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Reactive oxygen species (ROS) signalling in plant stress responses

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Supplementary Box 1. ROS production and scavenging pathways of plants.

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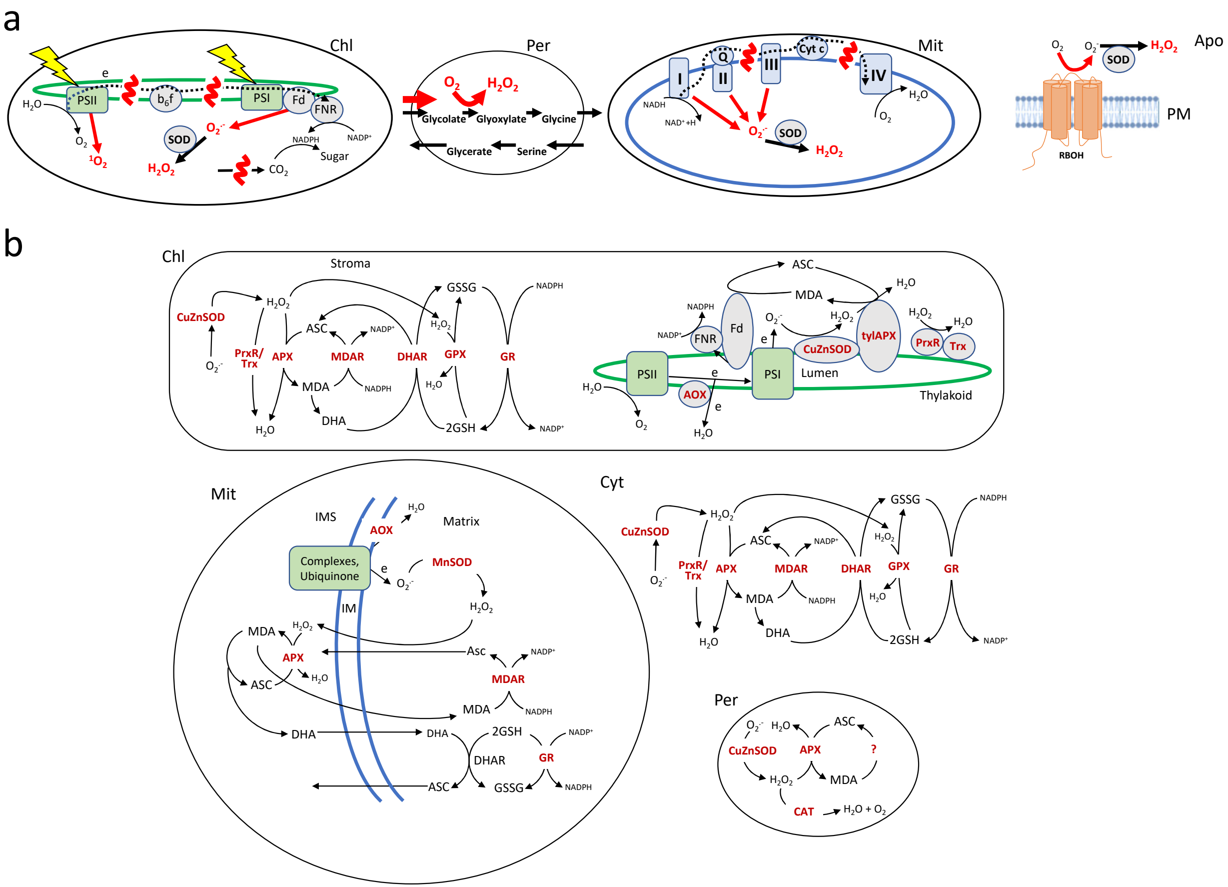
Table S1. Biochemical, physical, and molecular properties of different ROS and RNS in cells.

Table S2. Plant (*Arabidopsis thaliana*) proteins and small molecules regulating ROS levels in cells

Supplementary Box 1. ROS production and scavenging pathways of plants.

