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Review

Redox control of plant growth and development

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ABSTRACT

Redox changes determined by genetic and environmental factors display well-organized interactions in the control of plant growth and development. Diurnal and seasonal changes in the environmental conditions are important for the normal course of these physiological processes and, similarly to their mild irregular alterations, for stress adaptation. However, fast or large-scale environmental changes may lead to damage or death of sensitive plants. The spatial and temporal redox changes influence growth and development due to the reprogramming of metabolism. In this process reactive oxygen and nitrogen species and antioxidants are involved as components of signalling networks. The control of growth, development and flowering by reactive oxygen and nitrogen species and antioxidants in interaction with hormones at organ, tissue, cellular and subcellular level will be discussed in the present review. Unsolved problems of the field, among others the need for identification of new components and interactions in the redox regulatory network at various organization levels using systems biology approaches will be also indicated.

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Abbreviations: Asc, reduced ascorbate; DHA, dehydroascorbate; GSH, reduced glutathione; GSSG, glutathione disulphide; RNS, reactive nitrogen species; ROS, reactive oxygen species.

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1. Introduction

The characteristic pattern of growth and development of sessile plants is a genetically determined process that can be substantially modified by changes in temperature, light (intensity and spectrum) and water availability. The physiological processes are fine-tuned by a circadian clock, which synchronizes gene expression, protein synthesis and activity, and the synthesis and degradation of various compounds with the regular daily alterations in environmental conditions, consequently ensuring fitness and optimal growth [1]. Plants also have to adapt to weekly, seasonal and annual changes in the environment and to achieve the appropriate mass production necessary for successful reproduction. The seasonal alterations in growth and development are probably regulated by changes in day length and temperature, leading to the reprogramming of the metabolism.

An important consequence of the annual, seasonal or irregular fluctuations in environmental conditions is the alteration of the cellular redox state (Fig. 1). Redox changes may also be genetically determined. Both the level of reactive oxygen species (ROS) and antioxidants shows diurnal changes [2,3]. Abrupt changes in temperature and light intensity may lead to the accumulation of ROS due to the disturbed function of the photosynthetic and respiratory electron transport chains. Only the damaging effect of ROS

on macromolecules was emphasized in early studies. However, over the last 15 years increasing attention has been devoted to their regulatory and signalling role in plant growth and development and in adaptation to changing environmental conditions [4–8]. ROS concentrations are partly regulated by antioxidants, which usually prevent their accumulation at toxic levels. The involvement of H₂O₂ in signalling has been investigated very intensively [5,6]. Its concentration is regulated by a cascade of redox pairs of reduced glutathione (GSH)–glutathione disulphide (GSSG), reduced ascorbate (Asc)–dehydroascorbate (DHA), and kept in balance by metabolically produced NADP⁺ and NADPH. Changes in the ratio and amount of reduced and oxidised forms affect the cellular combined reduction potentials [9] and consequently the accumulation of transcripts encoding several redox-responsive proteins (Fig. 2) [10]. It is important to study not only the ratios, but also the total pool of these compounds, referred to as glutathione (GSH + GSSG) and ascorbate (Asc + DHA), since this is characteristic for their cellular redox buffering capacity. Although protein thiols have been suggested to be the major cellular redox buffers based on a recent redox proteomic approach [11] the non-protein thiols, especially GSH have important role in the cellular redox control since GSH/GSSG may reduce/oxidise or de/glutathionylate protein thiols. The control of glutathione and ascorbate levels appears to be coordinated, since an increased ascorbate level in transgenic

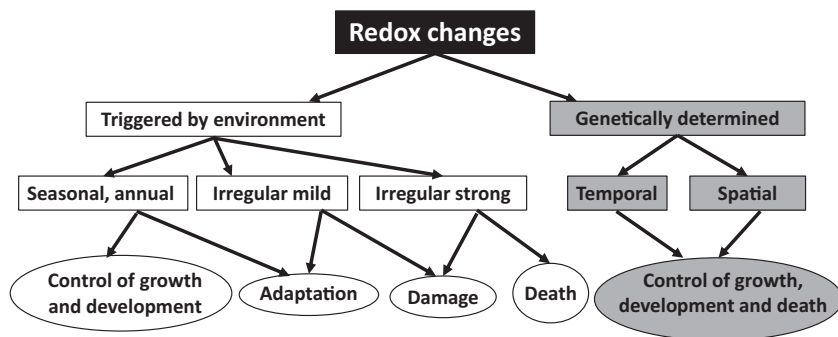


Fig. 1. Physiological effects of redox changes. Redox changes are either triggered by the environment or are genetically determined. The actual redox state is a result of the interaction of the two effects. Seasonal and annual changes in the environmental conditions are important for the normal course of growth and development and, similarly to their mild irregular alterations, for stress adaptation. However, large environmental changes may lead to injury or death of the plants. Certain part of the spatial and temporal redox changes is genetically determined. The redox changes modify or induce the various physiological processes through regulatory networks including ROS, RNS and antioxidants through the reprogramming of transcriptome, proteome and metabolome.

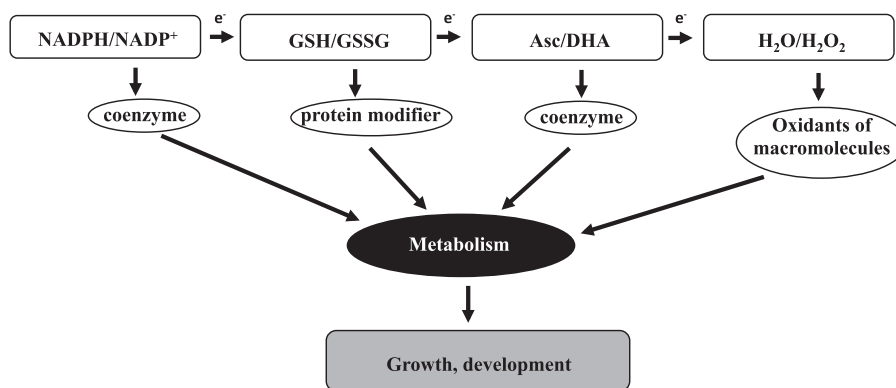


Fig. 2. Specific functions of the components of the ascorbate-glutathione cycle in the regulation of metabolism. NADPH and Asc serves as coenzyme, GSH is a posttranslational modifier (thiol/disulphide conversion, de/glutathionylation) and H_2O_2 is an oxidant (damage of macromolecules). They can modify metabolism, and subsequently growth and development due these functions. Asc: reduced ascorbate, DHA: dehydroascorbate, GSH: reduced glutathione, GSSG: glutathione disulphide.

plants is accompanied by higher glutathione content [7]. All components of the Asc-GSH cycle have specific roles in the regulation of metabolism, which affect growth and development (Fig. 2). In addition to H_2O_2 , other ROS (hydroxyl and superoxide radicals, singlet oxygen) are also involved in redox signalling [5,6,12]. Interestingly, redox (thiol-disulphide) and sugar signals have common metabolic targets, among others the components of the starch synthesis, which indicate the interconnection of the sugar and redox signalling pathways [10]. A model of the hierarchical and highly interconnected dithiol-disulphide network involving sensors, redox input elements, transmitters, targets and final electron acceptors were also elaborated [4]. In this network the NADPH-dependent thioredoxins and glutaredoxins play an important role due to their involvement in redox control and the posttranslational modification (thiol-disulphide conversion, (de)glutathionylation) of proteins [13].

As in the case of ROS, reactive nitrogen species (RNS) may have both a disadvantageous, direct effect in the form of nitrosative stress, and advantageous effects due to their antioxidant nature

and involvement in signalling mechanisms [14]. The antioxidant function of NO depends on the concentration and site of action and it is based first of all on chain breaking of free radical-mediated lipid peroxidation. NO in plants can be generated enzymatically by nitric oxide synthase-like enzyme, nitrate reductase or nitrite-NO reductase and by peroxisomal xanthine oxidase under anaerobic conditions, while NO generation may also be associated with polyamine catabolism (Fig. 3). In plant tissues non-enzymatic reduction of NO_2^- to NO has been shown through light-mediated reaction by carotenoids, by ascorbic acid at acidic pH and by the mitochondrial electron transport chain [15]. The oxidative metabolism of NO generates further RNS, including NO_2 , N_2O_3 , peroxynitrite (ONOO^-), S-nitrosothiols and S-nitrosoglutathione (GSNO). NO and GSNO induce the S-nitrosylation of target proteins by the transfer of NO moiety to reactive cysteines that are surrounded by a consensus grouping of amino acids (the acid-base motif theory). The S-nitrosothiol formation was shown to impact the structure of target proteins (Fig. 3) [16,17]. These redox-based modifications can be reversed and can serve as a redox switch

