Ph.D. Thesis

Overexpression of the Arabidopsis glutathione peroxidase-like 5 gene (*AtGPXL5*) resulted in altered redox status, plant development and salt tolerance

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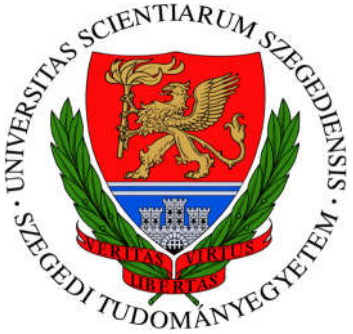

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Introduction

Environmental stresses, mainly salinity, severely inhibit plants growth and productivity. Irrigation substantially expands growing seasons and increases crop yields in many regions. Sodium chloride gradually accumulates in irrigated soils and is toxic to most crops; sodium chloride accumulation is particularly detrimental in leaves. According to the published report in 2020, approximately 40% of irrigated lands worldwide are affected by the problem of increased salt levels and spread of soil salinization is a major threat to crop performance. Salt stress can induce ROS production in different organelles of the plants, whereas inequality between the rate of ROS generation and detoxification can create oxidative stress. Accumulation of ROS in excessive amounts is deleterious to the plants and can lead to uncontrolled oxidation of membranes, proteins and DNA, causing oxidative stress and cells death. To avoid this, Plants elicit a complex and unique cellular and molecular response in response to various stresses in order to prevent the damage and ensure survival. In order to maintain the cellular homeostasis under abiotic stresses, plants possess a powerful and multifaceted antioxidant system that is composed of enzymatic and non-enzymatic components, with various kind of biochemical properties and distinct subcellular localization, which are involved in sensing, detoxification, elimination and/or neutralization of ROS overproduction.

Plant's glutathione peroxidase-like (GPXL) enzymes are thiol-based peroxidases catalysing the reduction of H_2O_2 or hydroperoxides to water or alcohols using reduced glutathione (GSH) or thioredoxin as an electron donor. The plant GPXLs enzymes are closely related to animal phospholipid hydroperoxide glutathione peroxidases, and it was reported that plant isoenzymes reduce more efficiently peroxides different from H_2O_2 such as lipid peroxides. They are commonly considered as one of the key players in the enzymatic defence system of plants because they can reduce peroxide with higher efficiency (sometimes exclusively) by the TRX system rather than by using GSH as a reducing agent. The *Arabidopsis* genome encodes 8 GPXL isoforms, which have been predicted to be localized in different subcellular compartments. AtGPXL1 and AtGPXL7 are chloroplastic proteins, AtGPXL2 and AtGPXL8 are localised in cytosol as well as in nucleus, AtGPXL6 can be found in cytosol and mitochondria, AtGPXL3 is a transmembrane protein of the secretory pathway, while AtGPXL4 and AtGPXL5 are associated to the inner side of the plasma membrane. we examine the effect of the elevated *AtGPXL5* expression level on the growth and salt stress

tolerance of seedlings. In addition, we investigate the antioxidant defence mechanisms in 6-week-old hydroponically grown plants were treated with 100 mM NaCl and the main enzymatic- and non-enzymatic antioxidants, the redox status of ASC and glutathione were analysed.

Aims

The function of AtGPXL5 in the stress response, regulation of growth and development and in signalling is not fully known. Our aims were to investigate the role of AtGPXL5 isoenzyme in the salt stress response of *Arabidopsis* plants. To check the involvement of the enzyme in the oxidative stress responses, firstly the ROS levels and vitality of the *Arabidopsis thaliana* ecotype Columbia (Col-0) and a glutathione peroxidase-like 5 T-DNA insertional mutant (*Atgpxl5*) seedlings were compared after applying NaCl stress, then the *AtGPXL5* gene was overexpressed in *Arabidopsis* Col-0. In the Ph.D. dissertation, we were looking for the answers on main questions as follows:

1) Has the AtGPXL5 role in the regulation of ROS and redox homeostasis and in the maintenance of the cell's vitality?

2) What is the effect of the decreased or increased AtGPXL5 expression on the activity of antioxidant mechanisms in control conditions and in the short-term salt stress response? Have the AtGPXL5-overexpressing or Atgpxl5 mutant plants altered glutathione redox potential?

3) Has the AtGPXL5 any kind of function in the growth and development of Arabidopsis seedlings under control conditions and in the presence of 100 mM NaCl?

Material and methods

In the present study, two different experimental systems were applied. Firstly, the growth and development of wild type, *Atgpxl5* mutant, and two overexpressing lines (OX - AtGPXL5-1 and OX-AtGPXL5-2) seedlings were compared *in vitro* under control conditions and in the presence of NaCl.

The superoxide anion level, total ROS level and the cell viability were detected by fluorescent microscope (Zeiss Axiowert 200 M microscope (Carl Zeiss, Jena, Germany)) in the 12-day-old *Arabidopsis thaliana* Col-0 and *Atgpxl5* insertional mutants after applying 7 days treatment with the concentration of 50 mM and 100 mM NaCl. The rate of germination

