

DOI: 10.5586/asbp.3567

**Publication history**

Received: 2017-07-03

Accepted: 2017-11-30

Published: 2017-12-29

**Handling editor**

Mirela Tulik, Faculty of Forestry,  
Warsaw University of Life  
Sciences – SGGW, Poland

**Authors' contributions**

ER and KJD wrote the  
manuscript

**Funding**

This research was supported  
by the Polish Ministry of  
Science and Higher Education  
and the National Centre of  
Sciences (grant number N 309  
070536). Additional funding  
was provided by the Institute of  
Dendrology, Polish Academy of  
Sciences.

**Competing interests**

No competing interests have  
been declared.

**Copyright notice**

© The Author(s) 2017. This is an  
Open Access article distributed  
under the terms of the [Creative  
Commons Attribution License](#),  
which permits redistribution,  
commercial and non-  
commercial, provided that the  
article is properly cited.

**Citation**

Ratajczak E, Dietz KJ. Regulatory  
redox state in tree seeds. *Acta  
Soc Bot Pol.* 2017;86(4):3567.  
[https://doi.org/10.5586/  
asbp.3567](https://doi.org/10.5586/asbp.3567)

**Digital signature**

This PDF has been certified using digital  
signature with a trusted timestamp to  
assure its origin and integrity. A verification  
trust dialog appears on the PDF document  
when it is opened in a compatible PDF  
reader. Certificate properties provide  
further details such as certification time  
and a signing reason in case any alterations  
made to the final content. If the certificate  
is missing or invalid it is recommended to  
verify the article on the journal website.

**MINIREVIEW**

# Regulatory redox state in tree seeds

Ewelina Ratajczak<sup>1\*</sup>, Karl Josef Dietz<sup>2</sup><sup>1</sup> Institute of Dendrology, Polish Academy of Sciences, Parkowa 5, 62-035 Kórnik, Poland<sup>2</sup> Department of Biochemistry and Physiology of Plants, Bielefeld University, University Street 25, Bielefeld 33501, Germany\* Corresponding author. Email: [eratajcz@man.poznan.pl](mailto:eratajcz@man.poznan.pl)**Abstract**

Peroxiredoxins (Prx) are important regulators of the redox status of tree seeds during maturation and long-term storage. Thioredoxins (Trx) are redox transmitters and thereby regulate Prx activity. Current research is focused on the association of Trx with Prx in tree seeds differing in the tolerance to desiccation. The results will allow for better understanding the regulation of the redox status in orthodox, recalcitrant, and intermediate seeds. The findings will also elucidate the role of the redox status during the loss of viability of sensitive seeds during drying and long-term storage.

**Keywords**

orthodox, recalcitrant, intermediate seeds; peroxiredoxins; thioredoxins; redox state

**Introduction**

The regulatory redox signaling network in plant cells senses metabolic imbalances and transduces this information into rapid modulation of transcription, translation, and metabolism. Using this mechanism, plants can quickly adapt to changing environmental conditions, maintain high fitness, and produce high-quality seeds. In plants, redox regulation is frequently based on Cys redox modifications that control the activity of enzymes and numerous additional biological processes [1,2]. Thiol homeostasis and thiol-based mechanisms are essential for plant performance and tolerance to abiotic stress as well as for plant development in relation to meristem function, reproductive development, life cycle, and metabolism [3,4].

Seeds of woody plants present a great variation in dehydration tolerance. Orthodox seeds tolerate desiccation to a very low water level (0.3–7.0%) and storage at low temperature for many years, e.g., seeds of *Betula* spp., *Acer platanoides* L., and *Ulmus americana* L. In contrast, recalcitrant seeds are sensitive to desiccation, so they are extremely difficult to store without a rapid loss of viability. These seeds are not suitable for long-lasting storage. They are characterized by a high initial moisture content, where lowering below a relatively high threshold level contributes to a gradual loss of vitality and finally to seed death [5]. This group includes *Acer pseudoplatanus* L., *Acer saccharinum* L., and *Quercus rubra* L. Suborthodox seeds are intermediate between orthodox and recalcitrant categories. *Fagus sylvatica* L., *Populus* spp., and *Juglans* spp. are important examples of trees with this intermediate behavior.

**Roles of reactive oxygen species**

Reactive oxygen species (ROS), primarily the superoxide anion radical (O<sub>2</sub><sup>•-</sup>) and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), are generated during seed development and germination and play important roles in signaling and regulation of metabolic processes [6]. However,

an excess of ROS, which is observed during long-term storage of seeds, disturbs the redox homeostasis of the cell and initiates oxidative stress, thus leading to a reduction of seed viability. These alterations are accompanied by membrane lipid peroxidation and changes in enzymatic antioxidant systems and the state of nucleic acids [7,8]. These observations and hallmarks of changes indicate that the redox and ROS network decisively control seed viability.