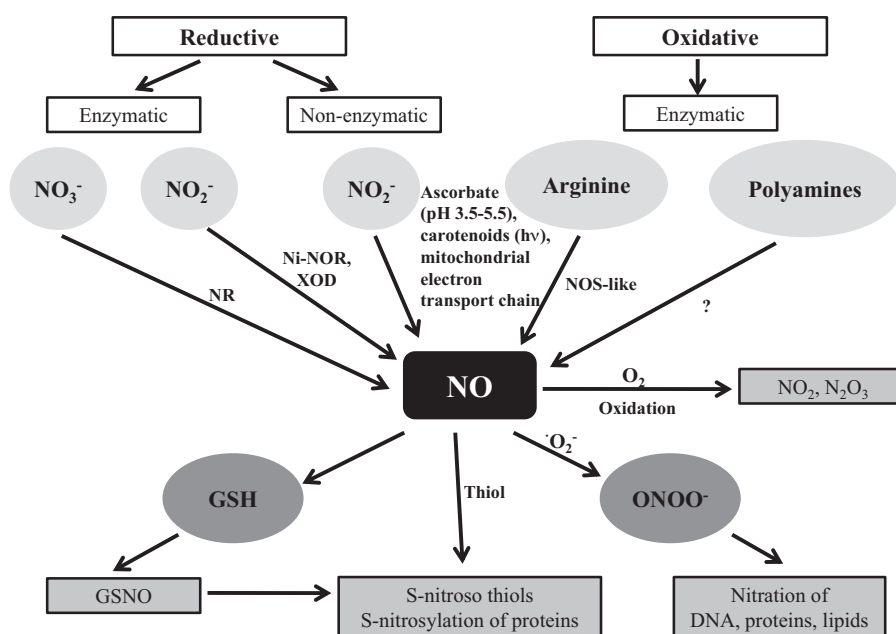


Fig. 3. Generation of reactive nitrogen species and their effect on posttranslational modification of proteins. NO can be formed both in enzymatic and non-enzymatic reactions and can be the precursor of other reactive nitrogen species. These molecules are involved in the protein nitration and S-nitrosylation which affect metabolism, signal transduction and indirectly the growth and development of plants. Asc: reduced ascorbate, GSH: reduced glutathione, GSNO: S-nitroso glutathione, $\text{O}_2^{\bullet-}$: superoxide radical, ONOO^- : peroxynitrite, Ni-NOR: nitrite-NO reductase, NOS: NO synthase, NR: nitrate reductase, XOD: xanthine oxidase.

responsive to the cellular redox status. The reaction of NO with the superoxide anion leads to the formation of a strong oxidizing agent, ONOO⁻. This compound can modify proteins by a generally irreversible nitration of tyrosine or tryptophan residues and it also reacts with DNA, forming 8-nitroguanine. Up to 1 mM concentration peroxyxynitrite does not induce cell death in plant tissues, however it is involved in the posttranslational modification of proteins. Tyrosine nitration may have both stimulatory and inhibitory effect on enzyme activities, the latter being the most common consequence but it may also have toxic effects and may contribute to cell death [15,18].

The subcellular distribution of ROS, antioxidants and the redox state in cell compartments is of great importance for proper plant growth and development, as the activity of genes and enzymes have specific redox requirements. Small shifts in these parameters can induce changes in gene expression, defence signalling and cell death. In this context, the changes in the levels of ROS and antioxidants are involved in the control of cell proliferation in the nuclei [19], and of cell death and senescence in mitochondria, chloroplasts and peroxisomes [20–23]. The vacuoles seem to be involved in the sequestration of ROS and GSSG in the case of severe oxidative stress [24]. It thus becomes obvious that the subcellular distribution of ROS, and of antioxidants such as ascorbate and glutathione, and the redox state of certain cell compartments play a key role in the fine tuning of plant growth and development.

Antioxidants, ROS and RNS interact with various plant hormones in the regulation of growth and development, as well as in biotic and abiotic stress responses [5,25–28]. The cross-talk between redox systems and hormones is very intensive. ROS affect the biosynthesis of hormones and serve as secondary messengers in their signalling pathways. On the other hand, various hormones control ROS generation and stimulate the expression of redox system-related genes.

The redox control of stress responses has been discussed in several recent reviews [5,6,29]. In the present review the regulatory cross-talk of antioxidants, ROS and RNS with hormones, is discussed mainly in plants grown under optimal environmental conditions.

2. Participation of reactive oxygen species and antioxidants in the regulation of growth and development

2.1. Redox control of metabolism

The redox environment is generally highly reducing under optimal conditions in plant cells, which ensures the appropriate metabolism in organisms living in an oxidising atmosphere. The redox couples of the Asc–GSH cycle contributes to the maintenance of this reducing environment since they are maintained in a generally reduced state in plants growing under optimal conditions as indicated by the high ratio of the reduced forms (Table 1). A very high ratio of GSH to GSSG exists in unstressed plants, which was 20 for wheat and 50 for olive [30,31]. Interestingly this ratio was much lower (varied between 1.3 and 2) for the Asc/DHA, NADPH/NADP⁺ and NADH/NAD⁺ couples (Table 1). The electron flow between

these redox couples is ensured by the increasing reduction potential (NAD(P)⁺ < glutathione < ascorbate) [2].

The antioxidants maintaining this reducing environment are under the control of light quality signals, including red/far red ratio as shown for ascorbate in *Phaseolus vulgaris* [2]. A higher H₂O₂ content coincides with a greater GSH level during the day due to this regulation and the level of both compounds shows diurnal light/dark cycling [3,32]. The generation of reducing power in plants during the light-driven electron transfer from water to NADP⁺ in the course of photosynthesis also shows daily fluctuations. Changes in the NADPH/NADP⁺ ratio affect both the redox state of antioxidants and ROS formation, which in turn leads, through a redox signalling pathway, to the reprogramming of the metabolism of many compounds, including carbohydrates, sulphur and nitrogen containing organic compounds [4].

The redox regulation of the whole metabolome was demonstrated in *Arabidopsis* plants by treatment with the strong reductant dithiothreitol in the light [10]. Carbohydrate metabolism was affected since ¹⁴C-glucose uptake and its flux into sucrose decreased. In addition, the synthesis of cell wall constituents, amino acids, organic acids and starch increased. To see whether these changes were due to the redox-dependent changes in the activity of the related enzymes because of their posttranslational modifications or due to the redox control of gene expression, a transcriptome analysis has also been done. Altered expression of genes coding components of redox regulation, transport processes and the cell wall, protein and amino acid metabolism was observed [10]. The redox control of transcript profile can be based on shift in reduction potential of the various redox couples or on changes in ROS concentrations. The specific effect of the individual ROS (¹O₂, O₂^{•-}, H₂O₂) was checked by comparing their transcriptomic footprints in *Arabidopsis* mutants, transgenic lines or in plants treated with inducers of ROS [12]. Besides genes responding to all ROS, the level of several transcripts was only influenced by one of the ROS. In addition, a high number of receptor-like kinase genes [33] were responsive to ozone-induced oxidative stress (formation of various ROS), while much less [21] to the accumulation of H₂O₂ in catalase-deficient mutants in *Arabidopsis* [8]. Similarly, many receptor-like kinase genes [34] were affected by low ascorbate, but only five by low glutathione [8]. These results indicate the high specificity of the H₂O₂⁻, ascorbate- and glutathione-dependent regulation of gene expression. In contrast to these observations, treatment with hydrogen peroxide and induction of superoxide radical formation by methyl viologen resulted mostly in the oxidation of the same proteins (involved in carbohydrate metabolism, photosynthesis, redox homeostasis and nitrogen assimilation) in *Arabidopsis* chloroplasts [11]. This contradiction observed at proteome and transcriptome level can be explained by the different experimental approaches. In the investigation of the redox transcriptome several genotypes and treatments were included [8,12] while in the investigation of the redox proteomes only one genotype was subjected to two different treatments [11]. In the latter study ribulose-1,5-bisphosphate carboxylase oxygenase, a primary oxidation target was suggested to be redox buffer due to its high concentration. Thus, besides non-enzymatic antioxidants, proteins are also very important in the maintenance of cellular redox homeostasis. Tyrosine phosphatases are also important in the control of cellular redox state. They are inactivated by oxidative stress and activated by reduction [35]. These phosphatases regulate the MAP kinases involved in various signalling pathways [4] and may be involved in perception of redox changes (Fig. 4). Other possible redox sensors are peroxiredoxins, glutathione peroxidases and GSSG/glutaredoxins [4]. The redox gradient across the plasma membrane, deriving from the highly reducing intracellular redox environment and the highly oxidising one in the apoplast has also been suggested to be a redox sensor [8]. The changes in this

Table 1
Redox state of various redox pairs under optimal and salt stress conditions in olive.

	Asc/DHA	GSH/GSSG	NADPH/NADP ⁺	NADH/NADP ⁺
Control	2.0	50.7	1.9	1.3
200 mM NaCl	2.7	6.7	2.1	1.2

Concentrations under control conditions in nmol (gFW)⁻¹: Asc: 2180, GSH: 158, NADPH: 14, NADH: 9. The amount of Asc decreased to 80%, that of DHA to and GSH to 60%, while GSSG concentration increased 4.5-fold after salt treatment. The concentrations given in the figure legend were taken and the ratios of the reduced and oxidised forms were calculated from the data published in Tables 3 and 4 of the article by Valderrama et al. [31].

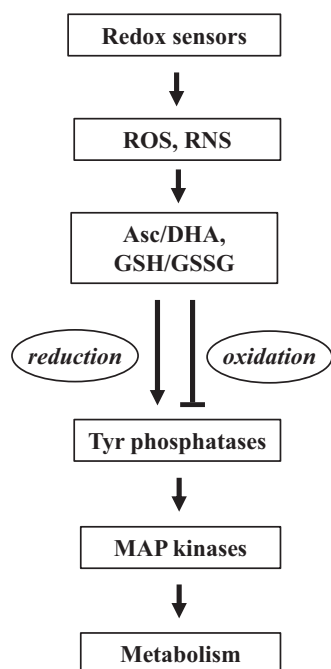


Fig. 4. Redox control of growth and development through the mitogen-activated protein kinase cascade. The redox sensor may be an unidentified protein kinase which may affect protein phosphatases through a redox signalling pathways. These phosphatases regulate protein kinase cascade and subsequently effect metabolism.

gradient may regulate receptor-like protein kinases of the plasma membranes which also participate in the control of growth and development.

