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EFFECT OF PRESOWING HYDRATION TREATMENT ON DNA REPLICATION ACTIVITY IN THE EMBRYO OF SUGAR BEET (*BETA VULGARIS* L.)

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ABSTRACT

The effect of presowing hydration treatment on DNA replication activity in the embryo of three sugar beet cultivars was studied using flow cytometry. The treatment protocol consisted of soaking the seeds for 2 hours in tap water and then for 2 hours in 0.2% NaOH solution. After drying, a slurry treatment with Oxafun T was applied. The applied treatment was aimed at removing soluble inhibitors from the pericarp and to control of damping off. Seedling length vigour index (SLVI), germination capacity and the relative proportions of the DNA replication stages were determined in untreated and treated seeds. Hydration treatment increased seed performance, in particular the vigour index. A higher G_2/G_1 ratio was noted for treated seeds than the untreated ones, which suggests that cells of the former seeds had entered the synthetic phase of the cell cycle. A positive correlation was found between the G_2/G_1 ratio and laboratory test parameters (SLVI and germination capacity). The results indicate that flow cytometry can be helpful in following the progress of cell cycle activity resulting from seed treatment.

Key words: cell cycle, DNA replication, flow cytometry, seed treatment, vigour

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INTRODUCTION

Flow cytometry, a method for measuring DNA content, also gives information about the cell cycle. Non-cycling cells are said to be in the G_0 stage. For cycling cells, it is usual to define four distinct phases of the cell cycle. Mitosis (M phase) is followed by the G_1 phase (gap 1). During this phase the cell continuously grows but does not replicate its DNA. When the cell starts to make new DNA it has entered the S (DNA synthesis) phase. The completion of DNA synthesis is followed by the G_2 phase (gap 2), during which cell growth continues and proteins are synthesised in preparation for mitosis [1]. Flow cytometry has been shown to be very suitable for determining the DNA replication stages in seeds [2,3,10,11,16,17,18].

In commercial practice, sugar beet seeds are often washed/soaked and treated with fungicides before sowing. These treatments are intended to leach out the soluble inhibitors from the pericarp and to control damping off, which, in turn, improve seed performance in the field [8,15]. It is also suggested that the presowing treatment (called usually 'priming') is related to activation of enzymes, which allows the repair of membranes and DNA damage, as well as metabolic advancement due to activation of the processes involved in germination [4,13]. Redfearn and Osborne [14] found that the 'advancement treatment' of sugar-beet seeds (according to Durrant and Mash [5]) causes an increase in RNA and DNA and that the cells of the treated seeds showed more progress in the cell cycle than the untreated ones. Similarly, an increase in nuclear DNA content has been demonstrated in primed tomato and pepper seeds [2,3,10,11,12]. However, Saracco et al. [16] showed that priming of pepper seeds, which improved germination performance, does not always affect nuclear replication activity.

In the present paper, we report the effect of a presowing hydration treatment, to sugar-beet seeds, on nuclear replication stages in the embryo. The changes in cell cycle activity due to this treatment were followed by means of flow cytometry. The aim of the study was to answer the questions as to whether the hydration treatment, which improves vigour (estimated on the base of SLVI), influences the embryo cell cycle directly and whether flow cytometry could be useful in predicting the effectiveness of the presowing treatment.

MATERIALS AND METHODS

Commercial sugar-beet seeds of the triploid cultivar Kawejana and two diploid cultivars, Jastra and PN Mono 1, were investigated. The seeds were kindly provided by the Polish company Kutnowska Hodowla Buraka Cukrowego (Kutno). Seed samples of each cultivar were divided into two parts. One of them was analysed as the untreated control and the other one was treated as follows: seeds were soaked for 2 h in tap water and 2 h in 0.2% solution of NaOH (in both cases water/solution: seed ratio as 5:1, on a weight basis). Soaking in NaOH is routinely applied in our laboratory because it has been proven to effectively decrease the content of both organic and inorganic inhibitors and thus improves the vigour of the seed [15]. After drying the seeds for 24 h at room temperature the slurry treatment, aimed at controlling damping off, was applied [8].

Laboratory germination test was performed according to ISTA rules [7] with some minor modifications (e.g. pleated filter paper at 65% relative substrate moisture content, in darkness, at 20°C). The germination capacity after 4, 7, and 14 days as well as the seedling length vigour index (SLVI; the percentage of seeds with radicle/hypocotyl axis over 15 mm long at 20°C, after 96 hours) were determined. Three replicates of 100 seeds were tested for each seed sample.

The true seeds were removed from the pericarp and used for flow cytometric analysis. Samples of individual seeds were prepared according to Galbraith et al. [6], with some minor modifications. Seeds were chopped with a sharp razor blade in a Petri dish containing 0.5 ml nuclei isolation buffer (Chemunex, Moisons-Alfort, France) supplemented by 4,6-diamidino-2-phenylindole (DAPI). After chopping, 1 ml of buffer was added and the mixture was filtered through a 30-micrometer mesh nylon filter. DNA content was measured after about half an hour in a Partec CA II flow cytometer (Partec, Münster, Germany). In each sample about 5000 nuclei were analysed. The Partec DPAC V2.1 computer programme was used for peak analysis. For each seed sample 30 replications were analysed.

