Study of storage compounds in beech embryos during dormancy breaking

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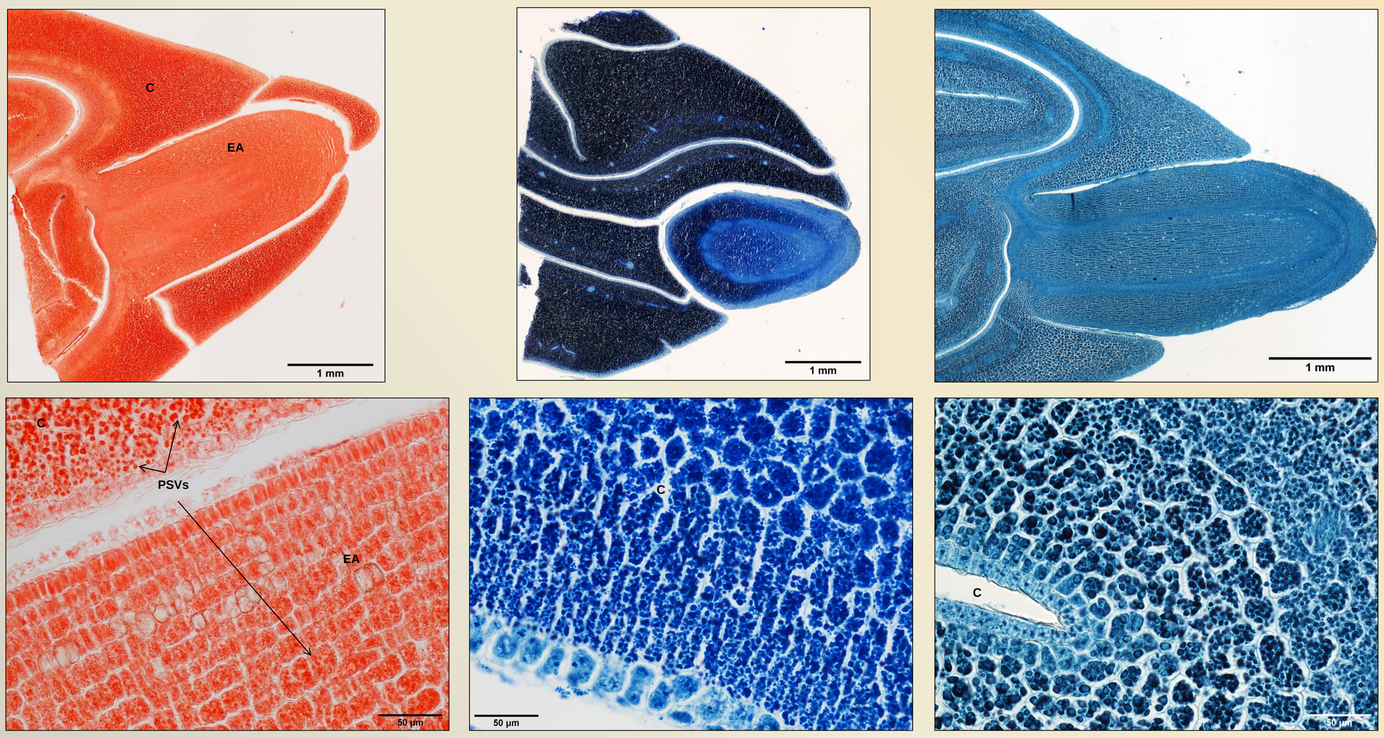
Introduction

The common beech (*Fagus sylvatica* L.) is one of the most important broadleaved species in European forestry. At harvest, beechnuts are in deep physiological dormancy as seeds of many temperate trees. Dormancy as a mechanism preventing germination during unsuitable ecological conditions is controlled by the genetic factors and regulated by phytohormones. It seems to be especially linked to the balance between abscisic acid and gibberellins. Seeds can be stimulated to dormancy breaking and to germination by cold stratification. The control of dormancy breaking is recently studied in the different levels – environmental, biochemical, molecular etc.

Our study is aimed to the changes in the amount and distribution of storage compounds in the beech embryos during stratification. Beechnuts belong to the group of non-endospermic seeds with reserves stored predominantly in the cotyledons (C), but also in embryonic axes (EA). The major mobilization of storage compounds as starch, proteins and oils within storage tissues commences after protrusion of the radicle. Nevertheless, some partial mobilization of storage proteins starts with the uptake of water by imbibition of the dry seed at the beginning of germination. This process is partially mimicked by rehydration of dried stored seeds before chilling treatment, which leads to the releasing of seeds from the dormancy. We follow the changes in storage compounds in beechnuts during the breaking of dormancy at the histological level.

