**Final report of postdoctoral research study supported by Bilateral State Scholarship (Agreement no. AK-00205-004/) for 2020/2021 academic year**

Glutathione peroxidases (GPXs) are important antioxidant enzymes in animals, however, similar enzymes identified in plants are termed as GPX-like (GPXL) because of the utilization of thioredoxin as a co-factor instead of glutathione. GPXs are non-heme thiol peroxidases that catalyze the reduction of $H_2O_2$ or organic hydroperoxides to water or corresponding alcohols using reduced glutathione (GSH) or thioredoxin (TRX). The mammalian GPXs are central components of processing ROS and lipid peroxides and thus participate in the maintenance of the membrane integrity. Plant's GPXL enzymes are thiol-based peroxidases that catalyze the reduction of $H_2O_2$ or hydroperoxides to water or alcohols using reduced glutathione (GSH) or thioredoxin as an electron donor. These enzymes are commonly considered as one of the key players in the enzymatic antioxidant defence system of plants as these can reduce peroxides with higher efficiency (sometimes exclusively) by the thioredoxin (TRX) system rather than using GSH as a reducing agent. The Arabidopsis genome encodes 8 GPXL isoforms, of which AtGPXL5 is associated with the inner side of the plasma membrane.

**Performed research work**

**According to the work plan**, we had three main aims to identify the correlations between the hormones and redox state and the role of GPXL5 in 2-week-old seedlings of *AtGPXL5*-overexpressing line and its knockdown *Atgpxl5* mutant under control conditions and after application of different phytohormones such as auxin (naphthyl acetic acid, NAA), strigolactones (SLs), abscisic acid (ABA) and brassinosteroids (BRs). I performed the experiments and successfully accomplished all the aims with the support of the extended Bilateral State scholarship. The results obtained can be summarized as follows:

**1.** We measured the growth data of 2-week-old seedlings such as root length, number of lateral roots and calculated the lateral root density in *Arabidopsis thaliana* wild type (Col-0) and transgenic lines with or without the phytohormones using Image J software. Overall, the results indicate that membrane localized AtGPXL5 enzyme regulates the growth of primary root and development of lateral roots under normal as well as after treatment of different phytohormones. Moreover, the seed size of *Atgpxl5* mutants was decreased as compared to Col-0 and overexpressing lines. The decreased root length and seed size of the *Atgpxl5* mutant indicates that this protein has a function even in normal development.

**2.** To detect the *in vivo* changes in redox status in roots, we expressed the cytoplasmic GRX1-roGFP2 redox sensor protein in Col-0, *Atgpxl5* mutant, and OX-AtGPXL5 by *Agrobacterium*-mediated floral dip transformation. After successful transformation, positive lines were selected on ½ MS media containing kanamycin as a selection marker. Following antibiotic selection, the transformants were identified using an UV stereomicroscope (Olympus SZX12, Hamburg, Germany) by visualizing the expression of the roGFP2 protein.

**3**. Further, we measured the $H_2O_2$ and superoxide radical levels under control conditions and after applying phytohormones in the transgenic as well as wild-type plants using fluorescent dyes (DHE and resorufin, respectively). The superoxide radical and $H_2O_2$ levels, analyzed by Zeiss Axiowert 200M fluorescent microscope, changed differently along with the roots in the Col-0, *Atgpxl5,* and OX-AtGPXL5 genotypes upon phytohormone treatment.

