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## Redox Regulation of Plant Development

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## ABSTRACT

All over their life cycle, plants are subjected to environmental constraints which affect many aspects of their developmental processes. Reactive Oxygen species are important signaling molecules integrating environmental stimuli and mediating redox regulation of plant development and stress response. Plant growth and formation of new organs is dependent on cell proliferation and differentiation occurring in meristematic tissues. In this chapter, we first overview how redox regulation maintain proper functioning of meristematic tissues. Then, we show that redox regulation controls major developmental processes like seed development, shoot and root architecture, flower development and gamete formation. Redox regulation targets key actors of hormone signaling and transcriptional networks, through sophisticated post-transcriptional modifications of proteins. Thus, we highlight how changes in ROS and redox homeostasis influence plant growth and development in response to environmental constraints.

## Introduction

The development of plant organs is dependent on the maintenance and the sequential activation of cell stem embedded in meristematic tissues. Cell division and differentiation cycles determine the shape and the functions of the different plant organs. While endogenous factors like plant hormones are major determinant of plant development programs, they are also largely influenced by their environment. Understanding the mechanisms by which plant perceives their environment to adapt its development is a major question for plant biologist. One of these mechanisms relies on regulation by oxidoreduction (redox). Redox regulation is mediated by Reactive Oxygen Species (ROS), which are by-products of oxygen metabolism. ROS include free radical species like superoxides ( $O_2^{\bullet-}$ ), hydroxyl radicals ( $OH^{\bullet}$ ) or nitric oxide ( $NO^{\bullet}$ ), and nonradical species like hydrogen peroxide ( $H_2O_2$ ) and peroxynitrite ( $ONOO^-$ ) (Sies et al., 2017). In plants, major sources of ROS are photosynthetic and respiratory chains in chloroplasts and mitochondria (Figure 1). ROS are also generated by plasma membrane NADPH oxidases and peroxisomal xanthine oxidases and exert fine tune regulation of developmental process depending on the location of their generation. ROS generation in challenged tissues can trigger oxidative distress, damaging cell or inducing cell death (Foyer and Noctor, 2016; Choudhury et al., 2017). Oxidative eustress is also playing important signaling functions by inducing post-translational modifications (PTM) and by regulating protein redox state. Plant cells display a large panel of ROS scavenging enzymes like catalases, peroxidases and superoxide dismutases. They also generate compounds that reverse ROS-induced oxidations. Among these compounds are antioxidant molecules like glutathione and ascorbate, which both play important roles as cofactors for thiol reduction enzymes like peroxidases and reductases (Noctor, 2017; Rahantaniaina et al., 2017). Glutathione and ascorbate are themselves reduced by glutathione reductases (GRs) and dehydroascorbate reductases (DHARs). Thioredoxins (TRXs) and glutaredoxins (GRXs) are key thiol reduction enzymes. They act as reducing power of metabolic enzymes and ROS scavenging systems but they also regulate thiol-based post-

transcriptional redox modifications in proteins (Meyer et al., 2012). Oxidized TRXs are generally reduced by NADPH-dependent thioredoxin reductases (NTRs), whereas the reduction of GRXs is dependent on glutathione. Due to their multifunctional thiol reduction capacities, TRXs and GRXs have been involved in many metabolic functions, controlling plant developmental programs and acting as key signaling molecules in response to abiotic and biotic stresses (Meyer et al., 2009; Rouhier et al., 2015).

In this chapter, we summarize the current knowledge on how redox regulation influences plant development programs and how it mediate plant perception of environmental constraints (Table 1).

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### **1. Cell division and differentiation / Meristems**

Plant growth and development is intimately dependent on founder cell proliferation in meristematic zones. The Shoot Apical Meristem (SAM) is established during the embryogenesis and gives rise to all above-ground tissues of a plant. The SAM is composed of different cell layers with contrasted cell division capacities. The central zone (CZ) contains three layers (L1, L2 and L3) of slowly dividing stem cells, while the organizing center (OC) found underneath maintains none dividing stem cells. Surrounding the CZ, the peripheral zone (PZ) contain rapidly dividing cells and gives rise to new boundary organs, like leave primordia (Figure 2A). By perceiving the environmental constraints, ROS and the redox status are involved in both triggering cell proliferation and regulating cell cycle progression. Oxidative stress conditions are known to block cell cycle progression at specific checkpoints (Reichheld et al., 1999; Vernoux et al., 2000) and different forms of ROS have antagonistic roles in plant stem cell regulation.  $O_2^{\bullet-}$  enriched in stem cells activate *WUSCHEL* to inhibit division of stem cells, whereas  $H_2O_2$  is more abundant in the peripheral zone and promote stem cell differentiation (Zeng et al., 2017). A balance between  $O_2^{\bullet-}$  and  $H_2O_2$  is also observed in the root apical meristem (RAM).  $O_2^{\bullet-}$  are enriched in the cell

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proliferation zone, whereas H<sub>2</sub>O<sub>2</sub> accumulate in the cell differentiation zone (Dunand et al., 2007; Tsukagoshi et al., 2010) (Figure 2B). In the root meristem transition zone, the transcription factor UPBEAT1 (UPB1) directly regulates the expression of a set of peroxidases that modulate the O<sub>2</sub><sup>•</sup>/H<sub>2</sub>O<sub>2</sub> balance (Tsukagoshi et al., 2010). Further emphasizing the redox regulation of meristematic

80 cell proliferation, the DNA binding activity TEOSINTE BRANCHED/CYCLOIDEA/PCF (TCP) transcription is affected by a Cys oxidation in its binding and dimerization domain. TCP transcription factors are regulating meristematic cell proliferation by inducing expression of cell cycle genes like CYCA2, CYCB1 and RETINOBLASTOMA-RELATED genes (Li et al., 2005; Li et al., 2012; Viola et al., 2013).

85 Indeed, very little is known about the redox-sensitive proteins that influence cell proliferation and cell cycle progression. Based on Cys residues accessibility predictions, different bioinformatics and redox proteomics approaches have identified potential redox-sensitive cell cycle regulators (Foyer et al., 2018; Martins et al., 2018). Some candidates are the DNA synthesis enzyme RIBONUCLEOTIDE REDUCTASE subunits which need disulfide reduction for its catalytic activity in

90 different organisms *E. coli*, yeast and mammals (Gon et al., 2006; Zahedi Avval and Holmgren, 2009), or the CYCLIN-DEPENDENT KINASE A (CDKA1) which activity on cell cycle progression is inhibited by Tyr-nitration by RNS in maize (Méndez et al., 2020).

Studies on proteins regulating thiol redox status have also highlighted their role in maintaining meristem activity. Among key actors are thiols reduction systems for which glutathione and

95 thioredoxin play major functions. For example, mutants deficient in glutathione biosynthesis or reduction mutant show major meristematic defects. The ROOTMERISTEMLESS1 (*rml1*) mutant accumulating low level of glutathione show an aborted RAM unable to maintain stem cell fate, associated with down-regulation of cell cycle gene expression and an arrest of cell cycle progression at the G1/S transition (Howden et al., 1995; Vernoux et al., 2000; Schnaubelt et al.,

100 2015) (Table 1). Other evidence point to the key role of glutathione nuclear/cytosolic distribution

and redox cycle to regulate cell cycle progression (Pellny et al., 2009; Diaz Vivancos et al., 2010; Diaz-Vivancos et al., 2015; García-Giménez et al., 2013a; Schnaubelt et al., 2015).

While mutants inactivated in glutathione biosynthesis affect root meristem development, TRX are able to replace glutathione for SAM development. In Arabidopsis, a *ntra ntrb* mutants inactivated  
105 in both thioredoxin reductases or mutants *cad2* having lower capacities of glutathione have a normal vegetative development. However, the *ntra ntrb cad2* mutant combining both thioredoxin reductase and glutathione biosynthesis mutation abolish flower meristem development, producing PIN-like meristem structure similar to auxin transport mutants (Bashandy et al., 2010). Similarly, association of the *ntra ntrb* mutant with the strong glutathione biosynthesis mutant  
110 ROOTMERISTEMLESS1 (*rml1*) completely abolish SAM development (Reichheld et al., 2007).

