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Response of *Abies alba* embryonal-suspensor mass to various carbohydrate treatments

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Abstract

Cultures of embryonal-suspensor mass of European silver fir (Abies alba) were screened for their response to various sucrose concentrations (29-175 mM) and to equimolar (100 or 200 mM) concentrations of glucose, fructose, galactose, sucrose, lactose and soluble starch. The higher concentrations were combined with 3.78 μM abscisic acid. While fructose was ineffective in promoting any somatic embryo maturation glucose, sucrose and galactose promoted early maturation stages. Somatic embryo development up to the torpedo stage was observed only on media with soluble starch or lactose. Lactose was superior to other carbohydrate treatments and led to more synchronized maturation and more normally structured embryos. In comparison with the pronounced carbohydrate effect abscisic acid at this concentration proved to be of less importance. A sequence of culture steps was established in order to stimulate differentiation and maturation.

Abbreviations: ABA, (±) 2-cis-4-trans-abscisic acid; BAP, N6-benzylaminopurine; ESM, embryonal-suspensor mass; L.S.D., Least Significant Difference; SE, somatic embryo(s); SH, Schenk and Hildebrandt.

Introduction

ESM cultures of Abies alba have been subcultured continuously for 2 1/2 years (Schuller et al. 1989). A high number of SE at early developmental stages are formed in the ESM, whereas mature SE with cotyledons appeared only sporadically. Promotion of SE maturation by reduction of the BAP concentration and/or addition of ABA failed (data not presented). In order to promote the maturation of Abies alba SE the effect of different carbohydrates was studied. Carbohydrates influence SE maturation in various species (Babbar and Gupta 1986; Brown et al. 1989, Button 1978; Kinnersley and Henderson 1988; Kochba et al. 1982; Strickland et al. 1987). In addition, ABA is considered to be required for inhibition of cleavage of conifer embryos, for culture synchronization and for enhancement of embryo maturation (Durzan 1988). Von Arnold and Hakman (1988) reported that ABA-treated SE accumulated lipids, became firm and developed a smooth surface. Therefore in our investigation different sucrose concentrations and a sequence of culture media supplemented with different carbohydrates in combination with or without ABA were studied in order to promote SE maturation in Abies alba.

Materials and Methods

The induction of ESM and the subculturing on a SH-medium modified with 50 mg l^{-1} myo-inositol has been reported previously (Schuller et al. 1989). In intervals of 3-4 weeks ESM of 3 genotypes (No. 2,3 and 7) has been subcultured continuously. Material from genotype 2 with 3 repetitions in subculture generation 22, 23 and 24 was used in sucrose concentration experiments on SH-medium modified by the addition of 29-175 mM sucrose with or without 2.22 μM BAP. The response was determined by fresh and dry weight measurements and by macroscopic observations of the developmental stages of the SE.

The influence of various carbohydrate sources on somatic embryogenesis was studied with all genotypes with 2 repetitions in subculture generations 29 and 30. A sequence of culture steps was used in order to stimulate maturation processes. ESM cultures grown on proliferation medium were transferred to the same medium but with the BAP concentration of 0.89 μM (step 1). In step 2 various carbohydrate sources, such as glucose, fructose, galactose, sucrose, lactose and soluble starch in equimolar concentrations (100 mM) were employed in combination with 0.44 μM BAP. The final

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step consisted of complete omission of BAP and addition of 3.78 μM ABA and the doubled concentration (200 mM) of the respective carbohydrates.

Fresh weight of the ESM inoculum was approximately 250 mg and in each step the cultures were kept for 4 weeks in dim light (white fluorescent tubes: 4 μmol $m^{-2}s^{-1}$) at 23°C.

In 15 cultures per treatment elongated SE with a minimum size of 0.5 mm were counted. Presented are the results of genotpye 2.

For binocular observation and documentation, 4 ESM samples per treatment, each of 50 mg fresh weight, were stained with 10 % acetocarmine for 30 min and dispersed in a defined area on a slide.

For histological investigations 15 μm sections of parafin embedded SE stained with haematoxylin have been used.

