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Phytohormones and structure of cells of *Acer saccharinum* seed embryo

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Abstract

Endogenous phytohormone levels and cell structure of *Acer saccharinum* embryos were studied during seed development. Mature seeds had high water content (50 %) and were able to germinate immediately after fruit abscission. The submicroscopic cell structure was similar to the structure of functionally active cells. Free and conjugated indole-3-acetic acid (IAA), abscisic acid (ABA), zeatin riboside and dihydrozeatin content and gibberellin-like substances (GLS) activity were determined during embryo maturation. Decrease in ABA, free IAA and cytokinin levels was observed at the end of maturation. Mature seeds contained considerable amounts of conjugated IAA and had high GLS activity.

Key words: abscisic acid, cytokinins, gibberellins, indole-3-acetic acid, silver maple.

Introduction

Seeds of plant species can be classified as orthodox or recalcitrant depending on their water content at the final stages of their development. Seeds of an orthodox type retain the embryo viability after a complete dehydration. Seeds of the recalcitrant type contain 40 - 60 % of water after maturation and they lose their viability when dried (Chin *et al.* 1984). Even when such seeds are kept in moist conditions their period of life is rather short and does not exceed several months (Bewley and Black 1982).

The leading role in regulation of seed development is played by phytohormones. Though many data concerning the function of hormones in seeds were obtained, only a few works were devoted to the investigation of phytohormones in recalcitrant seeds (Tomaszewska 1979, Dathe *et al.* 1981). The purpose of the present study was to clarify changes in hormone concentrations during the development of a recalcitrant seed embryo (*A. saccharinum*) as linked to the cell structure changes.

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Materials and methods

Acer saccharinum L. seeds for all analyses were collected from trees in city park 20, 26, 36, 43 and 48 d after anthesis. The embryos were isolated from seeds and divided into axes and cotyledons. A middle part of the cotyledons and embryo axes in the hypocotyl section were fixed with 3 % glutarate aldehyde and 1 % OsO_4 and after that they were dehydrated using alcohols at gradually increasing concentrations and were placed in the mixture of epoxy resins epon-araldit. Ultra-thin cross-sections were than analysed by means of *JEM 1200 EX* microscopy. To study the embryo organs cell structure, 1 μm thin cross-sections were coloured with 1 % toluidine blue and were analysed by means of *NU-2* microscopy.

To study phytohormones content embryos were isolated from seeds and homogenized in 80 % ethanol. GLS were extracted with ethylacetate and butanol at pH 3 and fractionated on column with *DEAE A-25* resin. To determine the biological activity of GLS the dwarf rice bioassay was used. IAA and ABA were extracted with diethylether at pH 3 and purified by thin layer chromatography (TLC). The content of conjugated IAA was determined after alkaline hydrolization of water fraction. Cytokinins were extracted with butanol at pH 8, purified on *Dovex 50Wx8* column and TLC. IAA, ABA and cytokinin levels were determined by means of high performance liquid chromatography (*Pye Unicam 4000*).

All experiments consisted of 3 replicates and were repeated at least 3 times.

Results and discussion

Studies of *A. saccharinum* embryo cell structure and phytohormones were started on the 20th d after anthesis. At that time seed size reached 1/3 of its final one. The most rapid seed growth (changes in seed and embryonic axes length and in total seed fresh mass) was observed during the first half of embryogenesis (Fig. 1).