Accumulation of oxidized glutathione in distinct GLUTATHIONE REDUCTASE (GR) mutants affect different aspects of plant development. Knock-out mutation of the chloroplastic GR2 isoform lead to embryoletality in Arabidopsis while the mitochondrial and the cytosolic glutathione reduction can be backed up by thioredoxin reductases and only triple mutants *ntra ntrb gr* exhibit  
115 developmental defects (Marty et al., 2009; Marty et al., 2019). Interestingly, the knocked down EMS-mutant *miao* with the GR activity being reduced to ~50% displays strong inhibition of root growth and severe defects in the RAM by affecting the auxin/PLETHORA (PLT) signaling pathways (Yu et al., 2013). Pointing to the role of ascorbic acid (AsA) in meristem development, the AsA biosynthesis mutant *vtc1* with higher H<sub>2</sub>O<sub>2</sub> level, exhibits abnormal QC and periclinal cell division  
120 patterns in the RAM (Kka et al., 2018; Figure 2B).

The contribution of thiols reductases thioredoxin and glutaredoxins in meristematic activities has been documented. In Arabidopsis, the involvement of the chloroplastic TRXm3 in meristem maintenance was attributed to its role in the redox regulation of callose deposition and symplastic permeability. Here, the *trxm3* mutant could not maintain a functional SAM because of callose  
125 accumulation in the meristem (Benitez-Alfonso et al., 2009). In Arabidopsis, an atypical

mitochondrial thioredoxin DCC1 was shown to affect shoot regeneration by interacting with CARBONIC ANHYDRASE2 (CA2), an essential subunit of the respiratory chain NADH dehydrogenase complex I. Inactivation of DCC1 results in mitochondrial ROS accumulation supposed to affect shoot regeneration. Although, the disulfide reduction activity of DCC1 is not established yet, the exact nature of the redox regulation is still to be established (Zhang et al., 2018) (Table 1).

Glutaredoxins which use glutathione as a co-factor have also been involved in SAM functions. For example, Arabidopsis plants deficient in the iron-sulfur cluster containing GRXS17 are sensitive to high temperatures and long-day photoperiods, resulting in compromised shoot apical meristem and auxin transport (Cheng et al., 2011; Knuesting et al., 2015). GRXS17 also interacts with the NF-YC11/NC2a transcription factor which participates in plant developmental processes in relation to the photoperiod duration and *nf-yc11/nc2a* and *grxS17* plants share similar developmental characteristics in long-day conditions (Knuesting et al., 2015). In maize, the glutaredoxin ABBERANT PHYLLTAXY (ABPHYL2) regulates shoot meristem and phyllotaxy and interacts with the b-zip TGA transcription factor FASCIATED EAR4 (Pautler et al., 2015; Yang et al., 2015).

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## 2. Redox regulation of reproductive development

Works on reproductive development have define redox status as a determinant of germ cell fate (Zafra et al., 2016; Luria et al., 2019; García-Quirós et al., 2020). In maize, hypoxia naturally occurring in growing anther tissues is necessary to maintain germ cell fate. Decreased oxygen (or H<sub>2</sub>O<sub>2</sub>) activates germ cell formation while, oxidizing environments inhibit germ cell specification and cause ectopic differentiation in anther tissues. The plant specific glutaredoxin MSCA1 protects redox environment in anther cells (Kelliher and Walbot, 2012).

Indeed, different members of the same GRX clade are acting in distinct aspects of flower development. In Arabidopsis, ROXY1/GRXC7 regulate petal formation and growth (Xing et al., 2005) and is taking part in the ABCE flower development model which specify the development of

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the different flower whorls through the activity of specific MADS-box transcription factors (Coen and Meyerowitz 1991). In the one hand, the *ROXY1* gene is a direct target of the floral development master regulators MADS-box *APETALA3*, *PISTILLATA* and *AGAMOUS* (Wuest et al., 2012; ÓMaoiléidigh et al., 2013). In the other hand, *ROXY1* redox-regulates the activity of the TGA transcription factor *PERIANTHIA*, possibly through reduction of a specific Cys localized in a transactivation domain (Li et al., 2009; Li et al., 2011).

In *Arabidopsis*, *ROXY1/GRXCx* and *ROXY2/GRXCx* are redundantly involved in anther development by reducing specific Cys residues in *TGA9* and *TGA10* transcription factors (Xing and Zachgo, 2008; Murmu et al., 2010). In the *roxy1 roxy2* mutant, both anther lobe and pollen mother cell differentiations are defective. Lack of *ROXY1* and *ROXY2* function affects a large number of anther genes at the transcriptional level downstream of the early-acting anther gene *SPOROCTELESS/NOZZLE* and upstream of *DYSFUNCTIONAL TAPETUM1*, controlling tapetum development (Xing and Zachgo, 2008).

Thiol reductase systems also act on other aspects of pollen development. In *Arabidopsis*, the *ntra ntrb* mutant has a decreased pollen fitness rather than a pollen development deficiency (Reichheld et al., 2007). Further works on the NTR-dependent nuclear thioredoxin *NRX1* also revealed decreased pollen fertility in the *nrx1* mutant, which were caused by defective pollen tube growth in the pistil (Qin et al., 2009; Marchal et al., 2014). Finally, the *ntra ntrb gr1* triple mutant is fully pollen sterile, suggesting that both TRX and GSH reduction systems acts redundantly on pollen fertility (Marty et al., 2009).

### **3. Redox control of seed establishment, dormancy and germination**

In flowering plants, double fertilization occurring into the ovule gives rise to a diploid embryo developing from the zygote, surrounded by a triploid storage tissue, the endosperm. Seed development can be divided into three phases, starting with an active phase of cell divisions that

builds up the body plan of the embryo and structures the endosperm. During mid-phase, intense and numerous biochemical activities allow the accumulation of food reserves, dehydration permissive compounds and toxic protective molecules. Finally, seeds of most Angiosperm species desiccate and acquire dormancy, characterized by a very low residual metabolic activity. After seed  
180 dispersion and dormancy release, imbibition strongly reactivates metabolism and the breakdown of food reserves. This allows germination to take place, and the subsequent embryo development to a novel autotrophic seedling (Figure 3).

Glutathione plays essential functions during seed maturation, since null alleles of *GR2* (encoding the plastid localized glutathione reductase), *GSH1* (encoding the first limiting step of glutathione  
185 biosynthesis), and of both *GRXC1* and *GRXC2* (encoding redundant glutaredoxins) are embryo-lethal in *Arabidopsis* (Tzafrir et al., 2004; Cairns et al., 2006; Riondet et al., 2012; Marty et al., 2019). *gsh1* homozygous embryos can initiate their developmental program and grow normally but start bleaching after torpedo stage. This suggests a redox control of the metabolic machinery responsible for the accumulation of reserves and protective compounds. Such regulation also  
190 occurs in the rice endosperm, where the first committed enzyme in starch synthesis, ADP-glucose pyrophosphorylase, is activated by reduction, probably via a TRXh member (Tuncel et al., 2014). Accordingly, seed tissues display increasing reducing conditions while accumulating reserves (De Gara et al., 2003; Ferreira et al., 2012).

During the ultimate phase of seed development, dehydration and acquisition of dormancy are  
195 accompanied by the dramatic drop of metabolic activities, including reducing pathways. Dry dormant seeds are characterized by an overall oxidized status, while residual ROS production are thought to be very limited in both embryo and endosperm (De Gara et al., 2003; Bailly et al., 2008).