Results

The concentration of sucrose in the SH-medium had a pronounced effect on the dry matter (Fig. 1) and the developmental stage (Fig. 2) of the ESM cultures. Since the ESM cultures consist only of highly vacuolated suspensor and densely cytoplasmic embryonal cells, the dry matter in these culture is strictly correlated with the portion of embryonal cells. With increasing sucrose the dry matter production increased. Cultures grown on hormone-free medium attained approximately 30 % higher dry matter than cultures grown on BAP-supplemented media. Proliferation of the ESM was promoted on media with BAP and low sucrose. Maturation processes, however, were stimulated only on media without BAP and particularly, with a higher sucrose content. Combined with a degradation of the suspensor cells a high number of globular structures was formed, but most of them arrested in this stage (Fig. 2). Elongated SE, partly with cotyledons and always with chlorophyll appeared sporadically on hormone-free media low in sucrose.

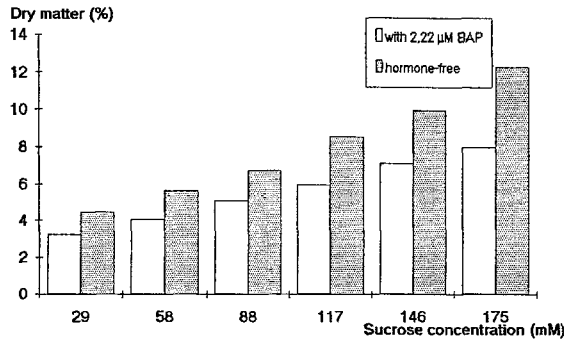

Fig. 1: Correlation between sucrose concentration (mM) and dry matter (%) on media supplemented with 2.22 μM BAP and on hormone-free media. All means significantly different by Duncan's multiple range test. L.S.D. (5 %)

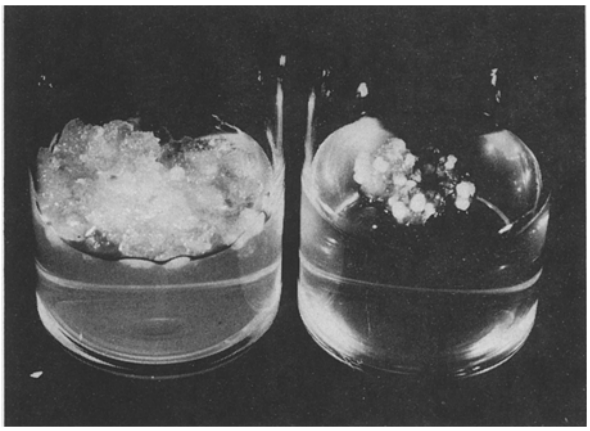

Fig. 2: Influence of sucrose concentration on ESM-cultures of Abies alba on hormone-free media. Left jar 29 mM sucrose, right jar 175 mM sucrose.

On the basis of these results the BAP concentration was reduced and various carbohydrate sources at 2 concentration levels were investigated in the sequence of the three culture steps. For further promotion of maturation 3.78 μM ABA was added in the third step. The influence of different media on the course of SE maturation at each subculture was evaluated by binocular observation.

With macroscopic and binocular control marked differences in the structures of SE between the various carbohydrate sources were observed (Fig. 3). Whereas fructose was ineffective in promoting SE maturation, glucose, sucrose and galactose promoted the early stages in culture step 2. In the final step these structures showed aberrant development with the same carbohydrate compounds. Enlarged globular stages were observed with no signs of further differentiation. Histological investigation of these structures revealed, that the inner tissue consisted of a homogenous cell type and was partly lacerated (Fig. 4).

According to quantitative evaluation of the SE development the highest number of torpedo stages occcured only on media with lactose and soluble starch (Fig. 5). In contrast to the results with soluble starch, the lactose treatment led to more synchronized maturation. Longitudinal (Fig. 6) of the SE in torpedo stage on lactose-containing media showed a differentiation in the following types of tissues: a one-layer protoderm in the apex region (pd) with lateral cotyledonary primordia (co), the hypocotyl (hy) with procambium (pr) and the root cap (rc) with the root initial inside (ri). Remarkable were the structural abnormalities of the tissue in the root initial zone and in the root cap. However, SE elongation was always correlated with chlorophyll formation in the apex region.