The investigation of the redox control at transcriptome, proteome and metabolome levels indicate both common and specific roles of the individual ROS. The synthesis of the results obtained using omics approaches by systems biology tools can be applied for determination of hubs and bottlenecks in the redox regulatory network [4].

2.2. Redox control of growth and development at cellular level

The redox-dependent reprogramming of the transcriptome, proteome and metabolome leads to changes in growth and development. The basis of plant growth is cell proliferation, which is under redox control. A dual role of ROS was proposed for the regulation of the division of alfalfa mesophyll cells, depending on their concentration, duration and site of action [36]. Low level of ROS or shorter exposure to a certain concentration promotes cell division while their excess or longer exposure may lead to cell death. The alteration in ROS concentrations in cells or cell compartments may also influence cell division. Such regulatory role was described for antioxidants since changes in the nuclear GSH concentration and the redox state of ascorbate were described during the cell cycle [7,19]. Asc induces mitosis, while DHA inhibits it. It is not clear whether the individual ROS and antioxidants have special functions in the regulation of mitosis or whether there is a redox checkpoint where the various regulatory paths converge, as has also been suggested [7]. The switch from mitosis to meiosis is very important in sexually reproducing eukaryotes and ROS or antioxidants may be also involved in the control of this process. This hypothesis is supported by the observation that the transition to meiosis was affected by a putative glutaredoxin in rice anthers [37]. However, further studies are necessary for the verification and detailed characterization of the redox control of meiosis.

The redox regulation of cell differentiation was demonstrated during the transition of mesophyll cells to tracheary elements in *Zinnia* cell cultures and during the development of *Arabidopsis* roots. The GSSG concentration transiently increased and the transcription of the *GLUTATHIONE REDUCTASE* gene was inhibited during this process [38]. The overexpression of the *GLUTATHIONE REDUCTASE* gene in *Arabidopsis* delayed tracheary element formation in the root, and the exogenous application of GSH had a similar effect. Changes in the ascorbate metabolism were also associated with cell differentiation in plants [7]. Results cited in the previous and this section indicate that a more reducing cellular redox environment may promote cell division, and a more oxidising environment cell differentiation.

2.3. Redox control of growth and development at tissue and organ levels

The redox control of growth and development was observed not only at the cellular, but also at the tissue and organ levels. Both $O_2^{\bullet-}$ and H_2O_2 may have special functions in the control of root growth as indicated by the localization of $O_2^{\bullet-}$ in the transition zone between the meristem and rapidly elongating cells and by the presence of H_2O_2 in the wall of the fully elongated cells in the region of root hair formation [39]. ROS and antioxidants may be interconnected during the regulation of growth through NADPH which serves as a substrate for NADPH oxidases producing $O_2^{\bullet-}$ and as an electron donor for GSSG. The involvement of glutathione in the control of root growth due to its redox buffering capacity and its specific functions was shown in *Arabidopsis* [40]. Treatment of wheat and maize with various reductants and oxidants affected the total amount of non-protein thiols, the ratio of their reduced and disulphide forms and the development of roots and shoots (Kocsy G., unpublished results). Changes in the concentration and in the reduction potential of the GSH/GSSG and other redox couples make possible the fine regulation of the cellular redox environment and consequently the growth of the plants.

The redox regulation of embryo development was described in spruce and canola [41]. Greater amounts of Asc and GSH were necessary for appropriate cell proliferation during early embryo development, while greater levels of DHA and GSSG were subsequently required for cell elongation and the proper organization of the root apical meristem [41]. In the root apex glutathione and ascorbate are mainly found in oxidized forms in the very slowly dividing cells of the quiescent centre, while they are in reduced forms in the adjacent, rapidly dividing cells of the root meristem [25]. Compared to the surrounding tissues, the quiescent centre is enriched in transcripts related to hormones, signalling, metabolism and cell division in *Arabidopsis*, though mutants for these genes show no change in phenotype [42]. This can be explained by functional redundancy, which may ensure the normal development of the root apex even if certain genes encoding transcription factors (bZIP, AP2, NAC, Zinc finger, Myb, MADS) related to flowering exhibit mutations. The triple mutant *ntra ntrb cad2*, which is defective in thioredoxin reduction and GSH synthesis, lost apical dominance and had vasculature defects and reduced secondary root formation [13]. This mutant has a higher percentage of GSSG due to the reduced amount of the total glutathione. Since the reduction of both thioredoxins and GSSG are NADPH-dependent, the changes observed in the mutant may indicate that NADPH plays an important role in the redox control of root development. Certain antioxidants and ROS may coordinate the development of roots and shoots due to their transport between the two organs as described for ascorbate in poplar [7]. Based on the observations cited in this section it is feasible that the alteration in the redox state of GSH/Asc

is not only an indicator of the general redox changes, but it is part of a redox regulatory mechanism.

Besides the vegetative organs, characteristic changes in the ROS level were found during the growth of the flower buds, their opening and after fertilization [43]. Until the opening of flowers H_2O_2 was the most abundant ROS in stigma of olive, however H_2O_2 levels decreased parallel to the increase in $\text{O}_2\cdot^-$ concentration after the adhesion of pollen grains. An increase in H_2O_2 and $\text{O}_2\cdot^-$ concentrations was found during the release of mature pollen grains in anthers of olive. These observations indicate that the various ROS may have specific functions during the flower bud formation, opening and after fertilization in stigmas and anthers. However, it would be interesting to know which spatial and temporal changes occur in ROS level in these parts of the flower at subcellular level, and which effects do they have on metabolism.

2.4. Redox control of senescence and cell death

The redox control of senescence and death processes was also shown in plants [9,44]. The mechanism of the redox regulation of apoptosis was clarified in animals, where GSH depletion was found to precede ROS accumulation [45]. This is an active process, since the activation of death receptors led to GSH extrusion across the plasma membrane. GSH may affect apoptosis through the posttranslational modification of target proteins and may subsequently activate caspases and inactivate anti-apoptotic signals. The signalling pathways of apoptosis-like processes and the involvement of ROS and antioxidants need to be clarified in plants [44]. Cell death is an integral part of plant growth and development, as observed in the tapetum cells of anthers, during the differentiation of tracheary elements in the xylem, during the elimination of suspensor cells, and in senescing leaves and petals. The redox control of senescence was shown by the artificial ageing of *Lathyrus pratensis* seeds, in which a decrease in seed viability was correlated with the half-cell reduction potentials of thiols [9]. Thus, this parameter is a good marker for viability. Although the determination of the reduction potential in tissue extracts is possible, its measurement would be even more important at cellular or subcellular level for the better understanding of redox processes. The redox-sensitive GFP can be used for the general characterization of the redox environment, but not for that of the individual redox pairs.

2.5. Redox control of stress response

ROS and antioxidants have an important role in the reprogramming of the metabolism in stressed plants, leading to a reduction in growth and development and to the activation of defence processes [2,6]. The regular daily diurnal fluctuations in antioxidants disappear under stress conditions, as observed for the activity of the enzymes involved in the GSH metabolism [46]. Thus, the activity of adenosine phosphosulphate reductase required in the synthesis of the GSH precursor Cys, GSSG reductase and GSH S-transferase remained continuously high during chilling of maize. Interestingly, a similar effect was observed for nitrate reductase, indicating the coordinated control of sulphate and nitrate reduction. A correlation was found between freezing tolerance and the H_2O_2 concentration, and the amounts and redox state of ascorbate and glutathione in wheat genotypes with different levels of freezing tolerance after 3 weeks of cold hardening [30]. The redox environment became more oxidising after 3 weeks at 2 °C as shown by the lower Asc and GSH contents and GSH/GSSG and Asc/DHA ratios. Similarly, salt stress decreased the GSH/GSSG ratio in salt-stressed olive, however the NAD(P)H/NAD(P)^+ ratios were not affected, and the Asc/DHA ratio even slightly increased (Table 1, 31). The modification of the redox environment by salt treatment affected embryo development in *Dactylis glomerata* suspension

cultures, where more reducing conditions induced the proliferation and formation of pro-embryogenic masses, while more oxidizing conditions led to differentiation and to the formation of somatic embryos [47]. Depending on the intensity and length of the unfavourable environmental conditions, different modifications in growth and development will be induced ranging from the stress adaptation through the stress-induced early flowering until the death of the plants (Fig. 1). Consequently, the individual ROS and antioxidants and the changes in their concentrations may have specific roles in the determination of the alterations in growth and development of stressed plants. However, this hypothesis should be clarified in further studies in which ROS and antioxidants will be monitored at different organization levels.

2.6. Model of the redox control of growth and development

Redox changes in the plants are either triggered by the environment or are genetically determined (Fig. 1). The actual redox state is a result of the interaction of the two effects. Seasonal and annual changes in the environmental conditions are important for the normal course of growth and development and, similarly to their mild irregular alterations, for stress adaptation. However, large environmental changes may lead to injury or death of the plants due to the damage to macromolecules (lipids, proteins, nucleic acids). Certain part of the spatial and temporal redox changes is genetically determined; among others cell-specific differences in root apex or shifts in the redox environment during development (see Sections 2.3 and 2.4). The different types of redox changes affect growth and development through signalling networks due to the reprogramming of the transcriptome, proteome and metabolome. In this network changes in the combinations of different redox states of various redox pairs and in the concentration of various ROS and RNS could be the basis of a very complex redox regulatory system, affecting gene expression and enzyme activities and subsequently energy use and the metabolism.

