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TRANSCRIPTOME OF THE ARABIDOPSIS THALIANA CHERNOBYL ECOTYPE SEEDLINGS: SIMULATING OF THE SPACE RADIATION ACTION AND MICROGRAVITY

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Introduction

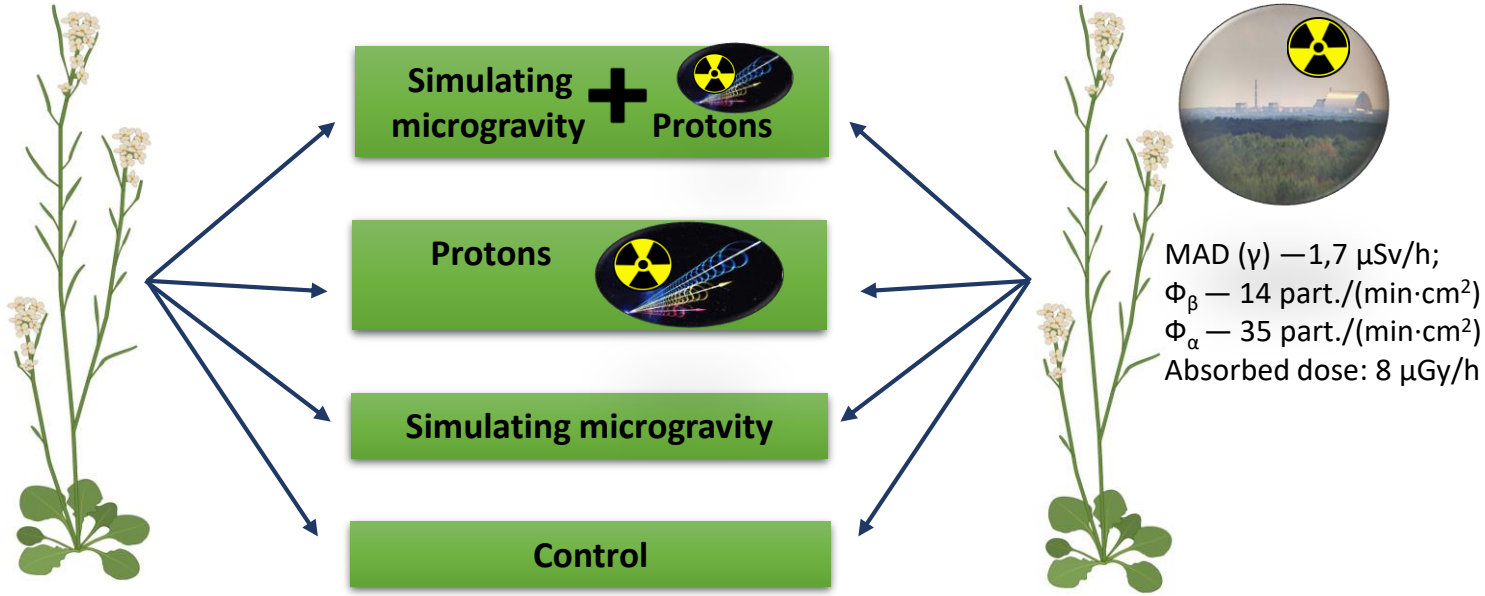
In the field of space biology, the rapid development of omics technologies has made a significant contribution to the discovery of differentially expressed genes, which are involved in the plant adaptive reactions that are activated under stressful conditions of space flight. However, the molecular pathways and mechanisms responsible for the changes are still not fully understood. In addition, the search for opportunities to increase plant tolerance to space conditions is especially important at the present time.

Research Objective

is to study the transcriptome of the chronically irradiated (the Chernobyl ecotype) and wild-type Col-8 *A. thaliana* plants exposed to the conditions of simulating space flight. The results of this study will gain new knowledge about the molecular mechanisms of the action of cosmic radiation and microgravity on plants and will help to understand whether the stress tolerance of the Chernobyl ecotype are increased.