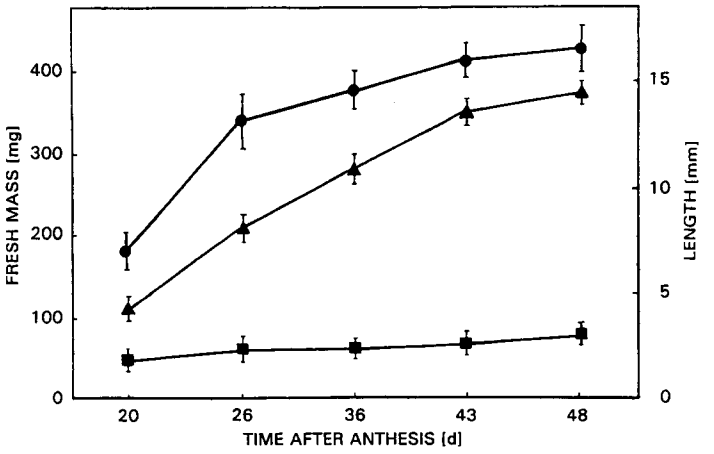

Fig. 1. Changes in fresh mass (*triangles*) and length of cotyledons (*circles*) and embryo axis (*squares*) during *Acer saccharinum* seeds development.

The embryo cell structure during the period of intensive seed growth (20 - 26 d after anthesis) was similar to the structure of meristematic tissue cells. Embryo cells were characterized by a thin cell wall, central position of nucleus and presence of juvenile organelles. Rather high content of ABA and conjugated IAA and comparatively high GLS activity were determined in embryos at the this growth stage. Zeatin riboside and dihydrozeatin were identified as well, their levels were low (Fig. 4, 5).

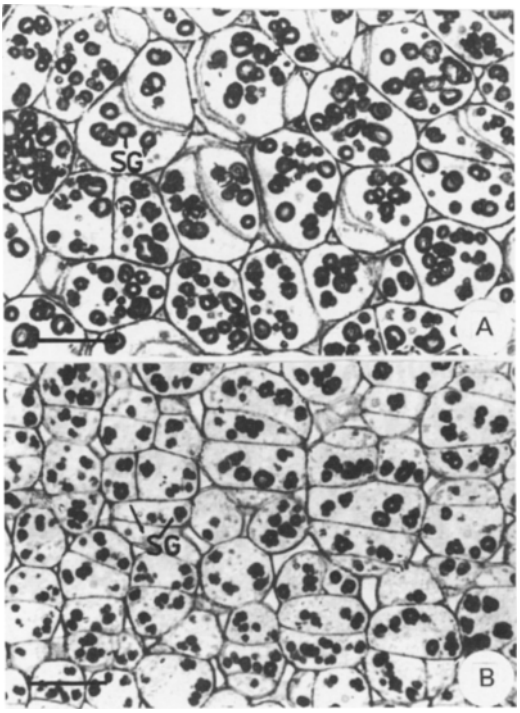

Fig. 2. Parenchyma cells of cotyledon (*A*) and cortical parenchyma of embryonic axis (*B*) in *Acer saccharinum* mature seeds (25 × 24), SG - starch grains. *Bars* = 30 μm.

36 d after anthesis the mitotic activity was generally retarded in embryo cells. Embryo organs main parenchyma cells increased in their sizes a little, but a clear transition to cell elongation was not observed. The characteristic feature of this stage was the decline of the ABA level and increase in cytokinins content. Activity of free and bound GLS was at minimum.

43 and 48 d after anthesis embryo fresh mass continued to increase, in spite of gradual decrease in water content, which dropped to 50 % in seed after fruit abscission. This level was enough for seed germination even without an additional moistening. 43 d after anthesis synthesis and accumulation of storage substances began in embryo cells. The storage lipid pool was the first among other storage

substances to be formed as lipid bodies. 48 d after anthesis an intensive accumulation of starch, storage proteins and lipids occurred (Fig. 2).

The analysis of the mature embryo cells submicroscopic structure showed that lipid bodies had a globular shape with diameter of 0.6 - 0.8 μm (Fig. 3A). At the

