The effect of salt stress on the expression of the brassinosteroid biosynthesis genes

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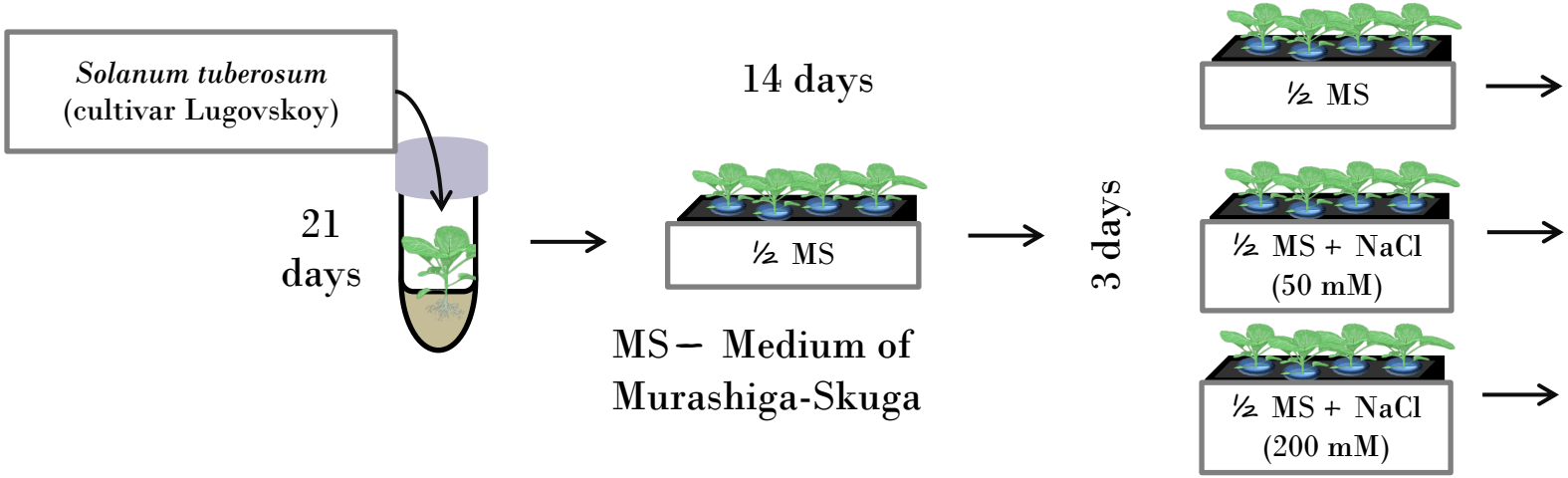
Soil salinization is one of the most unfavorable environmental factors caused by the increased anthropogenic load on the environment. Excessive sodium chloride content in the soil negatively affects the implementation of many physiological processes in plants, largely due to the generation of reactive oxygen species and the development of oxidative stress.

Components of a phytohormonal nature, such as brassinosteroids (BRs), are considered as a regulator of plant resistance. Their protective properties under abiotic stress are currently being actively studied. However, the mechanisms underlying the protective effect have not yet been established.

The purpose of this work: to study the effect of several concentrations of sodium chloride (**50 and 200 mM**) on the level of expression of genes for the biosynthesis of brassinosteroids.

Model of experiment



❖ **Gene expression** levels of brassinosteroid synthesis genes (CYP450 90A1, CYP450 85A1, DET2 and CYP450 90B1) were evaluated by **real-time PCR** using **highly specific primers** and the intercalating dye - **SYBRGreen I**.

❖ For normalization a reaction used **two** housekeeping genes which encode cyclophilin and elongation factor –1α.

