

Review Article

Production, functions and scavenging mechanisms of reactive oxygen species in plants under low-temperature stress

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Abstract: Under low-temperature stress, reactive oxygen species (ROS) metabolism in plants becomes dysregulated, predisposing them to oxidative bursts and accumulation of oxygen radicals within plant cells. At this point, perturbations in cellular redox states can elicit varying degrees of oxidative damage to plant tissues. Comprehending the production, functions and scavenging mechanisms of ROS under low-temperature stress is pivotal for studying the mechanism of plants' cold tolerance. This review provides a comprehensive overview of advances in understanding the mechanisms underpinning ROS changes in plants subjected to low-temperature stress, which include (1) ROS production, (2) ROS functions and (3) ROS scavenging mechanisms. The review systematically summarises the physiological responses of plants to low-temperature stress, with a particular focus on ROS scavenging mechanisms. It also analyses current research limitations in this realm and proposes recommendations for future research directions.

Keywords: low-temperature stress, ROS production, ROS functions, enzymatic antioxidants, non-enzymatic antioxidants

INTRODUCTION

Plants growing in natural environments inevitably suffer the effects of environmental stresses. Environmental factors have an integrated effect on plant growth and development. Temperature is an important factor influencing their growth and development. Low-temperature stress affects plant growth, development, physiology, biochemistry, hormone homeostasis, and other biological processes [1]. Low-temperature stress also affects photosynthesis, disease resistance and growth duration of crops, and ultimately affects the quality and yield of crops,

thereby influencing agricultural productivity [2]. Similarly, in ornamental plants low-temperature stress has adverse effects on plant growth and development, reducing the ornamental and economic value of roots, stems, leaves, flowers and fruits [3].

Under low-temperature stress, plants' defense mechanisms are rapidly activated in response to stress and plant tissue structure may change to some extent, exemplified by denser arrangements of leaf tissues and mesophyll cells, reduced intercellular spaces, decreased stomatal density, and thickened cell walls [4]. In addition, the roots of maize and wheat are damaged by the excessive accumulation of reactive oxygen species (ROS), which affects root proliferation and leads to a decrease in crop yield [5]. Physiological and biochemical properties of plants may change under low-temperature stress, including enhanced superoxide dismutase (SOD) activity, peroxidase (POD) activity, catalase (CAT) activity, and increased malondialdehyde (MDA) content, free proline content, soluble sugar content, soluble protein content, and other physiological changes [6]. Accumulation of ROS in cells partially accounts for low temperature-induced damage to plants. To maintain ROS homeostasis, the antioxidant enzyme systems in plants must scavenge excessive ROS to sustain normal physiological functions of cell membranes [7, 8]. Therefore, systematically analysing ROS production, functions and scavenging mechanisms in plants is crucial for gaining deeper insights into plant responses to low-temperature stress.

This review systematically summarises plant physiological and biochemical hormone responses to low-temperature stress, and systematically analyses ROS production pathways, ROS functions, and enzymatic and non-enzymatic ROS scavenging mechanisms in plants under low-temperature stress (Figure 1). The physiological and molecular mechanisms underlying plant responses to low-temperature stress are closely associated with ROS production and elimination. This review should serve as a reference for scholars investigating strategies to improve plants' cold tolerance. Additionally, it can also be of benefit for genetic engineering approaches to enhancing plants' cold resistance by optimising ROS scavenging systems.