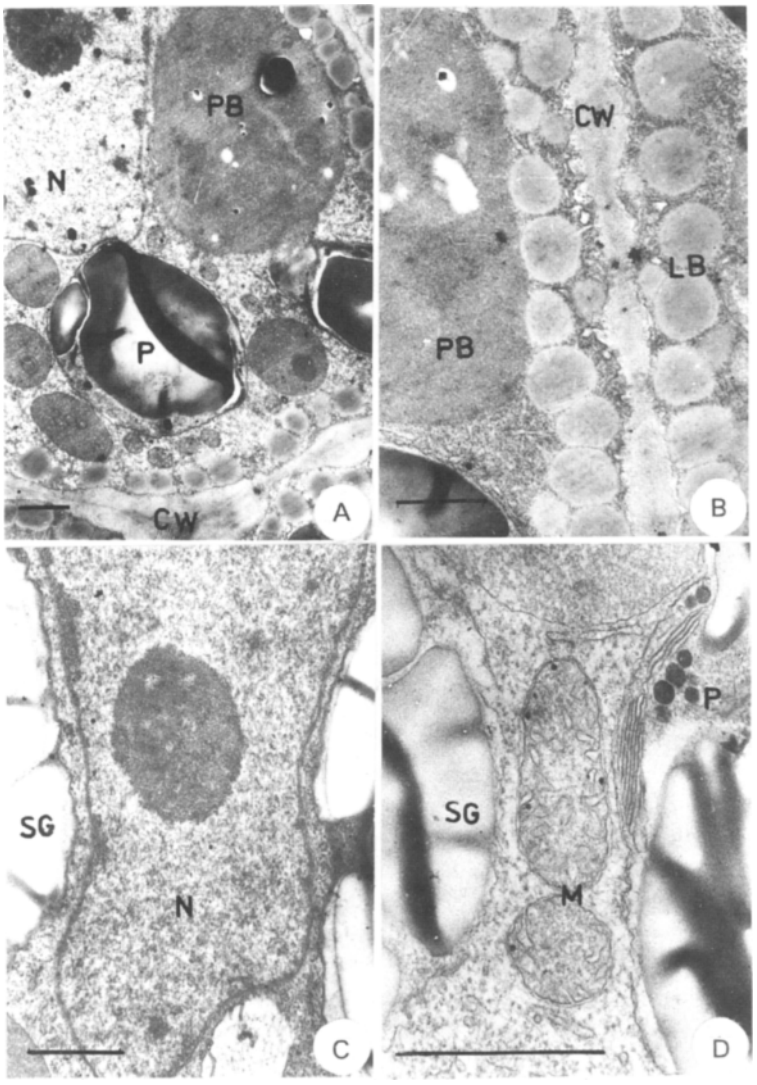

Fig. 3. Submicroscopic structure of embryo organs cells in *Acer saccharinum* mature seeds: A - general view, B - protein body, C - nucleus, D - mitochondria. CW - cell wall, LB - lipid bodies, M - mitochondria, N - nucleus, P - plastid, PB - protein bodies, SG - starch grains. *Bars* = 1 μm.

rapid growth stage they were found in all cytoplasm volume, at the final stage of seed development lipid bodies were located in cell peripheral sites, and in mature seed cells they were situated immediately near plasmalemma (Fig. 3B). Amyloplasts were distributed in cytoplasm evenly. Nuclei were located in the cell centre and contained a small heterochromatin inclusions, homogenous karyoplasm and pronounced two-membrane wall. Nucleoli had a fine-granular structure and rather a high electronic density (Fig. 3C). A considerable number of mitochondria (8 - 12 in each cell cross-section) with a structure typical of functionally active organelles were found in cells (Fig. 3D). They were characterized by wide cristas and electronically dense fine-granular matrix with some osmiophylous granules. Agranular endoplasmic reticulum had a shape of short channels and cisterns without a special orientation in the cytoplasm. Cells also contained single dictyosomes of the Golgi apparatus.

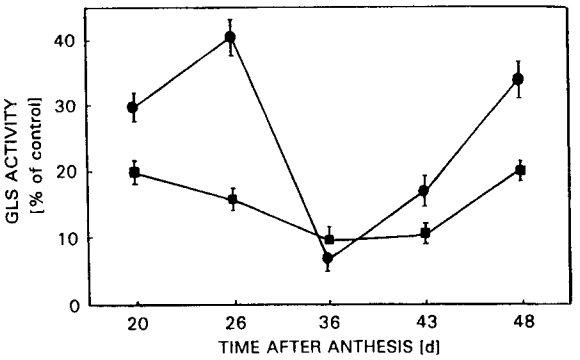

Fig. 4. Changes in free GLS (ethylacetate fraction, *circles*) and conjugated GLS (butanol fraction, *squares*) activity in *Acer saccharinum* seed embryos during development (as dwarf rice hypocotyl elongation, % of control).