The results shown are limited to the response of genotype 2. Genotypes 3 and 7 showed a similar response when first transferred to lactose but the ESM was less segregated into defined structures. Only few

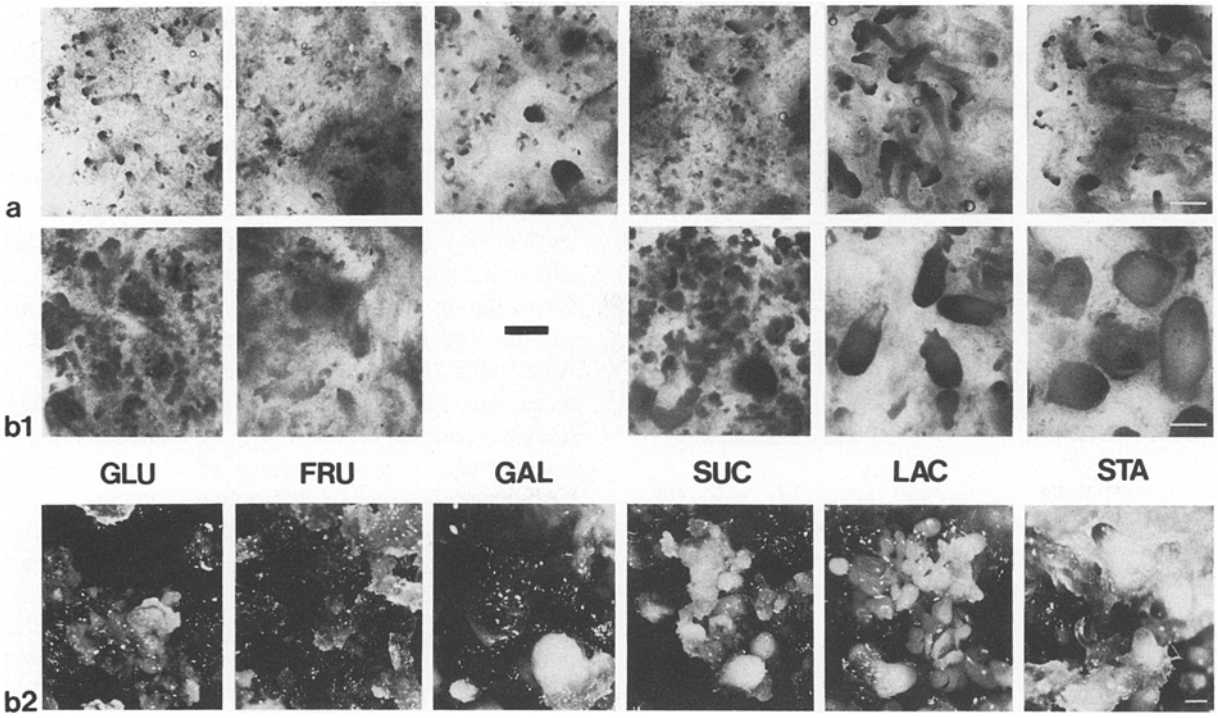

Fig. 3: Influence of various carbohydrate sources on SE maturation in Abies alba. The proliferation medium was modified as described in Materials and Methods; step 2 refer to a, step 3 refer to b1 (binocular observation) and b2 (macroscopic observation). Bar = 1 mm; Abbreviations: GLU: glucose; FRU: fructose; GAL: galactose; SUC: sucrose; LAC: lactose; STA: soluble starch.

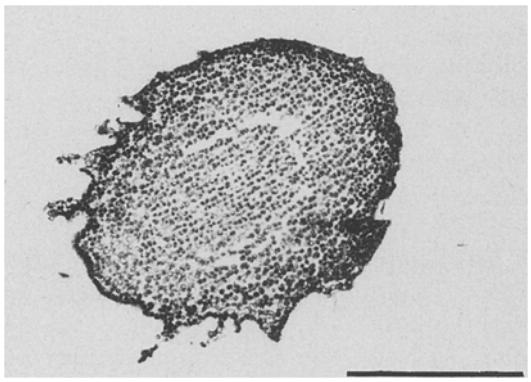

Fig. 4: Enlarged globular stage. Inner tissue partly lacerated and consisting of a homogenous cell type. Bar = 1 mm

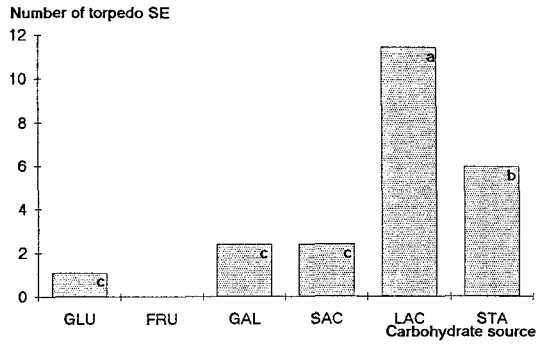

Fig. 5: Effect of different carbohydrates on the number of torpedo SE of the genotype 2 (size ≥ 0.5 mm). Means from 15 cultures per treatment, same letters are not significantly different by Duncan's multiple range test. L.S.D. (5 %). Abbreviations see Fig. 3

elongated SE have so far been observed in these genotypes. In contrast to genotype 2 it was possible to establish these genotypes on lactose-containing media under proliferation conditions (data not presented).