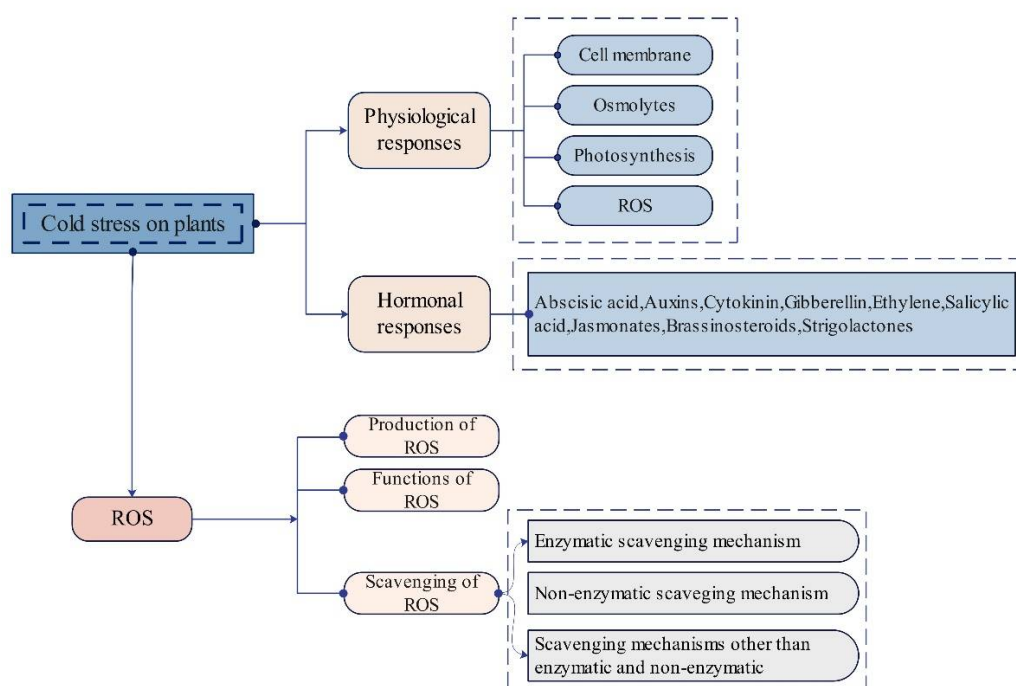


Figure 1. Effects of low temperature stress on plants

EFFECTS OF LOW-TEMPERATURE STRESS ON PLANTS

Plants' Physiological Responses to Low-temperature Stress

Under low-temperature stress, plant growth and development slow down, and physiological and biochemical indexes and molecular regulatory processes also change. With the persistence and intensification of the low-temperature environment, plants will make a series of stress responses including changes in cell membrane structure, osmoregulatory substances, photosynthesis, plant hormone levels and ROS [9]. Plants' cell membrane biophysical properties can also change. Lipid molecules in the cell membrane may become unstable, potentially decreasing cell membrane's fluidity and influencing the fluidity and function of many functional membrane proteins [4]. Low-temperature may also disrupt membrane lipid interactions and arrangements, thereby modifying cell membrane structure and stability.

Membrane lipid peroxidation can be enhanced by low temperature, resulting in cell membrane damage and rupture that irreversibly impairs the cell membrane system [10]. Studies have demonstrated that MDA is produced as a by-product of lipid peroxidation in plant membranes. MDA level increases in the leaves of various plants, as does cell membrane permeability. Therefore, MDA can be employed as an indicator of the extent of cell membrane lipid peroxidation [11]. When affected by chilling injury, plant cell membranes are transformed from a liquid crystalline phase into a gel phase. Cell membrane fluidity can decrease, the cytoskeleton can rearrange, electrolytes and soluble substances can leak, plasma membrane ATPase activity can decrease, and cellular ion balance can be disrupted [12]. It appears that cell membranes can rapidly respond and adapt to low-temperature conditions.

Under low-temperature stress, plants can produce osmotic adjustment substances to modify cell fluid concentration, decrease intra- and extra-cellular osmotic potential and maintain cell morphology and structural stability [13]. Osmotic adjustment substances primarily encompass inorganic ions such as K^+ , Ca^{2+} and Mg^{2+} , as well as organic compounds which include soluble proteins, soluble sugars, betaine and free proline [14]. Plants can modulate inorganic ion channel activity, sustain or elevate intracellular inorganic ion concentrations, avert disruptions in cellular ion homeostasis and enhance cold tolerance [15]. The biosynthesis of organic compounds such as soluble proteins, soluble sugars, betaine and free proline can aid plants in sustaining turgor pressure and safeguarding cell membrane integrity under low-temperature stress. Concurrently, these organic molecules may also stabilise membrane proteins and attenuate membrane lipid peroxidation, thereby shielding plant cell membranes from damage [16].