Plants contain multiple pathways that produce or scavenge ROS[1-3], and the interplay between these pathways determines the localization, duration and intensity of different ROS signals generated under normal growth conditions or during different environmental stresses. Selected pathways for ROS production and removal in plants are shown below. **a** | Selected ROS production pathways at the chloroplast, peroxisome, mitochondria and apoplast. The production of ROS in these compartments is enhanced during stress or pathogen infection. **b** | Selected ROS scavenging pathways at the chloroplast, peroxisome, cytosol and mitochondria. Most of these pathways are always present in cells and scavenge ROS produced during normal metabolism or stress. APX, ascorbate peroxidase; ASC, ascorbate; AOX, alternative oxidase; Apo, apoplast; b6f, cytochrome b6f; CAT, catalase; Chl, chloroplast; Cyt, cytosol; Cyt c, cytochrome c; DHA, dehydroascorbate; DHAR, dehydroascorbate reductase; e, electron; Fd, ferredoxin; FNR, ferredoxin NADPH reductase; GPX, glutathione peroxidases; GR, glutathione reductase; GSH, glutathione; GSSG, oxidized glutathione; IM, inner membrane; IMS, inner membrane space; MDA, monodehydroascorbate; MDAR, monodehydroascorbate reductase; Mit, mitochondria; Per, peroxisome; PM, plasma membrane; PrxR; peroxiredoxin; PSI, photosystem I; PSII, photosystem II; RBOH, respiratory burst oxidase homolog; SOD, superoxide dismutase; TRX, thioredoxins. Figure modified with permission from[3].



Supplementary Box 2. Evolution of ROS metabolism in cells.

It is generally believed that due to the highly reduced environment on Earth, billions of years ago, almost any O_2 molecule produced within a biological system, at the time, would have been turned into a ROS and reacted with various cellular substances[4,5]. This point is critical to our understanding of current ROS physiology in different organisms because it highlights the fact that aerobic life on Earth evolved in the presence of ROS. The successful evolution of aerobic life depended on the ability of cells to scavenge ROS, and ROS-related pathways and genes were integrated into the genomes of all aerobic organisms[3-7]. Once cells acquired the ability to defend themselves against ROS toxicity, ROS took on a major regulatory role. The evolution of ROS metabolism in cells is depicted in the graph below. Genes encoding different ROS metabolizing enzymes are thought to have emerged in the genomes of unicellular organisms long before the great oxidation event, suggesting that the appearance of oxygenic photosynthesis was made possible due to the presence of these genes[3-7]. Bya, Billion years ago; e, electron; ROS, reactive oxygen species; SOD, superoxide dismutase. Figure modified with permission from[4].

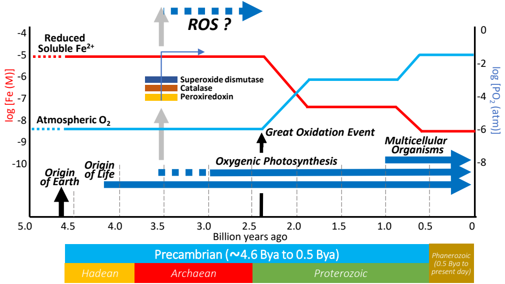


Table S1. Biochemical, physical, and molecular properties of different ROS and RNS in cells.