Upon dormancy release and imbibition, a brutal and dual redox switch occurs. On the one hand,  
200 ROS and RNS concentrations increase rapidly, via the recovery of metabolism and the activation of

dedicated producing systems (Bailly et al., 2008). This rise in ROS and RNS is critical for dormancy breakdown and for germination to take place (Lozano-Juste and León, 2010; Mhamdi and Van Breusegem, 2018). ROS allow mRNA oxidation, which in turn regulates translation, and selective protein oxidation, thus adapting the proteome for successful germination (El-Maarouf-Bouteau et al., 2013). In contrast, an excess of ROS could be toxic and alter the embryo viability. ROS and RNS homeostasis is therefore tightly regulated inside the borders of an “oxidative window” convenient for the germination process (Bailly et al., 2008; Bailly, 2019). On the other hand, a massive reduction of thiol-containing proteins also occurs during seed rehydration, mainly through the action of the TRX system, both in cytosol and mitochondria (Alkhaloui et al., 2007; Nietzel et al., 2019). Hence, the subtle redox control of seed proteome, by the concomitant oxidation and reduction of different subsets of proteins, is a central component of seed to seedling transition (Figure 3).

Abscisic Acid (ABA) is a key phytohormone that promotes seed maturation and dormancy, and inhibits germination. ROS / RNS and ABA negatively control each other to fine tune seed germination. Indeed, RNS deficient seeds are hypersensitive to ABA (Lozano-Juste and León, 2010), while the master ABA-activated transcription factor ABI5 directly binds to the promoter of *CATALASE1* to regulate H<sub>2</sub>O<sub>2</sub> homeostasis (Bi et al., 2017). In contrast, H<sub>2</sub>O<sub>2</sub> has also been reported as an important second messenger in ABA signaling, involving its production by NADPH oxidases (Pei et al., 2000). The negative regulators of ABA signaling, ABI1 and ABI2 phosphatases, are inactivated by the oxidation of some of their cysteines. They could be direct targets of H<sub>2</sub>O<sub>2</sub>-release, thus allowing ABA responses to take place (Meinhard and Grill, 2001; Meinhard et al., 2002).

#### **4. Redox control of root development**

The development and adaptation of an optimal root system is critical for plant anchoring and nutrition. Canonical root system architecture depends on root growth and root branching. Cell

divisions in the root apical meristem and cellular elongation both support root growth, and precede cellular differentiation that allows the root to become mature and fully functional. Root branching first relies on the specification and initiation of lateral root primordia (LRP) mainly in the pericycle layer that surrounds the central stele. The development of the primordium and its protrusion through overlying tissues leads to the emergence of the new lateral root from the primary root. In addition to this canonical root system, adventitious roots develop from aerial tissues, the most often from stems, and help the plants to adapt to their environment. Finally, while it is not the case for *Arabidopsis*, most of plant species develop root-derived symbioses with microbial partners, such as mycorrhiza or nodulation.

Many studies support a critical role for ROS in regulating most of the steps of root system development. In the root apical meristem, the balance between the two major ROS, superoxide and hydrogen peroxide, is associated with the transition from the distal meristematic zone, where  $O_2^{\bullet -}$  is predominant, to the basal maturing zone, in which  $H_2O_2$  predominates (Dunand et al., 2007). This gradient regulates the proper transition from cellular proliferation to differentiation and is tightly controlled by the bHLH transcription factor UPBEAT1 that down-regulates the expression of peroxidases in the transition zone (Tsukagoshi et al., 2010; Figure 2B). The role of ROS in regulating cell differentiation is also demonstrated in trichoblasts development, since RBOHC localization at the tip of the root hair drives ROS and calcium gradients, thus allowing exocytosis and cellular growth (Foreman et al., 2003). In addition, ROS and peroxidases are involved in the regulation of LRP development and emergence, but not their initial specification (Manzano et al., 2014; Fernández-Marcos et al., 2017). Moreover, LRP protrusion requires cell wall remodeling of surrounding tissues, that is controlled by NADPH oxidases-derived ROS production (Orman-Ligeza et al., 2016). Adventitious roots development also depends on ROS homeostasis in rice. In response to the pressure of the growing root, mechanosensing of surrounding tissues leads to ethylene signaling via ROS production that initiates cell death to let the root protrude (Steffens et

al., 2012). Finally, RBOH-derived ROS, downstream of Nod Factor perception, regulate nodulation in legumes via the control of infection thread progression, nodule organogenesis, function and senescence (reviewed in Montiel et al., 2016).

Glutathione is also critical for root system development. This is supported by the very severe root development phenotypes of the *rml1* (*root meristemless*) allele of *GSH1* (Vernoux et al., 2000), and of the mutant in the plastidial GR2 (Yu et al., 2013). The glutathione-deficient *cad2* mutant (weak allele of *GSH1*) is impaired in lateral root and root hair growth in response to the auxin Indole Butyric Acid (Trujillo-Hernandez et al., 2020). Some mutants in glutathione-dependent pathways also confirm its role during root development, such as the Glutathione Peroxidases GPX1 and GPX7 that mediate auxin control of LR development, or the Glutaredoxin GRXS17 that regulates root apical meristem (Passaia et al., 2014; Martins et al., 2020). LR analyses also establish a link between strigolactones (SLs) and the GSH pool that occurs in a MAX2-dependent manner (Marquez-Garcia et al., 2014). The *ntra ntrb cad2* triple mutant also displays an important reduction in root growth, affecting both primary and secondary roots more severely than *cad2* or *ntra ntrb* mutants (Bashandy et al., 2010). This indicates that thiol reducing systems redundantly control root developmental pathways.

Many works have reported redox regulation systems as pivotal regulators of root development in response to endogenous or exogenous signals. First, the plastidial NTRC controls root growth and LR formation, thus adapting the root system development to the primary metabolism of plants (Kirchsteiger et al., 2012).

Glutathione and glutaredoxins appear as critical modulators of root system development in response to nutrients availability. Indeed, glutathione controls the regulation of root hair elongation in response to phosphorus starvation (Trujillo-Hernandez et al., 2020). Moreover, a cluster of glutaredoxins from the class III subfamily (ROXY class) redundantly mediate nitrate-inhibition of primary root growth, downstream of cytokinins perception (Patterson et al., 2016).

Recent works based on ROXY15 overexpression lines tend to suggest that this control of nitrate-dependent transcriptional and developmental responses occurs via binding to the transcription factors TGA1 and TGA4 (Ehrary et al., 2020). More generally, the ROXY genes could be major adaptors of root development to nutrients availability since nitrate starvation, but also  
280 phosphorus, potassium, sulfur and iron deprivations transcriptionally regulate some of these genes (Jung et al., 2013). Very recently, ROXY7 and ROXY8 have been proposed as mobile signals regulated by the nitrogen status of shoots, that enhance nitrate transport by regulating the expression of NRT2.1 in roots (Ota et al., 2020). Altogether, these data suggest that ROXY glutaredoxins are very promising redox components that adapt root development and functions to  
285 nutrients availability in soils and nutrition status of plants. Finally, ROXY1 was recently found to act also in the RAM, where it interacts with RNA polymerase II subunits (Maß et al., 2020).

Redox systems are also involved in mediating root responses to other stress conditions. In Arabidopsis, the NADPH oxidases RBOHD and RBOHF mediate the ABA-dependent reduction of root sensitivity to auxin, while the rice OsTRXh1 is required for root system adaptation to salt  
290 stress and ABA by regulating the apoplastic redox homeostasis (Jiao et al., 2013; Zhang et al., 2011). The GRXS17 control of root development is exacerbated and extended to lateral root growth in case of heat stress, by protecting root meristems from cell death through its holdase activity (Martins et al., 2020). Finally, oxygen-deficient conditions activate RBOHH-dependent production of ROS in rice, via the ethylene-dependent CDPK5 and CDPK13 kinases. These ROS  
295 trigger programmed cell death in cortical cells to create the adapted aerenchyma (Yamauchi et al., 2017).