Photosynthesis constitutes the fundamental life process underlying normal plant growth and development. Low-temperature stress can impair photosynthesis in plants, thereby directly impacting normal growth and development [17]. Chloroplast is the place where plants carry out photosynthesis. It can detect low temperature and represents the organelle most profoundly impacted by chilling. Chloroplast response to chilling stress can also be detected by lipid membrane systems and photoreceptors, and can induce plants to produce excessive ROS, which in turn impairs chloroplast lipid membrane integrity [18]. Chloroplast thylakoid membrane and nucleus constitute primary targets of regulatory proteins and metabolites governing the photosynthetic pathway. Low-temperature stress can inflict damage upon the constituents of thylakoid membranes, the photosynthesis-associated organelles as well as the chloroplast structure. The structure and function of photosynthetic components also become impaired and compromised. Chilling injury can also induce structural alteration in the stomata, which impedes the transport of photosynthetic products

such as sucrose and starch, thereby compromising photosynthesis [19].

In plants ROS primarily occurs as free radicals and non-free-radical species including hydrogen peroxide (H₂O₂), singlet oxygen (¹O₂), hydroxyl radical (·OH) and superoxide radical (O₂⁻) [20]. The overabundance of these radical species can elicit programmed cell death in plants and serve as a crucial indicator for assessing and determining chilling resistance when exploring plant low-temperature response [21]. In the long process of evolution, plants have formed mechanisms for scavenging ROS, including enzymatic scavenging mechanism (antioxidant enzyme system), non-enzymatic scavenging mechanism (antioxidant) and other scavenging mechanisms [22].

Plant Hormonal Responses to Low-temperature Stress

Phytohormones are signalling molecules that govern the functioning of plant organs and play a pivotal role in responding to low-temperature stress. Major plant hormones comprise abscisic acid, auxins, cytokinins, gibberellins, ethylene, salicylic acid, jasmonates, brassinosteroids and strigolactones [23].

Studies have demonstrated that abscisic acid, which governs foliar senescence, accumulates in leaves and roots of *Iris pseudocorus* under low-temperature stress [24]. Low-temperature stress suppresses the biosynthesis and excretion of auxin, hampering plant growth and morphogenesis. However, auxin can respond by interacting with other phytohormones to govern plant cell differentiation and organogenesis [25]. cytokinin response factors, *CRF2* and *CRF3*, govern lateral root formation in *Arabidopsis* under low-temperature stress [26]. Overexpression of *Cytokinin response factor 4 (CRF4)* in *Arabidopsis* presumably plays a role in transient cold acclimation, resulting in enhanced freeze tolerance in *Arabidopsis* [27]. *WRKY53* can negatively regulate the cold tolerance of rice during the panicle initiation stage by regulating gibberellin levels in rice anthers [28]. Transgenic *Arabidopsis* overexpressing ethylene response factor *CdERF1* from *Cynodon dactylon* exhibits enhanced cold tolerance [29].

Salicylic acid can potentiate the chilling tolerance of *Vitis riparia* × *V. labrusca* by modifying photosynthesis, antioxidant systems and expression of chilling-responsive genes [30]. Jasmonate cooperates with other phytohormone signalling cascades such as auxin, ethylene and gibberellin to reinforce plant chilling tolerance [31]. In addition to potentiating basal chilling tolerance, brassinosteroid also contributes to attained freeze tolerance in *Arabidopsis*, which encompasses intricate molecular and biochemical shifts elicited by chilling yet non-freezing temperature [32]. Glutathione reductase 24 is a synthetic strigolactone analog. Investigations have demonstrated that compared to untreated rapeseed seedlings, those treated with the reductase can upregulate their cell viability, soluble protein and proline levels, boost antioxidant enzyme activities, thus suppressing the production of ROS, enhance photosynthesis, decrease the relative conductance of the seedlings, and substantially mitigate the impact of chilling stress on the seedlings [33]. Numerous studies have shown that hormones play an important role in plant response to low-temperature stress, but improving plant cold tolerance requires complex physiological, biochemical and molecular mechanisms.

PRODUCTION AND FUNCTIONS OF ROS UNDER LOW-TEMPERATURE STRESS

ROS Production

ROS refers to several metabolically produced oxygen derivatives with relatively high

reactivity. A multitude of ROS exist in nature, but those commonly examined in studies generally encompass O_2^- , 1O_2 , $\cdot OH$ and H_2O_2 . [34]. Under low-temperature stress, plants generate a plethora of ROS intracellularly. ROS refers to a class of oxygen derivatives with unstable electronic configurations which possess oxidative capacity to react with molecules such as proteins, nucleic acids, lipids and enzymes within the plant system, demolishing their structures and functions and eliciting oxidative impairment in cells [35]. Therefore, ROS production constitutes one of the pivotal causal factors in low-temperature stress-induced damage in plants.