3. Participation of reactive nitrogen species in the regulation of plant growth and development

3.1. General effect of reactive nitrogen species

In contrast to ROS, there are only few data on diurnal alteration of reactive nitrogen species (RNS). NO emission exhibited a typical diurnal cycle in an antisense nitrite reductase transformant tobacco plants which accumulated nitrite 5-fold over wild type and emitted much more NO during the whole light/dark cycle [48]. The diurnal cycle in NO content may be due to the diurnal alterations in NR activity [46] which enzyme is involved in the NO formation. NO generation may also depend on light in the chloroplast [14]. In addition, some RNS are formed by the interaction of NO with ROS or antioxidants, thus RNS control several metabolic pathways having regular temporal fluctuations.

Nitric oxide is an important signal in various physiological events in plants, such as germination, root development, stomatal closure, flowering, nodule formation, leaf senescence, cell death, and the acclimation of plants to abiotic and biotic stresses [49].

NO integrates into plant signal transduction and interferes with other signalling pathways by the S-nitrosylation of specific signalling intermediates, protein kinases, phosphotransfer proteins and transcription factors. Due to NO-induced activation of guanylate cyclase, there are transient increases in cyclic guanosine monophosphate levels that are also implicated in NO-induced signalling. The other reactive form of nitrogen, ONOO^- may control signal transduction through the nitration of specific tyrosines which otherwise are targets of phosphorylation [50].

3.2. Effect of NO on germination

The NO-induced breakage of seed dormancy can be detected in several species, and this is coupled with reduced abscisic acid accumulation. NO may regulate respiratory oxygen consumption in germinating seeds. Nitrite, as an alternative electron acceptor, was found to generate NO as an alternative electron acceptor in the mitochondrial electron transport chain. This process maintains the oxidation state of the accumulated NAD(P)H and contributes to ATP synthesis under hypoxic conditions in the seeds. Both non-enzymatic and enzymatic NO production is of special importance during germination which acts in correlation with ethylene, carbon monoxide, H₂O₂ and gibberellic acid in breaking seed dormancy [51]. A NO-releasing compound, NOC-18 markedly increased GSH accumulation in recalcitrant seeds of *Baccaurea ramiflora*, and enhanced the activities of the antioxidant enzymes involved in the glutathione-ascorbate cycle. It also decreased the H₂O₂ content in the embryo, which improved the germination percentage and chilling tolerance of seeds [52]. NO enhanced osmotic stress tolerance and promoted the germination of wheat under high salinity by increasing the respiration rate and ATP synthesis [53]. These observations indicate that there is a cross-talk between NO, hormones and ROS signalling and the details need to be clarified by following the temporal and spatial regulation of ROS and RNS production in tissues, cell types and cell compartments of seeds during dormancy release.

3.3. Effect of NO on growth and organ development

NO is involved in the control of the elongation growth of plant organs. The hypocotyl length of *Arabidopsis* seedlings was reduced by GSH treatment and by the inhibition of nitrate reductase and NO synthase [54]. The authors concluded that well-balanced pools of reductants/oxidants are essential for hypocotyl elongation and that this process is controlled by NO in etiolated *Arabidopsis* seedlings [54]. These results indicate that it would be interesting to dissect the role of the individual RNS, ROS and antioxidants in the apoplast in order to elucidate their role in the control of elongation growth.

NO was observed to affect growth not only under optimal conditions, but also in stressed plants. Exogenous NO generated from sodium nitroprusside [Na₂[Fe(CN)₅NO] alleviated salt stress injury in cucumber [55]. The NO donor enhanced the elongation of hypocotyls and radicles, stimulated ROS-scavenging enzymes, decreased the H₂O₂ content and reduced lipid peroxidation in tissues exposed to high salinity. Salt-induced ultrastructural damage in the mitochondria and cell walls of root tip cells could also be diminished by NO treatment [55]. The protective effect of NO against salt stress was mediated by antioxidants, suggesting an interaction between RNS, ROS and antioxidants during the stress response.

The development of root system and the formation of lateral and adventitious roots are regulated by hormonal and environmental factors. Auxin-induced lateral root development is mediated by NO, which is associated with the activity of nitrate reductase in *Arabidopsis* [56]. Auxin-mediated differentiation of adventitious roots in the hypocotyl base of sunflower can be divided into an NO-independent induction phase and NO-dependent initiation and extension phases. Treating hypocotyl explants with 1-naphthylphthalamic acid, an inhibitor of basipetal auxin transport, prevented NO accumulation and adventitious root development. This suggests that polar auxin transport and the appropriate location of auxin efflux carriers (PIN proteins) are necessary for NO-mediated root initiation [57]. The involvement of NO in the development of reproductive organs was shown in olive, in which its level increased both in stigmas and anthers when the mature pollen grains were released [43].

3.4. Role of NO in the senescence and plant cell death

NO is an anti-senescence agent and the mechanism through which NO may counteract senescence is related to preventing the formation of deleterious ROS, lipid peroxidation and chlorophyll degradation. NO may inhibit ethylene biosynthesis, a senescence promoting factor thus prolongs the post-harvest life of horticultural plants. An NO-deficient mutant (*nos1 noa1*—the corresponding protein is not a NO synthase as it was originally thought but a GTPase, therefore it effects NO levels only indirectly) of *Arabidopsis* has an early senescence phenotype [58]. Both chlorophyll degradation and the up-regulation of senescence marker genes were inhibited and senescence was delayed in *ein2-1/nos1 noa1* double mutants, suggesting that EIN2, a positive regulator of ethylene signalling, is involved in the early senescence of *nos1 noa1* mutants caused by NO deficiency [58]. NO can modify fruit ripening, a specific type of organ senescence by the direct control of ROS-scavenging enzymes or by interfering with the relevant signalling cascades. In kiwi fruit ROS effects were reversed by NO via the up-regulation of genes coding for superoxide dismutase and catalase and via the suppression of lipoxygenase [59].

The role of NO in cell death programme was studied as well. The NO and GSNO generated during nitrosative stress may participate in deleterious nitrosylation reactions. Rice *noe1* mutant was identified due to a higher S-nitrosothiol content, an increased expression of nitrate reductase and an increased generation of NO. Moreover, the mutant proved to be catalase deficient, contained more H₂O₂ in leaf tissues and exhibited a leaf cell death phenotype after high light exposure. Removal of NO reduced the cell death suggesting that NO is an important endogenous mediator of H₂O₂-induced leaf cell death [60]. In animal cells glyceraldehyde-3-phosphate dehydrogenase was identified as one of the first targets of S-nitrosylation. This modification facilitated the nuclear transport of the glyceraldehyde-3-phosphate dehydrogenase complex, leading to the initiation of apoptosis [60]. A similar nitrosylation of glyceraldehyde-3-phosphate dehydrogenase occurred in tobacco suspension culture cells but S-nitrosylation affected only a small proportion of the glyceraldehyde-3-phosphate dehydrogenase population [61]. The S-nitrosylation of the proenzyme form of metacaspase 9 (AtMC9), an apoptosis-associated cysteine-protease in plants, keeps the enzyme in the inactive, unprocessed form suggesting that there is a NO-dependent control of protein degradation during cell death [17]. The uncontrolled accumulation of ROS and NO may result in detrimental, irreversible processes. A simultaneous increase in NO and H₂O₂ led to the activation of cell death in tobacco BY-2 cells [44] and in tomato cell suspension cultures during salt stress [62]. Several forms of reactive nitrogen and oxygen can chemically react with each other or can mutually promote the synthesis of one another suggesting that RNS and ROS may function in combination to initiate cell death.

3.5. The source of NO during various developmental processes and environmental conditions

It seems likely that plants use alternative NO-producing systems in various physiological contexts. The distinct regulatory characteristics of the NO producing systems in various cell compartments determine the interaction with reactive oxygen forms. Arg-dependent NO generation can be detected in plastids, mitochondria and peroxisomes during salt stress, P-starvation and pathogen defence [15]. Nitric oxide synthase-like activity contributes to NO production in response to high temperature stress, drought, wounding and in the stomata of *Brassica juncea* [14,63]. In contrast, nitrate reductase-dependent NO production could be observed during the abscisic acid-induced closure of the stomata in *Arabidopsis thaliana* [64]. Apoplastic NO may be generated by