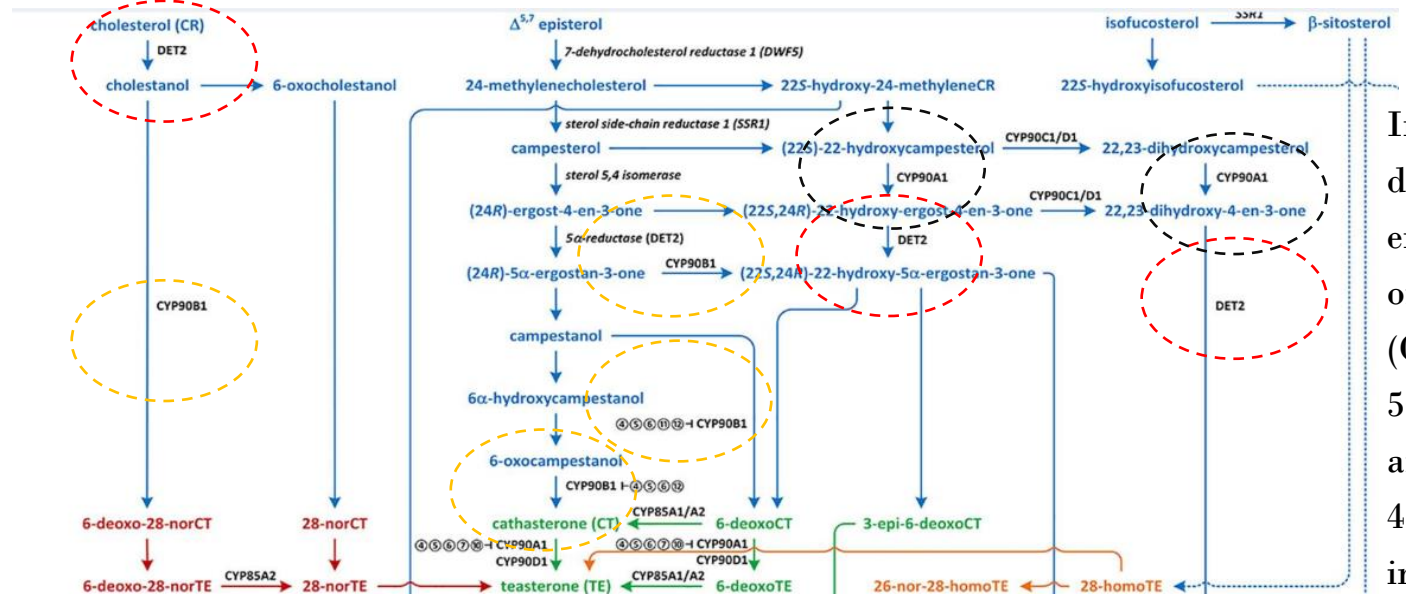
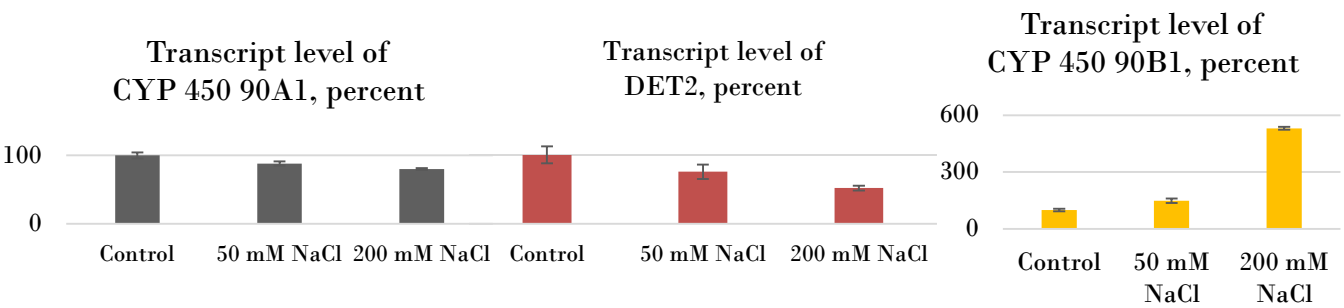
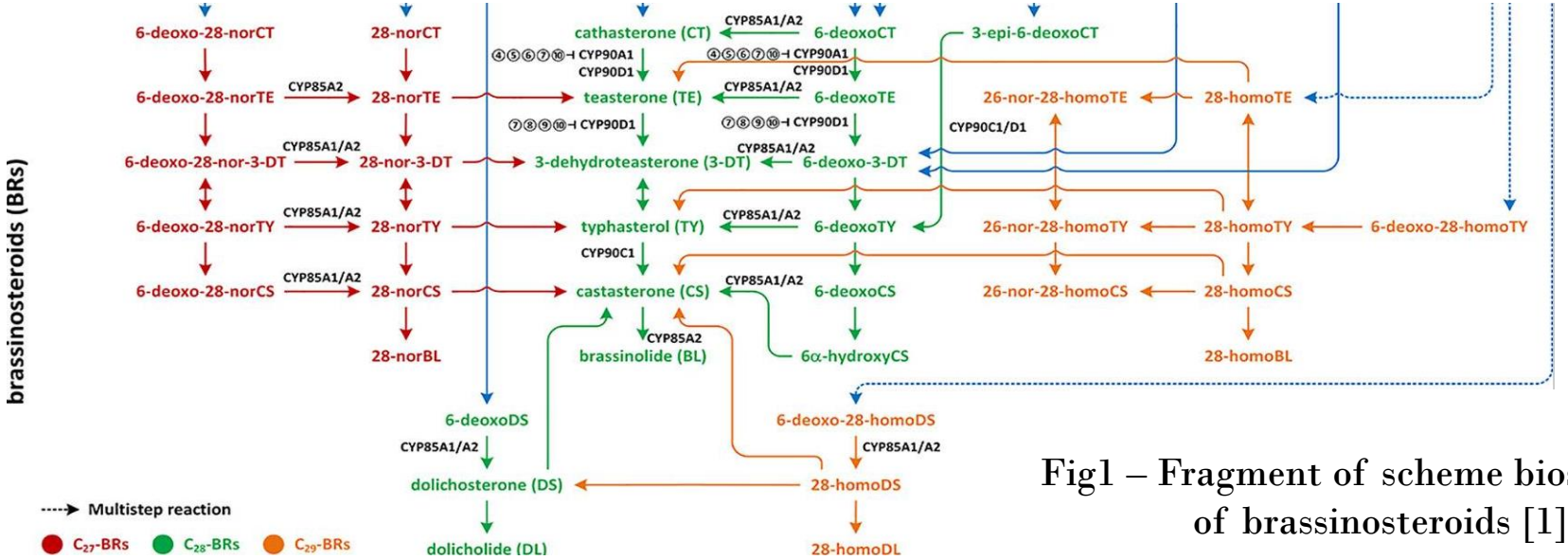


