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FLOW CYTOMETRIC ANALYSIS OF NUCLEAR REPLICATION STAGES DURING GERMINATION OF SUGAR-BEET SEEDS DIFFERENT IN VIGOUR

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ABSTRACT

The nuclear replication stages in dry and germinating seeds of two sugar-beet cultivars were determined by flow cytometry. For each cultivar two seed lots of similar, high germination capacity, but different in seedling length vigour index (SLVI; applied as quality marker) were studied. Analyses were performed every 12 hours during 72 hours of germination and early seedling growth at 20°C and 15°C. Before start of germination, in all seed lots, about 90% of cells were arrested in the G_1 phase of the cell cycle. The proportion of G_1 cells decreased with time

of germination, faster in higher quality seed lots than in those of lower quality. The G_2/G_1 ratio in dry seeds was slightly higher for the seeds of higher SLVI, and it increased during germination in all seed lots. However, the difference between higher and lower quality seeds of the same cultivar did not become more distinct during germination. The differentiation in cell cycle activity was more evident when seeds of one of the cultivars, characterised by a very high SLVI, were compared with seeds of another with lower SLVI.

Key words: *Beta vulgaris* L., cell cycle, DNA replication, flow cytometry, germination, seed, vigour

INTRODUCTION

Flow cytometry, a method widely used for plant ploidy estimation, can be applied to the study of the cell cycle in the seeds [2,3,7, 12, 14, 15, 16, 17, 18]. The cell cycle of proliferating cells is divided into four phases. After mitosis (M), there is a period of normal cell growth (G_1). When the cell starts to make new DNA it has entered the S (DNA synthesis) phase of the cell cycle. The completion of DNA synthesis is followed by a second, preparative to mitosis, growth period (G_2) [1]. Deltour [5] found that mature embryos of some species contain both G_1 and G_2 nuclei while other species contain G_1 nuclei only. Flow cytometric analysis of the seeds of tomato [2,14], pepper [12,13], maize [7] and sugar beet [15] has shown an increase of the proportion of G_2 cells during germination; therefore DNA synthesis precedes the completion of germination, which is marked by emergence of the radicle, and subsequent seedling growth.

High quality commercial seed lots are demanded by farmers for planting in the field. Usually the germination capacity of sugar-beet lots should reach at least 95% to be competitive on the market. In addition to seed lots having a high germination capacity it is also very important that they have uniform field emergence, since this tends to improve plant establishment and uniformity of the crop at harvest. Thus there is considerable interest in developing an effective method for predicting seed vigour.

In our previous investigations [16,18] we reported on our research to adapt flow cytometry to sugar-beet seed testing. A significantly higher G_2/G_1 ratio was found in dry mature seed lots of low vigour index than in the corresponding seed lots of high vigour index [16]. This was most probably due to a high proportion of undeveloped seeds that are characterised by high cell cycle activity in the embryo [17] and show lower germination capacity [8]. Application of presowing treatments, such as priming or soaking, to the fully developed seeds can also increase the G_2/G_1 ratio, due to germination [2,12,13, 18]. It was suggested that flow cytometry provides useful information about the physiological status of the seed during development and germination.

In the present study the cell cycle in sugar-beet seed lots of similar, high germination capacity (97-99%), but different in SLVI, has been investigated under optimal and lower temperature to find the differences in the distribution of nuclear replication stages of particular seed lots during germination. Detection of such differences could be helpful in predicting the ability of the seeds to germinate quickly.

MATERIALS AND METHODS

Cleaned and sized untreated seeds of two sugar-beet cultivars, Formula (2x) and Matador (3x), were used. Two seed lots of each cultivar were investigated.

A laboratory germination test was performed for 14 days in pleated filter paper at 20°C [8] and 15°C, with 65% substrate moisture [10], in darkness. The germination capacity after four and 14 days, as well as the seedling length vigour index (SLVI; the percentage of seeds with

radicle/hypocotyl axis over 15 mm at 20°C and over 5 mm at 15°C, after 96 h), were determined.

Field tests were performed at Polanowice, central Poland (4 replicates of 100 naked, slurry treated [11] seeds for each seed lot). Field emergence was determined by counting the number of plants established at three days (first count) and 21 days (final count) after the first seedlings emerged from the soil. When the seedlings reached the 2-4-leaf stage, they were collected from the field, cleaned and the fresh weight of 100 seedlings was determined.