### Peroxiredoxin and thioredoxin

Published data indicate that the thiol peroxidases Prxs are important for regulation of the redox state and modulation of cell signaling pathways. Prxs participate in the regulation of the redox state of seed cells during seed development and adaptation to environmental conditions [9,10]. The Prxs family can be divided in six groups, named A, B, C, D, E, and F, based on sequence and structure similarities and positions of conserved cysteinyl residues. According to the more commonly used nomenclature, the A-type Prx corresponds to the (typical) 2-Cys peroxiredoxin (2-CysPrx), the B-type Prx to the (typical) 1-Cys peroxiredoxin (1-Cys-Prx), the C-type Prx to peroxiredoxin Q (PrxQ), and the D-type Prx to type II peroxiredoxins (PrxII) [10]. Prxs undergo conformational changes based on their redox state [11].

Ratajczak et al. [12] reported results indicating an important role of the mitochondrial PrxIIF in the control of redox balance in seed cells and in defining physiological differences between orthodox and recalcitrant seeds. PrxIIF plays a crucial role in maintaining the redox balance in mitochondria, which may be important for seed viability during drying. The study involved two species of the genus *Acer*: Norway maple (*A. platanoides* – orthodox) and sycamore (*A. pseudoplatanus* – recalcitrant) seeds during drying. Mitochondrial PrxIIF was detected in seeds of both species, but its level was higher in the orthodox seeds of Norway maple than in the recalcitrant seeds of sycamore. Moreover, in Norway maple seeds, PrxIIF decreased significantly after seed desiccation. Semi-quantitative analysis (RT-PCR) showed that the PrxIIF transcript level declined during seed drying in both species but more strongly in sycamore seeds. With the use of 2D-Western blotting, the values of isoelectric point (pI) of PrxIIF were determined: a single value for Norway maple (5.7), while two values for sycamore (5.7 and 6.0). PrxIIF in sycamore seeds may be phosphorylated completely, whereas in sycamore seeds, it is only partly subject to post-translation modification. The identified PrxIIF gene and amino acid sequences were compared with those of other plants and a high sequence identity between PrxIIF sequences of *Populus trichocarpa* L. and *Arabidopsis thaliana* (L.) Heynh. was revealed.

The protein complement of developing seeds of Norway maple and sycamore differs in the amounts of several Prxs, namely 2-Cys-Prx, 1-Cys-Prx, PrxIIE, PrxIIC, PrxIIF, and PrxQ (unpublished – article in review). The application of diagonal 2D-redox SDS-PAGE makes it possible to assess protein redox states in seeds and embryos [13]. The role of Prxs in redox control and regulation in seeds during development was evidenced by their presence (confirmed by identification) outside the diagonal 2D-redox SDS-PAGE; in Norway maple seeds (tolerant to desiccation), both 1-Cys-Prx and 2-Cys-Prx were particularly active, while in sycamore seeds (sensitive to desiccation), only 2-Cys-Prx was active (unpublished – article in review).

Oxidized forms of Prxs lose their enzymatic activity and must be regenerated by the action of thioredoxin (Trx), glutaredoxin, glutathione, and/or ascorbate [10]. The most important among them are Trx proteins. They are small, 12-kDa proteins containing a redox-active disulfide group [14]. The active site of Trx proper has two redox-active cysteine residues in a highly conserved sequence [-Trp-Cys-Gly(Pro)-Pro-Cys-]. Thioredoxin proteins play an important role in seed physiology and are substrates for enzymes, which catalyze the reduction of ribonucleotides, methionine sulfoxide, and hydrogen peroxide [15]. The proteins also play a key role in the control of the redox state of proteins containing disulfide bonds and thus in the regulation of seed development and germination [3]. Trxs are classified based on structures and subcellular localization into Trx *f*, *m*, *o*, *x*, *y*, *z*, and *h* [16]. *Trxh* is important for regulation of enzymatic function, the deactivation of enzymes involved in protein and carbohydrate degradation during seed development, and the activation of these enzymes during germination [2]. These

proteins are electron donors for antioxidant enzymes, especially for peroxiredoxins. Thus, it can be assumed that *Trxh* and peroxiredoxins may be involved in redox signal modulation during various metabolic processes.