**4.** We also measured the non-enzymatic components of the antioxidant defense mechanism. According to our findings, there were significant changes in the reduced ascorbate (ASC) or oxidized dehydroascorbate (DHA) levels among the investigated 2-week-old seedlings under control conditions. The higher levels of ASC were detected in *Atgpxl5* and OX-AtGPXL5, while more DHA level was detected in OX-AtGPXl5 plants than in Col-0. However, ABA, NAA, BRs, and SLs were able to alter the ASC levels in the investigated transgenic lines compared to the control condition. In addition, GSH concentrations also varied among different samples. Under control conditions, GSH content was

significantly reduced in the *Atgpxl5* mutant roots, while its amount was significantly elevated in the OX-AtGPXL5 seedlings. Exogenous application of ABA, NAA, BRS, and SLs induced accumulation of GSH in the shoots of Col-0 and *Atgpxl5* lines, however, phytohormones significantly reduced its accumulation in the seedlings of OX- AtGPXL5 plants. Interestingly, among the treated roots, the lowest level of GSH was found in the OX-AtGPXL5 seedlings after the application of ABA as compared to other investigated lines. The calculation of the redox potential based on reduced GSH is currently underway. The reduction potential of the GSH/GSSG couple (half-cell reduction potential; $E_{hc}$) would be determined with the Nernst equation using the formula of Schafer and Buettner: Ehc= –240 – (59.1/2) log([GSH]$^2$/[GSSG]) mV.

**5.** RNA was isolated from two-week-old seedlings using a RNA extraction kit (NucleoSpin RNA Plant and Fungi, Macherey, Germany), following the instructions. After synthesis of cDNA, we determined the transcript amount of *AtGPXL5* and several other genes coding important proteins in the signaling of different hormones by quantitative real-time PCR (RT-qPCR) under normal conditions as well as after applying hormone treatments. We found the alteration of the several genes including *AtGPXL5* and others involved in phytohormone signalling under control conditions as well as after application different hormones.

As per my knowledge, we are the first to unravel the role of the AtGPXL5 isoenzyme in the development of roots under different phytohormones treatments in Arabidopsis plants. At present, compilation of all the data is being done to write a high-impact research manuscript which would be a milestone for my scientific career.

**Professional activities:**

With the support of the Bilateral State scholarship, I was able to continue my research work. However, due to the fund scarcity, I could not attend any offline international conference, but I presented my work at several online international conferences. I attended two virtual conferences organized via Baltic redox as co-author and had lecture on two Hungarian conferences. In addition, I also got the opportunity to participate in four manuscripts as a leading author, and a book chapter as a co-author. I have published following papers during this fellowship tenure:

**1.** Crosstalk between the membrane localised Arabidopsis glutathione peroxidase-like isoenzyme (AtGPXL5) and ethylene. **Riyazuddin Riyazuddin**, Krisztina Bela, Péter Poór, Edit Horváth, Ágnes Szepesi, Gábor Rigó, László Szabados, Attila Fehér, Jolán Csiszár (Submitted in Antioxidant Journal (IF: 6.312))

**2.** Mutations in glutathione peroxidase-like enzymes affect root abiotic stress responses in *Arabidopsis thaliana*. Krisztina Bela, **Riyazuddin Riyazuddina,** Edit Horváth, Ádám Barnabás Hajnal, Ágnes Hurton, Ágnes Gallé, Sajid Ali Khan Bangash, Jolán Csiszár (Submitted in Acta Physiologiae Plantarum (IF: 2.354)).

**3.** Functions of Polyamines in Abiotic Stress Tolerance in Plants. Péter Pálfi, **Riyazuddin Riyazuddin**, László Bakacsy and Ágnes Szepesi* (**Taylor & Francis (CRC Press) Advancements in Developing Abiotic Stress-Resilient Plants: Basic Mechanisms to Trait Improvements-Under publication**).

**4. Riyazuddin, R.** and Gupta, R., 2021. Plausible Involvement of Ethylene in Plant Ferroptosis: Prospects and Leads. Frontiers in plant science, 12. (Accepted, IF: 5.753)

**5. Riyazuddin, R.,** Nisha, N., Singh, K., Verma, R. and Gupta, R., 2021. Involvement of dehydrin proteins in mitigating the negative effects of drought stress in plants. Plant Cell Reports, pp.1-15. (Accepted, IF: 4.570)

Kind regards
29-09-2021, Szeged