For flow cytometry, the true seeds were removed from the pericarp prior to the start of germination (dry seeds) and after 12, 24, 36, 48, 60, and 72 hours from the start of imbibition. Samples were prepared according to Galbraith et al. [6], with some minor modifications, following a one-step protocol [19]. Fluorescence of 4',6-diamidino-2-phenylindole (DAPI) was measured in a Partec CA II flow cytometer (Partec GmbH, Germany) equipped with an HBO 100W/2 lamp, KG1, B38, UG1 filters, a CG435 barrier filter, a TK420 dichroic mirror and a 40 x 0.8 quartz objective. In each sample 5,000 to 10,000 nuclei were measured and analysed using the Partec DPAC V2.1 computer programme. For each period of germination the weighted mean of 30 replications was calculated to determine the percentage share of particular replication stages. The G_2/G_1 ratios were calculated based on absolute values.

A Student's *t*-test for independent samples was performed on the G_2/G_1 ratios and, after angular transformation, on laboratory and field test parameters.

RESEARCH RESULTS AND DISCUSSION

Laboratory germination tests showed that the seed lots of particular cultivars were equal in germination capacity after four and 14 days, but different with respect to SLVI (Table 1). This index has been recognised as having a good correlation with seed vigour [10], and therefore it was used here as a marker of seed quality. The seeds of cv. Formula demonstrated a lower SLVI than those of cv. Matador. Similarly, in the field cv. Matador demonstrated a higher field emergence at the first count and a greater fresh weight of 100 seedlings at the 2-4-leaf stage. However, the final count showed the same field emergence for all samples of both cultivars (Table 2). There were no statistically significant differences between the two investigated seed lots of particular cultivars in respect of any field parameter. Thus, the difference in vigour observed in the laboratory was not confirmed under field conditions.

Table 1. Seedling length vigour index and germination capacity of different seed lots of sugar-beet diploid cultivar Formula and triploid cultivar Matador

Cultivar	Temperature	Seed lot	Seedling length vigour index (%)	Germination capacity after 4 days (%)	Germination capacity after 14 days (%)
Formula	20°C	1	38 a*	96 NS	98 NS
		2	44 b	94	98
	15°C	1	1 a	56 NS	97 NS
		2	3 b	64	98
Matador	20°C	1	76 a	98 NS	99 NS
		2	90 b	98	98
	15°C	1	29 a	92 NS	99 NS
		2	48 b	93	99

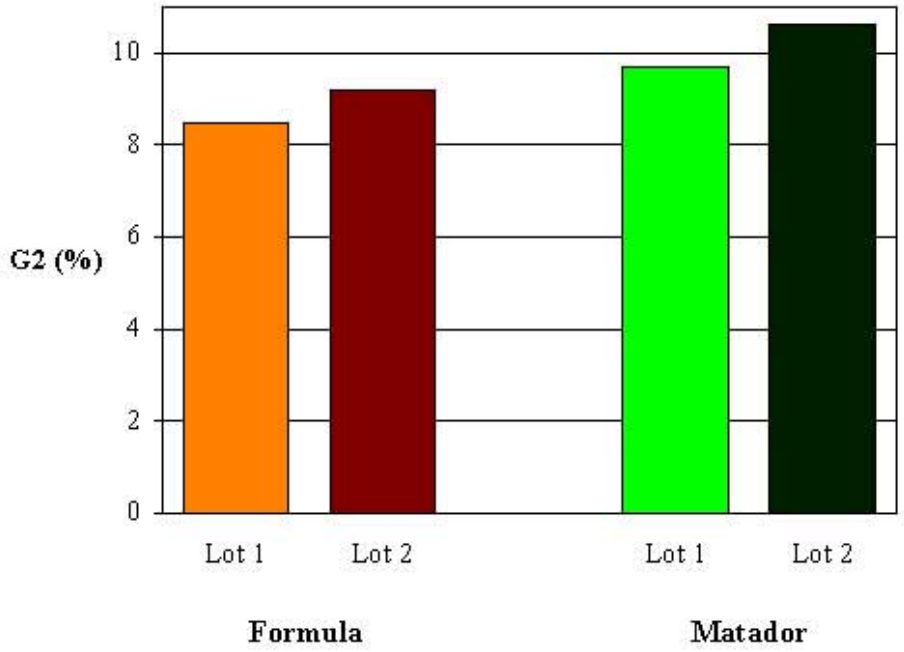
*values for particular seed lots followed by different letters are significantly different at P=0.05 (Student's *t*-test for independent samples)
NS no significant differentiation

Table 2. Field emergence and fresh weight of 100 seedlings in stage 2-4-leaf of different seed lots of sugar-beet diploid cultivar Formula and triploid cultivar Matador

