The biochemical characteristics of the physiological activity of beech and spruce embryos

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We try to find convenient and measurable criteria for the evaluation of seeds and embryos quality with the aim to estimate the properties of seeds harvested from different sources and/or the properties of the somatic embryos developed in different embryogenic cultures. We selected 3 substances to measure: **ABA** (abscisic acid) – the phytohormone which controls the dormancy in seeds and which regulates the maturation of somatic embryos; **IAA** (indolyl-3-acetic acid) - the phytohormone which regulates the growth and the development of embryos and whole seedlings; **fumarase** – the enzyme which is often correlated with the dormancy and the germination of seeds. Fumarase is a key enzyme in mitochondrial metabolism. Its activity indicates the respiration rate and the ability of seeds to use stored reserves.

Conclusions:

ABA – accurate method with excellent results

- The endogenous level of ABA can indicate the depth of dormancy and the effect of stratification on beech embryos
- ABA can characterize the ability of somatic embryos to germinate

IAA – accurate and very sensitive method

- The endogenous level of IAA does not correlate with the dormancy of beech embryos and/or stratification
- The endogenous level of IAA increases during polarization of spruce somatic embryos
- The increase of IAA level correlates with the start of somatic embryos germination

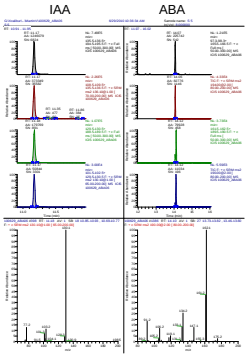
Fumarase – more complicated method, sensitive to external conditions

- The rate of fumarase activity can clearly distinguish the living and dead cells or tissue.
- The fumarase activity could be the promising method for testing dormancy – after rigorous optimization of the method
- The fumarase activity is not a suitable criterion for the evaluation of spruce somatic embryos quality

Methods adapted for seeds and embryos:

IAA and ABA

The weighted plant material (around 0.1 g FW) was milled on a DNA mill and extracted in a modified Bieleski solution. The extract was centrifuged and dried on a rotary vacuum concentrator at room temperature.
Dried samples were dissolved in 15 vol % solution of acetonitrile in water, injected into HPLC and precleaned on C-18 with gradient elution and fractionation on fraction collector. Fraction at time 23.05 min was collected for 1 min and dried. After drying collected fraction was derivatized by diazomethane solution in ether, dried, and dissolved in 100 µl of acetone. 8 µl of redissolved sample was injected into GC-MS/MS and analyzed by Ion trap in MS/MS scan mode (MS1: full scan 50-300 amu; MS2 IAA: precursor 130.1 amu, product full scan 65-200 amu; MS2 labeled IAA: precursor 136.1 amu, product full scan 70-200 amu; MS2 ABA: precursor 190.2 amu, product full scan 65-200 amu; MS2 labeled ABA: precursor 194.2 amu, product full scan 70-200 amu).

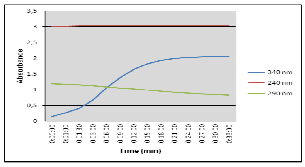


Fumarase

The seeds stored at -80ºC were used. The weighted 5 pieces of embryonal axis (or adequate amount of somatic embryos) was milled on a DNA mill and extracted by extraction buffer. Solution was centrifuged and pure extract was filtered by a 0,2 µm microfilter. 150 µl of filtered extract was added to a reaction mixture (HEPES buffer, malat dehydrogenase, NADP+, $MgCl_2$, KH_2PO_4). After 3, 6, 9 and 12 minutes of reaction the increase of absorbance at 340 nm (NADPH formation) is measured in 50 mm cuvette. It is proportional to the amount of fumarase in sample (method adapted according Hatch 1978 and Shen and Oden 2000).

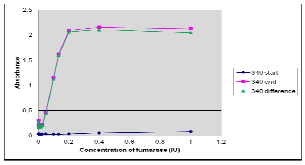
The optimization of the enzymatic reaction:
1.The timing of the reaction using pig fumarase from Sigma
2.The effect of fumarase concentration on absorbance

1.



Optimum for measurement: 340nm; 1 – 18 minutes of reaction

2.

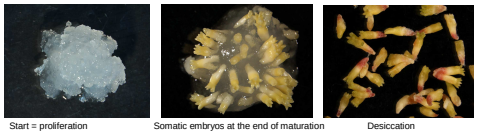


The maximum of measurement sensitivity: between 0 and 0,25 IU of fumarase

Fagus sylvatica L.