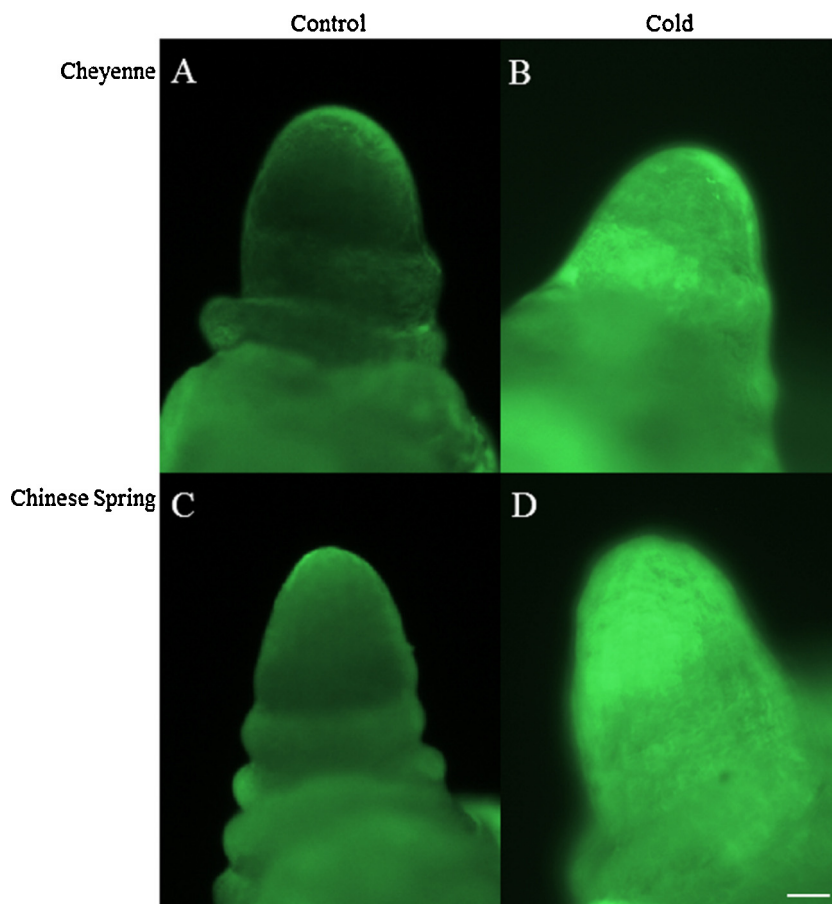


Fig. 5. Cold-induced NO accumulation in shoot apex of wheat. NO was investigated in apices of 5-week-old seedlings of the freezing-tolerant winter wheat (*Triticum aestivum*) variety, Cheyenne (A and B) and the moderately freezing-sensitive spring wheat variety, Chinese Spring (C, D) cultivated at 20/17 °C (A and C) or at 5 °C (B and D) for additional two weeks. NO was detected by 4-amino-5-methylamino-2'-7'-difluorofluorescein (DAF-FM) dye using a Zeiss Axiowert 200 M fluorescent microscope. Bar represents 50 μm .

plasma membrane-bound nitrate reductase and nitrite-NO reductase in roots [65]. In the case of oxygen deficiency the main NO producers are the mitochondria [14]. The low-temperature-induced accumulation of NO was observed in shoot apices (being in the vegetative phase) of 5-week-old winter wheat, the freezing-tolerant variety Cheyenne and in the moderately sensitive variety Chinese Spring (Fig. 5; control staining: Supplementary Fig. S1). The origin of the NO in cold-treated wheat could be the subject of future experiments.

RNS, like ROS, are involved in the mediation of regular and irregular environmental fluctuations and the appropriate adjustment of the metabolism, growth and development of plants, but the molecular mechanisms underlying several important events in plant life remain to be discovered. In contrast to ROS, our knowledge on the implication of NO or other nitrosylated or nitrated derivatives in cell cycle re-entry, cell cycle progression and cell division is still only fragmentary. However, the results obtained in mammals provide promising guidelines for plant research.

4. Cross-talk between hormones and redox systems during growth and development

4.1. Interactions between the redox systems and hormones

Plant growth and development as well as plant interactions with the environment are regulated by a complex network involving not only ROS and RNS, but also plant hormones. As in the case of ROS, daily fluctuations also occur in the levels of most hormones [e.g., 66]. Thus, hormone metabolism is adjusted to daily changes

in light and temperature and also to long-term seasonal changes in environmental conditions. Apart of these regular rhythms, hormone metabolism is affected by actual developmental stage and environmental factors.

ROS and RNS are integral parts of the plant hormone signalling pathways. Dynamic relationship between plant hormones and ROS is indicated by the fact that ROS induce the synthesis of several hormones, such as ethylene, salicylic acid, jasmonate and brassinosteroids [67]. On the other hand, some hormones (e.g. abscisic acid, salicylic acid or auxin) also induce ROS production. Plant hormones also affect the redox equilibrium by stimulation of the transcription of genes encoding components of the redox system, e.g. glutathione S-transferase [68]. Cytokinins stimulate the NO catabolism, thus suppressing the responsiveness to NO [69]. Peroxynitrite, a product of NO interaction with superoxide, was reported to react directly with *trans*-zeatin *in vitro*. Several nitro- and nitroso-*trans*-zeatin and isopentenyladenine derivatives were identified in *Arabidopsis continuous NO-unstressed cnu1-1* and *1-2* mutants having elevated levels of cytokinins. N⁶-nitro- and N⁶-nitroso-zeatin exhibited similar biological activity as *trans*-zeatin, 8-nitro-zeatin was much less effective. The occurrence of 8-nitro-*trans*-zeatin in plant tissues suggests that the reaction of cytokinins with ONOO⁻ may control the RNS level *in vivo*.

4.2. Coordinated redox and hormonal regulation of metabolism and development

Cross-talk between ROS and hormones regulates many metabolic and developmental processes in plants. Brassinosteroids

were reported to induce an increase in hydrogen peroxide, which accumulated on the cell walls of mesophyll cells facing the inter-cellular spaces [70]. Appropriate hydrogen peroxide accumulation resulted in an increase of the ratio of GSH to GSSG, elevation in the content of Rubisco activase, activation of Rubisco and enhanced CO₂ assimilation [70]. Cell cycle progression from the G₀ to the G₁ phase requires both ROS generation and auxin [36]. The cell elongation associated with cell wall loosening is associated with ROS production, after being stimulated by auxins and brassinosteroids [71]. Nodulation is a complex process requiring both ROS and a number of hormones (cytokinins, auxins, gibberellins, abscisic acid, ethylene, salicylic acid, jasmonate and strigolactones) [72]. Brassinosteroids promote cadmium stress tolerance by elevating the activities of antioxidant enzymes [73]. A similar inducing effect on the activity of antioxidant enzymes is exhibited by cytokinins, which delay chlorophyll degradation and preserve chloroplast integrity, e.g. during dark-induced senescence [34]. These results show that many effects of hormones are mediated by ROS. ROS may enable a convergence of different hormone signalling pathways (e.g. stimulation of stomatal closure by both abscisic and salicylic acid). Control of NO level, which is up-regulated by abscisic acid and down-regulated by cytokinins, may underlie the antagonistic relationship between these two hormones.

One of the best characterized interactions between hormones and the redox system is the control of root apical meristem organization. As mentioned above, the roots contain a quiescent centre, a group of cells necessary for the maintenance of meristematic activity in the root tip. The slowly dividing cells of the quiescent centre, which remain predominantly in the G₁ phase, are in a highly oxidized status (high content of DHA and GSSG; high level of superoxide and hydrogen peroxide) together with a very high level of auxin [25]. In contrast, the adjacent proximal meristem cells have a reduced redox state and rapidly dividing cells. The redox and auxin gradients ensure the optimal size of the quiescent centre. NADPH-dependent thioredoxin and glutathione systems strongly affect the auxin metabolism and transport. The down-regulation of these processes has severe developmental consequences, resulting in smaller root meristems and the pin-like shoot phenotype [13]. GSH levels influence the expression of the auxin efflux carriers PIN1, PIN2, PIN3, PIN4 and PIN7 [13,40].

Root apical meristem behaviour is very dynamic, responding to multiple environmental stimuli, especially to nutrient availability and abiotic stresses. Phosphate deficiency induces a reducing environment in the quiescent centre, coinciding with cell differentiation and leading to determinate primary root growth [74]. Simultaneously, the stimulation of lateral root formation is initiated in areas rich in phosphate.

4.3. Cross-talk between plant hormones and ROS during plant interactions with the environment

Intensive cross-talk between ROS, RNS and abscisic acid occurs in developmental stages associated with dehydration (e.g., during seed desiccation), during diurnal temperature variations (in order to maintain leaf water potential), or during the responses to abiotic stresses, especially those associated with dehydration (drought, salinity and cold). Abscisic acid stimulates the production of ROS and RNS in the guard cells, resulting in stomatal closure as well as big changes of the transcriptome. Hydrogen peroxide production is mediated by the NADPH oxidase catalytic subunits AtrbohD and AtrbohF [75]. Hydrogen peroxide activates the Ca²⁺ channels in guard cell plasma membranes, thus contributing to stomatal closure. Abscisic acid also triggers generation of NO, which is indispensable for stomatal closure, stimulating both MAPK activity and cyclic guanosine monophosphate production [64].

Hydrogen peroxide activates, usually after biotroph attack, synthesis of salicylic acid, which in turn induces H₂O₂ accumulation [26]. This self-amplifying loop is necessary for the formation of leaf necroses, which limits pathogen spreading in the plant. Lipopolysaccharides, e.g., from gram-negative bacteria, cause NO generation [76]. Salicylic acid and NO function in a positive feed-back loop. Both induce changes in the cellular redox state, which result in the monomerization of NONEXPRESSOR OF PATHOGENESIS-RELATED PROTEINS1 (NPR1) oligomers held in cytosol together by disulphide bridges. NONEXPRESSOR OF PATHOGENESIS-RELATED PROTEINS1 monomers are translocated into the nucleus, where they interact with TGA transcription factors and induce a transcription cascade [26]. NONEXPRESSOR OF PATHOGENESIS-RELATED PROTEINS1 is essential for salicylic acid signal transduction, being the key regulator of systemic acquired resistance.