Histochemical detection of storage proteins in dormant beechnut embryos

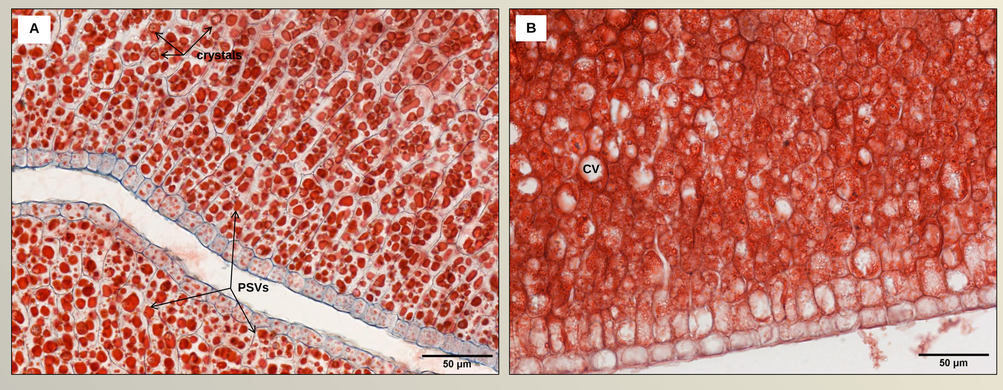
Ponceau xylidin | Commassie brilliant blue R250 | Amido black 10B



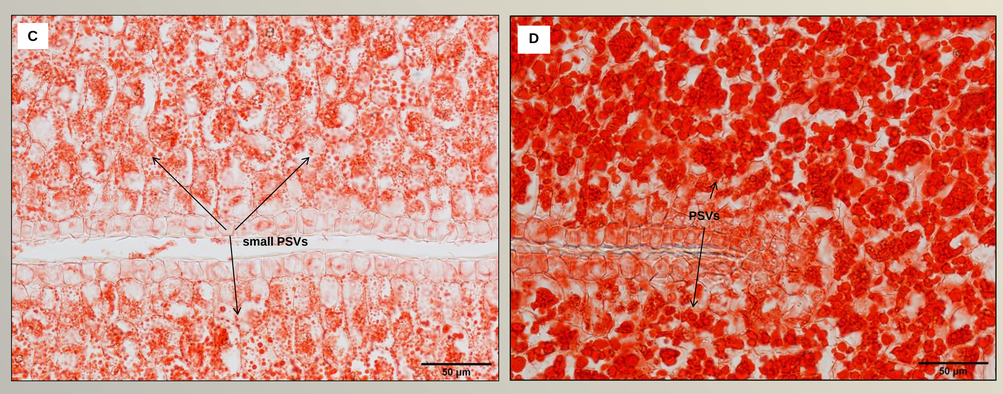
Staining of proteins with Ponceau xylidin was confirmed with two other protein – specific dyes: Commassie brilliant blue and Amido black.

Storage proteins in cotyledons of stratified embryos (Ponceau xylidin)

