Polyamine metabolism and autophagy in plants

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INTRODUCTION and AIMS

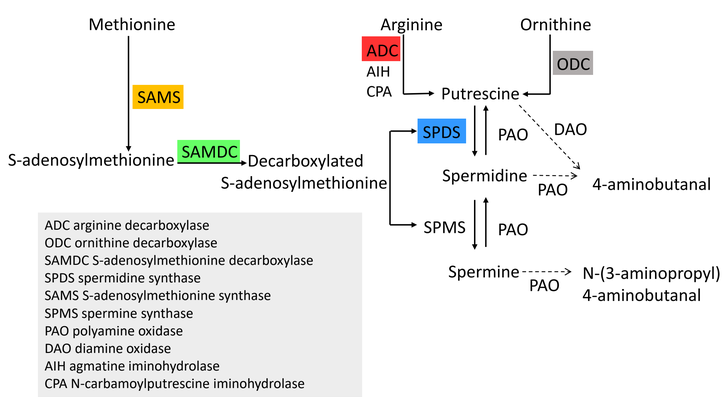
Polyamines putrescine (Put), spermidine (Spd) and spermine (Spm) are ubiquitous, small aliphatic polycations found in eukaryotic organisms, which regulates key developmental and physiological events. They play an important role in diverse plant growth and developmental processes as well as in adaptation to environmental stresses. Among other functions spermidine has been shown to stimulate the process of autophagy across species including yeast, animals and even humans.

The **AIM** of our study was to follow changes in polyamine metabolism after autophagy induction. We have used two experimental plant systems.

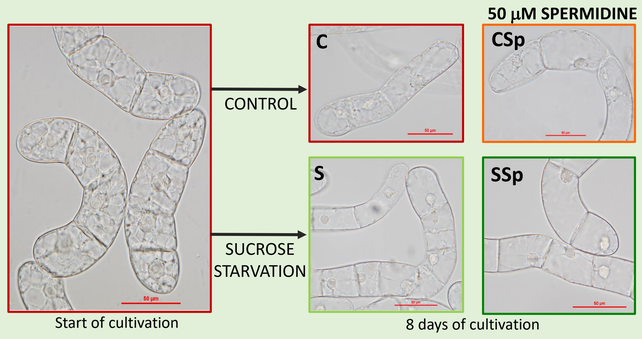
1) ***Nicotiana tabaccum*** suspension cell culture: autophagy is induced by sucrose starvation. Key role of autophagy in plant response to various environmental cues has been proved (e.g. Masclaux-Daubresse *et al*. 2017).

2) ***Picea abies*** embryogenic cultures: autophagy is activated in the phase of suspensor cell disintegration, what is the process essential for proper embryo maturation (e.g. Minina *et al*. 2013).