## **5. Developmental adaptation to stress**

Adaptation of plant development to environment constrains involves redox regulation as well.  
300 Light and temperature have a major impact on physiological and metabolic changes in plants.

Similarly to immune system, they trigger local and systemic signals and induce defense responses. ROS and redox regulation are involved in both signaling and defense priming. Upon various stress conditions, generation of ROS in the apoplast is challenging local responses which are likely initiated by extracellular sensor of hydrogen peroxide like the recently characterized HYDROGEN  
305 PEROXIDE-INDUCED  $Ca^{2+}$  INCREASES 1 (HPCA1) receptor kinase (Wu et al, 2020) and by diffusion of  $H_2O_2$  through aquaporins (Rodriguez et al 2017) during stomatal closure (Figure 1). Stress-induced oxidative burst are also initiating systemic responses called systemic acquired resistance (SAR) in the case of biotic stress or systemic acquired acclimation (SAA) for abiotic stress. A number of different signaling mechanisms were implicated in this response, including a cell to cell  
310 transmission of ROS, calcium ( $Ca^{2+}$ ) and electric signals (Gilroy 2016).

RESPIRATORY BURST OXIDASE HOMOLOG (RBOH)-type of NADPH oxidases are key players of both local and systemic responses. The well-characterized members RBOHD and RBOHF are implicated in a variety of stress-related and developmental responses in plants, including stomatal closure induced by abscisic acid, pathogenesis-related responses, and systemic signaling to stresses such  
315 as wounding, drought, and salt (Chaouch et al., 2012; Kwak et al., 2003; Miller et al., 2009) They are also both required for local and systemic ROS signaling at the vascular bundles during light stress (Zandalinas 2020).

The role of ROS scavenging systems in relaying environmental signals is highlighted by inactivating the peroxisomal  $H_2O_2$ -scavenging enzyme CATALASE2 (CAT2). The *cat2* mutant exhibits a  
320 conditional light-dependent phenotype (Queval 2007). In the *cat2* mutant, light intensity affects leaf bending revealing a crosstalk between  $H_2O_2$  and auxin signalling which is mediated by changes in glutathione redox status (Gao 2014; Kerchev 2015). While the glutathione redox pool is perturbed under both under long-day (LD) and short-day (SD) conditions, only LD triggers a salicylic acid (SA)-dependent programmed-cell death (PCD) (Queval 2007; 2009; 2012). Interestingly, a  
325 mutation in SHORT-ROOT (SHR) rescued the cell death phenotype of *cat2* plants, further

integrating developmental and stress response pathways (Waszczak et al., 2016). Plants deficient in the chloroplastic thioredoxin reductase NTRC exhibit stunted growth particularly under SD, due to the fact that metabolic and antioxidant reactions performed by NTRC are particularly important during the night (Michalska 2009; Lepisto, 2009, 2012). Accordingly, NTRC also maintain redox  
330 homeostasis in non-photosynthetic chloroplasts in the root and *ntrc* mutant exhibits lack in root auxin biosynthesis and lateral root growth inhibition (Kirschsteiger 2012).

As light, temperature changes are major factors signaling daily and seasonal changes and leading to ROS generation (Mittler et al., 2012; Martins et al., 2016; Choudhury et al., 2017). Indeed, plants are capable of integrating signals simultaneously generated during light and temperature stress  
335 combination and systemic ROS signaling is involved in transmitting those stress (Zandalinas 2020). Interestingly, thiol reductases like the glutaredoxin GRXS17 regulate meristem development and flowering time in response to temperature and photoperiod-dependent redox regulation, potentially through interaction with a transcription factor NF-YC11 (Knesting et al 2015). GRXS17 was also involved in heat stress tolerance through its redox-dependent holdase activity (Martins et  
340 al., 2020), a function shared with other thiol peroxidases and reductases (Muthuramalingam 2009; Lee 2009; Park 2009; Chae 2013). The paradigm for a single protein playing a dual role in regulating plant development and in stress defence responses was also revealed for the auxin biosynthesis monooxygenase YUCCA6 (YUC6) which display a thiol-reductase activity and improve plant drought stress tolerance (Cha 2015).

345 Oxygen sensing by the N-end rule pathway play important role in signalling various environmental constraints like hypoxia occurring in waterlogged plants or during photomorphogenesis processes following seedling establishment following germination or in shoot apical meristem activity (Licausi et al., 2011; Gibbs et al., 2011; Abbas et al., 2015; Weits et al., 2019). In this pathway, a penultimate N-terminal Cys residue (Cys2) is exposed by removal of the initial methionine by a Met  
350 aminopeptidase. In the presence of nitric oxide or oxygen, the Cys can be oxidized by plant

cysteine oxidases and, further arginylated by Arg-aminotransferases (Weits et al., 2014; Graciet et al., 2009). Finally, proteins labelled with an N-terminal Arg can then be ubiquitinated by the single-subunit E3 ligase PROTEOLYSIS 6 (PRT6), and thereby targeted to proteasomal degradation. Hypoxia inhibits the N-rule pathway and the proteolysis of substrates of transcription factors  
355 regulating water stress, photomorphogenesis shoot meristems activities.

## 6. Epigenetic and redox regulation

The crucial role of epigenetic in plant development is exemplified by the embryo lethality of null mutants and the dramatic and pleiotropic developmental defects of many mutants affected in  
360 epigenetic processes. However, redox regulation of epigenetic processes has mostly been addressed in mammals, but this field is still poorly explored in plants (Delorme-Hinoux et al., 2016; Shen et al., 2016; García-Giménez et al., 2017). The conservation of these epigenetic mechanisms in living organisms, makes it likely that such regulation also occurs in plants. In mammals, oxidative stress alters global histone modification, histone acetylation and DNA methylation, affecting  
365 chromatin conformation and transcription (Ito et al., 2004; Chen et al., 2006; Ago et al., 2008; Nott et al., 2008; Zhou et al., 2008; Doyle and Fitzpatrick, 2010; Niu et al., 2015). During brain development, neurotrophic factors induce S-nitrosylation at conserved Cys of HDAC2 in neurons, resulting in changes of histone modification and gene expression (Nott et al., 2008). Upon cardiac hypertrophy, a ROS/TRX-dependent redox switch of key Cys residues affects nuclear trafficking of a  
370 class II HDAC and subsequent gene expression (Ago et al., 2008). In plants, among the large family of HDAC (Pandey et al., 2002), members of the class I RPD-3 like HDAC were identified as redox-sensitive proteins during early salicylate- and flg22-response (Liu et al., 2015). NO-induced HDAC inhibition is proposed to operate in plant stress response by facilitating the stress-induced transcription of genes (Mengel et al., 2017).

375 Some key metabolic enzymes involved in DNA methylation are candidates for redox regulation. S-Adenosyl Methionine (SAM) cycle which provide precursors for DNA and histone methylation are among these enzymes (Shen et al., 2016). DNA demethylases Repressor of Silencing1 (ROS1) and Demeter-like (DME, DML2, DML3) enzymes which remove methylated bases from the DNA backbone are other nuclear candidates (Zhu, 2009). These enzymes contain an iron-sulfur (Fe-S) cluster which might be susceptible to oxidation by ROS. Moreover, different members of the cytosolic Fe-S cluster assembly machinery (i.e. MET18, AE7) are involved in DNA methylation, likely because they affect the nuclear DNA demethylases Fe-S cluster metabolism (Liu et al., 2015; Duan et al., 2015). Therefore, all these examples show an emerging link between redox regulation and epigenetic regulation.