Plants are capable of generating ROS in several sites including chloroplasts, mitochondria, peroxisomes, photosynthetic membranes, apoplasts and cell walls. Likewise, plants produce ROS through diverse locations and pathways, but the types of ROS generated are primarily O_2^- and H_2O_2 (Table 1). Under light conditions, chloroplasts and peroxisomes are the major producers of ROS. Under dark conditions, mitochondria are the primary ROS generators [36]. Chloroplasts and mitochondria are pivotal sites for photosynthesis and respiration in plants. They transduce light or chemical energy into ATP and nicotinamide adenine dinucleotide phosphate (NADPH) via electron transport chains (ETC). The ETC represents one of the primary metabolic pathways for generating cellular ATP, with oxygen being the ultimate electron acceptor. Under low-temperature stress, mismatching between the rates of photosynthesis and respiration leads to electron accumulation or leakage in the ETC, enabling oxygen molecules to capture certain electrons and generate superoxide anion [37]. 1O_2 is a natural by-product of photosynthesis, formed primarily in photosystem II even under low light conditions. On the surface of the outer mitochondrial membrane, CuZn-SOD decomposes O_2 into H_2O_2 spontaneously. Complexes I and III of the mitochondrial electron transport chain can generate O_2^- . In aqueous solutions, O_2^- can be further reduced by SOD dismutation to H_2O_2 [38].

Table 1. Pathways of ROS production in plants

ROS production pathway	Location of ROS production	ROS type	Reference
Photosynthesis ETC, photosystems I and II	Chloroplast	O_2^- , 1O_2 , H_2O_2	[39, 40]
Respiration ETC, complex I and III of mitochondrial ETC, SOD dismutation	Mitochondria	O_2^- , H_2O_2	[41]
Polyamine oxidase	Peroxisome	O_2^- , H_2O_2	[42]
Fatty acid β -oxidation	Peroxisome	H_2O_2	[43]
Glycolate oxidase	Peroxisome	H_2O_2	[44]
Flavin oxidase	Peroxisome	H_2O_2	[45]
Xanthine oxidase	Peroxisome	O_2^-	[36]
NADPH oxidase, germin-like oxalate oxidases	Cell wall	O_2^- , H_2O_2	[35]
Cytochrome P450	Cytoplasm and endoplasmic reticulum	H_2O_2	[46]
Amine oxidase	Apoplast	H_2O_2	[47]

Peroxisomes are the major sites for decomposing peroxides in plants. They contain enzymes such as CAT that can convert peroxides to H_2O and O_2 [42]. Fatty acid, glycolate oxidase and flavin oxidase can also react with O_2^- to produce H_2O_2 [43-45]. Under low-temperature stress, due to the imbalance of peroxide concentrations inside and outside the peroxisome, it undergoes an autocatalytic reaction to release peroxides. POD is an important antioxidant enzyme in cells that can catalyse the degradation of ROS such as H_2O_2 . Under low-temperature stress, decreased POD activity leads to H_2O_2 accumulation, stimulating ROS accumulation and oxidative stress responses [48].

NADPH oxidase is an important enzyme system on the cell membrane that can convert substrates such as O_2 and NADPH into O_2^- and $NADP^+$ in plants. Studies have shown that under

low-temperature stress, increased NADPH oxidase enzyme activity exacerbates ROS accumulation, leading to oxidative stress responses [49]. The cell membrane is an important interface for plants to exchange substances and information with the external environment. They contain various membrane-bound proteins and membrane-bound enzymes involved in signal transduction and metabolic regulation. Under low-temperature stress, decreased membrane fluidity and increased permeability can lead to enhanced or aberrant expression of some membrane-bound enzymes such as NADPH oxidase on the cell membrane, promoting the production of ROS [50].