The scheme of polyamine metabolism

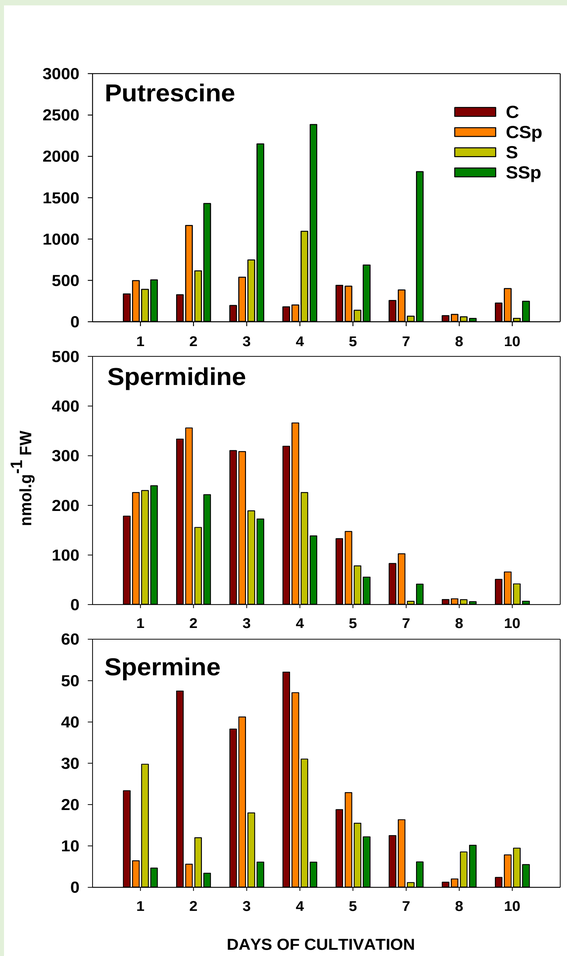


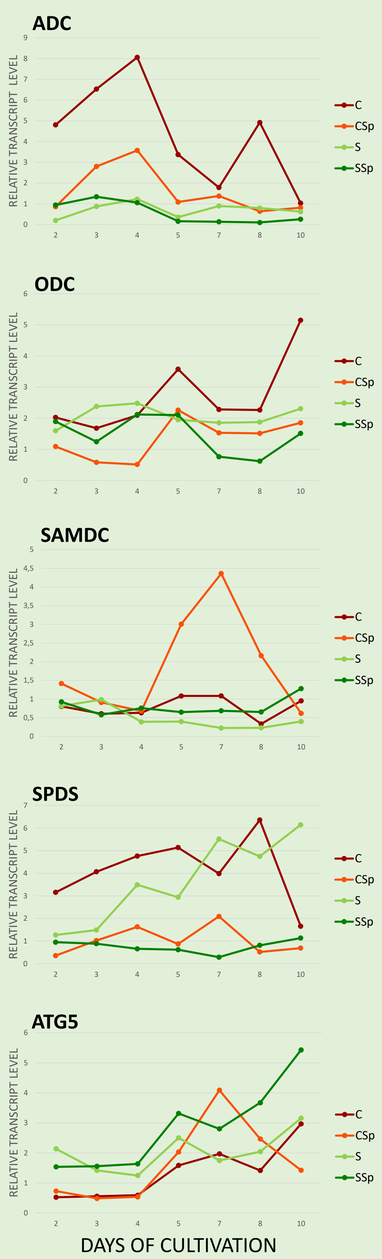
1) TOBACCO BY-2 CELL CULTURE



Experimental scheme – Tobacco BY-2 suspension culture was cultivated in control (C) and sucrose starvation (S) conditions, with (CSp and SSp) or without (C and S) external application of 50 µM Spermidine.

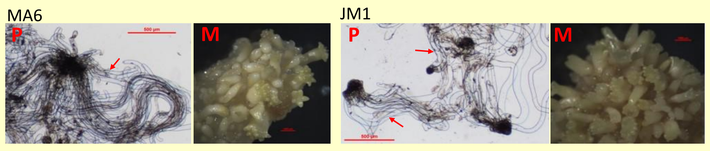
The content of free polyamines in tobacco BY-2 cells



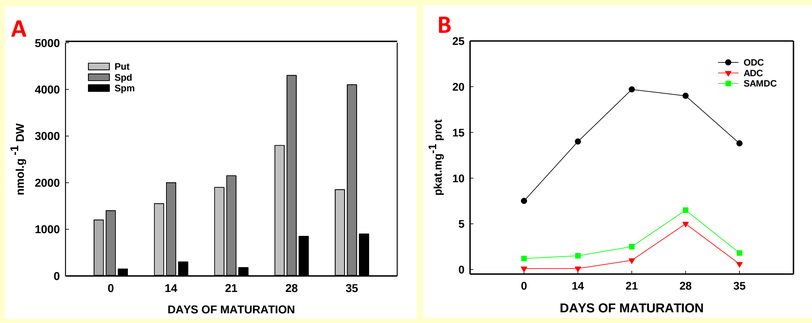


The expression of polyamine biosynthetic enzyme genes and Autophagy related gene 5 (ATG5)
ADC arginine decarboxylase;
ODC ornithine decarboxylase;
SAMDC S-adenosylmethionine decarboxylase;
SPDS spermidine synthase

2) SPRUCE EMBRYOGENIC CULTURES

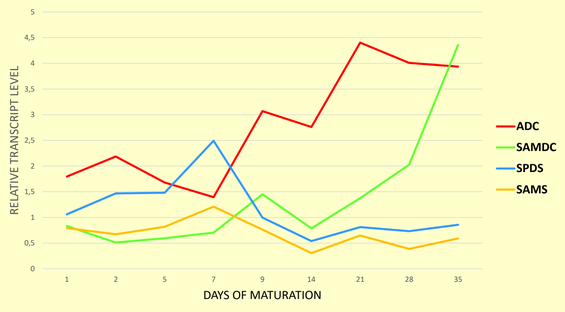


Proliferation (**P**) and the end of maturation phase (**M**) in Norway spruce embryogenic lines MA6 and JM1. Maturation of embryos is trigerred by abscisic acid. Arrows indicate suspensor cells.