Cultivar	Seed lot	Field emergence - first count (%)	Field emergence - final count (%)	Fresh weight of 100 seedlings in 2-4-leaf stage (g)
Formula	1 2	59^{NS} 54	78^{NS} 81	189^{NS} 170
Matador	1 2	68^{NS} 74	79^{NS} 81	274^{NS} 324

^{NS} no significant differentiation between the lots of particular cultivars (Student's *t*-test for independent samples)

Figure 1. Proportion of G_2 cells in the dry seeds of different quality seed lots of two sugar-beet cultivars (Lot 1 – lower quality, Lot 2 – higher quality)



Flow cytometric analysis showed that the seeds contained only a few endosperm cells (data not shown), which suggests that the seeds were fully developed [17]. Before germination, in all seed lots, about 90% of the embryo cells were arrested in the G_1 phase of the cell cycle (Table 3 and Table 4). For both cultivars dry seeds of the higher SLVI lots showed a slightly higher proportion of G_2 cells (Figure 1). This was statistically significant for the cv. Formula only, however, which indicates a higher cell cycle activity in these seeds. This, in turn, means that they should possess the ability to germinate faster. Differences between seed lots in the cell cycle activity, estimated by flow cytometry, becomes more distinct when the radicle tip, instead of the whole true seed, is analysed [16, 18]. To determine whether during germination the better quality seeds can synthesise DNA more rapidly than the ones of lower SLVI, changes in the G_2/G_1 ratio were followed during 72 hours from the start of imbibition, at 20°C and 15°C (Figure 1 and Figure 2). At an optimal temperature (20°C), an emergence of the radicle was observed after 72 h for the cv. Formula and after 60 h for the cv. Matador. The

G_2/G_1 ratio clearly increased with the time of imbibition. In addition to the G_1 and G_2 cells, those with higher amounts of DNA occurred in the embryo (up to 16C in the diploid cultivar and 24C in the triploid cultivar; Table 3 and Table 4), which was due to endoreplication. The cells of the triploid cultivar went through this process earlier during germination, and with higher frequency, than those of the diploid cultivar. This appears to confirm the results of Butterfass and Schlayer [4] who induced mitosis to young sugar-beet roots, and found a higher level of endopolyploidy in the triploid roots than in the diploid ones. One of the reasons for this phenomenon could be aneuploidy that sometimes occurs in triploid hybrids.

Figure 2. Changes in the G_2/G_1 ratio in the embryo of two sugar-beet cultivars during germination and early seedling growth at 20°C (Lot 1 – lower quality, Lot 2 – higher quality; * - mean values for particular cultivars at particular germination time are significantly different at *P*=0.05; Student's *t*-test for independent samples)

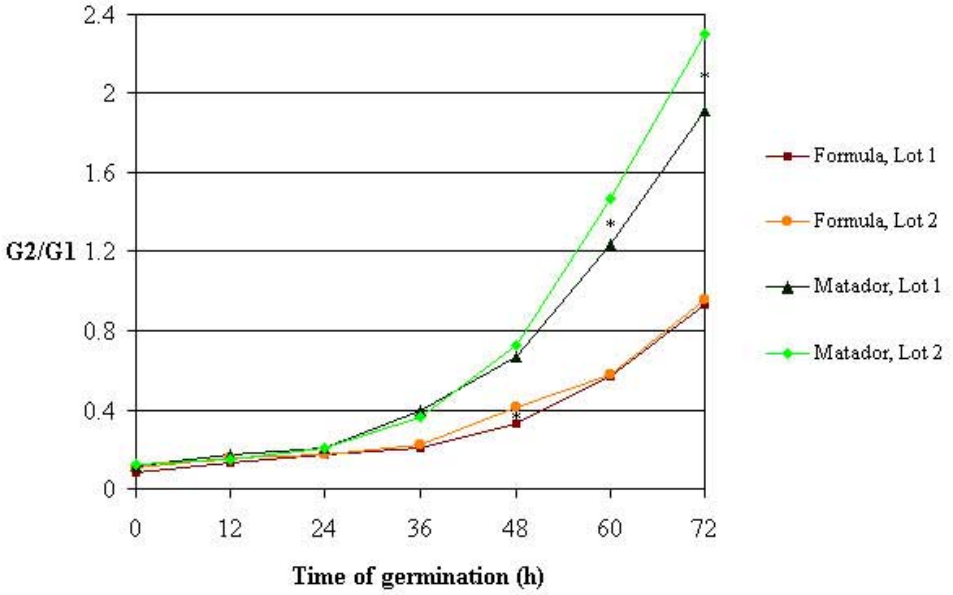


Table 3. Percentage share of nuclei with different DNA content expressed as C values in germinating seeds of two seed lots different in seedling length vigour index (SLVI) of diploid sugar-beet cultivar Formula

