BRIEF COMMUNICATION

# Effect of paclobutrazol on soluble sugars and starch content of *de novo* regenerating potato stem explants

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## Abstract

Changes in the content of soluble sugars and starch were determined during the first phase of *de novo* organogenesis in stem internode segments (SIS), taken either from control plants or from the plants grown on medium with $10^{-8}$ M paclobutrazol (PBZ). Transient accumulation of soluble sugars was observed in both variants during the first two days. Control SIS accumulated higher amount [22.0 mg $g^{-1}$(f.m.)] of soluble sugars than the PBZ pretreated SIS [15.2 mg $g^{-1}$(f.m.)]. PBZ variant showed four times higher starch content at the start of experiment in comparison with the control. Both variants accumulated starch during cultivation until the beginning of regeneration.

*Additional key words*: growth retardants, organogenesis, stem internode segments.

In potato, great differences were found in regeneration capacity among different genotypes (Opatrná 1990). Many procedures were used, based mainly on the changes in hormonal composition of culture media and/or insertion of bacterial oncogenes (Wheeler *et al.* 1985, Coleman *et al.* 1990, Ovesná *et al.* 1993). Other factors, which can improve *de novo* regeneration, are growth retardants, from which paclobutrazol (PBZ) seems to be the most effective (Sankhla *et al.* 1992). PBZ acts as an inhibitor of the three oxidative steps of the gibberellin precursor *ent*-kaurene to *ent*-kaurenoic acid (Hedden and Graebe 1985). Thus PBZ blocks synthesis of gibberellin in early step of its biosynthetic pathway. It results in reduction of stem internode growth, which is the most widely reported morphological response to the PBZ application. Recently, the evidence for more pleiotropic effect of PBZ has been reported, including the effect on the biosynthesis of sterols, ABA and cytokinins (Rademacher 1991), photosynthesis and dark respiration (Vu and Yelenosky 1992), and enhancement of tolerance to environmental stresses (Yelenosky *et al.* 1987, Abou El-Khasab *et al.* 1997), which might be related to increased antioxidant content. PBZ can be applied either directly into the regeneration medium, or on donor plants, to influence the quality of primary explants. In our previous paper (Opatrná *et al.* 1997), increase in the percentage of regenerating explants originated from PBZ pretreated plants were registered. No positive effect was observed in case of direct PBZ application into regenerating media.

It was demonstrated that PBZ can affect sugar content and partitioning between soluble sugars and starch (Okuda *et al.* 1996, Mehouachi *et al.* 1996). Because of the great role which sugars can play in growth and developmental processes of plant tissues, we tried to characterize changes in content of soluble sugars (glucose, fructose, sucrose) and starch, during the *de novo* regeneration in stem internode segments (SIS) of potato which were pretreated with PBZ. The aim of this study was to find the possible correlation between sugar content and differences in the regeneration capacity of the explants.

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*Abbreviations*: ABA - abscisic acid; BAP - benzylaminopurine; IAA - indole-3-acetic acid; MS medium - Murashige and Skoogs medium; PBZ - paclobutrazol; SIS - stem internode segments.

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Potato (*Solanum tuberosum* L.) plants of genotype Klára were micropropagated on the Murashige and Skoog (1962; MS) medium with or without $10^{-8}$ M paclobutrazol (PBZ) at temperature 22 °C, irradiance of 80 μmol $m^{-2}$ $s^{-1}$, 16-h photoperiod. After four weeks of cultivation, approximately 7 mm long stem internode segments (SIS) were cut from donor plants. SIS were cultivated on inductive MS1 medium containing BAP (2.1 mg $dm^{-3}$), IAA (0.1 mg $dm^{-3}$) and adenine (40 mg $dm^{-3}$). During four weeks, the meristematic centres were initiated. In the second period, SIS were transferred onto MS2 medium supplemented with IAA (0.1 mg $dm^{-3}$) only (Opatrný and Müllerová 1986). On this medium, already initiated meristematic structures developed into shoots within 2 - 4 weeks. Soluble sugars (glucose, fructose, sucrose) were determined by HPLC (*Shodex RI-71*, *Spectra-Physics*, USA) with refractometric detection. Starch content was determined by anthrone method (Yemm and Willis 1954).

The *de novo* regeneration was stimulated by PBZ pretreatment of donor plants in the way as previously published by Opatrná *et al.* (1997). After 7 weeks of cultivation, 70 % of PBZ explants regenerated in comparison with only 53 % of the control ones. No significant differences were found in the contents of soluble sugars between control and PBZ pretreated SIS at the start of the experiment (Fig. 1*A*). During the first two days of cultivation both variants accumulated high amount of sugars. Maximum concentration in control SIS was 22.0 mg $g^{-1}$(f.m.) in comparison with the concentration in PBZ variant which was 15.2 mg $g^{-1}$(f.m.). After 2 d of cultivation the sugar content began to decrease and it reached nearly starting content in both variants after two weeks. Then, content of soluble sugars remained unchanged during the rest of the experiment. Even the onset of regeneration did not influence content of soluble sugars significantly (data not shown).