in the presence of 100 mM NaCl or without salt using a small stereo microscope (Carl Zeiss Jena 402339) was monitored daily for 7 days. We measured the root length, no of lateral roots and the fresh weight of plants. We determined the morphological parameters (rosette size, convex area, convex percentage) and pigment contents (chlorophyll and anthocyanin contents) of *in vitro* grown 15-day-old Col-0 wild type, *Atgpxl5* mutant and OX-AtGPXL5 seedlings were investigated using PlantSize (http://www.brc.hu/pub/psize/index.html) software. For gene expression analysis, RNA was isolated by LiCl-method and after cDNA writing, quantitative RT-PCR was performed using primers designed by Primer3 program. To measure the H_2O_2 and malondialdehyde contents, the antioxidant enzyme activities (glutathione peroxidase, thioredoxin peroxidase, superoxide dismutase, catalase, ascorbate peroxidase, guaiacol peroxidase, glutathione transferase, glutathione reductase) and the amount of non-enzymatic antioxidants were determined spectrophotometrically. The reduction potential of the GSH/GSSG couple (half-cell reduction potential; E_{hc}) was determined with the Nernst equation using the formula of Schafer and Buettner (2001): E_{hc}= -240 - (59.1/2) log([GSH]^2/[GSSG]) mV; where –240 mV is the standard reduction potential of glutathione on 25°C, pH = 7.0. Statistical analysis was performed using the SigmaPlot12.0 software.

Summary

Among all AtGPXLs, AtGPXL5 is a poorly known plasma membrane-associated enzyme, although its role in salt stress tolerance was suggested. We have constitutively overexpressed the *AtGPXL5* cDNA and investigated the role of AtGPXL5 in response to NaCl treatment and in development. Experiments were performed by using *AtGPXL5*-overexpressing lines (OX-AtGPXL5) and *Atgpxl5* mutant plants. Based on our results, we have made the following observations:

1) 12-day-old *Arabidopsis thaliana Atgpxl5* insertional mutants had higher level of superoxide radical anion and total ROS in untreated roots and shoots, respectively compared with Col-0. The higher level of ROS decreased the cells' vitality in the shoot of *Atgpxl5* seedlings even under control condition. After applying 7-day treatment with the concentration of 100 mM NaCl, the $O_2^{\bullet-}$ level in the root was elevated further and reached higher level than in the wild type. These indicate that AtGPXL5 might play an important role in the ROS homeostasis and maintaining the cell's vitality.

2) The antioxidant mechanisms of the 6-week-old plants have altered, especially in the *Atgpxl5* mutants compared to OX-AtGPXL5 plants. Several ROS processing enzymes worked in elevated level in *Atgpxl5* mutant, but OX-AtGPXL5 plant exhibited similar activity to the Col-0 wild type. The GPOX activity was elevated in the lowest extent in *Atgpxl5* plants while GPOX and TPOX enzymes in the *AtGPXL5*-overexpressing plants worked about on the level of wild type.

3) Under control conditions, significantly lower GSH was found in the *Atgpxl5* mutant roots while its amount was elevated in the OX-AtGPXL5 shoot. The applied salt stress caused accumulation of the highest amount of reduced glutathione and the less oxidized form (GSSG) in the *AtGPXL5*-overexpressing plants among the investigated lines, while the GSSG increased most in the *Atgpxl5* roots. The amount of reduced glutathione was higher and the calculated redox potential was more negative in the overexpressed line than in Col-0. The result confirms that AtGPXL5 has function in regulating the redox state, through which they can also influence the growth and development.

4) AtGPXL5 enzymes are required for healthy growth and development of the *Arabidopsis thaliana* seedlings. Deficiency of AtGPXL5 led to reduce the length of primary roots, biomass, rosette size, convex area, chlorophyll and anthocyanin contents compared to other investigated lines under normal conditions. In the presence of 100 mM NaCl, *Atgpxl5* mutant and the Col-0 wild type seeds showed delayed germination, while germination of the OX-AtGPXL5 lines was not inhibited in the presence of 100 mM NaCl. Untreated OX-AtGPXL5 lines exhibited similar phenotype as Col-0, however the overexpressing plants grew better in the presence of 100 mM NaCl: they had larger rosettes, larger convex area and lower convex percentage values with higher content of chlorophyll and anthocyanin than that of the wild type and *Atgpxl5* plants. The reduced development of the shoots and decreased root length of the *Atgpxl5* mutant indicate that this protein has a function even in the normal development.

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Publications

Riyazuddin, R., Bela, K., Horváth, E., Rigó, G., Gallé, Á., Szabados, L., Fehér, A., Csiszár, J., 2019. Overexpression of the Arabidopsis glutathione peroxidase-like 5 gene (AtGPXL5) resulted in altered plant development and redox status. Environmental and Experimental Botany, 167, 103849. https://doi.org/10.1016/j.envexpbot.2019.103849. **IF: 3.712 (2019)**

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Riyazuddin, R., Bela, K., Horváth, E., Rigó, G., Gallé, Á., Szabados, L., Fehér, A., Csiszár, J., 2019. Overexpression of the Arabidopsis glutathione peroxidase-like 5 gene (AtGPXL5) resulted in altered plant development and redox status. Environmental and Experimental Botany, 167, 103849. https://doi.org/10.1016/j.envexpbot.2019.103849. **IF: 3.712**

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Statement

As the corresponding author of the following scientific publications, I declare that authors have no conflict of interest and Riyazuddin Riyazuddin Ph.D. candidate has a great contribution to the published results. Results discussed in his Ph.D. dissertation are regarded as outcomes of his own scientific work and they were not used to acquire any Ph.D. degree previously and will not be used in future either.

Riyazuddin, R., Bela, K., Horváth, E., Rigó, G., Gallé, Á., Szabados, L., Fehér, A., Csiszár, J., 2019. Overexpression of the *Arabidopsis* glutathione peroxidase-like 5 gene (*AtGPXL5*) resulted in altered plant development and redox status. Environmental and Experimental Botany, 167, 103849. https://doi.org/10.1016/j.envexpbot.2019.103849.

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