Temperature of germination	Time of germination	Relative nuclear DNA content (%)			
		2C	4C	8C	16C
Seed lot of lower SLVI (1)					
20°C	12	87.0	13.0	0	0
	24	85.5	14.5	0	0
	36	83.3	16.7	0	0
	48	72.6	24.2	3.1	0
	60	59.3	32.1	8.6	0
	72	45.3	39.3	12.4	3.0
15°C	12	91.0	9.0	0	0
	24	87.2	12.8	0	0
	36	85.9	14.1	0	0
	48	85.5	14.5	0	0
	60	81.7	15.9	2.4	0
	72	77.7	19.3	3.0	0

Seed lot of higher SLVI (2)					
20°C	12	86.9	13.1	0	0
	24	85.5	14.5	0	0
	36	81.8	18.2	0	0
	48	66.7	27.1	6.1	0
	60	57.7	32.8	9.5	0
	72	42.7	38.8	14.5	4.0
15°C	12	90.1	9.9	0	0
	24	85.2	14.8	0	0
	36	85.7	14.3	0	0
	48	84.1	15.9	0	0
	60	80.7	19.3	0	0
	72	73.7	22.6	3.7	0

Table 4. Percentage share of nuclei with different DNA content expressed as C values in germinating seeds of two seed lots different in seedling length vigour index (SLVI) of triploid sugar-beet cultivar Matador

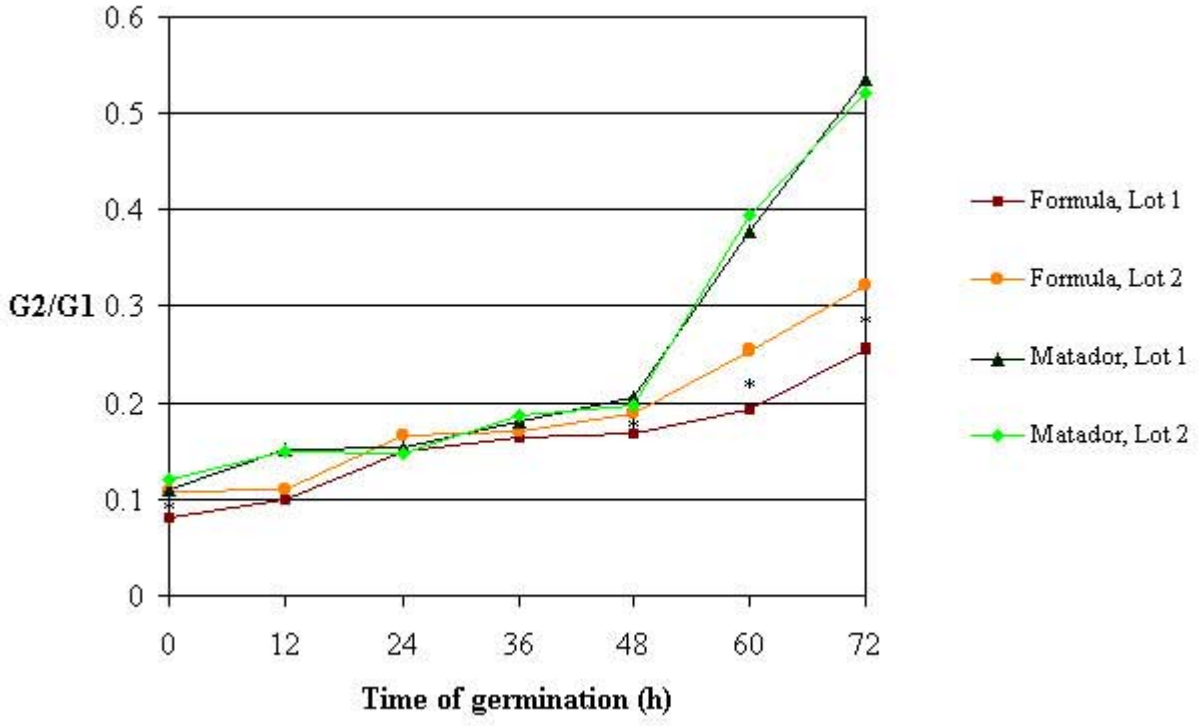
Temperature of germination	Time of germination	Relative nuclear DNA content (%)			
		3C	6C	12C	24C
Seed lot of lower SLVI (1)					
20°C	12	84.3	13.9	1.8	0
	24	81.0	16.3	2.7	0
	36	68.7	26.9	4.4	0
	48	55.2	35.6	7.9	1.3
	60	35.5	42.7	18.0	3.8
	72	24.2	44.0	25.1	6.7
15°C	12	87.1	12.9	0	0
	24	86.5	13.5	0	0
	36	85.3	14.7	0	0
	48	82.5	17.5	0	0
	60	70.8	25.3	3.8	0
	72	61.5	32.1	6.4	0
Seed lot of higher SLVI (2)					
20°C	12	85.8	12.7	1.4	0
	24	82.8	16.0	1.2	0
	36	71.6	24.9	3.5	0
	48	52.6	37.2	9.1	1.1
	60	32.3	46.4	17.7	3.6
	72	20.8	47.2	23.9	8.1
15°C	12	87.0	13.0	0	0
	24	87.0	13.0	0	0
	36	84.4	15.6	0	0
	48	84.0	16.0	0	0
	60	69.9	26.1	4.0	0
	72	62.2	32.0	5.8	0