ROS Functions

When ROS levels exceed the maximum tolerance of plant cells, they can cause varying degrees of damage to cells, such as lipid, protein and DNA damage [51]. Cell membranes or organelle membranes are particularly susceptible to ROS damage known as lipid peroxidation due to high polyunsaturated fatty acid content. ROS-induced lipid peroxidation plays a key role in cell death including apoptosis, autophagy and ferroptosis. When pathogens attack plants, plant cells have higher levels of cellular oxidation than normal. High levels of cellular oxidation induce plants to activate their own protective mechanisms. At this time, cells undergo a series of allergic reactions, causing programmed cell death or necrosis of plant cells, eventually preventing plant pathogens from further invading plant tissues [52]. In rice researchers found an immune suppression network centred on Ca^{2+} sensors. The RESISTANCE OF RICE TO DISEASES1 (*RODI*) gene promotes ROS scavenging by stimulating CAT activity and enhancing rice resistance [53]. In corn the mitogen-activated protein kinase gene *ZmMKK1*, ectopically expressed in tobacco, increases the activity of antioxidant enzymes in mutants, increases the accumulation of osmotic regulators, and stimulates the up-regulation of ROS-related genes, thereby enhancing tolerance to low temperatures [13].

In *Arabidopsis* *CAT* genes catalyse the decomposition of H_2O_2 . *CAT2* is an important participant in removing H_2O_2 produced under abiotic stress in *Arabidopsis*. *CAT3* and *CAT2* are the main scavengers of H_2O_2 . *CAT3* promotes the balance of ROS in plants under light conditions while *CAT2* promotes ROS homeostasis in the dark [54]. The SOD gene family is involved in scavenging ROS in tea trees under low-temperature stress and plays an important role in responding to low-temperature stress. It is speculated that the microRNA398 family genes of *Camellia sinensis* (csn-miR398a-3p-1) negatively regulating the expression of *Cu/Zn-SOD4* may be a key regulatory mechanism for tea trees under low-temperature stress [55]. Ascorbate peroxidase (APX) is indispensable in the synthesis of L-ascorbic acid (AsA), which plays an important role in plant resistance to adversity stress by scavenging ROS. Under low-temperature stress, the *CaAPX* gene in *Camellia azalea* is ectopically expressed in tobacco. The gene expression is significantly up-regulated and the tolerance to cold damage is enhanced. In addition, the *CaAPX* gene could have coordinated ROS signal transduction under low-temperature stress and increased the activity of ROS scavenging enzymes [56].

In *Malus baccata* ectopic expression of the *MbCBF2* gene in *Arabidopsis* significantly enhances the cold tolerance of transgenic *Arabidopsis*. The high expression of *MbCBF2* also promotes increase in chlorophyll and proline content in transgenic *Arabidopsis*, increases the activities of SOD, POD and CAT, and reduces MDA content. This indicates that the *MbCBF2* gene can positively regulate *Arabidopsis* tolerance to low temperature [57]. In addition, *CBF* genes can also bind to cis-elements in the *COR* gene promoter to activate the expression of *COR* genes and participate in the plant's response to low-temperature stress [58].

AsA is an antioxidant implicated in *Actinidia eriantha* as a basic leucine zipper domain transcription factor *AcePosF21*, which participates in AsA biosynthesis. It can promote the production of more AsA in plants, thereby neutralising excess ROS under low-temperature stress [59]. In *Chrysanthemum morifolium* overexpression of the glutathione peroxidase gene (*DgGPXI*) results in increased activity of glutathione peroxidase (GPX/GSH-Px), which helps scavenge excess ROS in plant tissues, thereby enhancing the cold tolerance of transgenic chrysanthemum [48]. In addition, ROS can also act as signalling molecules which affect the oxidation-reduction state inside and outside plant cells and participate in plant response to low-temperature stress [60].

In summary, the functions of ROS in plants under low-temperature stress are mainly manifested in four aspects: (1) as signalling molecules, participating in low-temperature-induced signal transduction pathways such as calcium signalling and mitogen-activated protein kinase signalling, regulating the expression of downstream genes; (2) as transcription factors, directly or indirectly affecting the transcription levels of genes related to low temperature responses, such as antioxidant enzyme genes APX, CAT, SOD, and cold-induced genes CBF and COR; (3) as antioxidants, protecting cells from damage by scavenging excess ROS such as AsA and glutathione; and (4) as regulatory factors which affect cell physiological metabolism by altering the oxidation-reduction state inside and outside cells.