Salicylic acid stimulates the production of ROS by peroxidases as well as of NO [77] in guard cells, which results in stomatal closure. Salicylic acid increases plant tolerance not only by regulating stomata, but also by stimulating the activity of the antioxidant system. Exogenous application of salicylic acid resulted in an elevation in the level of hydrogen peroxide and in the activities of antioxidant enzymes in maize [27]. An increase in the activities of a number of antioxidant enzymes was also observed after pre-soaking of pea seeds in salicylic acid [29].

The interplay between ROS and hormones is also very intensive in the case of the exposure to ozone. ROS generated from ozone degradation may either cause direct necrotic damage or induce apoptosis. Salicylic acid and ethylene control and promote ROS formation and lesion propagation, while jasmonate is involved in limiting lesion spread [78]. The aerenchyma formation, associated with the response to flooding or sulphur deficiency, requires both ethylene function and ROS formation [79].

4.4. A model for the cross-talk between hormones and redox systems

These data clearly show that the integration of developmental and environmental signals is modulated by cross-talk between ROS, RNS and plant hormones, which significantly affects both developmental changes and stress responses [28]. ROS and NO occupy a central hub position in this cross-talk and mediate the effects of various hormones on metabolic and physiological processes (Fig. 6). The spatial and temporal changes in the levels of ROS, NO and hormones at various organization levels are very important in the operation of this regulatory network. The concentration-dependent effect of ROS and their interaction with auxin was described in the case of cell cycle [36]. Similar effect can be supposed in the control of growth and development in general, including both activation and inhibition of certain signalling pathways depending on the concentration of ROS, NO and hormones. The scheme corresponds to the recent state of knowledge; it is likely that new interactions and/or additional players will be described in the future.

5. Subcellular importance of oxidants and antioxidants for plant growth and development

5.1. Chloroplasts

Plant growth and development depend greatly on the subcellular distribution and accumulation of ROS, antioxidants such as ascorbate and glutathione, and the redox state of certain cell compartments. In this context the state of the chloroplasts is decisive for plant growth and development: during photosynthesis the electron transport chain delivers the reducing power for the plant

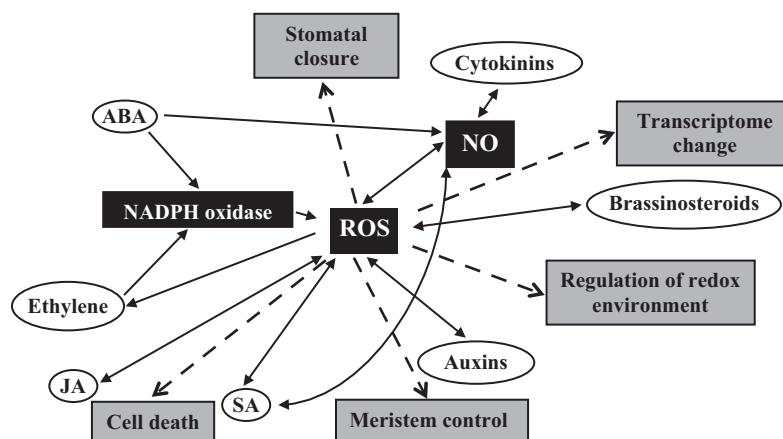


Fig. 6. Cross-talk between hormones, ROS and NO during the regulation of the redox environment. According to this simplified model, ROS and NO have a central role in the mediation of hormonal effects on the cellular redox environment as well as various physiological and molecular processes influencing plant growth and development. The spatial and temporal changes in the levels of ROS, NO and hormones at various organization levels are very important in the operation of this regulatory network. The concentration-dependent effects of ROS and their interactions with hormones are supposed to control growth and development in general, including both activation and inhibition of certain signalling pathways. Interactions between ROS, hormones and NO occur in this network. ABA: abscisic acid, JA: jasmonate, ROS: reactive oxygen species, SA: salicylic acid.

metabolism. The photosystems within the thylakoids of the chloroplasts are the major sources for ROS production in plants [4]. So far most research has focused on investigating the direct toxic effects of the ROS in the chloroplasts on the plant metabolism, but it has been recently acknowledged that ROS accumulation in the chloroplasts during stress situations also activates signalling events and causes changes in the redox state that have a great impact on the metabolism as well as on gene transcription and protein synthesis [4,21]. In this context the accumulation of ROS in the chloroplasts seems to be involved in the regulation of cell death events [80]. In *flu* mutants the generation of singlet oxygen in the chloroplasts within the first minute of illumination correlated with the inhibition of plant growth and the development of necrotic lesions [80]. Based on the study of gene expression patterns in *flu* mutants and further studies involving the *EXECUTER1* gene the authors concluded that changes in the growth and development of these mutants after illumination did not reflect the direct toxic effects of singlet oxygen but rather its role as a signal initiator that activated several stress-response pathways [80]. In this context the *EXECUTER1* protein, which is a highly conserved protein in plastids, seems to be essential as it allows plants to sense singlet oxygen as a stress signal which leads to the activation of a genetically determined stress response program [80]. These results support the conclusion that cell death events during stress conditions are not only caused by the direct damage of an excess of ROS in the chloroplasts, but are also indirectly triggered by signalling events induced by ROS in the chloroplasts (or in other cell compartments). Singlet oxygen signalling is interconnected with other ROS signals as it was shown for H_2O_2 [6]. In a *flu* mutant overexpressing the gene of thylakoid-ascorbate peroxidase the activation of nuclear genes related to the *EXECUTER1* pathway was strongly decreased [6]. The involvement of H_2O_2 and $O_2^{\bullet-}$ in the redox regulation in chloroplasts was clarified by redox proteomic approach and several common target proteins of the two ROS have been identified [see Section 2.1, 11]. A regulatory role for NO was also shown in the chloroplasts, where a large part of the cellular NO is localized [81]. NO inhibits the photosynthetic electron transport; it can therefore regulate photooxidative stress if the rate of NADPH and ATP production exceeds the demand for the carbon reducing cycle [82]. In order to understand how the interplay of ROS and antioxidants in the chloroplasts affects plant growth and development, future research should focus on unfolding redox signalling networks regulated by ROS and antioxidants in the chloroplasts

and on identifying the role of the components involved in these pathways.

5.2. Vacuoles

The vacuoles have an important role in the regulation of the subcellular redox environment and metabolism. During the degradation of H_2O_2 by vacuolar peroxidases phenols are oxidised to form phenoxyl radicals [83]. These phenols are then reduced by Asc, which was detected in elevated concentrations in vacuoles subjected to high light stress [84]. The sequestration of GSSG in the vacuoles (10-fold increase in its concentration) seems to be an important mechanism by which the *cat2* mutant (20% catalase activity compared to the control) avoids the negative effects of GSSG accumulation, such as lesion formation, dormancy or cell death [24]. The sequestration of GSSG and the detoxification of H_2O_2 in the vacuoles may be involved in the control of the cytosolic redox potential and the redox state of target molecules, and subsequently in the regulation of cell division, differentiation and defence. Future research should focus on resolving the extent of H_2O_2 leakage into and accumulation in the vacuoles and to what extent the vacuoles act as a sink for GSSG in the case of oxidative stress. Such research is needed in order to clarify the importance of these pathways for plant growth and development.

5.3. Mitochondria

The glutathione content and redox state in the mitochondria are also important for proper plant development. Growth defects in glutathione-deficient *rml1* mutants, such as lack of root meristem, development of short shoots, and smaller rosettes, inflorescences, and flowers, were correlated with low levels of glutathione (3% of wild type plants) in the mitochondria [85]. In contrast, the glutathione deficient *pad2-1* mutant, which develops a phenotype similar to the wild type, contains wild type glutathione levels in the mitochondria despite a strong decrease of glutathione levels (of up to 90%) in all the other cell compartments [86]. Thus, it is obvious that high, stable levels of glutathione (and ascorbate) in the mitochondria are essential for proper plant growth, development and survival, as they keep ROS levels under control in this cell compartment. The depletion of antioxidants in the mitochondria favours the accumulation of ROS, which can trigger cell death [20]. Thus, it is not surprising that the levels of antioxidants

such as ascorbate and glutathione in the mitochondria can reach concentrations of up to 10.4 and 15 mM, respectively, and that the glutathione contents are higher in the mitochondria than in any other cell compartments [84,87]. There is a strong drop of ascorbate and glutathione contents during darkness-induced senescence and a shift towards their oxidized forms were correlated with a great accumulation of H₂O₂ in the mitochondria of pea leaves [88]. The importance of the mitochondria in this context is further supported by the observation that distorted leaf development during aging in *Arabidopsis* mutants lacking a mitochondrial protease was correlated with elevated levels of ROS, increased amounts of oxidatively damaged mitochondrial proteins and the impairment of organelle development due to mitochondrial dysfunction [89]. Thus, it seems that the drop in antioxidant contents in the mitochondria during aging favours the accumulation of ROS in this cell compartment, leading to mitochondrial dysfunction and senescence. Future research in this area should concentrate on the role of interplay between antioxidants and ROS in the mitochondria in cell death events and the importance of high, stable levels of glutathione in the mitochondria for plant growth and development.

