: germination capacity after 14 d at 15°C
4: seedling (radicle or radicle / hypocotyl axis) length vigour index at 20°C
5: germination capacity after 4 d at 20°C
6: germination capacity after 14 d at 20°C
7: field emergence – first count
8: field emergence – final count
9: weight of 100 seedlings in the two- to four-leaf stage
* Significant at $P = 0.05$
** Significant at $P = 0.01$ (Student's t test)

Accumulation of β-tubulin

No β-tubulin could be detected in the root tip samples from the dry seeds. However, it could be detected in germinating seeds, especially after 48 h of imbibition, having the same 55 kDa molecular mass as the control bovine brain tubulin (Fig. 1). The β-tubulin identity of these signals was confirmed, by a negative control, in which the primary monoclonal anti-β-tubulin antibody was omitted (data not shown).

During imbibition, β-tubulin showed a similar accumulation trend in all samples, occurring before radicle protrusion. Although the western blotting technique was not sufficient for detailed quantification of β-tubulin, the analysis showed clearly that the accumulation was faster with the late mature seeds and especially with those seeds which had received a soaking treatment.

Solubilization of the B-subunit of 11 S globulin

SDS-PAGE analysis of total protein extracts from seeds of both cultivars showed an accumulation of storage proteins from the immature to the more mature stage. In particular, a major protein of 20 kDa, which corresponded to the B-subunit of the 11 S globulin, was present in higher amounts in total protein extracts from late mature seeds when compared to those from

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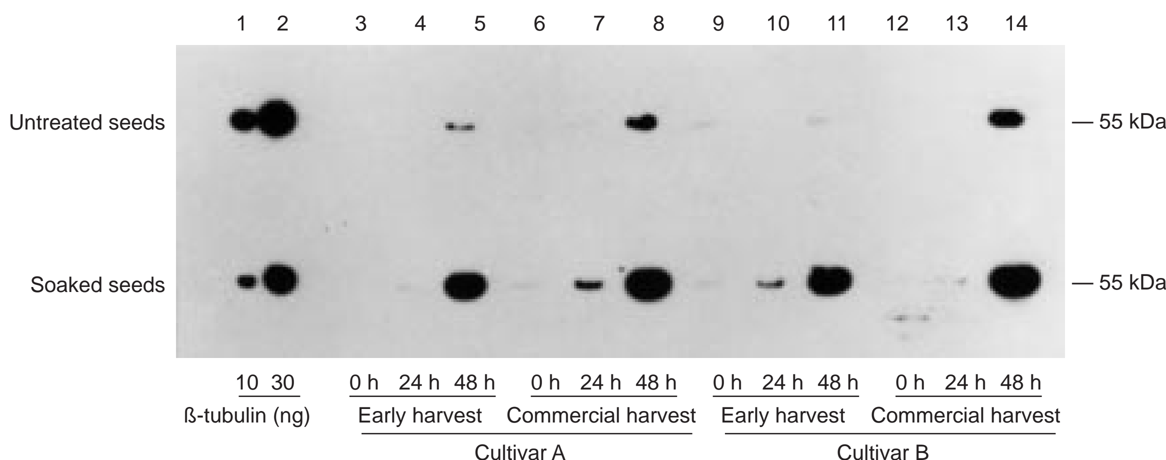

Figure 1. Detection by western blotting of β-tubulin in radicle tips of untreated and soaked seeds of sugarbeet cultivars A and B. Extracts were prepared from radicle tips isolated from dry seeds and from seeds germinated for 24 and 48 h. For the seed samples 50 μg of protein was loaded. Pure bovine brain β-tubulin was loaded as control in amounts of 10 and 30 ng (lanes 1 and 2). The position of the bands correlates with a molecular mass of 55 kDa as indicated on the right.

early harvested seeds (Fig. 2A). When soluble protein extracts from the various seed samples were analysed by western blotting, a low amount of soluble B-subunit was detected in immature seeds of both cultivars (Fig. 2B and C, lanes 2 and 4). This markedly increased in soluble protein extracts from the mature seeds (lanes 1 and 3). Thus, concomitant with accumulation within protein bodies during maturation, a substantial part of the 11 S globulin was mobilized, resulting in the solubilization of part of the B-subunit, as normally occurs during seed priming and early germination. The latter was confirmed by the control commercial sample from mature and primed seeds, which showed a much higher amount of 11 S globulin B-subunit in soluble protein extracts in the primed seeds, compared to that in the untreated seeds (Fig. 2B and C, lanes 5 and 6).

Discussion

sugarbeet seed quality increases towards the end of the maturation period (Grimwade *et al.*, 1987). Also, in the present experiments the quality of the seeds harvested at commercial harvest time was higher than that of the early harvested seeds. For both cultivars, the early harvested seeds performed poorer with respect to vigour, germination capacity and field emergence. Nevertheless, even for the seeds harvested at the commercial harvest time, the germination capacity was lower than is characteristic for commercial lots, which is usually over 90%. This is because in our experiment the seed lots were only pre-cleaned with no further processing. The seed lots still contained considerable quantities of empty seeds which are normally removed during commercial processing of sugar-beet seeds. Consequently, one of the reasons for the higher seed quality of cv. A than cv. B must be the lower percentage share of empty seeds in the former, most likely as a result of more favourable weather conditions in France during the flowering period.