Fig1 – Fragment of scheme biosynthesis of brassinosteroids [1]



In our study, the salinity descent the level of gene expression for encoding enzymes of earlier stages of biosynthesis (CYP 450 90A1 and DET2). At 50 mM NaCl the decrease was 12 and 24.5%, at 200 mM - 20 and 48%, respectively. A significant increase in the expression level (by 1.5 and 5 times) in response to chloride salinity was noted for the CYP 450 90B1 gene encoding DWARF4.

[1] - Bajguz, A., Chmur, M., & Gruszka, D. (2020). Comprehensive Overview of the Brassinosteroid Biosynthesis Pathways: Substrates, Products, Inhibitors, and Connections. Frontiers in plant science, 11, 1034. https://doi.org/10.3389/fpls.2020.01034



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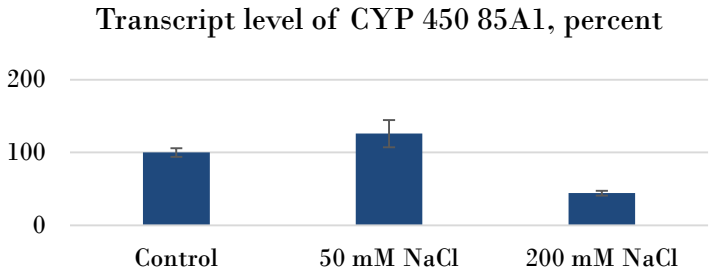

The versatile effect of sodium chloride on the expression level of the CYP 450 85A1 gene has also been showed. Low concentration (50 mM) promoted the accumulation of transcripts by 25%. Moderate concentration (100 mM) by 39% (data not showed). However, relative to 200 mM NaCl, the expression level decreased twofold.

[1] - Bajguz, A., Chmur, M., & Gruszka, D. (2020). Comprehensive Overview of the Brassinosteroid Biosynthesis Pathways: Substrates, Products, Inhibitors, and Connections. Frontiers in plant science, 11, 1034. https://doi.org/10.3389/fpls.2020.01034

Conclusions

- The low concentration of sodium chloride in the nutrient medium reduces the expression of genes involved in the realization of early stages of brassinosteroid biosynthesis. However, an increase in the expression level of the CYP 450 90B1 and CYP 450 85A1 genes may indicate on enhance of C27-, C28-brassinosteroids synthesis.
- High salt concentration significantly reduced the expression of genes which responsible for early and late stages of brassinosteroid biosynthesis. A 5-fold increase in the expression of the CYP 450 90B1 gene, as well as a 2-fold decrease in the level of CYP 450 85A1 gene transcripts, possibly indicates the accumulation of intermediate forms of brassinosteroids (catasterones) or a change in the pathway of the biosynthesis of brassinosteroids from C-27 (Norbrassinolide) or C-28 (Brassinolide) to C-29 (Homobrassinolide).