385 In mammalian, histone H3, the core constituent of nucleosome, is glutathionylated on a conserved Cys residue (García-Giménez et al., 2013b). Histone H3 glutathionylation fluctuates with cell proliferation and aging. This produces structural changes which affects nucleosome stability and leads to more open chromatin structures and modifications of gene expression (Xu et al., 2014; García-Giménez et al., 2017; García-Giménez et al., 2013b). Recently, Attar et al. (2020) revealed another role of eukaryotic histone H3-H4 tetramer than chromatin compaction or epigenetic regulation. In *Xenopus laevis*, the H3-H4 tetramer binds  $\text{Cu}^{2+}$  through its Cys residue and acts as a  $\text{Cu}^{2+}/\text{Cu}^{1+}$  oxidoreductase dependent on NADPH, and usable for mitochondrial respiration and ROS detoxification enzymes (Attar et al., 2020).

Small RNAs (siRNA and miRNA) are other important regulators of gene expression, and are involved in various developmental and stress response processes in eukaryotic cells (D'Ario et al., 395 2017; Leisegang et al., 2017). The biogenesis of small RNAs is coordinated by DICER-LIKE (DCL) and RNASE THREE-LIKE (RTL) endonucleases which process almost all class of double-stranded RNA precursors. In *Arabidopsis*, members of the DCL and RTL families were shown to be redox-regulated through disulfide bond or S-glutathionylation of conserved Cys in their RNA binding

400 domains, therefore affecting their RNase III activity. Indeed, RTL1 activity can be restored by GRXs, indicating that redox switch controls small RNA biogenesis (Comella et al., 2008; Charbonnel et al., 2017).

## Conclusion

405 Research performed since the last two decades have evidenced the panel ROS effects on plant growth and development. This has been mainly been highlighted by the characterization of knock-out mutants in the large panel ROS metabolism, scavenging or oxidoreduction systems. The function of almost all plant organs, tissues and cell types as well as principal plant development programs are affected by oxidoreduction processes. This gives high credence to the notion of redox control as a major hub regulating plant growth and development in response to environmental constraints. However, much is still to least about the targets of oxidoreduction regulation. This is a challenging task, mostly due to the high dynamic of redox modifications. Techniques dedicated to analyse redox modifications on proteins like redox proteomics are still difficult to manage *in planta* due to high fluctuations of the modifications in different tissues or under various stress conditions and due to the limitation in detection of low abundance target proteins like receptors, transcription factors and other signaling molecules. Interestingly, recent breakthrough research have been generated by forward genetic screens, like the recent identification of the HCPA receptor kinase/sensor of H<sub>2</sub>O<sub>2</sub>. By the way, genetically-based fluorescent imaging is a promising field to design new genetic screen and to decipher redox modifications at the cellular and sub-cellular level. Much is still to discover on the crosstalks between different redox pathways but also on the redundancy between different members of multigenic families in the regulation of plant development and stress response pathways. Good examples are within the multigenic families of thioredoxin, glutaredoxins or peroxidases which exhibit several dozen of genes, most of them being uncharacterized yet.

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**Table 1 : Development defect in redox mutants**

Protein	Gene locus	Plant species	Subcellular localization	Phenotype	References
<b>ROS and NOS metabolism</b>					
RBOHC	At5g51060	<i>A. thaliana</i>	Plasma membrane	Root hair defective	Foreman et al., 2003
RBOHD	At5g47910	<i>A. thaliana</i>	Plasma membrane	Tubulin formation	Yao et al., 2011
RBOHD/RBOHF	At5g47910/ At1g64060	<i>A. thaliana</i>	Plasma membrane	Early emergence of LR and enhanced density of LR	Li et al., 2015
RBOHE	At1g19230	<i>A. thaliana</i>	Plasma membrane	Aborted pollen and reduced fertility	Xie et al., 2014
RBOHH/RBOHJ	At5g60010/ At3g45810	<i>A. thaliana</i>	Plasma membrane	Root hair defect. Reduced fertility and impaired pollen tube growth	Mangano et al., 2017 ; Kaya et al., 2014
CAT2	At4g23100	<i>A. thaliana</i>	Peroxisome	Delayed growth and hyponastic leaves	Queval et al., 2007
MSD1	At3g10920	<i>A. thaliana</i>	Mitochondrion	Defect in embryo sac development	Martin et al., 2013
APX1	At1g07890	<i>A. thaliana</i>	Cytosol	Reduced growth and embryo defect	Pagnussat et al., 2005
APX6	At4g32320	<i>A. thaliana</i>	Cytosol	Reduced germination	Chen et al., 2014
GSNOR	At5g43940	<i>A. thaliana</i>	Cytosol	Reduced root length, defects in stem and trichome branching, reduced fertility, loss of heat acclimation loss of apical dominance, reduced hypocotyl elongation decreased silique size and seed production	Espunya et al., 2006 ; Xu et al., 2013 ; Lee et al., 2008 ; Kwon et al., 2012
NOX1		<i>A. thaliana</i>	Chloroplast	Reduced growth, late flowering	He et al., 2004
NIA1/NIA2/NOA1-2		<i>A. thaliana</i>	Cytosol	Reduced size, fertility, and seed germination. Increased dormancy and stomatal closure.	Lozano-Juste and León, 2010
<b>Ascorbate and glutathione metabolism</b>					
VTC1	At2g39770	<i>A. thaliana</i>	Cytosol/Nucleus	Early flowering and senescence	Barth et al., 2010
VTC2/VTC3/VTC4	At4g26850/ At3g02870	<i>A. thaliana</i>	Cytosol/Nucleus	Early flowering and senescence	Kotchoni et al., 2009
VTC1/VTC2	At2g39770/ At4g26850	<i>A. thaliana</i>	Cytosol/Nucleus	Seedling lethal	Dowdle et al., 2007
GSH1	At4g23100	<i>A. thaliana</i>	Chloroplast	Knock-out mutant embryo lethal, strong allele shows severely impaired post-embryonic growth and weak alleles defects in pollen and root development and auxins transport/signaling.	Cairns et al., 2006; Howden et al., 1995; Vernoux et al., 2000; Ball et al., 2004; Bashandy et al., 2010; Parisy et al., 2007; Shanmugam et al., 2012 ; Trujillo-Hernandez et al., 2020
GSH2	At5g27380	<i>A. thaliana</i>	Chloroplast/Cytosol	Seedling-lethal	Pasternak et al., 2008
GR1	At3g24170	<i>A. thaliana</i>	Cytosol/Peroxisome/ Nucleus	No growth phenotype. Slow growth in <i>gr1 cat1. gr1 ntra ntrb</i> is pollen lethal	Marty et al., 2009 ; Mhamdi et al., 2010
GR2	At3g54660	<i>A. thaliana</i>	Chloroplasts/ Mitochondrion	KO is embryo lethal. Weak alleles have defects in root growth and in root apical meristem maintenance. Inactivation of mitochondrial GR2 and NTRA/NTRB is plant lethal.	Tzafir et al., 2004 ; Yu et al., 2013 ; Marty et al., 2019
<b>Thioredoxins, Glutaredoxins and targets</b>					
NTRA/NTRB	At2g17420/ At4g35460	<i>A. thaliana</i>	Cytosol/ Mitochondrion/ Nucleus	Growth defect and reduced fertility	Reichheld et al., 2007
FTR catalytic subunit	At2g04700	<i>A. thaliana</i>	Chloroplast	Abnormal chloroplast development	Wang et al., 2013
NTRC	At2g41680	<i>A. thaliana</i>	Chloroplast	Retarded growth of shoots and roots, defects	Pérez-Ruiz et al., 2006