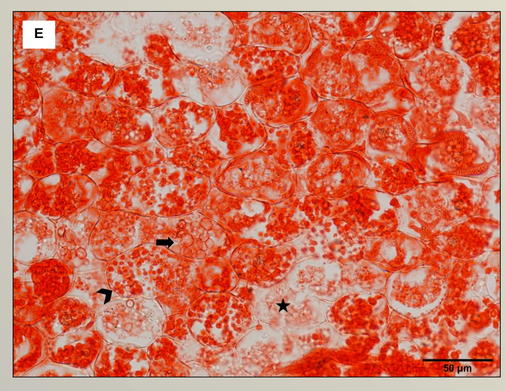
Dormant embryo | Non-dormant embryo



1 week of stratification



11 weeks of stratification

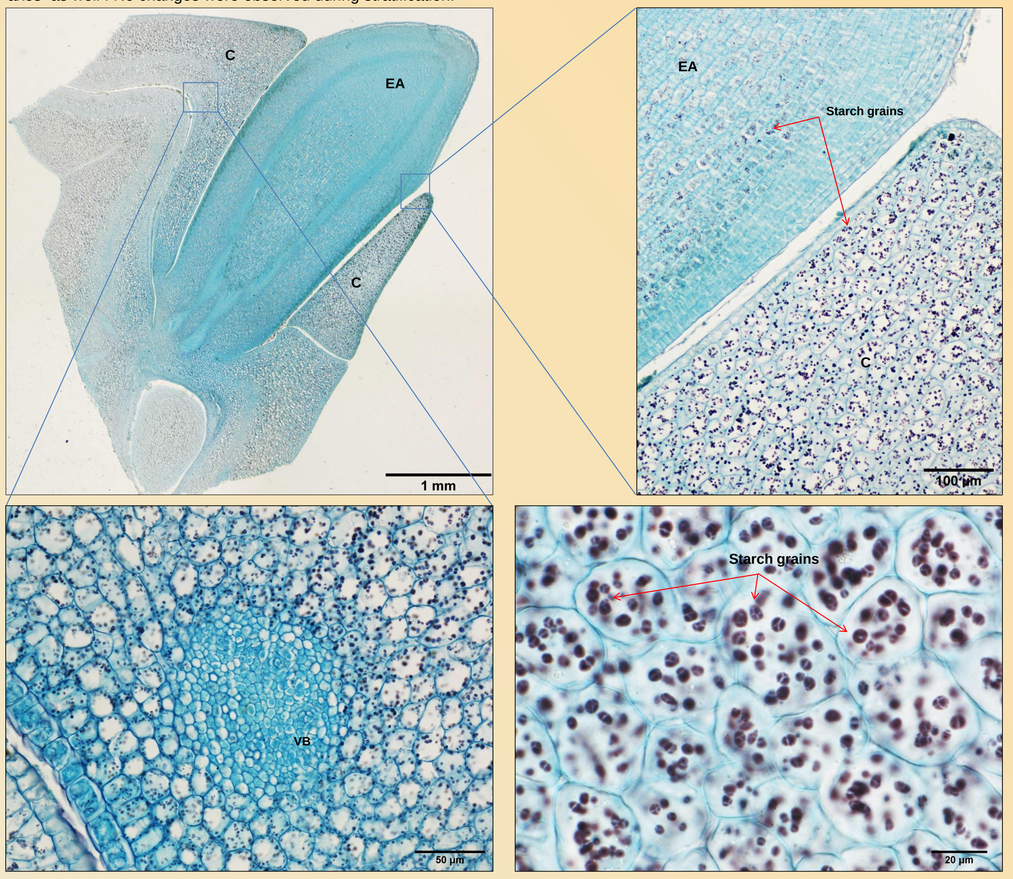


In dormant beechnut embryos storage proteins were deposited in protein storage vacuoles (PSVs), which filled mainly the cells of cotyledons (A). During moist cold stratification these vacuoles diminished, their proteinaceous content gradually disappeared and small vacuoles fused forming central vacuoles. Proteins were than detected in the cytoplasm (B). The first changes were observed after 1 week of stratification, shortly after seed imbibition. In the cells of external parts of cotyledons PSVs reduced or almost disappeared; central vacuoles enlarged (C). In the central inner parts of cotyledons PSVs stayed almost unchanged (D). Later on, as the mobilization of storage proteins continued, histological changes were detected in all studied parts of embryos. Utilization of storage proteins went on unequally, since we could observed different stages of PSVs changes in the neighbouring cells (E).