SCAVENGING MECHANISMS OF ROS UNDER LOW-TEMPERATURE STRESS

Enzymatic ROS Scavenging Mechanisms

In the enzymatic ROS scavenging system, the main antioxidant enzymes involved in scavenging ROS include SOD, POD, CAT, GPX/GSH-Px, APX, dehydroascorbate reductase (DHAR), glutathione reductase (GR), monodehydroascorbate reductase (MDHAR), glutathione S-transferase (GST), thioredoxins (TRX) and glutaredoxins (GRX) [61]. These ROS scavenging enzymes are distributed in different parts of plants and work together to scavenge ROS in plant tissues. The differences in ROS types scavenged by different enzymes and plant responses are shown in Table 2.

Under low-temperature stress, SOD in plant cells first acts to convert excess O_2^- into H_2O_2 and oxygen O_2 . If Cu^{+2}/Fe^{+3} exists in plant cells, O_2^- can be converted into $\cdot OH$ through the Haber-Weiss cycle (Figure 2). According to the different auxiliary metal factors, SOD can be divided into copper-zinc SOD (Cu/Zn-SOD), manganese SOD (Mn-SOD) and iron SOD (Fe-SOD), and their mechanisms of action in plants vary [70]. In *Festuca arundinacea*, overexpression of *CuZnSOD* and *APX* genes can increase the antioxidant enzyme activity in chloroplasts and scavenge excess ROS and enhance plant tolerance to abiotic stresses [71]. In *Daucus carota*, 2 *FeSODs*, 2 *MnSODs* and 5 *Cu/ZnSODs* (CuZnSOD) have been identified. The increased expression of SOD gene family indicates that it plays an important role in enhancing plant cold tolerance. POD can catalyse the oxidation reaction of various reductants involved in H_2O_2 and reduce H_2O_2 to H_2O (Figure 2). It can also coordinate with SOD and CAT to scavenge excess free radicals in plants [72].

Under low-temperature stress, the relative conductivity of H_2O_2 in *Triticum aestivum* ‘Dongnongdongmai 1’ increases significantly. ABA improves the cold tolerance of wheat leaves and stems by increasing the contents of CAT, SOD, POD, APX, GR, DHAR and MDHAR [73]. CAT is a heme-containing tetrameric enzyme. Under low temperature stress, CAT can decompose H_2O_2 in plant tissues into H_2O and O_2 ; it is an indispensable enzyme for scavenging ROS in plants under low-temperature stress (Figure 2) [74]. In sweet potato the activities of antioxidant enzymes such as

APX, SOD, CAT and GR increase rapidly in response to low-temperature stress to scavenge excess ROS and enhance cold tolerance [74].

Table 2. Main antioxidant enzymes scavenging ROS in plants

Antioxidant enzyme	Location where ROS scavenging occurs	ROS type	Description of ROS scavenging process	Reference
SOD	Chloroplast, Cytosol, Mitochondria, Peroxisome, Apoplast	O_2^- , H_2O_2	<i>MeCu/ZnSOD</i> and <i>MeCAT1</i> can significantly enhance ROS scavenging ability and thus reduce accumulation of H_2O_2 .	[62]
POD	Cell wall, Cytosol, Vacuole	H_2O_2	Transgenic <i>Ipomoea batatas</i> overexpressing lignin-forming peroxidase (<i>IbLfp</i>) gene has enhanced cold tolerance.	[63]
CAT	Peroxisome	H_2O_2	Overexpressing <i>ScCAT1</i> gene from <i>Saccharum spontaneum</i> in <i>Nicotiana benthamian</i> enhances plant stress resistance by removing excess toxic ROS.	[64]
GPX/ GSH-Px	Cytosol	ROOH, H_2O_2	Overexpressing <i>DgGPX1d</i> gene in <i>Chrysanthemum morifolium</i> increases GPX activity.	[48]
APX	Chloroplast, Cytosol, Mitochondria, Peroxisome, Apoplast	H_2O_2	In transgenic <i>Manihot esculenta</i> , co-expressing <i>SOD</i> , <i>MeCu/ZnSOD</i> and <i>MeAPX2</i> can activate cassava's antioxidant defense system by scavenging ROS and enhance its cold tolerance.	[65]
DHAR, GR, GST	Chloroplast	O_2^- , H_2O_2	Co-expressing DHAR, GST and GR in tobacco increases regeneration of reduced ascorbate and glutathione and participate in scavenging of O_2^- and H_2O_2 .	[66]
MDHAR	Chloroplast	O_2^- , H_2O_2	Transcription of <i>MDHAR</i> gene in <i>Trifolium repens</i> rapidly activates participation of plant's antioxidant defense mechanism, reducing its ROS content.	[67]
TRX	Chloroplast	O_2^- , H_2O_2	The h-type thioredoxin gene <i>LmTrxh2</i> isolated from <i>Lobularia maritima</i> enhances ROS scavenging ability.	[68]
GRX	Cell wall, Chloroplast	H_2O_2	Overexpressing <i>RtGRL1</i> from <i>Reaumuria trigyna</i> in <i>Arabidopsis</i> increases glutathione biosynthesis, glutathione-dependent detoxification of ROS, and proline content under stress.	[69]