				in lateral root formation	Lepistö et al., 2009 ; Kirchsteiger et al., 2012
TRXm3	At2g15570	<i>A. thaliana</i>	Chloroplast	Embryo-lethal, impaired meristem development	Benitez-Alfonso et al., 2009
TRXm	Os12g08730	<i>O. sativa</i>	Chloroplast	Abnormal chloroplast development	Chi et al., 2008
TRXm1/2/4	At1g03680/ At4g03520/ At3g15360	<i>A. thaliana</i>	Chloroplast	Pale-green leaves and reduced stability of PSII	Wang et al., 2013
TRXz	At3g06730 AY500242	<i>A. thaliana</i> <i>N. benthamiana</i>	Chloroplast Chloroplast	Albino phenotype Albino phenotype	Meng et al., 2010 ; Arsova et al., 2010
TRXh9	At3g08710	<i>A. thaliana</i>	Plasma membrane	Dwarf plants with short roots and small yellowish leaves	Meng et al., 2010
TRXo1	At2g35010	<i>A. thaliana</i>	Mitochondrion	Accelerated germination	Ortiz-Espín et al., 2017
NRX1	At1g60420	<i>A. thaliana</i>	Cytosol/Nucleus	Reduced pollen fertility and fitness	Qin et al., 2009 ; Marchal et al., 2014
THL1/THL2	AAB53694/ AAB53695	<i>B. napus</i>	Cytosol	Self-incompatibility rejection response with reduced pollen adhesion, germination and pollen tube growth	Cabrillac et al., 2001 ; Haffani et al., 2004
GRXC1/GRXC2	At5g63030/ At5g40370	<i>A. thaliana</i>	Cytosol	Embryolethal	Riondet et al., 2012
GRXS13	At1g03850	<i>A. thaliana</i>	Cytosol/Nucleus	Reduced plant growth	Laporte et al., 2012
GRXS17	At4g04950	<i>A. thaliana</i>	Cytosol/Nucleus	Compromised meristems and LR development, growth arrest and delayed bolting. Hypersensitivity to elevated temperature and altered auxin perception	Cheng et al., 2011 ; Knuesting et al., 2015 ; Martins et al., 2020
ROXY1	At3g02000	<i>A. thaliana</i>	Cytosol/Nucleus	Impaired petal primordia initiation and petal development	Xing et al., 2005
ROXY2	At5g14070	<i>A. thaliana</i>	Cytosol/Nucleus	Impaired anther development	Xing and Zachgo, 2008
ROXY4	At3g62950	<i>A. thaliana</i>	Cytosol/Nucleus	Impaired anther development	Hou et al., 2008
MIL1	Os07g05630	<i>O. sativa</i>	Cytosol/Nucleus	Defective meiosis	Hong et al., 2012
MSCA1	CAX52135	<i>Z. mays</i>	Cytosol/Nucleus	Impaired anther development	Kelliher and Walbot, 2012 ; Timofejeva et al., 2013
GRXS3/4/5/8 ROXY11/12/13/15	At4g15700/ At4g15690/ At4g15680/ At4g15660	<i>A. thaliana</i>	Cytosol/Nucleus	Increased PR length and decreased sensitivity to nitrate	Patterson et al., 2016
PDI1	At2g47470	<i>A. thaliana</i>	Cytosol	Defect in embryo development	Pagnussat et al., 2005
GPX3	Os02g44500	<i>O. sativa</i>	Mitochondrion	Reduced root and shoot development	Passaia et al., 2014
GPX5	At3g63080	<i>A. thaliana</i>	Plasma membrane	Arrested embryo development	Pagnussat et al., 2005
GPX1/GPX7	At2g25080/ At4g31870	<i>A. thaliana</i>	Chloroplast	Altered leaf cells and chloroplast morphology	Chang et al., 2009
MSRB2	EF144171	<i>C. annuum</i>	Chloroplast	Reduced shoot development	Oh et al., 2010
ECB1	At4g28590	<i>A. thaliana</i>	Chloroplast	Albino phenotype, impaired chloroplast development	Yua et al., 2014
HCF164	At4g37200	<i>A. thaliana</i>	Chloroplast	Impaired accumulation of cytochrome b6f complex subunits	Lennartz et al., 2001

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## Figure legends

### 440 **Figure 1: ROS-dependent cellular metabolism**

ROS generation by reduction of molecular oxygen occurs at several subcellular and extracellular sites. Extracellular ROS generated by RBOH and peroxidases play major role in cell wall flexibility and control cell elongation. The most stable ROS, H<sub>2</sub>O<sub>2</sub>, can move from apoplast to the intracellular space by diffusion through aquaporins. It can also be sensed by the HPCA1-type receptor kinase to  
445 trigger Ca<sup>2+</sup> translocation and signaling cascades. The chloroplast is the main site of singlet oxygen formation whereas superoxides are generated in all cell compartments where it is rapidly dismuted to H<sub>2</sub>O<sub>2</sub> by SODs. H<sub>2</sub>O<sub>2</sub>, can move from the different compartments in which it is mainly produced to alter cytosolic and nuclear redox states, which can be perceived by receptor proteins like transcription factors (yellow barrel) or chromatin modifiers (purple barrel) in the nucleus. Gene  
450 expression will be modified by altered activity or translocation of these factors that may or may not themselves be redox-modified. Extensive panels of plant antioxidative systems and thiols regeneration are found within the cell. The figure summarizes available information for Arabidopsis on the subcellular localization of antioxidative enzymes and related proteins. The information is not exhaustive, and other proteins may be involved.

455 APX, ascorbate peroxidase. CAT, catalase. CM, chromatin modifier. DHAR, dehydroascorbate reductase. GR, glutathione reductase. GRX, glutaredoxin. HPCA1, hydrogen peroxide-induced Ca<sup>2+</sup> increases 1. MDAR, monodehydroascorbate reductase. NTR, NADPH-thioredoxin reductase. PRX, peroxiredoxin. RBOH, respiratory burst oxidase homologue. SOD, superoxide dismutase. TF, transcription factor. TRX, thioredoxin.

460 O<sub>2</sub>, Oxygen. <sup>1</sup>O<sub>2</sub>, singlet oxygen. OH<sup>-</sup>, hydroxide ion. H<sub>2</sub>O<sub>2</sub>, hydrogen peroxide. SH, reduced thiol. S-S, disulfide bond. AsA, ascorbic acid. Ca<sup>2+</sup>, calcium cation. Car, carotenoid. GSH, glutathione. Toc, tocopherol.

Figure adapted from Noctor et al. (2018).

465 **Figure 2: Redox regulation of apical meristems.**

(A) In the SAM, high levels of  $O_2^{\cdot-}$  accumulate in the stem cells of the central zone (CZ, green and orange colors) by repressing superoxide dismutases (SOD) to maintain WUS expression and plant stem cell fate. Stem cell-specific peroxidases (PRX) are expressed to maintain the optimal low level of  $H_2O_2$  in stem cells and fine-tune the  $O_2^{\cdot-}$  levels by a negative feedback. In the peripheral zone  
470 (PZ),  $H_2O_2$  is generated by SOD to promote stem cell differentiation partially by repressing WUS activity. Green, Central zone/stem cells. Orange, organizing center. Brown, rib zone. Red, peripheral zone. Blue, leaf primordia.

