UV-B RADIATION INDUCES CHANGES IN POLYAMINE METABOLISM IN SOMATIC EMBRYOS OF NORWAY SPRUCE

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Introduction

UV-B radiation is a key environmental signal that affects metabolism, development and viability of plants. One of the most general plant response to stress conditions incl. UV radiation is directed to biosynthesis of polyamines (PAs). Two pathways for polyamine biosynthesis exist in plants, starting from either ornithine decarboxylase (ODC) or arginine decarboxylase (ADC). We intend to identify alterations in polyamine metabolism that might be indicative for resistance response of Norway spruce to UV-B radiation.

We used embryogenic spruce cultures exposed to 0.1; 0.6 and 6 W/m² of UV-B (312 nm) radiation in different stages of somatic embryo development. We observed the changes in the structure of embryogenic culture; in the yield of matured embryos and in the development of emblings after UV-B exposition.

We correlated morphological response of embryogenic culture during somatic embryogenesis with the changes in endogenous levels of polyamines, putrescine (Put), spermidine (Spd) and spermine (Spm) and/or the ratio Spd/Put.

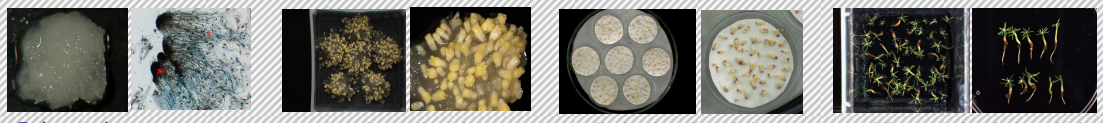
Methods

An embryogenic culture of *Picea abies*, genotype AFO 541, was obtained from AFOCEL, France. Cultivation in detail is described in : Gemperlová, L., Fischerová. L., Cvikrová, M., Malá, J., Vondráková, Z., Martincová, O., Vágner, M.: Polyamine profiles and biosynthesis in somatic embryo development and comparison of germinating somatic and zygotic embryos of Norway spruce. Tree Physiology 29(10): 1287-1298, 2009.

Extraction and HPLC analysis of benzoylated polyamines was performed according to : Slocum, R.D., Flores, H.E., Galston, A.W., Weinstein, L.H.: Improved method for HPLC analysis of polyamines, agmatine and aromatic monoamines in plant tissue, Plant Physiology 89: 512-517, 1989.

Somatic embryogenesis of *Picea abies*

Proliferation | Maturation (6 weeks) | Desiccation (3 weeks) | Germination



Embryogenic suspensor mass (ESM) = early embryos or polyembryogenic complexes; consisted of meristematic and suspensor cells

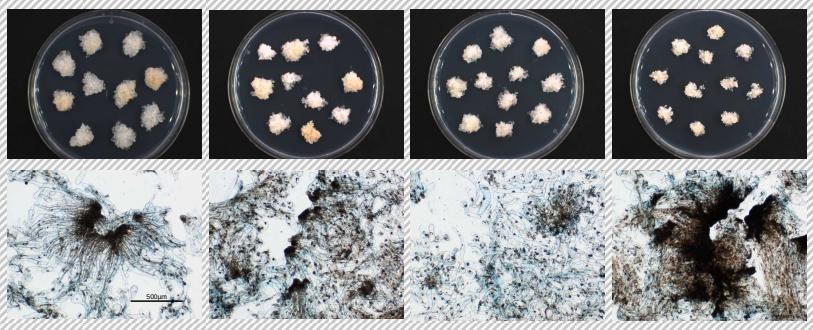
Matured somatic embryos

Fully developed cotyledonary embryos desiccated on the paper in 100% humidity

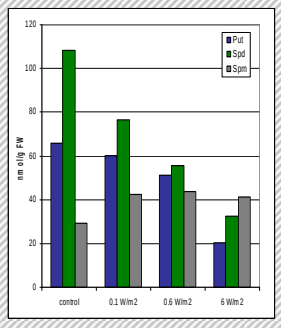
Emblings consisted of terminal bud, cotyledons, hypocotyl and root

The changes in the ESM after UV-B radiation in proliferation (7 days after the treatment)

control | 0.1 W/m² | 0.6 W/m² | 6 W/m²



The contents of endogenous polyamines in ESM



Exposure to UV-B irradiation altered substantially the anatomical structures of ESM and the process of proliferation.