ROS/RNS	Molecule	Migration distance	Non-radical / free radical	Membrane permeability	Lifetime ($t_{1/2}$)	Reactivity	Sources	Scavenging	Localization	References
Singlet oxygen	O_2	30 nm	Non-radical	Unlikely	1-4 µs	Oxidizes lipids, proteins (Trp, His, Tyr, Met and Cys residues), and G residues of DNA	Chl ETC PSII	Carotenoids Proline GSH	Chl	8,9,10
Superoxide radical	$O_2^{\cdot -}$	30 nm	Free radical	Unable	1-4 µs	Reacts with Fe-S proteins and dismutates to H_2O_2	Chl ETC PSI, PSII Ferredoxin Mit ETC CI, CII, CIII NADPH oxidase Class III Peroxidase	Class III peroxidase SOD	Chl Mit Ap CW Per	8,10,11
Hydrogen peroxide	H_2O_2	> 1 µm	Non-radical	Through aquaporins	> 1 ms	Reacts with proteins by attacking Cys and Met residues, and reacts with heme proteins and with DNA	Acyl-CoA oxidase Aldehyde oxidase Class III Peroxidase Copper amine oxidase Diamine oxidase ER oxidoreductin Farnesylcysteine lyase Flavin containing amine oxidoreductase Glycolate oxidase L-aspartate oxidase Oxalate oxidase Polyamine oxidase Sarcosine oxidase Sulfhydryl oxidase Sulfite oxidase Urate oxidase Xanthine oxidase SOD	Class III peroxidase APX CAT FAUE GPX PrxR PRX TPX AsA Flav GSH HRP Keto acids	Per Cyt ER Vac Mit Chl Ap CW	8,11,12
Hydroxyl radical	$OH^{\cdot}$	1 nm	Free radical	Unable	1 ns	Extremely reactive with all biomolecules including DNA, RNA, lipids and proteins	Haber-Weiss reaction Fenton reaction Class III Peroxidase UV (254 nm)	Proline AsA GSH	Ap Chl Mit Cyt?[2] Per	8,10,12
Hydroperoxyl radical	$HO_2^{\cdot}$	1 µm	Free radical	Yes	1 sec	Peroxidation of fatty acids	Cardiolipin (Myt), eq w/ $O_2^{\cdot -}$		Membranes Mit Cyt	8,12
Organic hydroperoxides	ROOH	> 1 µm	Non-radical	Yes	stable	Reacts with proteins and fatty acids	Protein oxidation Lipid peroxidation	Tocopherol GPX	Per	8

Peroxyl radical	$ROO^{\cdot}$		Free radical	Yes	7 sec	Reacts with fatty acids, proteins, DNA and carbohydrates	Lipid peroxidation Direct reaction of oxygen with alkyl radicals	Tocopherol GSH		8
Alkoxyl radical	$RO^{\cdot}$		Free radical	Yes	1 µs	Reacts with fatty acids	Lipid peroxidation	GSH		8
Hypochlorous acid	$HOCl$		Non-radical	Yes	stable (min)	Reacts with proteins and lipids. Attacks Cys, Tyr and Met residues, side chains amine groups, thiolate metal ions; oxidation of NAD(P)H; chlorinates DNA AND RNA bases; oxidizes thiocyanate	Myeloperoxidase	GSH Keto acids		8
Peroxynitrite radical	$ONOO^{-}$	1.9 µm	Non-radical	High diffusibility across cell membranes	0.05-1 sec	Reacts with proteins, lipids, nitrated amino acids and DNA	$NO^{\cdot} + O_2^{\cdot -}$	GSH PrxR; Keto acids Flavonoids	Chl Mit Per	8,12
Nitric oxide	$NO^{\cdot}$	2.21 µm	Free radical	Yes	1-10 sec	Reacts with proteins and DNA	L-arginine nitric oxide synthase Nitrate reductase	Nitrosylation PRX GSH	Per	8,12
Nitric dioxide	$NO_2^{\cdot}$	1.85 µm	Free radical		sec	Reacts with lipids and Asa	Reaction of peroxyl radical and NO	GSH	Per	8,12
Nitrate radical	$NO_3^{\cdot}$	1µm	Free radical		sec	Reacts with phenylalanine, proline	$NO^{\cdot} + O_2$		Per	8,12
Nitrous acid	HNO_2	1.85 µm	Non-radical		sec	Reacts with DNA (deamination of deoxyribonucleic acids)				8,12
Nitrosonium cation	NO^{+}	1.4 nm	Non-radical		1 ms	S-nitrosylation of proteins				8,12
Nitroxyl anion	NO^{-}		Non-radical		1 ms	Reacts with DNA	SOD Cytochrome c Nitric oxide reductase Hemoglobin Ligning peroxidase	SOD GSH Flavonoids		8
Dinitrogen tetroxide	N_2O_4	1µm	Non-radical		sec	Strong oxidizing agent		Decays by reaction with water to produce nitrite and nitrate		8,12
Dinitrogen trioxide	N_2O_3	1µm	Non-radical		sec	Strong oxidizing agent	$NO^{\cdot} + O_2$		Per	8,12