Statistical analyses showed that at an optimal germination temperature the cell cycle activity in the cv. Formula was increasing at a similar rate in both seed lots. The G_2/G_1 ratio for the lot

with the higher SLVI was significantly higher than that for the lot with the lower SLVI only after 48 hours. However, in the better quality seed lot of cv. Matador a more rapid increase in the G_2/G_1 ratio occurred after 60 hours and later when compared to the lower quality lot (Figure 2). As it was mentioned before, at that time the radicle was already visible. This suggests that the higher SLVI of this seed lot was due to a higher cell division rate during seedling growth rather than to faster DNA synthesis during germination sensu stricto. This assumption seems to be supported by the higher fresh weight of the seedlings obtained from very high SLVI seeds of cv. Matador growing in the field (Table 2).

At a lower germination temperature (15°C), no radicle emergence occurred and the G_2/G_1 ratio increased much more slowly. The increase was similar for all seed lots up to 48 hours of germination and than it became faster, especially in the seeds of cv. Matador (Figure 3). However, the two lots of this cultivar of different quality did not differ much from each other. In the case of cv. Formula the G_2/G_1 ratio in the better quality lot after 48, 60 and 72 hours of germination was significantly higher than in the lower quality lot. This is indicative of a higher cell cycle activity during germination sensu stricto in the first seed lot.

Figure 3. Changes in the G_2/G_1 ratio in the embryo of two sugar-beet cultivars during germination at 15°C (Lot 1 – lower quality, Lot 2 – higher quality; * - mean values for particular cultivars at particular germination time are significantly different at *P*=0.05; Student's *t*-test for independent samples)



It is noteworthy that in the very high SLVI seed lots (cv. Matador) the increase in cell cycle activity during germination was much faster than in the lower SLVI seed lots (cv. Formula; Figure 2 and Figure 3). Obviously, flow cytometric analysis can easy distinguish between seed lots if the difference in vigour is substantial. It seems that flow cytometric analysis of dry seeds is sufficient for such an estimation and study of the G_2/G_1 ratio after imbibition does not give much more information about the seed germination potential.

Since the results from flow cytometry can be obtained much faster and easier than those from laboratory germination tests, this method should be useful for seed producers if they need to know quickly the quality of the seed lots, originated from different growers. Nevertheless, the results demonstrate that estimation of seedling length vigour index (SLVI) can better differentiate between the seed lots of very similar high quality than the G_2/G_1 ratio. The results of flow cytometry fully correspond with those from field test that also showed only small differences between seed lots of the same cultivar.

CONCLUSIONS

1. The seeds of higher SLVI show higher cell cycle activity in the embryos of dry and germinating seeds. Flow cytometric analysis of germinating seeds does not provide more information about seed lot quality than the analysis of dry seeds and, being less time- and cost-effective, it is not recommended for routine work.
2. The difference between the G_2/G_1 ratio in higher SLVI seeds germinating at optimal temperature increases rapidly after the transition from the completion of germination sensu stricto to growth. We suggest that a higher cell division rate in the seedlings rather than a faster DNA synthesis in the germinating embryo is responsible for high vigour.
3. While flow cytometry cannot replace laboratory germination tests, it can be useful for the quick recognition of different quality seed lots. Nevertheless, further studies on a higher number of different quality lots, using only the radicle of dry seeds, would be advisable.

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