Dormant seed | Embryo (E) | Embryonal axis (EA) and cotyledons (C) | Embryonal axis (EA)

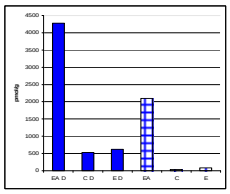
Picea abies (L.) Karsten



Start = proliferation | Somatic embryos at the end of maturation | Desiccation

The endogenous level of ABA

The ABA content in dormant and non-dormant beech embryos

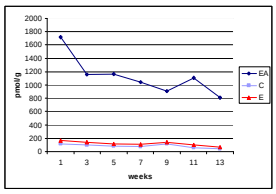


EA = embryonal axis; C = cotyledons; E = the whole embryo from the stored seeds before germination

EAD, CD, ED = from dormant seeds

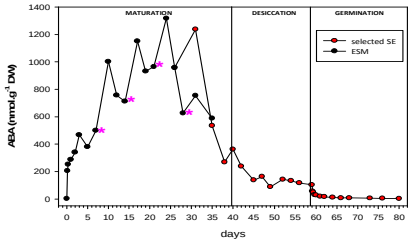
- The endogenous ABA is relatively high in beech embryos.
- The highest level of ABA was found in embryonal axis.
- The difference between the ABA content in dormant and non-dormant embryos is more than 50%.

The changes in endogenous ABA during stratification of beech seeds



- The endogenous ABA decreases during the whole process of stratification.
- The decrease of ABA content in embryonal axis and in the whole embryos is 50 - 60% during 13 weeks of stratification.
- The ABA level decreases more rapidly at the beginning of stratification.

The changes in endogenous ABA during spruce somatic embryogenesis



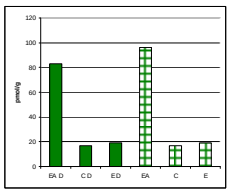
Maturation on the GD medium supplemented by ABA

* = passage on the fresh medium

- The endogenous ABA increases during 4 weeks of embryos maturation.
- The ABA level in embryos is higher than in ESM.
- During the end of maturation and during the whole desiccation the ABA level decreases.
- The somatic embryos are able to germinate when the ABA content is low.

The endogenous level of IAA

The IAA content in dormant and non-dormant beech embryos

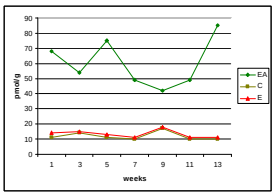


EA = embryonal axis; C = cotyledons; E = the whole embryo from the stored seeds before germination

EAD, CD, ED = from dormant seeds

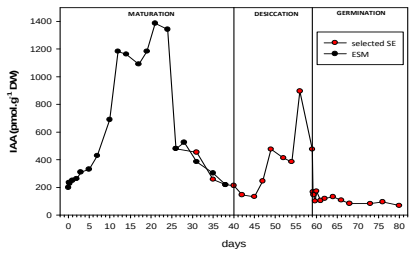
- The endogenous IAA is extremely low in beech embryos.
- The highest level of IAA was found in embryonal axis.
- The difference between the IAA content in dormant and non-dormant embryos was not found.

The changes in endogenous IAA during stratification of beech seeds



- The endogenous IAA fluctuates during the whole process of stratification.
- No differences between the level of IAA at the start and after 13 weeks of stratification were found.

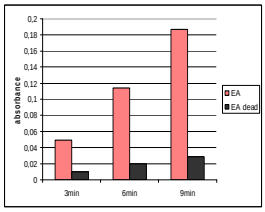
The changes in endogenous IAA during spruce somatic embryogenesis



- The maximum of IAA level is detected in the 3rd week of maturation when the root and shoot pole of embryos are formed.
- The 2nd maxima of IAA level is found at the end of desiccation – just before germination.
- The transient increase of IAA together with low ABA is necessary for successful germination of spruce somatic embryos.

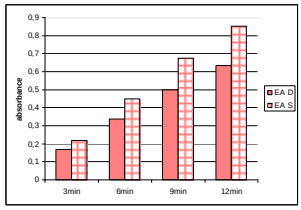
The fumarase activity

The fumarase activity in embryonal axis from stored seeds and from dead seeds of beech

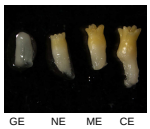


- The verification of the method:
- The enzyme fumarase is active in living cells only.
- The activity measured in dead embryonal axis represents the base level for measurement.
- It is possible to differentiate the living and dead embryos using this method.

The fumarase activity in embryonal axis from dormant beech seeds and from seeds after 15 weeks of stratification



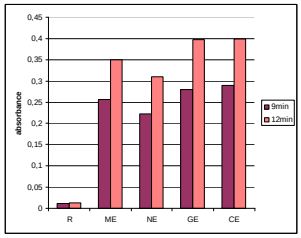
- The fumarase activity is lower in embryonal axis from dormant seeds.
- The higher activity of fumarase in embryonal axis from seeds just before germination is evident after 3 minutes of reaction. This difference increases during the whole measurement.



GE NE ME CE

GE = globular embryos
NE = non-matured embryos
ME = matured embryos
CE = malformed embryos + callus formation
R = rest of ESM

The fumarase activity in matured somatic embryos of spruce



-The rest of ESM consists of dead cells and embryos – without any activity of fumarase.

- The differences in fumarase activity among other somatic embryos are smaller.
- The higher fumarase activities were found in growing somatic embryos; ie. in developing globular embryos and in malformed embryos, where callogenesis starts.
- The lower fumarase activity was found in somatic embryos at the end of successfull maturation. The embryos finish their development before desiccation.

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