Table S2. Plant (*Arabidopsis thaliana*) proteins and small molecules regulating ROS levels in cells

Protein/small molecule name	Abbreviation	Reaction	Compartment*	Number of Genes
PRODUCTION				
Acyl-CoA oxidase	ACX	Acyl-CoA + O_2 → enoyl-CoA + H_2O_2	Per	6
Aldehyde oxidase	AAO	Indole-3-acetaldehyde + H_2O + O_2 → indol-3-acetate + H^+ + H_2O_2	Cyt	4
Chl ETC PSI	ETC PSI	O_2 → $O_2^{\cdot -}$	Chl	17
Chl ETC PSII	ETC PSII	O_2 → $O_2^{\cdot -}$ O_2 → $^1O_2$	Chl	28
Copper amine oxidase	CuAO	Amine + H_2O + O_2 → aldehyde + H_2O_2 + NH_4^+	Per, Ap	10
Diamine oxidase	DAO	Diamine + H_2O + O_2 → product + NH_3 + H_2O_2	Ap, Cyt	2
ER oxidoreductin	ERO	PDI + $2e^-$ + O_2 → H_2O_2	ER	2
Farnesylcysteine lyase	FCLY	H_2O + O_2 + farnesyl-cysteine → farnesal + H_2O_2 + cysteine	Vac	1
Ferredoxin	Fd	O_2 → $O_2^{\cdot -}$	Chl, Mit	9
Flavin containing amine oxidoreductase	PPOX	O_2 + protoporphyrinogen → H_2O_2 + protoporphyrin	Chl, Mit	2
Glycolate oxidase	GOX, HAOX	Hydroxycarboxylates + O_2 → 2-oxocarboxylate + H_2O_2	Per	5
L-aspartate oxidase	FIN4	Aspartate + O_2 → H_2O_2 + iminosuccinate	Chl	1
Mit ETC CI, CII, CIII	ETC CI, CII, CIII	O_2 → $O_2^{\cdot -}$	Mit	69
NADPH oxidase	RBOH, NOX	NAD(P)H + e^- + O_2 → NAD(P)$^-$ + $O_2^{\cdot -}$ + H^+	PM	10
Oxalate oxidase	OXO	Oxalic acid + O_2 → $2CO_2$ + H_2O_2	Ap	1
Polyamine oxidase	PAO	Polyamine + H_2O + O_2 → product + NH_3 + H_2O_2	Ap, Per, Cyt	5
Sarcosine oxidase	SOX	H_2O + O_2 + sarcosine → formaldehyde + glycine + H_2O_2	Per, Cyt	1
Sulfhydryl oxidase	QSOX	O_2 + R'C(R)SH → H_2O_2 + R'C(R)S-S(R)CR'	CW	2
Sulfite oxidase	SO	Sulfite + H_2O + O_2 → sulfate + H_2O_2	Per	1
Urate oxidase	UOX	H_2O + O_2 + urate → 5-hydroxyisourate + H_2O_2	Per	1
Xanthine oxidase	XO	Xanthine + H_2O + O_2 → urate + H_2O_2 NADH + O_2 → NAD^+ + $O_2^{\cdot -}$	Cyt, Per	2
PRODUCTION/ SCAVENGING				
Superoxide dismutase (Fe, Mn, Cu/Zn)	SOD	$2H^+$ + $2O_2^{\cdot -}$ → H_2O_2 + O_2	Cyt, Chl, Per, Mit, Ap, ER, Vac, PM, Nuc	8
Peroxidases	PRX	$NAD^{\cdot}$ + O_2 → NAD^+ + $O_2^{\cdot -}$ NADH + H^+ + $O_2^{\cdot -}$ → H_2O_2 + $NAD^{\cdot}$ 2Phenolic donor + H_2O_2 → 2phenolic radical donor + $2H_2O$	CW, Ap, Vac	73
YUCCA	YUC	H^+ + indole-3-pyruvate + NADPH + O_2 → (indol-3-yl) acetate + CO_2 + H_2O + $NADP^+$ O_2 + NAD(P)H → NAD(P)$^-$ + H_2O_2	Cyt, PM?, Per?	11
SCAVENGING				