(B) In the root tip,  $O_2^{\cdot-}$  accumulate in the meristem (blue area), whereas  $H_2O_2$  accumulates in the elongation zone (red area). UBP1 represses expression of peroxidases in the transition zone,  
475 regulating the ROS balance between the cell proliferation and differentiation zones. The activity of the apical meristem and the quiescent center (QC) are maintained by glutathione (GSH) and ascorbic acid (AsA) as well as different thiol reduction enzymes, thioredoxins (TRX) and glutaredoxins (GRX/ROXY) as well as their respective reductases (NTR and GR2), acting through the auxin pathway (Auxin/PLT). In the differentiation zone, root hairs (grey pins) elongation is

480 controlled by RBOHC which drives ROS and  $Ca^{2+}$  gradients in the root hair tip, and GSH in an IBA-dependent way. Lateral root (brown dome) development is controlled by a panel of peroxidases (PRX, GPX), oxidases (RBOH) and GSH, partially by affecting the auxin and strigolactone pathways. AsA, ascorbic acid.  $Ca^{2+}$ , calcium cation. CZ, central zone. GPX, glutathione peroxidase. GR, glutathione reductase. GRX, glutaredoxin. GSH, glutathione.  $H_2O_2$ , hydrogen peroxide. IBA, indole  
485 butyric acid. MDAR, monodehydroascorbate reductase. NTR, NADPH-thioredoxin reductase. PLT, plethora. PRX, peroxiredoxin. PZ, peripheral zone. QC, quiescent center. RBOH, respiratory burst

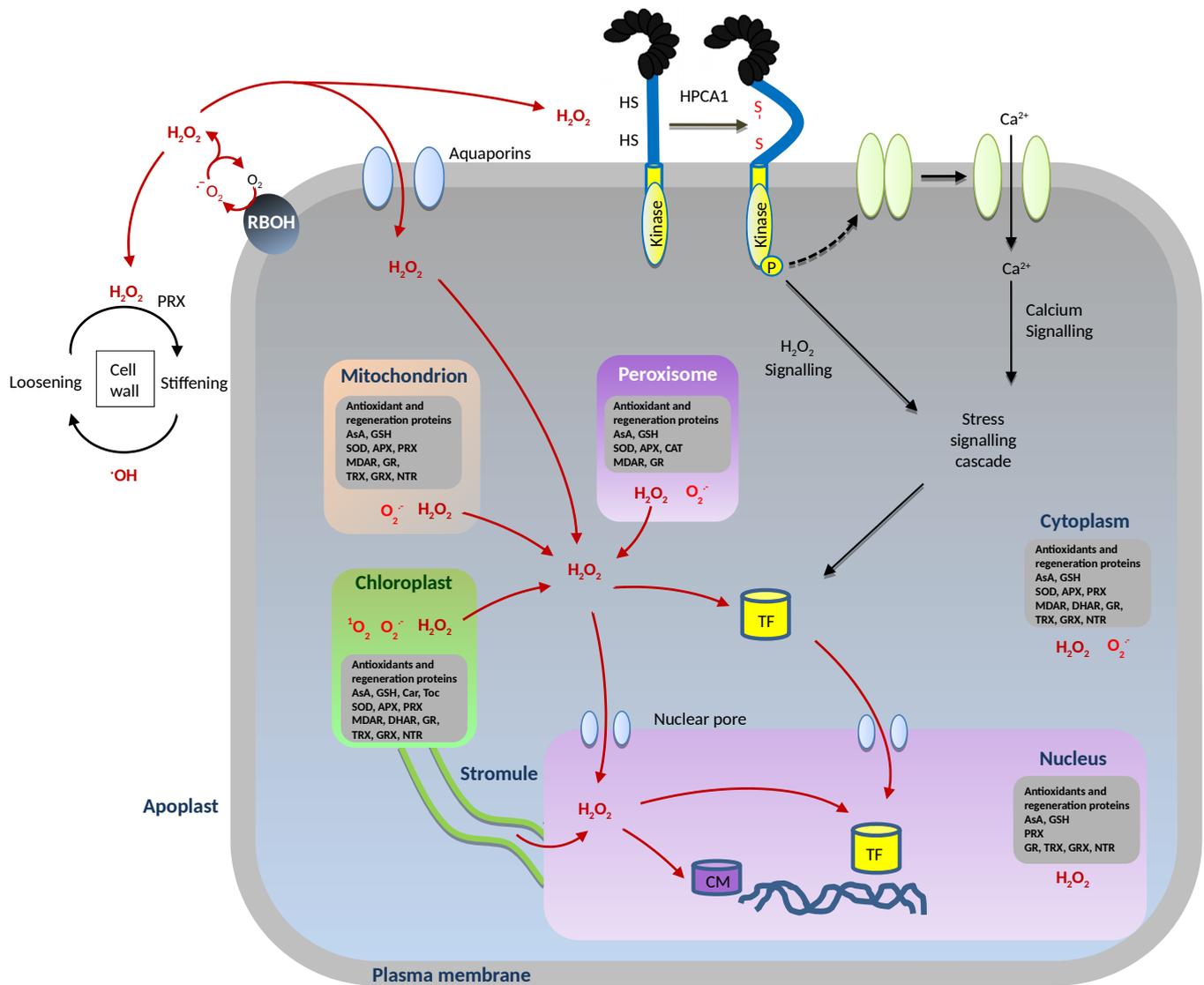
oxidase homologue. ROS, reactive oxygen species. SL, strigolactone. SOD, superoxide dismutase. TRX, thioredoxin. UPB1, UPBEAT 1. WUS, WUSCHEL.

490 **Figure 3: Redox regulation of seed development, dormancy and germination.**

ROS generation is proportional to metabolic activities, with high ROS levels accompanying seed development followed by a rapid drop during the latest stage of seed maturation. These low levels are characteristic from dry dormant seeds and the recovery of higher ROS production is necessary for dormancy breakdown and germination, although it should be kept inside a non-toxic, 495 permissive window. In parallel, reciprocal changes appear in the global reduction status of thiols-containing proteins, with an oxidized proteome being characteristic from dry dormant seeds. Finally, Abscisic Acid (ABA) is a critical plant hormone that promotes seed dormancy and negatively regulates seed germination. NO and H<sub>2</sub>O<sub>2</sub> modulate ABA biosynthesis or early signaling, while they are themselves regulated by ABA-dependent pathways. This allows the integration of different 500 exogenous and endogenous signals to properly adapt seed germination and the establishment of a novel autotrophic plantlet.

ABA, abscisic acid. ABI5, abscisic acid insensitive 5. CAT1, Catalase 1. H<sub>2</sub>O<sub>2</sub>, hydrogen peroxide. NO, nitric oxide. PP2C, protein phosphatase 2C. RBOH, respiratory burst oxidase homologue. RCAR/PYL, REGULATORY COMPONENTS OF ABA RECEPTORS (RCAR)/PYRABACTIN RESISTANCE1 505 (PYR1)/PYR1-LIKE (PYL). SnRK2, SNF1-related protein kinase 2.

Adapted from Bentsink and Kornneef (The Arabidopsis Book, 2008).