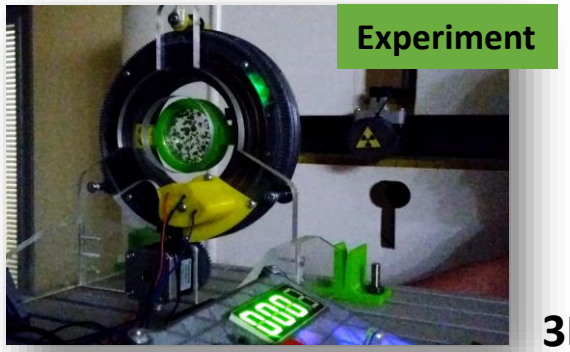
Materials and Methods

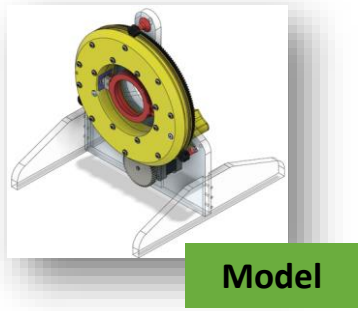
SCHEME OF THE EXPERIMENT



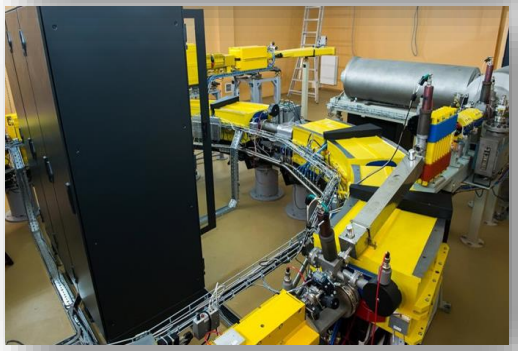
Transcriptomics

SIMULATIING OF THE SPACE FLIGHT CONDITIONS





3D-clinostat



The proton therapy complex «Prometheus» A. Tsyb MRRC

Experiment stages

Simulating of the space flight conditions

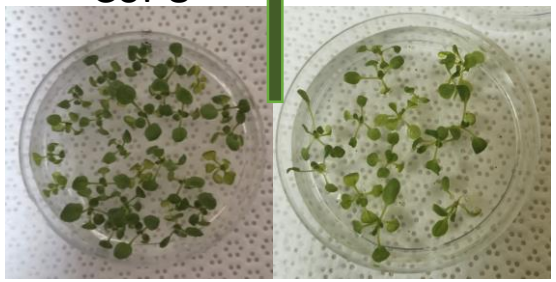
Planting seeds
Chernobyl ecotype (ChE)
and Col-8
Medium ½ MC, 0.3% sucrose
(16 dishes – 8 for ChE, 8 for Col-8;
30 seeds per dish)

Cultivation
13 days

Clinostating:
1 hour, the speed of rotation of the clinostat 60 rpm

Proton irradiation:
energy of 100 MeV, the total absorbed dose for each irradiated sample was 10 Gy

Col-8 ChE



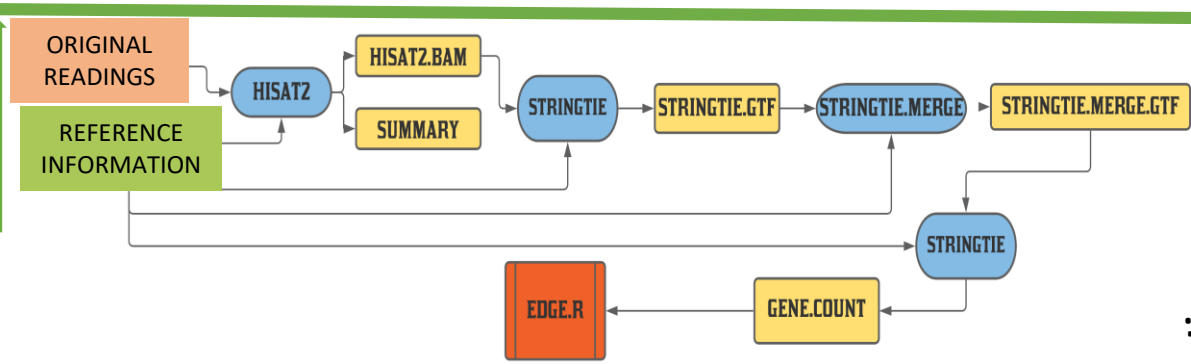
13-day-old seedlings *A. thaliana*

Proton irradiation + clinostating
Petri dishes with plants *A. thaliana*

Plant selection
for RNA
isolation

RNA isolation

RNA sequencing
on the Illumina NovaSeq 6000 platform (Eurogen).
The length of the reads obtained was 100 base pairs. 993 907 276 raw reads were obtained.



Bioinformaticsdata analysis
DEGs were detected
($log_2FC > 2$, FDR adjusted p-value < 0,05),
GO enrichment

Interpretation of the results

Results

The number of detected differentially expressed genes with increased expression in seedlings of the Chernobyl ecotype (ChE) and Col-8 subjected to simulated conditions, compared with control seedlings ChE and Col-8, respectively

Col-8	ChE
Clinostating+Protons/Control	Clinostating+Protons/Control
47	**44**
Protons/Control	Protons/Control
29	**32**
Clinostating/Control	Clinostating/Control
0	**0**

As the result of the clinostation only, there were no DEGs; however, for p-value < 0,05 и FDR = 1 genes potentially associated with the effect of simulated microgravity on plants were found.