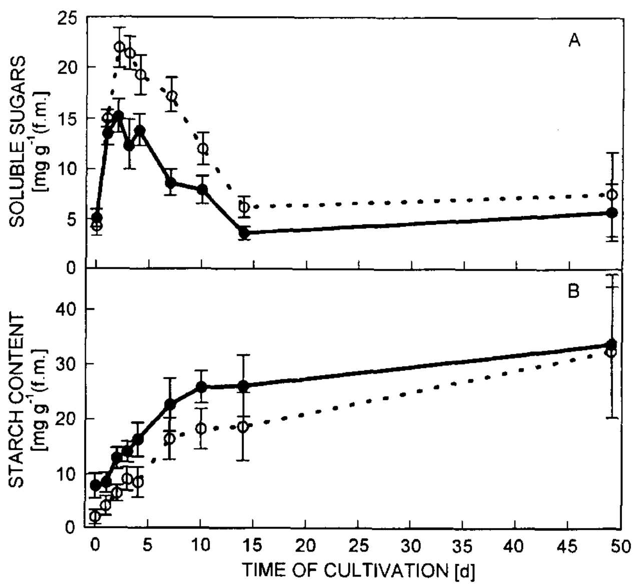


Fig. 1. Changes in the content of soluble sugars (*A*) and starch (*B*) in SIS during the cultivation *in vitro*. Only non-regenerating SIS were evaluated. *Open symbols* - control, *closed symbols* - paclobutrazol treated plants. *Bars* represent SE, $n = 6$.

On the contrary, there was a great difference in the starch content between variants at the beginning of the experiment. PBZ pretreated SIS had nearly four times higher concentration of starch than control SIS [7.9 mg $g^{-1}$(f.m.) and 2.1 mg $g^{-1}$(f.m.), respectively]. During the whole period of cultivation, until the onset of regeneration, starch content continuously increased in both variants and after the period of seven weeks, no significant differences were found between PBZ and control explants (Fig. 1*B*). The highest accumulation of starch was observed during the first two weeks of cultivation. This ceased at the moment when newly regenerated shoots appeared and starch began to be utilized for growth (Fig. 2).

The differences between variants in sugar content at the start of the experiment are in good accordance with the results of other authors. Mehouachi *et al.* (1996) found, that PBZ treatment enhanced starch accumulation in shoots and roots of citrus rootstock seedlings, but did not influence soluble sugars concentrations. In rice seedlings PBZ increased starch content and decreased soluble sugar content in shoots (Yim *et al.* 1997). The reason for starch accumulation is probably the stem growth retardation. Lower sink strength can result in lower assimilate consumption and higher deposition of storage material. Insufficient demand for carbon source (sucrose from the medium) is probably also the reason for steady increase in the starch content during the cultivation of nodal segments. The starch utilization starts as late as the new sink in the form of *de novo* regenerated shoot develops.

The cause of massive accumulation of soluble sugars during the first two days of cultivation is not quite clear. This can be the consequence of the presence of relatively high sucrose concentration in fresh medium and lack of barriers against its penetration inside the explant. The resulting good availability of sugar probably did not correspond to the ability of tissue to utilize it.

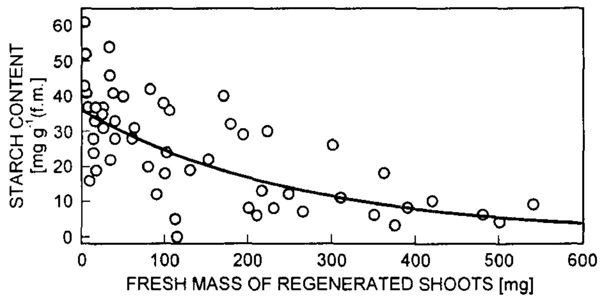


Fig. 2. Starch degradation in SIS after the onset of regeneration. The starch content in individual internode segments is expressed as a function of fresh mass of *de novo* regenerated shoots. Only data from control SIS are shown, PBZ variant was not significantly different.

We can conclude from our experiments that paclobutrazol can influence the plant soluble sugar and starch content. The differences, nevertheless, very soon disappear, and at the time of the meristematic centre formation (approximately after 4 weeks of cultivation) they are not significant any more. From the presented data it is impossible to deduce that the PBZ treatment improved regeneration *via* changes in sugar metabolism.

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