A Student's *t*-test for independent samples was performed on the G_2/G_1 ratios, and, after angular transformation, on laboratory test parameters. An analysis of correlation between studied traits was performed.

RESULTS AND DISCUSSION

The laboratory germination test proved that the presowing hydration treatment increased seedling length vigour index of all the seed samples (Table 1). The results obtained were in agreement with those of some previous

investigations [8,15,19]. However, in the present study the treated seeds did not show a higher germination capacity than the untreated ones. This agrees with the suggestion of Sadowski [15] that presowing treatments of sugar-beet seeds causes leaching out of germination inhibitors from the pericarp, thus improving vigour without influencing germination capacity. There was a tendency for improvement of germination capacity due to the hydration treatment, but it was statistically significant only for germination capacity after 4 days for the cv. PN Mono 1 seeds. Consequently, there was no correlation between SLVI and germination capacity (Table 2).

Table 1. Seedling length vigour index and germination capacity of untreated and treated seeds of three sugar-beet cultivars

Cultivar	Seed sample	Seedling length vigour index	Germination capacity (%)		
		(%)	after 4 days	after 7 days	after 14 days
Kawejana	untreated	29 a*	70 NS	77 NS	80 NS
	treated	59 b	77	82	84
Jastra	untreated	27 a	76 NS	86 NS	88 NS
	treated	46 b	82	89	91
PN Mono 1	untreated	31 a	85 a	92 NS	93 NS
	treated	55 b	91 b	94	94

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

Table 2. Coefficients of correlation between the studied traits of the seeds of three sugar-beet cultivars

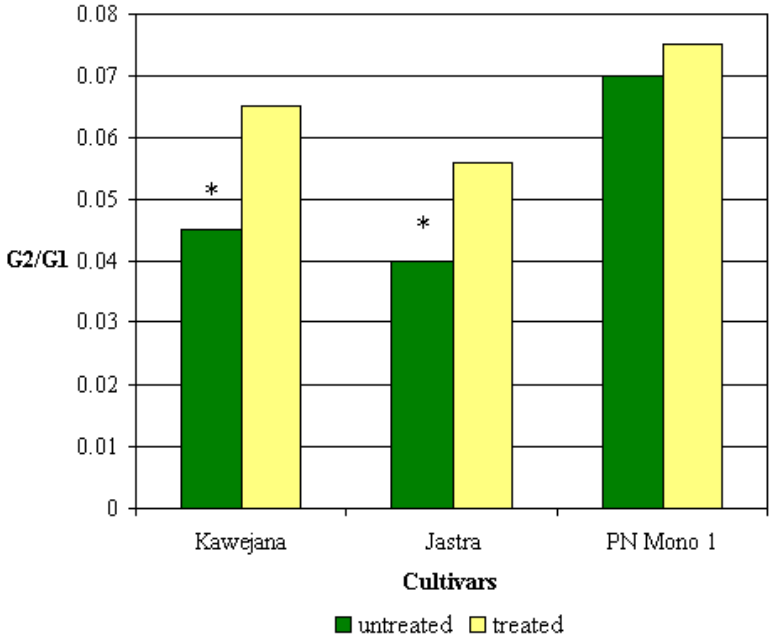
	G_2/G_1 ratio	Seedling length vigour index	Germination capacity after 4 d	Germination capacity after 7 d
Seedling length vigour index	0.635*			
Germination capacity after 4 d	0.874**	0.470		
Germination capacity after 7 d	0.721*	0.241	0.944**	
Germination capacity after 14 d	0.674*	0.189	0.911**	0.994**

* significant at P=0.05
** significant at P=0.01

Flow cytometric analysis demonstrated that in untreated as well as in treated seeds of all three cultivars most of the cells (over 90%) were arrested in the G_1/G_0 phase of the cell cycle. However, a considerable number of the cells arrested in the G_2 phases were also detected (Figure 1). A similar distribution of the nuclear replication stages in the seeds of sugar beet has been reported previously [17].

The proportion of G_2 cells increased after hydration treatment, especially for the cvs. Kawejana and Jastra. This increase was probably due to cells entering the synthetic phase of the nuclear division cycle. For the two above mentioned cultivars an increased G_2/G_1 ratio was evident in treated seeds (Figure 1), which may be related to the greater ability of these seeds for fast germination. The beneficial effect of the treatment was most probably due to completion of DNA repair processes and the completion of germination sensu stricto. This was not the case for the seeds of cv. PN Mono 1, where increased cell cycle activity was not statistically significant. This is in agreement with the results of Redfearn and Osborne [14] and Sliwinska et al. [18], who found that the 6-h-long steeping and soaking of sugar-beet seeds did not initiate DNA replication in the embryo cells. Apparently, the increase in SLVI in the cv. PN Mono 1 seeds was due only to decreased amounts of inhibitors in the pericarp, with no direct influence on the cell cycle in the embryo.