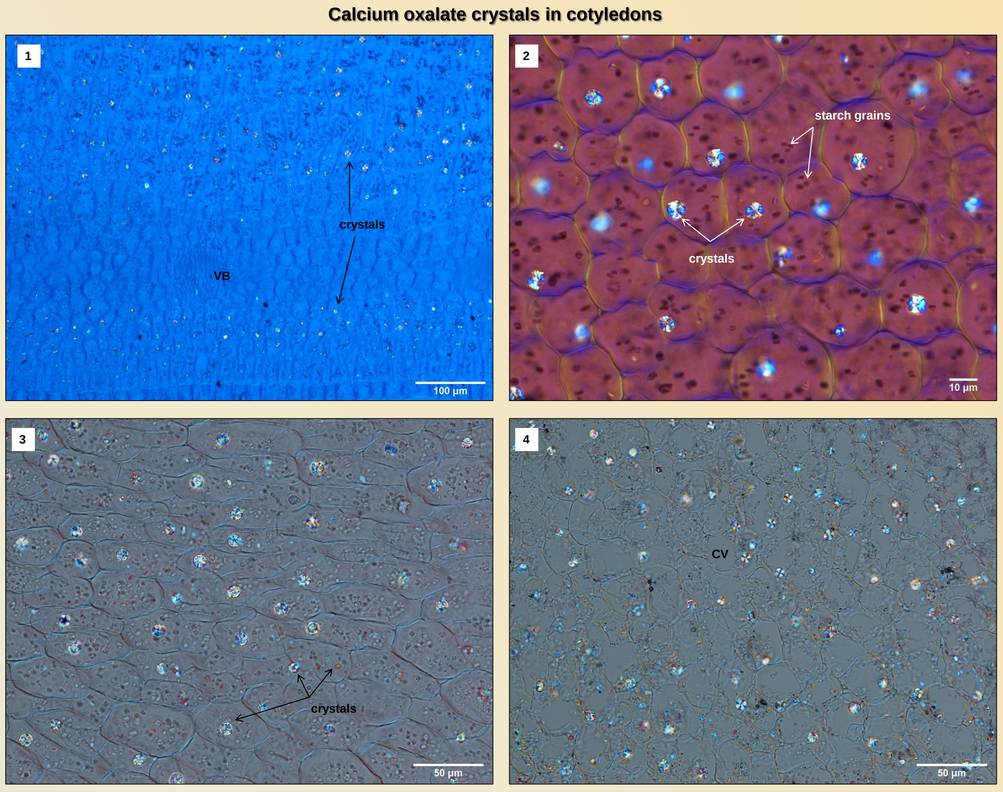
C – cotyledon, EA – embryonic axis, PSVs – protein storage vacuoles, CV – central vacuole
↰ - small PSVs, ➡ - empty PSVs, ★ - cells almost free of PSVs

Histochemical detection of starch grains (IIK/ Azur II)

Starch was detected in whole cotyledons , even in the region of vascular tissue s (VB = vascular bundle); it was present in embryonic axes as well . No changes were observed during stratification.



Calcium oxalate crystals in cotyledons



Large druses or small prismatic crystals of calcium oxalate (CaOx) were abundant in cotyledons , with the exception of the zone of vascular tissue (1 – notice the band with clear vascular bundle (VB) , but without CaOx crystals). Crystals were present in the storage vacuoles in the cells of the storage tissue together with starch grains (2) and storage proteins (A).

The distribution or amount of crystals within the storage tissue of cotyledons did not change during moist cold stratification. Nevertheless, after the uptake of water at the beginning of stratification crystals were pushed to the cell cortex by enlarged central vacuoles (3-dormant embryo; 4-stratified embryo). Calcium oxalate crystals provide a reservoir of calcium, which can be redissolved and used during the germination. In beechnuts CaOx could play also the defensive role against insects and herbivores.

Conclusions

- The main storage compounds (starch, proteins and CaOx) were found in beech embryos of dormant as well as stratified seeds.
- No differences in size and location of starch grains and/or CaOx crystals linked with stratification were detected. After imbibition large central vacuoles pushed the cytoplasm to the cell cortex.
- The storage proteins localized in vacuoles were exhausted during the process of stratification; the first histological changes were evident after the first week of moist chilling treatment

Material and methods

The seeds of common beech (*Fagus sylvatica* L) were obtained from the Forestry and Game Management Research Institute - Research Station Kunovice, Czech Republic.
Dormant seeds were analyzed just after harvesting; moist stratification (without any growing medium) at a low temperature (3°C) was applied for 15 weeks. Selected seed material was stored in plastic bags at -80ºC while awaiting analysis.
Histochemical analyses were conducted on longitudinal paraffin sections (12 µm) of seed parts (cca 5 mm), which contained embryonic axes and the adjoining portion of the cotyledons.
Histochemical confirmation of proteins was achieved using the protein-specific dyes Ponceau xylidine , Amido black 10B and Commassie brilliant blue R250. Starch grains were stained using Lugol solution (IIK). Cell walls were stained with Azur II. Crystals of calcium oxalate (CaOx) were detected using polarized light. CaOx was determined by HCl (dissolved the crystals in contrast to CH_3COOH).
Histological observations were made using a Jenaval microscope (Zeiss, Germany) equipped with a Nikon DS-5M digital camera and processed using the Nis-Elements AR 3.0 (Laboratory Imaging, Prague, Czech Republic) computer image analysis system.

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