The elongated SE of genotype 2 always turned brown when maintained on lactose-containing media. Survival and further development were suppported upon transfer to sucrose-containing media.

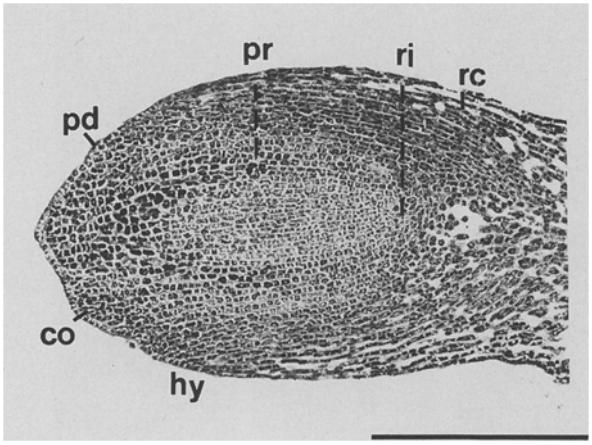

Fig. 6: Longitudinal of torpedo stage SE with the following types of tissues: one-layer protoderm in the apex region (pd) with lateral cotyledonary primordia (co), the hypocotyl (hy) with procambium (pr) and the root cap (rc) with the root initial inside (ri). Bar = 1 mm

Following a sequence of media from 88 mM sucrose + 2.22 μM BAP to 88 mM sucrose + 0.89 μM BAP (step 1) to 100 mM lactose + 0.44 μM BAP (step 2) to 200 mM lactose + 3.78 μM ABA (step 3) the genotype 2 responded at step 2 with a high regeneration rate of SE and at step 3 with further maturation.

Discussion

Since ABA was included in all carbohydrate variants in step 3 SE differentiation was promoted only by lactose and soluble starch. In comparison with the pronounced carbohydrate effect ABA at this concentration proved to be of less importance in this developmental step. The stimulation of SE maturation by lactose and soluble starch due to a specific osmotic effect could be excluded by the isomolar carbohydrate concentrations. The viability of torpedo stage SE could only be maintained when transferred from lactose- to sucrose-supplemented media. This could indicate nutritional deficiency during the preceding maturation process. An extremely reduced availability of the carbon source may have enhanced the SE maturation in Abies alba.

Higher sucrose concentrations led to enlarged globular SE in ESM cultures of Abies alba but also to SE maturation. Von Arnold (1987) found that increased sucrose concentration was important for further SE development in Picea abies and that the effect was not due to increased osmotic pressure. However, Lu and Thorpe (1987) reported that increasing osmolarity enhanced development and maturation of the SE in Picea glauca and in interior spruce low levels of mannitol (2-6 %) promoted the formation of globular embryos (Roberts 1991). In spite of the same osmolarity and comparable amounts of total carbohydrate in sucrose and lactose-containing media, sucrose stimulated only the enlargement of the globular stages in ESM cultures of Abies alba whereas lactose favoured elongation. A marked increase of organized embryos on lactose-containing media of Citrus ovular callus was observed by Button (1978). In accordance with our results he pointed out that the monomer components of lactose were ineffective. He presumed furthermore that lactose stimulated the formation of chlorophyll, leading to starch accumulation and organization. Chlorophyll formation was also observed in our experiments in elongated SE of Abies alba. This phenomenon is interpreted as a precocious ageing process due to nutritional deficiency. In subsequent experiments with combinations of lactose and sucrose, a great number of elongated SE were formed with no signs of chlorophyll formation.

The lactose mediated SE maturation in Abies alba can be due to a special lactose effect combined with a deficiency in carbohydrate supply. Preliminary analytical data of the lactose uptake and metabolism in the different stages of SE maturation indicate lactose uptake without metabolic degradation (data not presented). Further histological and biochemical studies will be necessary to describe the differentiation steps of somatic embryos in Abies alba.

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