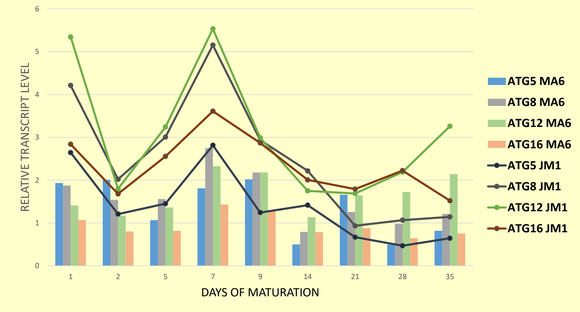


A Content of free polyamines putrescin (Put), spermidin (Spd) and spermin (Spm) during maturation of somatic embryos

B Activity of polyamine biosynthetic enzymes during maturation of somatic embryos: ODC ornithine decarboxylase; ADC arginine decarboxylase; SAMDC S-adenosylmethionine decarboxylase



The expression levels of putative polyamine biosynthetic enzyme genes during maturation of somatic embryos:
ADC arginine decarboxylase; SAMDC S-adenosylmethionine decarboxylase; SPDS spermidine synthase;
SAMS S-adenosylmethionine synthase



The expression levels of putative *ATG5*, *ATG8*, *ATG12* and *ATG16* genes during the maturation of somatic embryos. Lines represent embryogenic culture JM1, bars represent embryogenic culture MA6.

CONCLUSIONS

TOBACCO

Sucrose starvation

- affected levels of free polyamines, Put levels increased during first half of cultivation, Spd and Spm levels decreased as compared to control
- ceased expression of ADC and ODC genes, did not affect expression of SAMDC, SPDS and ATG5 genes

External spermidine application

- increased the level of free Put, mainly under starvation; the level of internal spermidin remained unchanged
- decreased expression of ADC, ODC and SPDS genes, slightly increased expression of ATG5 and under prolonged starvation highly increased expression of SAMDC

SPRUCE

- Content of free polyamines (especially Spd and Spm) increased in the course of embryo development; biosynthetic enzymes activities correlated with free polyamine content.
- Expression of putative ADC and SAMDC genes continuously increased during embryo development; expression of SPDS peaked after 7 days of maturation
- Expression of analyzed ATG genes decreased at the beginning of maturation, than transiently increased at day 7 in coincidence with the start of suspensor cell disintegration.

OBTAINED DATA INDICATE INTERCONNECTION OF POLYAMINE METABOLISM AND AUTOPHAGY INDUCTION IN OUR EXPERIMENTAL SYSTEMS

MATERIAL AND METHODS

Cultivation:
Nicotiana tabaccum BY-2 suspension cultures were cultivated in basal MS medium either with 3% (w/v) sucrose or without sucrose addition. Embryogenic cultures of Norway spruce (*Picea abies* L. [Karst.]) were cultivated on solidified (proliferation) resp. liquid (maturation) GD medium as described elsewhere (Gemperlova *et al.* 2009). The proliferation medium contained 5 µM 2,4-D, 2 µM BA and 2 µM kinetin, maturation medium was supplemented by 20 µM abscisic acid (ABA) and 3.75% (w/v) PEG 4000.

Morphology of cultures:
Light transmission microscope Jenaval (Carl Zeiss) with DS-5M Nikon camera was used to monitor phases of embryo development as well as the state of tobacco suspension cultures.

Polyamine content:
Polyamines were extracted and benzoylated according to the method of Slocum *et al.* (1989). Detection and quantification of benzoylamines were carried out using an HPLC/MS system.

Activity of polyamine biosynthetic enzymes:
Ornithine decarboxylase (ODC; EC 4.1.1.17), arginine decarboxylase (ADC; EC 4.1.1.19) and S-adenosylmethionine decarboxylase (SAMDC; EC 4.1.1.50] were determined by a radiochemical method as described by Tassoni *et al.* (2000).

Gene expression analysis:
The relative transcript levels of the genes of interest were analyzed by real-time PCR. RNA was isolated from 0.1g of frozen embryos by RNeasy Plant Kit (Qiagen) and subjected to DNaseI. cDNA was prepared using Revert Aid First Strand cDNA Synthesis Kit (Thermo Scientific). Quantitative RT-PCR was carried out in 12 µl PCR mixture containing 6 µl of PCR MasterMix (Generi Biotech); 3.5 µl of nuclease free water; 0.5 µl of mixture of forward and reverse primer (initial concentration 10 mM) and 2 µl of cDNA. *Picea abies* alpha-tubulin gene (GenBank: X57980.1) and *Nicotiana tabaccum* elongation factor (GenBank: AF120093) were used as reference genes. Primers for genes of interest were designed based on gene homology (tBLASTn). The relative transcript level expression was analyzed by the modified 2-ΔΔCT method using individual amplification efficiency for each gene (Schefe *et al.*, 2006) and compared relative to expression levels at day one of tobacco cell culture cultivation and spruce embryogenic culture proliferation, respectively (value1).

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