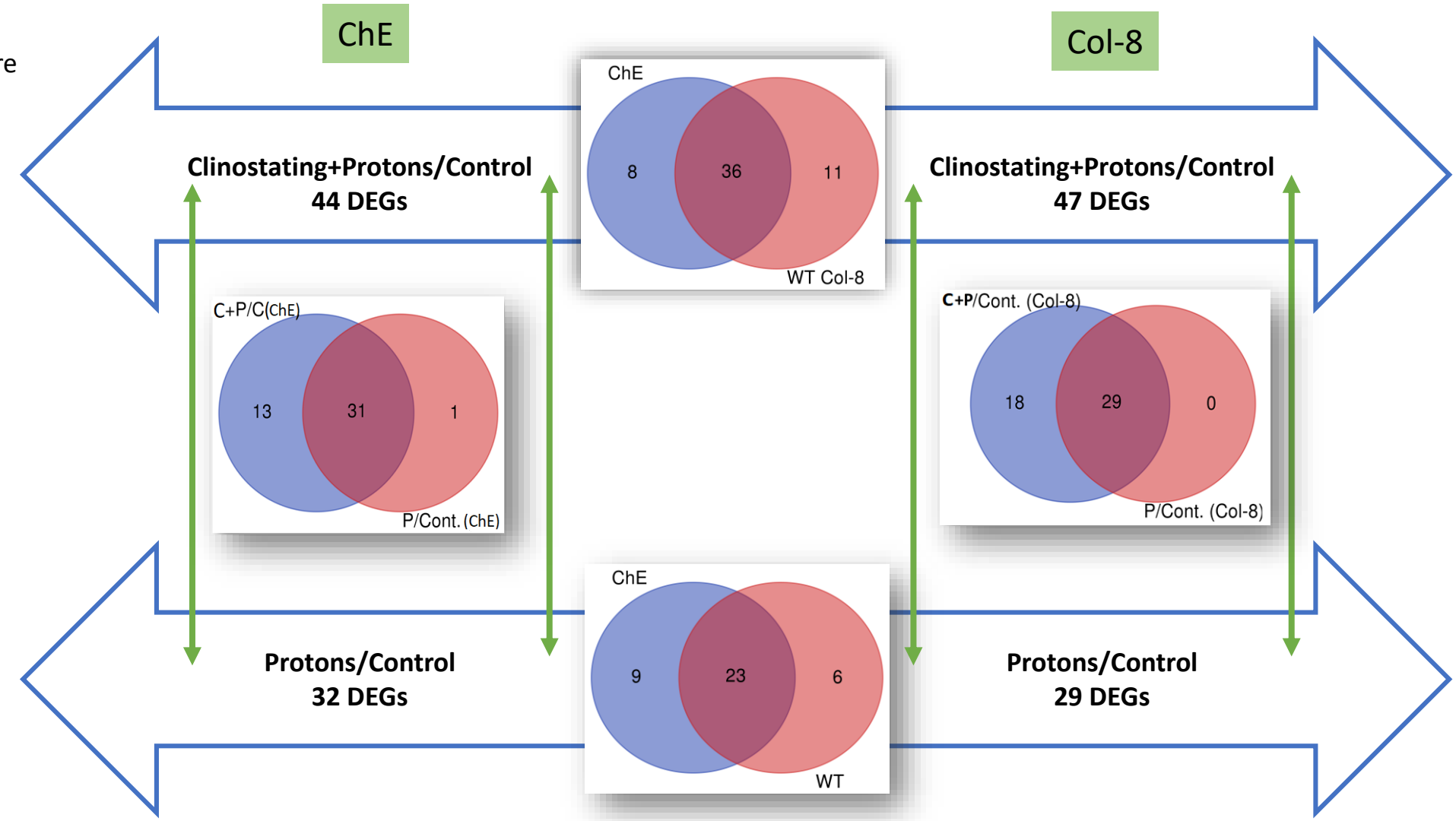
Comparison of ChE and Col-8 after to simulated space conditions

ChE/Col-8	
ChE (clinostating+Protons)/Col-8 (clinostating+Protons)	
165	**206**
ChE (control)/Col-8 (control)	
200	**220**
ChE (protons)/Col-8 (protons)	
264	**225**
ChE (clinostating)/Col-8 (clinostating)	
205	**215**