5.4. Nucleus

There is increasing evidence that the interplay of ROS and antioxidants in the nucleus has an important role in successful cell proliferation and subsequently in plant growth and development. In addition, the ROS in the nuclei are important for signal transduction between the nuclei, chloroplasts and cytosol, especially under stress conditions [21]. The ascorbate and glutathione contents in the nuclei of fully developed leaves can reach levels of up to 16.3 and 9.72 mM, respectively [84,87]. Whereas the ascorbate contents in non-stressed leaves were similar to those in the cytosol, the glutathione contents were as much as double those in the cytosol [87], indicating the importance of high antioxidant levels in the nuclei. Large amounts of GSH (up to 80% of the total cellular pool) co-localize with nuclear DNA during early stages of cell proliferation, and presumably at the G1 and S phases during the cell cycle [19]. The sequestration of GSH into the nucleus could not be prevented by H₂O₂ accumulation in the cytosol, indicating that even in situations of severe oxidative stress, GSH is transported into the nuclei at the expense of the cytosolic GSH pool [19]. The observed accumulation of H₂O₂ in the cytosol [19] was similar to the situation in animal cells, where oxidation events early in the G1 phase are an essential activator of signalling events leading to cell proliferation [90]. The depletion of GSH by buthionine sulfoximine inhibited the transition of the plant cells from the G1 to the S phase [91]. Low levels of ascorbate in the quiescent centre of the root seem to be responsible for keeping these cells in the extended G1 state [33]. Even though the latter two studies did not specifically evaluate the situation of antioxidants in the nuclei, it seems likely that the accumulation of GSH and high levels of ascorbate in the nuclei during the G1 phase represent an important strategy in the antioxidant defence mechanism, allowing the cell cycle to be continued [19]. If the redox balance in the nuclei is disturbed, the DNA is damaged possibly leading to mutations or eventually cell death [19].

5.5. Peroxisomes

Peroxisomes are a major source for ROS and RNS production especially during abiotic and biotic stress conditions [22,23]. Ascorbate and glutathione reach concentrations of 23 and 4 mM, respectively, under non stressed conditions in this cell compartment [84,91]. Ascorbate contents in peroxisomes are highest among all organelles indicating the importance of high levels of antioxidants in this cell compartment for proper cell metabolism.

A strong drop of antioxidative enzymes, an overproduction of singlet oxygen and H₂O₂ and a drop of NO in peroxisomes during senescence indicate an important function for this cell compartment in ROS-mediated senescence [22,23]. The upregulation of antioxidative enzymes in peroxisomes during abiotic stress conditions such as the exposure to cadmium and excess heat seems to be an important defense mechanism against ROS and RNS [22,23]. ROS and RNS produced in peroxisomes together with catalase and other players involved in the ascorbate and glutathione metabolism have also signaling functions in plants during abiotic and biotic stress conditions which can lead to physiological and developmental adaptations of plants and programmed cell death [22,23]. Thus, we can conclude that peroxisomes play an important role in cellular oxidative metabolism as they generate ROS and RNS during senescence, abiotic and biotic stress conditions and release them into other cell compartments where they lead to modifications in defense gene expression. Future research should focus on unraveling the exact mechanisms behind possible signaling functions of ROS/RNS and antioxidants produced in peroxisomes during abiotic and biotic stress conditions in order to reveal their importance for plant growth and development.

5.6. Integrated cell

It can thus be concluded that, the levels of ROS, RNS and antioxidants and the redox state of the chloroplasts, mitochondria, nuclei and peroxisomes have an important role in plant growth and development. ROS were suggested to participate in the signalling between the various organelles and nucleus and the cytosolic antioxidants may fine-tune these signals [6]. In addition, movements of chloroplasts, mitochondria and peroxisomes in the cytosol and interactions between the organelles may further affect the intracellular redox signalling.

Low levels of antioxidants in the nuclei inhibit cell proliferation, while low levels of glutathione in the mitochondria favour the accumulation of ROS, leading to growth defects and senescence. The ROS in the chloroplasts seem to be involved in signalling events that can trigger cell death. ROS and RNS in peroxisomes are involved in physiological adaptations during senescence and stress conditions and are used as signalling agents in order to modify defence gene expression. The sequestration of H₂O₂ and GSSG into the vacuoles is important in avoiding negative effects of these components in other cell compartments. Thus, the fine tuning of the antioxidants, ROS, RNS and redox state in these cell compartments is essential for proper plant growth and development under both non-stress and stress conditions. Future research in this area needs to clarify the interplay between ROS and antioxidants in individual cell compartments. It should be investigated how large changes in these parameters trigger signalling events that interfere with the growth and development of the whole plant.

6. Redox control of flowering time

6.1. Adjustment of flowering to environmental fluctuations

Organisms measure alterations in day lengths using their circadian clocks and by sensing light and temperature conditions for the best timing of vegetative reproductive transition. Various pathways help to adjust flowering to these fluctuations: the photoperiod pathway, the gibberellin pathway, the autonomous pathway, the vernalization pathway, and the most recently described 'aging' pathway [92]. Recent reviews have discussed the relationships between abiotic stress and the plant circadian clock, and between the circadian clock and photoperiodism, and have also dealt with the effect of the circadian clock on determination of flowering

time [1,92]. From these studies it became clear that not only are the phytochrome and cryptochrome photoreceptors important for oscillator control under diurnal cycles of light and dark, but that feedback from the levels of primary and secondary metabolites, including ROS, RNS and antioxidants, also appears to have an influence on oscillator function. At the same time, the circadian clock is responsible for controlling and amplifying output signals, determining the timing of various metabolic and developmental processes, including flowering.

6.2. Control of flowering by ascorbate and glutathione

The involvement of both the Asc/DHA and GSH/GSSG redox couples has been widely implicated in the control of the flower meristem initiation [93–95]. The reducing power required for the Asc-GSH cycle is ensured by NADPH, so the modification of its redox state by the overexpression or suppression of a calcium-dependent NADPH dehydrogenase present in the mitochondrial electron transport chain resulted in earlier or later bolting, respectively [96]. Alterations in the endogenous Asc content affected the flowering time in four Asc-deficient (*vtc*) *Arabidopsis* mutants [97], which flowered and senesced before the wild type, irrespective of the photoperiod. This difference was accompanied by the significantly higher expression of genes related to the circadian clock and the photoperiodic pathway in the mutants. In contrast, flowering was delayed when the Asc content was artificially increased, which was correlated with lower mRNA levels of circadian clock-related genes compared with control plants treated with water [97]. The authors argued that flower induction was affected by Asc itself, not by changes in the redox environment during plant development. However, a short day treatment of the same mutants delayed development and flowering in an experiment of other authors [98]. The main difference between these two studies was that the short day-cultivated plants grew in a much higher light intensity ($250 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) [98] than the first group ($160 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) [97]. Higher light intensity might change the redox conditions, which could be the reason for the contrasting phenotypes. It is also worth mentioning that the glutathione contents in the *vtc1* and *vtc2* mutant lines were significantly higher than in the wild type. Changes in the glutathione level were not monitored during plant development under lower light intensity [97], although the contribution of GSH to the promotion of flower induction cannot be ruled out. The mechanism by which Asc directly affects the flowering time is not clear. Several authors hypothesised an interaction between Asc and plant hormones, including gibberellin, abscisic acid, ethylene and salicylic acid [95]. The most likely mechanism linking the homeostasis of Asc or more generally that of antioxidants and flowering time induction is discussed in Section 6.3. The regulatory effect of GSH on flowering was demonstrated in two rosette species, *Arabidopsis* and *Eustoma grandiflorum*, both by chemical modification of its level and using mutants [93,94]. The flowering of a late-flowering *Arabidopsis* mutant, *fca*, having increased expression of the *FLOWERING LOCUS C (FLC)* gene and a high GSH level, was hastened by decreasing GSH levels [93]. When studying seeds of an *Arabidopsis* line overexpressing the first enzyme of GSH synthesis, the same research group reported [99] that *FLOWERING LOCUS C* transcript levels increased at room temperature without any cold treatment. Unexpectedly, in the seeds of this mutant the GSSG levels were significantly higher than in the wild type seeds, and these levels were comparable to that recorded in seeds imbibed at low temperature. From this, the authors hypothesized that GSSG might be a key component in modulating flowering [100], possibly through the oxidation or glutathionylation of Cys in regulatory target proteins.

6.3. Mechanism of the redox regulation of flowering

The most likely mechanism by which the Asc/DHA and GSH/GSSG redox couples or redox changes in general influence the flowering time has recently been reported. The circadian clock regulates ROS homeostasis and ROS production and scavenging have time-of-day-specific changes [3]. The use of circadian clock mutants (*elf3-1*, *lux-1*, *toc1-1*, *cca1-1/lhy-11*, and *CCA1-ox*) demonstrated that a functional clock is required for the time-of-day-specific regulation of ROS production (Fig. 7). The response to ROS was found to be regulated by diurnal cycles, depending on the time of *CIRCADIAN CLOCK-ASSOCIATED1* gene expression. The authors proposed that the *CIRCADIAN CLOCK-ASSOCIATED1* gene was the master regulator of ROS homeostasis through association with the *Evening element* in the promoters of ROS genes [3]. The ROS signals also have a feed-back effect on other metabolic processes controlled by the oscillator [101]. The expression of the *FLAVIN-BINDING KELCH-REPEAT F-BOX1 (FKF1)* gene, which is an output signal of the circadian clock, had dramatic phase delays and dose-dependent lengthening in response ROS treatments. This is a very exciting finding, because a direct connection between *FLAVIN-BINDING KELCH-REPEAT F-BOX1* and flower meristem initiation has just been discovered [99].