Early harvested seeds of cv. A contained more underdeveloped (full, but ungerminated) seeds than the lot harvested at commercial harvest time, whereas in cv. B harvest time did not influence the proportion of this seed fraction (Table 1). Most probably a substantial part of underdeveloped seeds collected during early harvest of cv. B had been taken away during sizing. This result confirmed the suggestion of TeKrony and Hardin (1969), that underdeveloped seeds can be removed by processing and sizing, but not from all seed lots.

The poorer quality of the 2-weeks-earlier harvested seeds is in agreement with previous investigations (Lexander, 1980 and references therein), showing an increase in germination percentage with increasing ripeness of sugar-beet seeds. This increase in germination capacity may also be related to a decrease in the concentration of germination inhibitors. Support for this presumption is the observation that soaking improved seed vigour, germination capacity and field emergence of almost all tested seed lots. Indeed, the beneficial effect of soaking was more evident for the early harvested seeds, and this treatment did not improve the germination capacity at 20°C or field emergence of cv. A mature seeds (commercial harvest time), nor the final germination capacity of the mature cv. B seeds.

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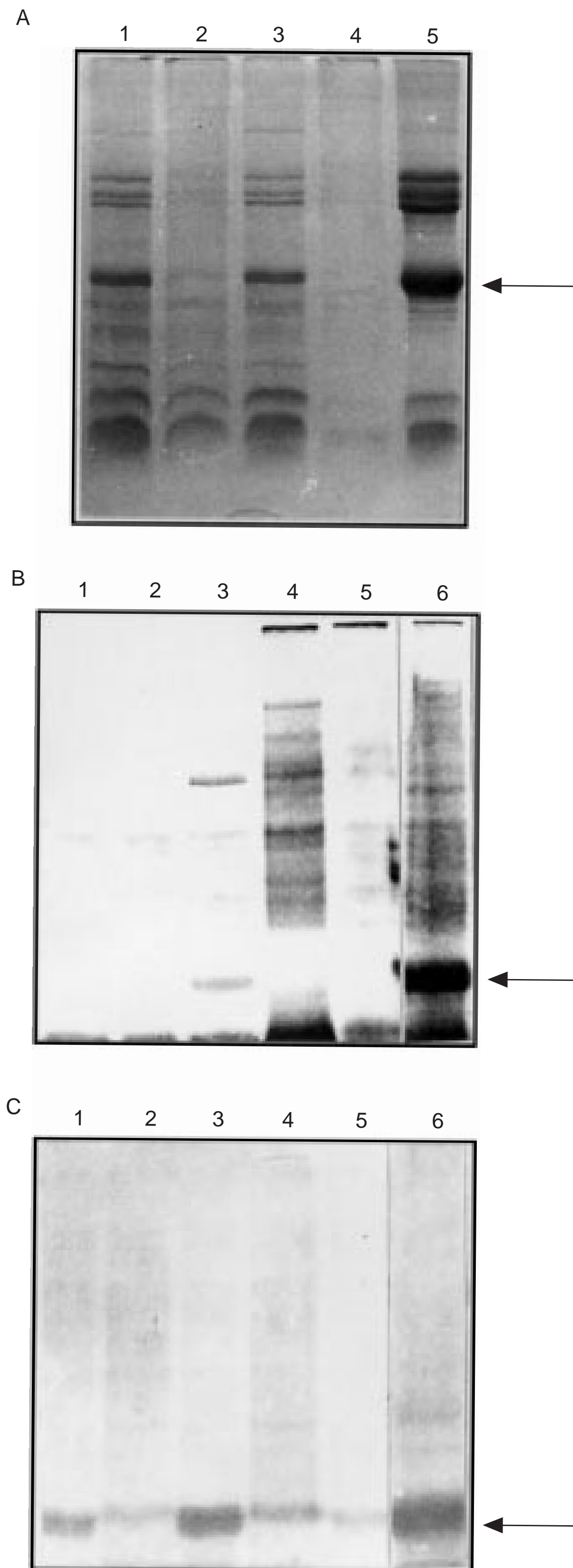

Most of the cells in the radicle tip of dry sugar-beet seed were arrested in the G_1/G_0 phase of the cell cycle, but with a considerable portion arrested in the G_2 phase. During sugar-beet seed development, after a period of intensive division during the first 4 weeks after pollination, the proportion of particular replication stages becomes stabilized (Śliwińska, 1998). Apparently, cell cycle activity was arrested, well before the early harvest in the present experiments. Based on these observations a change in the G_2/G_1 ratio during the last 2 weeks of seed maturation was unlikely. However, in immature seeds of both cultivars the G_2/G_1 ratio was lower than in mature seeds (Table 3). The higher share of 6C nuclei in the radicle tips from the late mature seeds indicates that cell cycle activity has resumed in the last 2 weeks before commercial harvest. For several seed species it has been demonstrated that initiation of cell cycle activity is an indication of the onset of germination (e.g. Bino *et al.*, 1995). The present study showed that cell cycle activation can be used as a marker for the potential onset of germination processes prior to harvest of sugar-beet seeds.