Figure 1. The G_2/G_1 ratio for untreated and treated seeds of three sugar-beet cultivars (* - mean values for particular cultivars are significantly different at *P*=0.05; Student's *t*-test for independent samples)



As shown in Table 2, the G_2/G_1 ratio can be correlated with the SLVI and germination capacity. However, the results of flow cytometric analysis also demonstrate that hydration treatment can affect the cell cycle in the seeds of different cultivars to different extents. Some seed samples respond with augmented cell cycle activity in the embryo even after short soaking, whereas in others the effect is not evident. This is in agreement with the results obtained for tomato and pepper seeds by Lanteri et al. [12], who found the dissimilar changes of G_2 cells proportion upon priming in different seed lots. These variations may result from specific differences in the reaction to the priming treatment among seed lots. Most probably during seed maturation cell cycle activity becomes suppressed at a certain developmental stage and seed lots may vary in their depth of dormancy. Thus, they can respond differently to the presowing treatments.

From the above results it is obvious that hydration treatment can influence the cell cycle activity in the embryo. However, further studies on more material are necessary to estimate the usefulness of flow cytometry for predicting seed treatment efficiency.

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REFERENCES

1. Bewley J.D., Black M., 1994. Seeds: physiology of development and germination. Plenum Press, New York and London.
2. Bino R.J., De Vries J.N., Kraak H.L., Van Pijlen J.G., 1992. Flow cytometric determination of nuclear replication stages in tomato seeds during priming and germination. Ann. Bot. 69, 231-236.
3. Bino R.J., Lanteri S., Verhoeven H.A., Kraak H.L., 1993. Flow cytometric determination of nuclear replication stages in seed tissues. Ann. Bot. 72, 181-187.
4. Dell'Aquila A., Savino G., De Leo P., 1978. Metabolic changes induced by hydration-dehydration presowing treatment in weat embryo. Plant Cell Physiol. 19, 348-354.
5. Durrant M.J., Mash S.J., 1992. Sugar-beet treatments, water supply and depth of sowing. Ann. Apl. Biol. 120, 151-159.
6. Galbraith D.W., Harkins K.R., Maddox J.M., Ayres N.M., Sharma D.P., Firoozabady E., 1983. Rapid flow cytometric analysis of the cell cycle in intact plant tissues. Science 220, 1049-1051.

7. ISTA (International Seed Testing Association), 1985. International Rules for Seed Testing. Seed Sci. Technol. 13, 322-447.
8. Jassem M., Sadowski H., 1990. Seed improvement as a factor increasing sugar beet growing (in Polish). Biul. IHAR 173–174, 155 - 165.
9. Jassem M., Sliwinska E., Zornow A., 1993. The influence of substrate moisture on germination capacity of sugar-beet seeds. Seed Sci. Technol. 21, 203-211.
10. Lanteri S., Belletti P., Marzach C., Nada E., Quagliotti L., Bino R.J., 1997. Priming-induced nuclear replication activity in pepper (*Capsicum annuum* L.) seeds. Effect on germination and storability. In: Basic and Applied Aspects of Seed Biology, Ellis R.H., Black M., Hong T.D. (eds). Kluwer Academic Publishers, Dodrecht, 451-459.
11. Lanteri S., Kraak H.L., De Vos C.H.R., Bino R.J., 1993. Effects of osmotic preconditioning on nuclear replication activity in seeds of pepper (*Capsicum annuum*). Physiol. Plant. 89, 433-440.
12. Lanteri S., Saracco F., Kraak H.L., Bino R.J., 1994. The effects of priming on nuclear replication activity and germination of pepper (*Capsicum annuum*) and tomato (*Lycopersicon esculentum*) seeds. Seed Sci. Res. 4, 81-87.
13. Osborne D.J., 1983. Biochemical control system operating in early hours of germination. Can. J. Bot. 61, 3568-3577.
14. Redfearn, M. and Osborne, D.J., 1997. Effects of advancement of nucleic acids in sugar beet (*Beta valgaris*) seeds. Seed Sci. Res. 7, 261-267.
15. Sadowski H., 1991. Quality improvement of sugar beet seeds by chemical methods (in Polish). Biul. IHAR 177, 71 - 82.
16. Saracco F., Bino R.J., Bergervoet J.H.W., Lanteri S., 1995. Influence of priming-induced replication activity on storability of pepper (*Capsicum annuum* L.) seed. Seed Sci. Res. 5, 25-29.
17. Sliwinska E., 1996. Flow cytometric analysis of the cell cycle of sugar-beet seed during germination. Gen. Pol. 37A, 254-257.
18. Sliwinska E., Jing Hai-Chun Job C., Job D., Bergervoet J.H.W., Bino R.J., Groot S.P.C., 1999. Effect of harvest time and soaking treatment on cell cycle activity in sugar-beet seeds. Seed Sci. Res. 9 (in press).
19. Wisniewski K., Sadowski H., 1985. Conditioning of sugar beet seeds (in Polish). Biul. IHAR 156, 151 - 160.

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