### Future perspectives

Some authors have expressed their concerns that trees will be unable to adapt to climate change because the rate of change is too rapid relative to the life-span of tree species and the germination and viability of their seeds [17–19]. The problem of seed aging is very important from both economic and scientific standpoints. Seed aging is linked with a decrease in metabolic rate and ends in seed death. The process of aging has been studied using various methods, considering that the maintenance of seed viability depends on genetic characteristics.

Analyses of redox conditions in seeds of different species have been carried out for many years. However, data on redox regulation in tree seeds differing in sensitivity to water loss are scanty. An accurate analysis of the redox state in seeds of trees classified as orthodox, recalcitrant, and suborthodox will help us to determine why seeds lose viability during long-term storage. Therefore, future research should explain the mechanism regulating the activity of Prx and redox homeostasis in these seeds. Pulido et al. [20] showed that the 1-Cys-Prx locates in cell nuclei of the aleurone layer and scutellum of wheat seeds, and that it plays an important role in protecting against oxidative stress and in maintaining redox homeostasis. Based on their results, those authors suggest that regeneration of the active form of 1-Cys-Prx depends on NADPH-thioredoxin reductase (NTR), colocalized with *Trxh* and 1-Cys-Prx. Apparently, NTR/Trx systems play an important role in cellular signaling and regeneration of active 1-Cys-Prx. Tovar-Méndez et al. [21] identified *Prx*, *Trx*, and *NTR* gene families of the model legume *Lotus japonicus*, and determined their expression profiles in plant tissues. Their results revealed that the expression profiles of the genes of Prxs are different in individual tissues in response to different signaling components. In seeds of *L. japonicus*, the highest level of expression was demonstrated for 1-Cys-Prx. The results confirm that Prxs play an important role in preventing the loss of redox homeostasis in plant tissues. An important finding of their study was that the regulation of Prx activity depends on tissues and signaling molecules, and is supported by the redox NTR/Trx system functioning in the cytosol, mitochondria, and plastids of nodules.

Regulation of the redox state by the NTR/Trx system takes place through their participation in the process of S-nitrosylation and denitrosylation and regulation of Prx activity. Thioredoxin and Prx can be S-nitrosylated to protect them from overoxidation and to allow cell signaling. S-Nitrosylation may regulate the Trx and Prx system, and may influence the change in activity of the Prx system [22]. We suggest that the NTR/Trx system is important for the development and germination of seeds, but the effect of these systems on seeds is more complex than was previously thought. To date, there have been no published data on the regulation of Prx activity and the role of the NTR/Trx systems in seeds of trees that differ in sensitivity to water loss. It is suggested that the Trx/NTR/Prx system regulates the redox state during seed maturation and that the level of this regulation affects the maintenance of seed viability during long-term storage. There is a need to assess the occurrence and profile of gene expression of Prx, Trx, and NTR in tree seeds classified as orthodox, recalcitrant, and suborthodox and to link their particular physiology to the regulation of the cell redox state and metabolism.

### References

1. Poole LB. The basics of thiols and cysteines in redox biology and chemistry. *Free Radic Biol Med.* 2015;8:148–157. <https://doi.org/10.1016/j.freeradbiomed.2014.11.013>
2. Häggglund P, Finnie C, Yano H, Shahpiri A, Buchanan BB, Henriksen A,