Red - up-regulated DEGs
Green - down-regulate DEGs

Venn diagrams showing unique and common differentially expressed genes

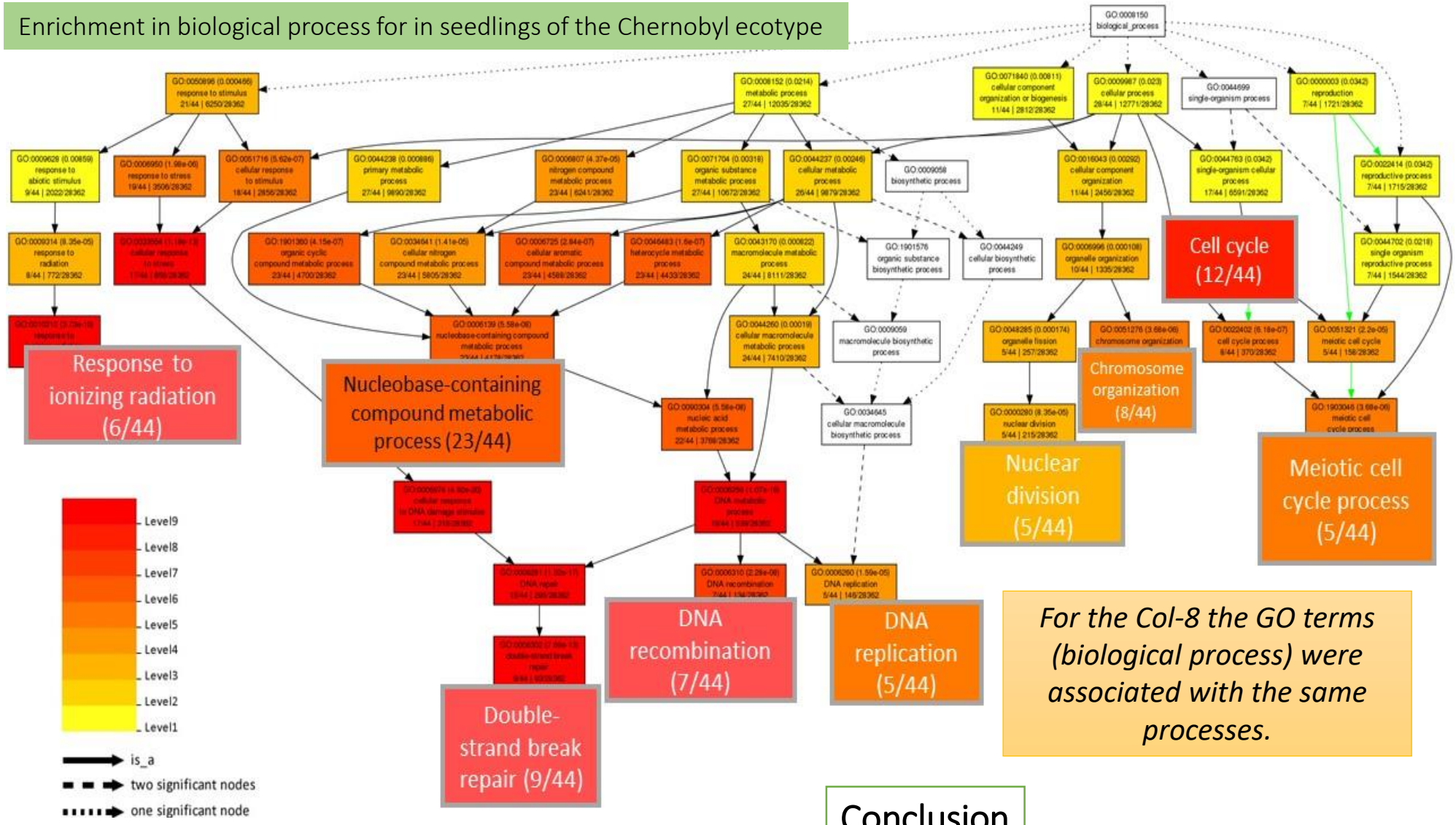
Among the 8 unique DEGs were identified associated with DNA repair, replication, and protein metabolism.



Among 11 unique genes were note genes whose products are responsible for the organization of the cell wall, associated with plant defense reactions, DNA repair.

The GO enrichment analysis (irradiation protons + clinostating)

Enrichment in biological process for in seedlings of the Chernobyl ecotype



For the Col-8 the GO terms (biological process) were associated with the same processes.

In terms of **cell localization** for two types of seedlings, enrichment was observed for GO terms associated with chromosomes.

In terms of **molecular function**, in A. thaliana of the Chernobyl ecotype, DEGs were associated with the building of DNA, ATP, and nucleoside triphosphatase activity, and for the Col-8 line, with the synthesis of ATP and with nucleoside triphosphatase activity.

Conclusion

The results of the work allowed us to identify and compare the most probable molecular pathways that respond to the simulated outer space conditions in the seedlings of the Chernobyl ecotype and wild type *A. thaliana*, which in the future will make it possible to obtain plants with increased stress resistance to space environmental conditions. Supported by RSF (No. 20-74-00101).