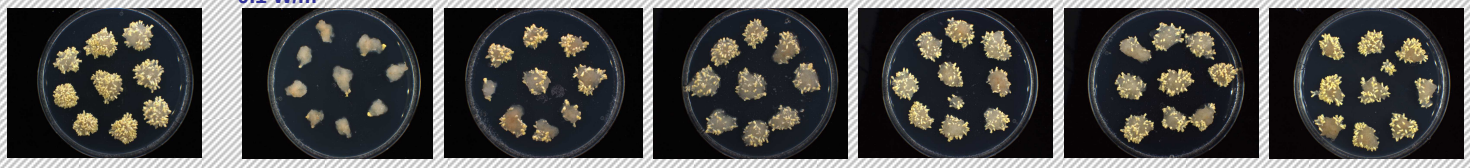
The radiation of 0.1 and 0.6 W/m² did not stop the growth of ESM, but the formation of embryogenic structures was blocked. The ESM radiated by 6 W/m² stopped its development – all visible polyembryogenic complexes were dead.

Marked decrease of polyamines occurred in response to UV-B radiation. In particular low levels of Spd, which represent a crucial role in both somatic and zygotic embryo development, were determined in irradiated ESM. Increased level of Spm represent a response to this abiotic stress.

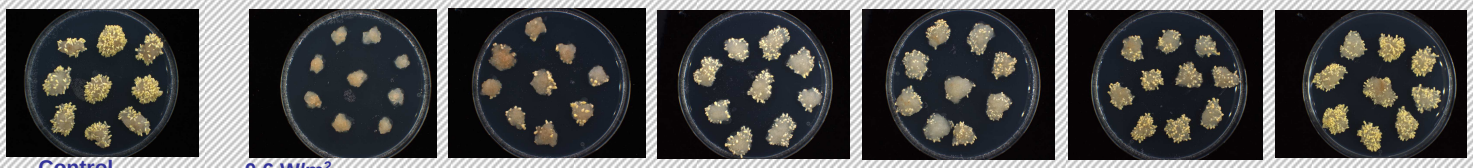
The effect of UV-B radiation timing on the yield of embryos

The embryogenic cultures were exposed to 0.1 or 0.6 W/m² radiation in different weeks of maturation. The response in polyamine contents was measured 7 days after the exposure. The effect of radiation on the embryos in different stage of development was characterised by the differences in the yield of embryos at the end of maturation in comparison to non-irradiated control.

Control | 0.1 W/m²



UV-B radiation during maturation: 1st week | 2nd week | 3rd week | 4th week | 5th week | 6th week



Control | 0.6 W/m²

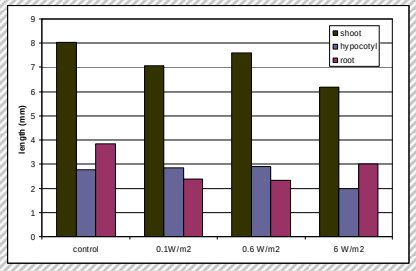
The effect of radiation depends strongly on the timing of the treatment. ESM at the start of maturation is more sensitive to the UV-B radiation than embryos at the end of maturation.

Spd/Put ratio 7 days after radiation

maturation	1st week	2nd week	3rd week	4th week	5th week	6th week
control	2.15	2.34	2.43	2.20	2.57	2.79
0.1 W/m²	1.54	1.23	1.35	1.22	1.99	2.42
0.6 W/m²	0.77	0.80	0.86	0.98	2.04	1.89

The changes in Spd/Put ratio indicated the intensity of embryogenic culture response to increasing UV-B dose radiation. The response of embryos at the end of maturation (5th and 6th week) was not so noticeable as in the beginning of maturation. Radiation of 0.6 W/m² resulted in much more significant stress reaction namely in the course of the first 4 weeks of maturation. At the end of maturation the values of Spd/Put ratios were close to the value of control.

The effect of UV-B radiation in desiccation on the development of emblings



Embryos were exposed to UV-B radiation in the 11th day of desiccation. The development of shoots (the length of hypocotyl, the length of the whole shoot) was not altered after 0.1 and 0.6 W/m² radiation exposures. However, 6 W/m² radiation slightly inhibited the growth of shoot. The root growth was slightly decreased as compared with non-irradiated control.

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