- et al. Seed thioredoxin *h*. *Biochim Biophys Acta*. 2016;1864(8):974–982.  
<https://doi.org/10.1016/j.bbapap.2016.02.014>
3. Rouhier N, Cerveau D, Couturier J, Reichheld JP, Rey P. Involvement of thiol-based mechanisms in plant development. *Biochim Biophys Acta*. 2015;1850(8):1479–1496.  
<https://doi.org/10.1016/j.bbagen.2015.01.023>
  4. Dietz KJ, Hell R. Thiol switches in redox regulation of chloroplasts: balancing redox state, metabolism and oxidative stress. *Biol Chem*. 2015;396(5):483–494.  
<https://doi.org/10.1515/hsz-2014-0281>
  5. Pukacka S, Hoffmann SK, Goslar J, Pukacki PM, Wójkiewicz E. Water and lipid relations in beech (*Fagus sylvatica* L.) seeds and its effect on storage behaviour. *Biochim Biophys Acta*. 2003;1621(1):48–56. [https://doi.org/10.1016/S0304-4165\(03\)00046-1](https://doi.org/10.1016/S0304-4165(03)00046-1)
  6. Miller G, Suzuki N, Ciftci-Yilmaz S, Mittler R. Reactive oxygen species homeostasis and signaling during drought and salinity stresses. *Plant Cell Environ*. 2010;33(4):453–446.  
<https://doi.org/10.1111/j.1365-3040.2009.02041.x>
  7. Ratajczak E, Małeczka A, Bagniewska-Zadworna A, Kalembe EM. The production, localization and spreading of reactive oxygen species contributes to the low vitality of long-term stored common beech (*Fagus sylvatica* L.) seeds. *J Plant Physiol*. 2015;174(1):147–156. <https://doi.org/10.1016/j.jplph.2014.08.021>
  8. Wojtyła Ł, Kubala S, Garnczarska M. Different modes of hydrogen peroxide action during seed germination. *Front Plant Sci*. 2016;7:66.  
<https://doi.org/10.3389/fpls.2016.00066>
  9. Dietz KJ. Peroxiredoxins in plants and cyanobacteria. *Antioxid Redox Signal*. 2011;15(4):1129–1159. <https://doi.org/10.1089/ars.2010.3657>
  10. Dietz KJ. Thiol-based peroxidases and ascorbate peroxidases: why plants rely on multiple peroxidase systems in the photosynthesizing chloroplast? *Mol Cells*. 2016;39:20–25.  
<https://doi.org/10.14348/molcells.2016.2324>
  11. Tripathi BN, Bhatt I, Dietz KJ. Peroxiredoxins: a less studied component of hydrogen peroxide detoxification in photosynthetic organisms. *Protoplasma*. 2009;235(1–4):3–15.  
<https://doi.org/10.1007/s00709-009-0032-0>
  12. Ratajczak E, Ströher E, Oelze ML, Kalembe EM, Pukacka S, Dietz KJ. The involvement of the mitochondrial peroxiredoxin PrxIIIF in defining physiological differences between orthodox and recalcitrant seeds of two *Acer* species. *Funct Plant Biol*. 2013;40(10):1005–1017. <https://doi.org/10.1071/FP13002>
  13. Ströher E, Dietz KJ. Concepts and approaches towards understanding the cellular redox proteome. *Plant Biol*. 2006;8(4):407–418. <https://doi.org/10.1007/s00709-009-0032-0>
  14. Wong JH, Cai N, Balmer AY, Charlene K, Tanaka CK, Vensel WH, et al. Thioredoxin targets of developing wheat seeds identified by complementary proteomic approaches. *Phytochemistry*. 2004;65(11):1629–1640.  
<https://doi.org/10.1016/j.phytochem.2004.05.010>
  15. Buchanan BB, Balmer Y. Redox regulation: a broadening horizon. *Annu Rev Plant Biol*. 2005;56:187–220. <https://doi.org/10.1146/annurev.arplant.56.032604.144246>
  16. Shahpiri A, Svensson B, Finnie CH. The NADPH-dependent thioredoxin reductase/thioredoxin system in germinating barley seeds: gene expression, protein profiles, and interactions between isoforms of thioredoxin *h* and thioredoxin reductase. *Plant Physiol*. 2008;146(2):789–799. <https://doi.org/10.1104/pp.107.113639>
  17. Aitken SN, Yeaman S, Holliday JA, Wang T, Curtis-McLane S. Adaptation, migration or extirpation: climate change outcomes for tree populations. *Evol Appl*. 2008;1(1):95–111.  
<https://doi.org/10.1111/j.1752-4571.2007.00013.x>
  18. Aitken SN, Bemmels JB. Time to get moving: assisted gene flow of forest trees. *Evol Appl*. 2015;9(1):271–290. <https://doi.org/10.1111/eva.12293>
  19. Hatfield JL, Prueger JH. Temperature extremes: effect on plant growth and development. *Weather Clim Extrem*. 2015;10:4–10. <https://doi.org/10.1016/j.wace.2015.08.001>
  20. Pulido P, Cazalis R, Cejudo FJ. An antioxidant redox system in the nucleus of wheat seed cells suffering oxidative stress. *Plant J*. 2009;57:132–145.  
<https://doi.org/10.1111/j.1365-313X.2008.03675.x>
  21. Tovar-Méndez A, Matamoros MA, Bustos-Sanmamed P, Dietz KJ, Cejudo FJ, Rouhier N, et al. Peroxiredoxins and NADPH-dependent thioredoxin systems in the model legume *Lotus japonicas*. *Plant Physiol*. 2011;156(3):1535–1547.  
<https://doi.org/10.1104/pp.111.177196>

22. Engelman R, Weisman-Shomer P, Ziv T, Xu J, Arner ESJ, Benhar M. Multilevel regulation of 2-Cys peroxiredoxin reaction cycle by S-nitrosylation. *J Biol Chem.* 2013;288(16):11312–11324. <https://doi.org/10.1074/jbc.M112.433755>