At the final stages of seed development activity of both free and bound GLS increased (Fig. 4). Concentration of free IAA gradually decreased during seed maturation, but the level of conjugated IAA remained high with the tendency to increasing to the end of embryogenesis (Fig. 5). Zeatin riboside and dihydrozeatin levels were rather low at the stage of seed maturation. ABA content was almost 10-fold less in mature seed than in growing one (26 d after after anthesis) (Fig. 5).

Analysis of *A. saccharinum* embryo cells showed that they possessed a number of structural similarities with seeds of other plant species. This concerns the localization and structure of protein and lipid bodies and amyloplasts (Wanner *et al.* 1981, Craig 1988). However, there were certain differences. Orthodox seed cell organelles are known to undergo some structural changes at the stage of dehydration (Hallam 1972). First of all dictyosomes, elements of endoplasmic reticulum and polyribosomes were not found in mature seed cells. Changes of nuclei, plastids and mitochondria shape and structure were observed (Musatenko *et al.* 1982). It is supposed that in this way the embryo cells form resistance to dehydration (Leprince *et al.* 1988). *A. saccharinum* seed cells retained structure of the cytoplasm.

Moreover, structure of the most of organelles was similar to the functionally active cell organelles. It is possibly caused by a rather high water content in the mature seed embryo since its decrease created a rapid lost of viability. Phytohormones content

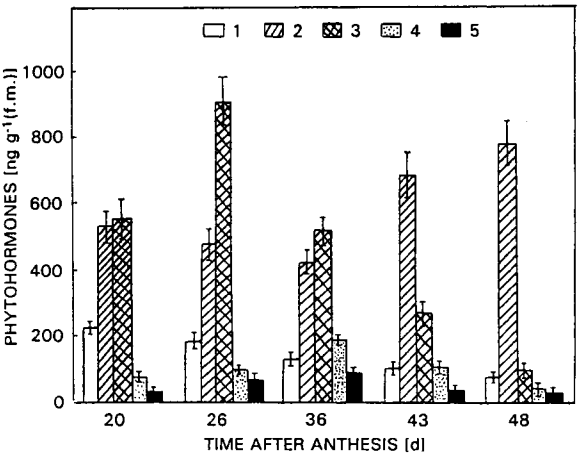

Fig. 5. Phytohormone levels in *Acer saccharinum* seed embryos during development: 1 - free IAA, 2 - conjugated IAA, 3 - ABA, 4 - zeatin riboside, 5 - dihydrozeatin.

and activity changed essentially during *A. saccharinum* seed maturation. However, differences in hormonal complex in recalcitrant and orthodox seeds were not so pronounced. ABA level decreased at the end of maturation in seeds with deep dormancy (Kefeli *et al.* 1989). High ABA level during the active seed growth period was assumed to prevent an early germination (Filkelstein *et al.* 1985). The same assumption can be made in the case of *A. saccharinum* seeds. Maturing *A. saccharinum* seeds were characterized by the decrease in free IAA level and parallel increase in concentration of conjugated IAA. The same tendency was observed in maturing orthodox seeds (Miller *et al.* 1987). Such transformation is physiologically justified because conjugated hormones are characterized by less activity, immobility and resistance to hydrolytic enzymes. At the same time high IAA concentrations are known to inhibit the germination (Polyakova *et al.* 1977). Content of stimulating hormones - cytokinins and gibberellins - in mature *A. saccharinum* seeds obviously is well balanced since exogenous treatment with GA and kinetin at different concentrations did not affect germination (data not shown).

Present study showed that *A. saccharinum* during a rather short time interval (less than 2 months) formed seeds with a high water content, specific cell structure and well balanced hormonal complex. These features allowed seeds to germinate immediately after fruit abscission and form new plants during the same vegetative season.

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