Alternative oxidase	AOX	$2e^-$ + $2H^+$ + O_2 → H_2O	Mit, Chl	6
Ascorbate peroxidase	APX	2Asc + H_2O_2 → $2H_2O$ + 2MDA	Cyt, Per, Chl, Mit, PD	8
Catalase	CAT	$2H_2O_2$ → $2H_2O$ + O_2	Per	3
Dehydroascorbate reductase	DHAR	2GSH + DHA → GS-SG + Asc	Cyt, Nuc, Per, Chl	4
F-box associated ubiquitination effector	FAUE	2P-SH+ H_2O_2 → P-S-S-P + H_2O	Cyt	1
Glutaredoxin	GRX	PS-SP + 2 GSH → P-SH + GSSG	Cyt, Golg, ER, Vac, PM, Chl, Nuc, Mit	41
Glutathione peroxidase	GPX	2GSH + H_2O_2 → GS-SG + $2H_2O$	Cyt, Nuc, Chl, ER, PM, Mit	8
Glutathione reductase	GR	GS-SG + H^+ + NAD(P)H → 2GSH + NAD(P)$^-$	Per, Mit, Chl, Cyt	2
Glutathione S-transferase	GST	GSH + RX → halide anion + *S*-substituted GSH + H^+	Cyt, Nuc, Chl, Per	54
Monodehydroascorbate reductase	MDHAR	H^+ + MDA + NAD(P)H → Asc + NAD(P)$^-$	Cyt, Per, Chl, Mit	5
NADPH-dependent thioredoxin reductase	NTR	2P-SH + $NADP^+$ → P-S-S-P + NADPH	Chl, Per, Mit	3
Nucleoredoxin	NRX	Prot-dithiol + NAD(P)$^+$ = Prot-disulfide + H^+ + NAD(P)H	Cyt, Nuc	2
Peroxiredoxin	PrxR	2P-SH + H_2O_2 → P-S-S-P + $2H_2O$	Chl, Mit, PM?, Cyt, Nuc	11
Thioredoxin-dependent peroxidase	TPX	2P-SH + H_2O_2 → P-S-S-P + $2H_2O$	Cyt, PM, Per?	2
Thioredoxin family	TRX	P-S-S-P + $2H^+$ → 2P-SH	ER, PM, Vac, Cyt, Nuc, Ap, Mit, Chl	34
TRANSPORT				
Aquaporin	AQP		ER, Gol, Mit, Nuc, Per, PM, Chl, Vac	19
Mitochondrial permeability transition pore	VDAC-mPTP		Mit	5
Nucleoporin	NUP		Nuc	30
Stromules, peroxules, matrixules, complexes			Chl, Mit, Per	
SMALL ANTIOXIDANTS				
Ascorbic acid	AsA	2Asc + H_2O_2 → $2H_2O$ + 2MDA	Chl, Cyt, Mit, Per, Ap, Nuc, Vac	
Carotenoids	Car	$^1O_2$ + Carotene → O_2 + Carotene + heat	Chl	
Flavonoids	Flav	H_2O_2+ Flavonoid → Flavonoid radical + H_2O	Vac, Chl, Nuc, CW, Ap, Cyt, ER	
Glutathione	GSH	2GSH + H_2O_2 → $2H_2O$ + GSSG	Chl, Cyt, Mit, Per, Ap, Nuc	
Proline	Pro	Proline + $OH^{\cdot}$ → Hydroxyproline Proline + $^1O_2$ → Proline radical + O_2	Chl, Cyt, Mit	
α-Tocopherol	α-TCP	Lipid peroxyl radical + α-Tocopherol → lipid hydroperoxide + tocopheroxyl radical	Membranes	

*Ap – Apoplast; Chl – Chloroplast; Cyt – Cytosol; CW – Cell Wall; ER – Endoplasmic Reticulum; Golg – Golgi apparatus; Mit – Mitochondria; Nuc – Nucleus; PD – Plasmodesmata; Per - Peroxisomes; PM – Plasma Membrane; Vac - Vacuole

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