The results obtained in the present experiments appear to confirm the suggestion of Deltour (1985) and Bino *et al.* (1993) that the external conditions during seed maturation, as well as the harvest time, affect the G_2/G_1 ratio of embryonic cells. It should be noted that at both locations, in France and Poland, heavy rainfalls and lower than optimal temperatures occurred during the last 2 weeks before commercial harvesting. The rain might have leached out some germination inhibitors from the fruit, and by simultaneously increasing the seed moisture levels, induced DNA replication which caused the augmentation of G_2 signals. The higher proportion of G_2 phase radicle tip cells in the late mature seeds indicates that germination processes had started within the seeds while still attached to the mother

Figure 2. SDS-PAGE analyses of seed storage proteins in two sugar beet cultivars. (A) SDS-PAGE analysis (18% homogenous polyacrylamide gel) of total protein extracts. Proteins were revealed by Coomassie blue staining. An equal amount of 10 µg protein was applied to each lane. Lane 1, mature seeds of cv. B; lane 2, early harvested seeds of cv. B; lane 3, mature seeds of cv. A; lane 4, early harvested seeds of cv. A; lane 5, mature seeds obtained from KWS. The arrow marks the position of the B-subunit of 11 S globulin. (B and C) SDS-PAGE analysis (12% homogenous polyacrylamide gel) of soluble protein extracts. Proteins were revealed by Coomassie blue staining. (B) The B-subunit of 11 S globulin was specifically identifed by Western blotting. (C) An equal amount of 40 µg protein was applied to each lane. Lane 1, mature seeds of cv. B; lane 2, early harvested seeds of cv. B; lane 3, mature seeds of cv. A; lane 4, early harvested seeds of cv. A; lane 5, mature seeds from KWS; lane 6, primed KWS seeds. The arrow marks the position of the B-subunit of 11 S globulin.

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plant. This assumption is supported by higher amount of the soluble B-subunit of the 11 S globulin in late harvested seeds, as compared to that present in the early harvested ones. Increased solubilization of part of the B-subunit normally occurs during seed priming and early germination (Job *et al.*, 1997), as was confirmed by the control samples from untreated and primed seeds from the commercial seed lot.

The results showing detection of β-tubulin are in an agreement with those of de Castro *et al.* (1995, 1998), who found that in imbibing tomato seeds the β-tubulin signal was first detectable between 24 and 48 h. Some previous investigations stated that during germination β-tubulin accumulation preceded the onset of DNA replication in tomato (de Castro *et al.*, 1995; Bino *et al.*, 1996) and in cabbage seeds (Górnik *et al.*, 1997). In the present experiments both cell cycle events seem to occur simultaneously during sugar-beet seed germination. However, during the onset of germination, prior to the later harvest, DNA replication had occurred without a detectable increase in β-tubulin. Both cell cycle processes may therefore be regulated independently, or the levels of β-tubulin associated with this replication were too low to detect.

Late mature harvested seeds exhibited increased germinability, vigour and field emergence. In commercial practice, sugar-beet seeds are frequently primed, to obtain such characteristics. It seems that the late mature harvested seeds used in the present experiments have received at least a partial priming treatment. This is in accordance with observations made for a number of other species, which found that hydration and subsequent dehydration of the seeds at early germination conferred increased vigour on the embryos (for a review see Hegarty, 1978). Dehydration after the radicle pierces the seed coats can kill the seed, however (Côme and Thévenot, 1982).

The soaking treatment applied in the present experiment did not initiate DNA replication in cells of the radicle. Similar observations were made by Redfearn and Osborne (1997), who found that a 6 hours-long thiram-steeping did not significantly increase the seed's nucleic acid contents. However, the effect of soaking on the cell cycle was observed during germination of the seeds. The higher G_2 / G_1 ratio and greater accumulation of β-tubulin in treated seeds was associated with a faster and earlier cell cycle activity in these seed lots. The results showed that an improvement of the seed quality by soaking is not only due to leaching of the inhibitors from the pericarp, but this treatment also influences cell cycle activity during subsequent germination.

It is concluded that combined analyses of the cell-cycle-related events in radicle tips of sugarbeet seeds, by flow cytometry, monitoring DNA replication, and by detection of β-tubulin accumulation, as well as analysis of the extent of the solubilization of 11 S storage protein in whole seeds, can be helpful in understanding and predicting sugarbeet seed quality.

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