Polyamine metabolism after induction of autophagy in tobacco BY2 cell culture

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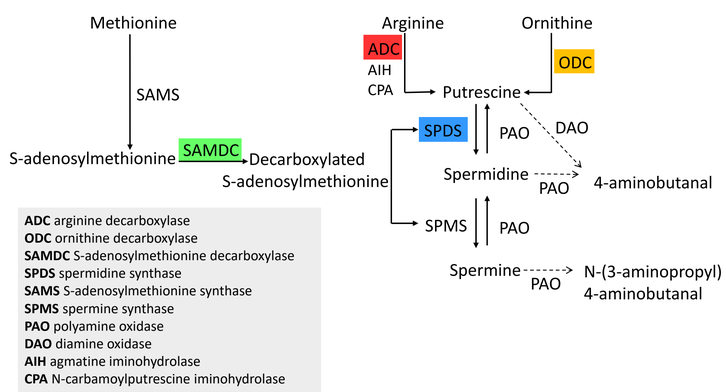
INTRODUCTION and AIMS

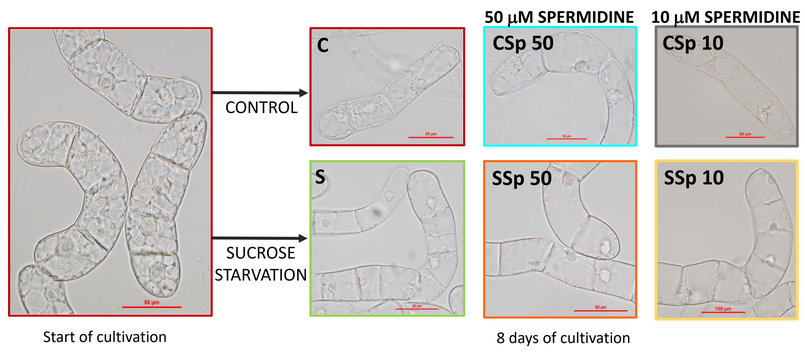
Polyamines putrescine (Put), spermidine (Spd) and spermine (Spm) are ubiquitous, small aliphatic polycations found in eukaryotic organisms, which regulates key developmental and physiological events. They play an important role in diverse plant growth and developmental processes as well as in adaptation to environmental stresses. Among other functions spermidine has been shown to stimulate the process of autophagy across species including yeast, animals and even humans (Madeo *et al*. 2010).

The **AIM** of our study was to follow changes in polyamine metabolism in *Nicotiana tabaccum* suspension cell culture after autophagy induction. We induced autophagy by sucrose starvation, since a central role of autophagy in plant response to various environmental cues has been proved (e.g. Masclaux-Daubresse *et al*. 2017).

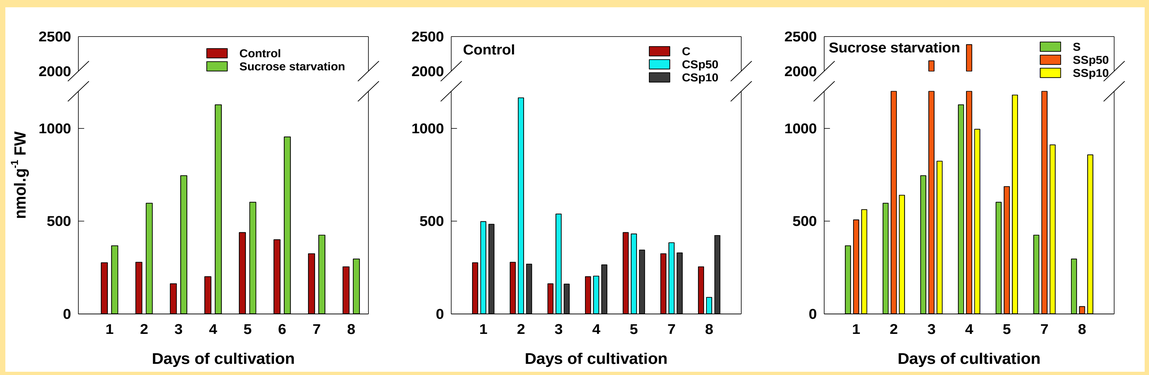
The scheme of polyamine metabolism



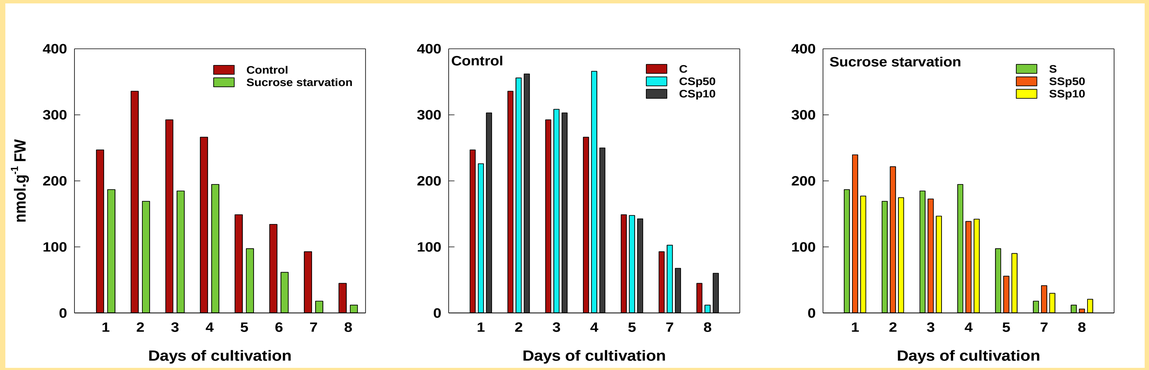
Experimental scheme: Tobacco BY-2 suspension culture was cultivated in control (C) and sucrose starvation (S) conditions, with (CSp and SSp) or without (C and S) external application of 50 μM or 10 μM spermidine, respectively.