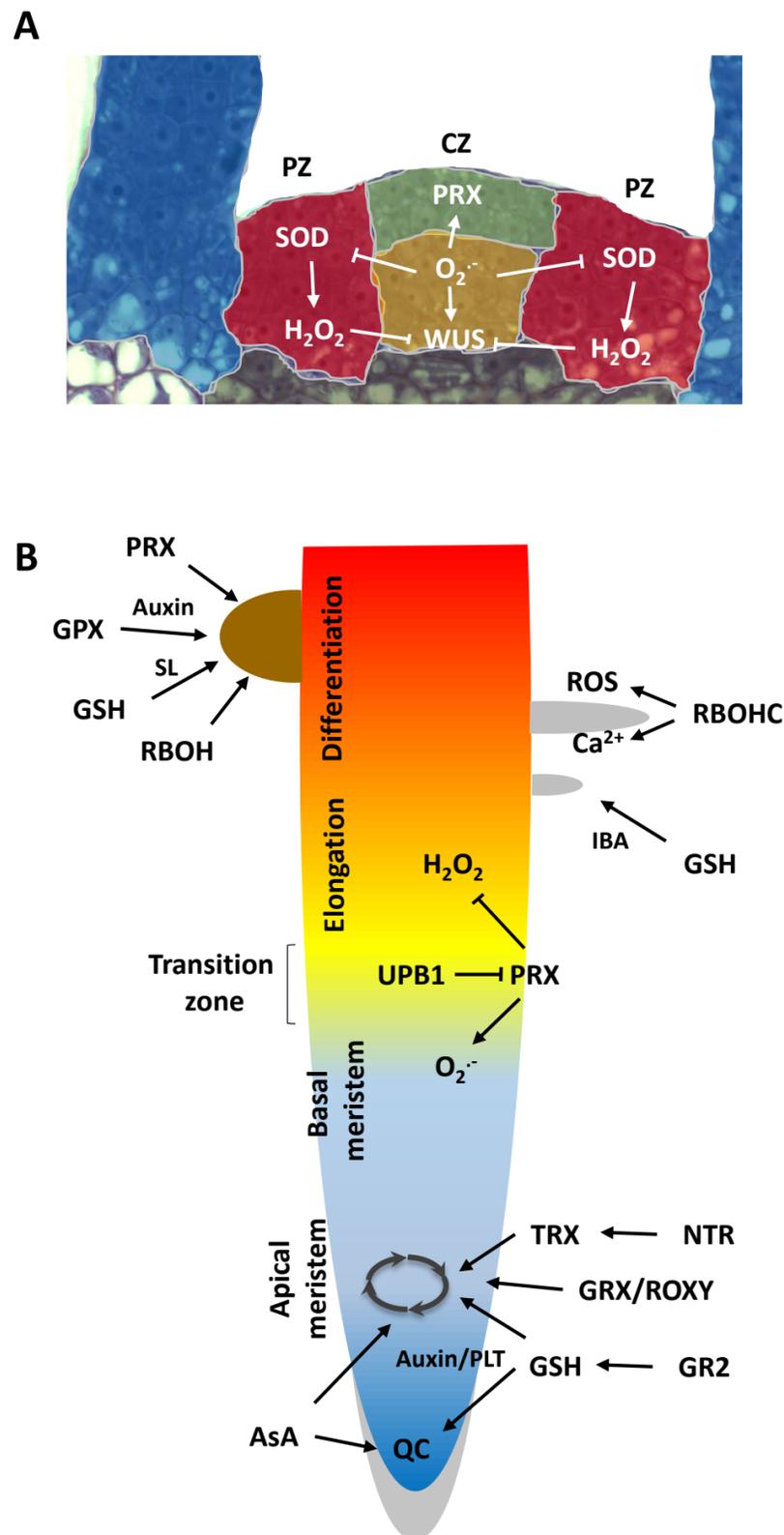
**Figure 1: ROS-dependent cellular metabolism**

ROS generation by reduction of molecular oxygen occurs at several subcellular and extracellular sites. Extracellular ROS generated by RBOH and peroxidases play major role in cell wall flexibility and control cell elongation. The most stable ROS,  $H_2O_2$ , can move from apoplast to the intracellular space by diffusion through aquaporins. It can also be sensed by the HPCA1-type receptor kinase to trigger  $Ca^{2+}$  translocation and signaling cascades. The chloroplast is the main site of singlet oxygen formation whereas superoxides are generated in all cell compartments where it is rapidly dismuted to  $H_2O_2$  by SODs.  $H_2O_2$ , can move from the different compartments in which it is mainly produced to alter cytosolic and nuclear redox states, which can be perceived by receptor proteins like transcription factors (yellow barrel) or chromatin modifiers (purple barrel) in the nucleus. Gene expression will be modified by altered activity or translocation of these factors that may or may not themselves be redox-modified. Extensive panels of plant antioxidative systems and thiols regeneration are found within the cell. The figure summarizes available information for Arabidopsis on the subcellular localization of antioxidative enzymes and related proteins. The information is not exhaustive, and other proteins may be involved.

APX, ascorbate peroxidase. CAT, catalase. CM, chromatin modifier. DHAR, dehydroascorbate reductase. GR, glutathione reductase. GRX, glutaredoxin. HPCA1, hydrogen peroxide-induced  $Ca^{2+}$  increases 1. MDAR, monodehydroascorbate reductase. NTR, NADPH-thioredoxin reductase. PRX, peroxidase. RBOH, respiratory burst oxidase homologue. SOD, superoxide dismutase. TF, transcription factor. TRX, thioredoxin.

$O_2$ , Oxygen.  $^1O_2$ , singlet oxygen.  $OH^{\cdot}$ , hydroxide ion.  $H_2O_2$ , hydrogen peroxide. SH, reduced thiol. S-S, disulfide bond. AsA, ascorbic acid.  $Ca^{2+}$ , calcium cation. Car, carotenoid. GSH, glutathione. Toc, tocopherol.

Figure adapted from Noctor et al. (2018).

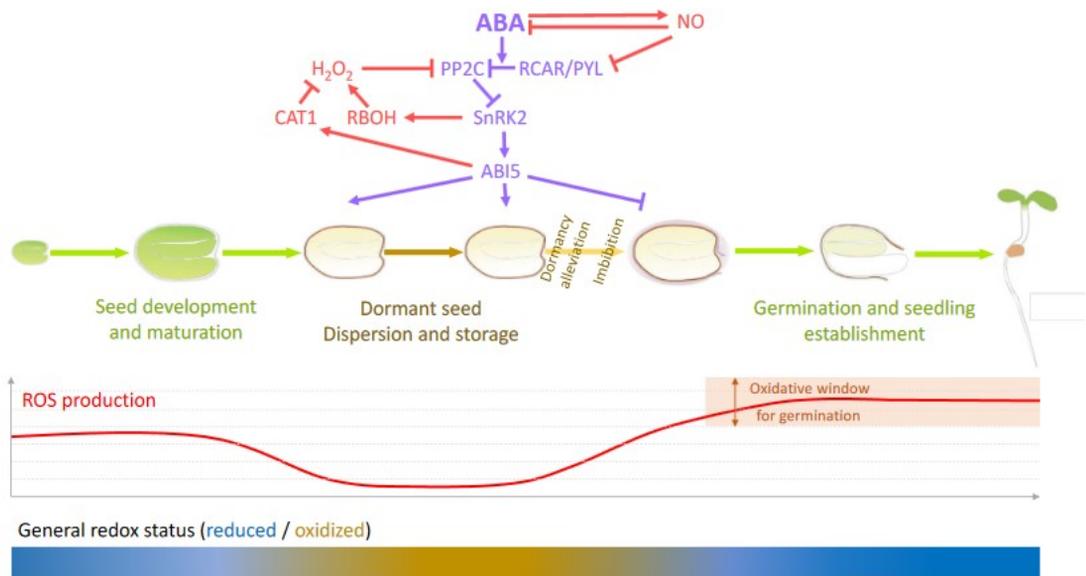


**Figure 2: Redox regulation of apical meristems.**

(A) In the SAM, high levels of  $O_2^{\cdot-}$  accumulate in the stem cells of the central zone (CZ, green and orange colors) by repressing superoxide dismutases (SOD) to maintain WUS expression and plant stem cell fate. Stem cell-specific peroxidases (PRX) are expressed to maintain the optimal low level of  $H_2O_2$  in stem cells and fine-tune the  $O_2^{\cdot-}$  levels by a negative feedback. In the peripheral zone (PZ),  $H_2O_2$  is generated by SOD to promote stem cell differentiation partially by repressing WUS activity. Green, Central zone/stem cells. Orange, organizing center. Brown, rib zone. Red, peripheral zone. Blue, leaf primordia.

(B) In the root tip,  $O_2^{\cdot-}$  accumulate in the meristem (blue area), whereas  $H_2O_2$  accumulates in the elongation zone (red area). UPB1 represses expression of peroxidases in the transition zone, regulating the ROS balance between the cell proliferation and differentiation zones. The activity of the apical meristem and the quiescent center (QC) are maintained by glutathione (GSH) and ascorbic acid (AsA) as well as different thiol reduction enzymes, thioredoxins (TRX) and glutaredoxins (GRX/ROXY) as well as their respective reductases (NTR and GR2), acting through the auxin pathway (Auxin/PLT). In the differentiation zone, root hairs elongation (gray pins) is controlled by RBOHC which drives ROS and  $Ca^{2+}$  gradients in the root hair tip, and GSH in and IBA-dependent way. Lateral root (brown dome) development is controlled by a panel of peroxidases (PRX, GPX), oxidases (RBOH) and GSH, partially by affecting the auxin and strigolactone pathways.

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**Figure 3: Redox regulation of seed development, dormancy and germination.**

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