The day length-specific expression of the *FLOWERING LOCUS T (FT)* transcription factor gene determines the timing of flower initiation both in dicot and monocot plant species (Fig. 7) [96]. Its transcription is directly activated by the *CONSTANS* transcription factor in the case of photoperiod-sensitive overwintering (biennial) plants. This occurs after fulfilment of the vernalization requirement under long days in the temperate zone of the northern hemisphere. The *LIGHT, OXYGEN, VOLTAGE* domain (a photoreceptor absorbing blue light domain which regulates the activity of other domains of the protein) of the *FLAVIN-BINDING KELCH-REPEAT F-BOX1* protein interacts with *CONSTANS* and stabilizes it in the afternoon on long days [101]. This protein is a blue light photoreceptor, and blue light stimulates this interaction. Moreover, it simultaneously removes the *CYCLING DOF FACTOR 1* which represses both *CONSTANS* and *FLOWERING LOCUS T* transcription. Constitutive *FLAVIN-BINDING KELCH-REPEAT F-BOX1* transgene expression stabilized the *CONSTANS* protein even in the early part of the day, while *FLOWERING LOCUS T* expression positively correlated with the *FLAVIN-BINDING KELCH-REPEAT F-BOX1* transcript level [102]. It is possible that ROS signals exert indirect effects on clock output pathways, thus *FLAVIN-BINDING KELCH-REPEAT F-BOX1* protein could be a potential link between the antioxidants and flowering. Based on these recent papers, a model of how alterations in ROS levels may affect the photoperiod-related flower induction pathway is suggested in Fig. 7.

6.4. Control of flowering by NO

NO also has a pivotal role in floral transition (Fig. 7). The induction of the reproductive phase was repressed by exogenous NO in *Arabidopsis*, while NO-deficient mutants flowered earlier. NO enhanced the expression of the *FLOWERING LOCUS C* gene coding for a MADS-box transcription factor, which is an inhibitor of flowering. Many factors, including the components of autonomous flowering, facilitate the transition to the reproductive phase by preventing *FLOWERING LOCUS C* mRNA accumulation. Moreover, NO suppresses *CONSTANS* expression, which mediates the effect of the circadian clock on flowering [103] (Fig. 7). *Arabidopsis thaliana* nitrate reductase-deficient double mutants (*nia1*, *nia2*) produced less NO, but had the same pattern of NO emission in the floral organs. NO accumulation exhibited tissue- and developmental phase-specific changes during floral development and occurred only in differentiated stigmatic papillae and in anthers

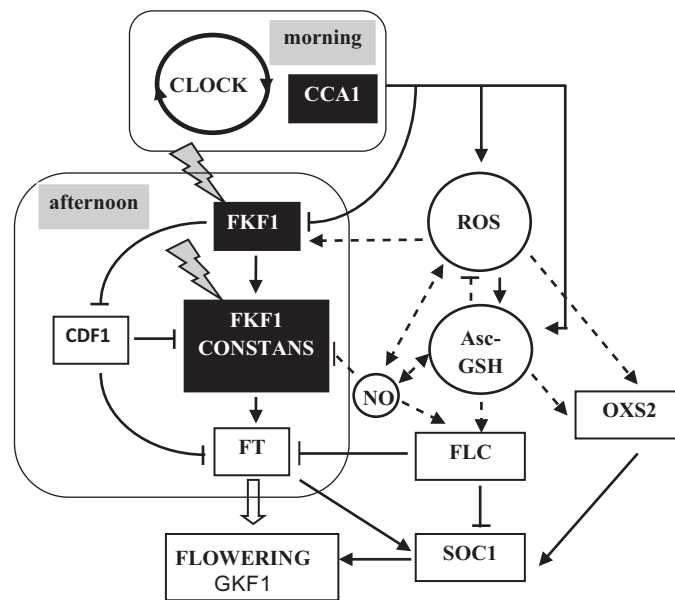


Fig. 7. Simplified model for the redox control of flowering. The left side of the figure shows the circadian clock-associated photoperiodic flowering pathway under long days. The *CIRCADIAN CLOCK ASSOCIATED 1* (*CCA1*) gene is expressed in the morning and represses the transcription of the *FLAVIN-BINDING KELCH-REPEAT F-BOX 1* (*FKF1*) gene. In the afternoon, when the proportion of blue light increases, *FKF1* degrades *CYCLING DOF FACTOR 1* (*CDF1*) and stabilizes *CONSTANS*. Together with *CONSTANS*, *FKF1* induces the expression of *FLOWERING LOCUS T* (*FT*) gene. The induction of flowering depends on the day length. *CCA1* also affects the transcriptional regulation of ROS-responsive genes as shown on the right side of the figure. Changing ROS levels affect *FKF1* expression independently of the clock regulation. ROS thus influence flower induction. The Asc-GSH cycle may also affect flowering time, most likely through the induction of *FLOWERING LOCUS C* (*FLC*) expression. Nitric oxide (NO) induces *FLC* expression and suppresses *CONSTANS* expression. The right side of the figure also shows the redox control of the stress-induced early flowering. Its central component is the redox-responsive *OXIDATIVE STRESS 2* (*OXS2*) transcription factor which regulates flowering due to its interaction with the *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1* (*SOC1*) gene. Solid lines indicate *CCA1*-, *FKF1*- and *OXS2*-dependent mechanisms. Dotted lines indicate Asc-, GSH-, ROS- and NO-dependent pathways.

producing pollen grains, while it could not be detected in sepals and petals. These nitrate reductase-deficient plants flowered precociously, indicating the role of nitrate reductase in flower induction [104]. In *Arabidopsis* plants, cadmium treatment resulted in earlier flowering due to the suppression of NO accumulation which in turn led to the up-regulation of the expression of the flowering promoters *CONSTANS* and *FLOWERING LOCUS T*, and to the inhibition of *FLOWERING LOCUS C* expression [105]. In addition, an NO donor delayed flowering, while an NO scavenger promoted it under Cd stress.

6.5. Role of RNS, ROS and antioxidants in abiotic stress-induced flowering

Although the results cited in Section 6 originated from carefully designed experiments, care must be taken when generalizing them from *Arabidopsis* for distant plant genera, because NO plays diverse roles in the growth and development of plants and in their responses to various abiotic stresses. Abiotic stressors may also promote the differentiation of reproductive organs by increasing ROS and NO production as well as by altering the Asc/DHA or GSH/GSSG ratios. The induction of early flowering will ensure the survival of the species under stress conditions; therefore the reproduction is a way of stress escape. Chilling stress increased the H₂O₂ and NO contents and induced panicle differentiation in mixed buds of the evergreen tree *Litchi chinensis* Sonn. [106]. This may be true for the Asc/DHA and GSH/GSSG redox couples as well. In wheat both the Asc and GSH concentrations and the Asc/DHA and GSH/GSSG ratios were correlated with the level of freezing tolerance after 3 weeks at 5 °C, but none of these parameters had any relationship with the developmental phase of the shoot apex when monitored for the determination of the vegetative/generative transition [30]. However, the treatment of a spring wheat variety with oxidants enhanced the speed of this transition (G. Kocsy, unpublished results).

The molecular background of the redox control of stress-induced early flowering was described recently in *Arabidopsis* [107]. The *OXIDATIVE STRESS 2* (*OXS2*) transcription factor affected by the thiol oxidizing agent diamide and the organic peroxide *tert*-butyl hydroperoxide controls abiotic stress-induced flowering by binding to the promoter of the *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1* (*SOC1*) gene (Fig. 7). The *OXIDATIVE STRESS 2* protein has dual roles. It is present in the cytoplasm and is necessary for normal growth while delaying flowering under optimal growth conditions. However, it was found to be transported to the nucleus after cold treatment where it interacted with the *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1* gene. This transport could also be induced by abscisic acid treatment, indicating the cross-talk between hormones and redox processes during stress-induced flowering [107]. The expression of the *OXIDATIVE STRESS 2* gene was not affected by day length, but diurnal changes in its transcript level due to indirect control by the circadian clock cannot be excluded (Fig. 7).

7. Conclusions and future directions

The redox changes in the plants are determined by an interaction of the genetic program and the environment. They lead to the reprogramming of the metabolism and consequently to alterations in growth and development. Although the participation of ROS, RNS and antioxidants in these processes has been intensively studied, there are still open questions in this field. According to a recent hypothesis, oxidants and antioxidants, which are opposing components of the redox signalling networks, may have individual signalling tasks within a given cellular compartment or niche [8]. The supposed compartment-specific functions have to be clarified. A more detailed study of the redox changes during cell growth, differentiation and division is necessary. What kind of effect do they have on the formation of cell compartments or at higher organization level on tissues and organs? The description of the specificity of

the individual ROS, RNS and antioxidants and of their interactions with hormone and secondary messengers would be also important. The study of the redox control of sexual processes especially that of the development of gametophyte would give many new results. The description of the redox-dependent spatial and temporal changes in the transcriptome, proteome and metabolome at various organization levels during growth and development and evaluation of the data by systems biology tools would provide new insights into converging and diverging redox signalling pathways. Besides its theoretical importance, a better understanding of the redox control of plant growth, development and flowering could be useful for the agriculture. Based on this knowledge, the modification of the cellular redox environment may be used to the increase the yield and stress tolerance.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.plantsci.2013.07.004>.

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