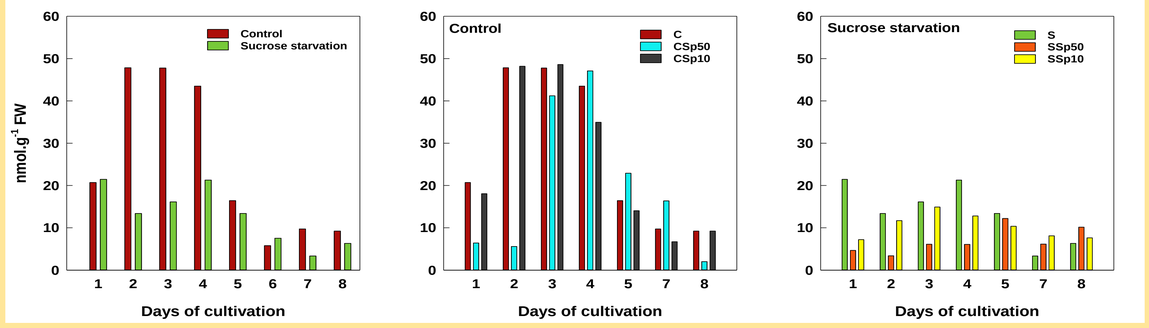
PUTRESCINE



SPERMIDINE



SPERMINE



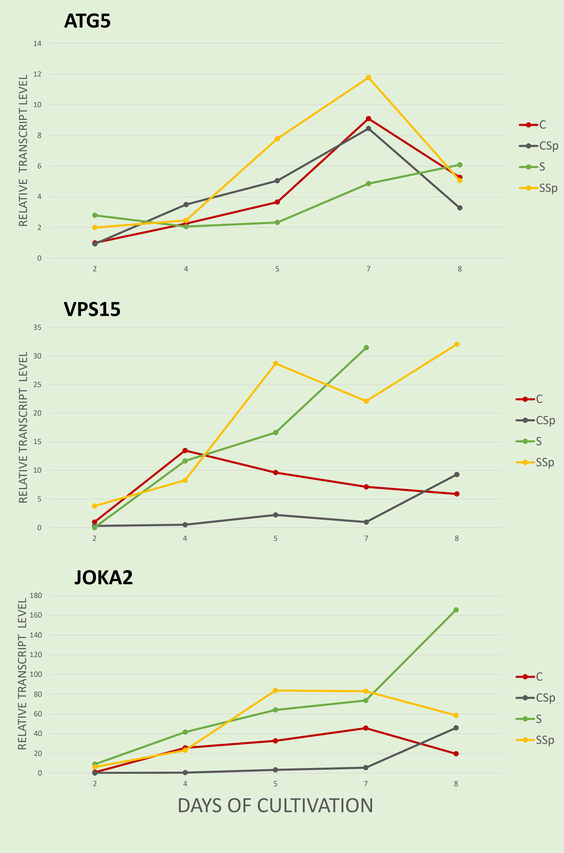
Content of free polyamines during cultivation in control and sucrose free medium.

Content of free polyamines during cultivation of cells in control conditions without (C) or with external application of 50μM (CSp50) or 10μM spermidine (CSp10).

Content of free polyamines during cultivation of cells in sucrose free medium without (S) or with external application of 50μM (SSp50) or 10μM spermidine (SSp10).

POLYAMINE METABOLISM ENZYME GENES

AUTOPHAGY RELATED GENES



The expression of polyamine biosynthetic enzyme genes and autophagy related genes during cultivation in control and sucrose free medium, with (CSp and SSp) or without (C and S) external application of 10 μM spermidine.
ADC arginine decarboxylase; **ODC** ornithine decarboxylase; **SAMDC** S-adenosylmethionine decarboxylase; **SPDS** spermidine synthase; **ATG5** Autophagy Related Gene 5; **VPS15** Serine/threonine-protein kinase VPS15; **JOKA2** tobacco hybrid homolog of two mammalian selective autophagy cargo receptors, p62 and NBR1

CONCLUSIONS

Sucrose starvation

- affected levels of free polyamines: Put levels increased during first half of cultivation, Spd and Spm levels decreased as compared to control.
- increased expression of *SPDS* and slightly decreased expression of *ODC* and *SAMDC*.
- did not affect expression of *ADC* and *ATG5* genes.
- massively increased expression of *VPS15* and *JOKA2*.

External spermidine application

- increased the level of free Put, mainly under starvation, whereas the level of internal Spd remained unchanged; this effect was pronounced by higher (50μM) Spd concentration.
- decreased expression of *ADC* and *SPDS* genes, did not affect expression of *ODC* and slightly increased expression of *ATG5* in starved culture.
- under prolonged starvation highly increased expression of *SAMDC*.
- in control conditions stopped expression of *VPS15* and *JOKA2* genes until 7th day of cultivation.

POLYAMINE METABOLISM OF TOBACCO CELL CULTURE IS AFFECTED BY AUTOPHAGY INDUCTION

MATERIAL AND METHODS

Cultivation:
Nicotiana tabaccum BY-2 suspension culture was cultivated for eight days in basal MS medium either with 3% (w/v) sucrose or without sucrose addition. Concurrently media were supplemented by spermidine (Sigma Aldrich), final concentrations was 10 μM or 50 μM.
Morphology of cultures:
Light transmission microscope Jenaval (Carl Zeiss) with DS-5M Nikon camera was used to monitor 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.
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 culture 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. *Nicotiana tabaccum* elongation factor (GenBank: AF120093) was used as reference gene. 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 (value1).

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