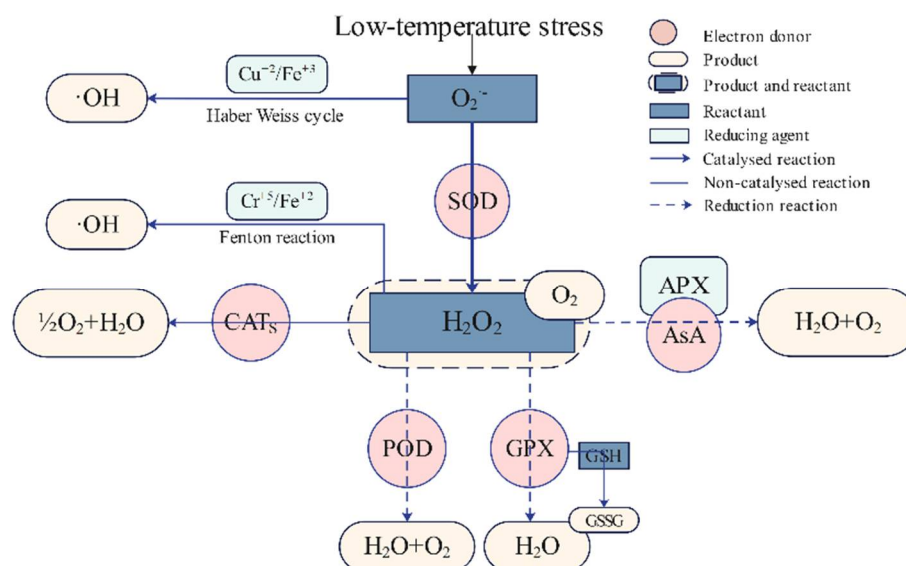


Figure 2. Enzymatic ROS scavenging mechanisms

In addition, H_2O_2 in plants can generate highly oxidising $\cdot OH$ through the Fenton reaction (Figure 2). Hydroxyl radicals have extremely strong oxidising capacity, which can damage plant

cell membranes, proteins, nucleic acids and other molecules, but can also kill invading pathogens or activate plant defense responses, thus helping plants cope with cold damage [75]. GPX can reduce H_2O_2 in plant tissues to H_2O and catalyse the conversion of reduced glutathione (GSH) to oxidised glutathione (GSSG), thereby reducing hydrogen peroxide in plant tissues to harmless hydroxy compounds, protecting biological membranes from damage by ROS, and maintaining normal cellular functions (Figure 2) [76]. APX is one of the main enzymes that scavenge H_2O_2 in plants. It uses reduced AsA as a substrate to decompose H_2O_2 that harms plant tissues into non-toxic H_2O and O_2 [77]. In rice overexpression of the ascorbate peroxidase gene *OsAPXa* can enhance the H_2O_2 scavenging ability and increase the cold tolerance of rice [78].

In addition, APX, MDHAR, DHAR and GR are involved in the AsA-GSH cycle. In this cycle APX acts as a deoxygenase to reduce H_2O_2 to H_2O , producing monodehydroascorbic acid (MDHA) molecules in the reduction process [79]. In photosystem I, MDHA can be converted to AsA and dehydroascorbic acid (DHA) via MDHAR or ferredoxin. DHA is reduced to AsA by DHAR, consuming GSH and producing oxidised glutathione, which is reduced to GSH by GR using NADPH as an electron donor. As reductases, DHAR and MDHAR play an important role in the AsA-GSH cycle and are important enzymes for scavenging ROS (Figure 3) [79].

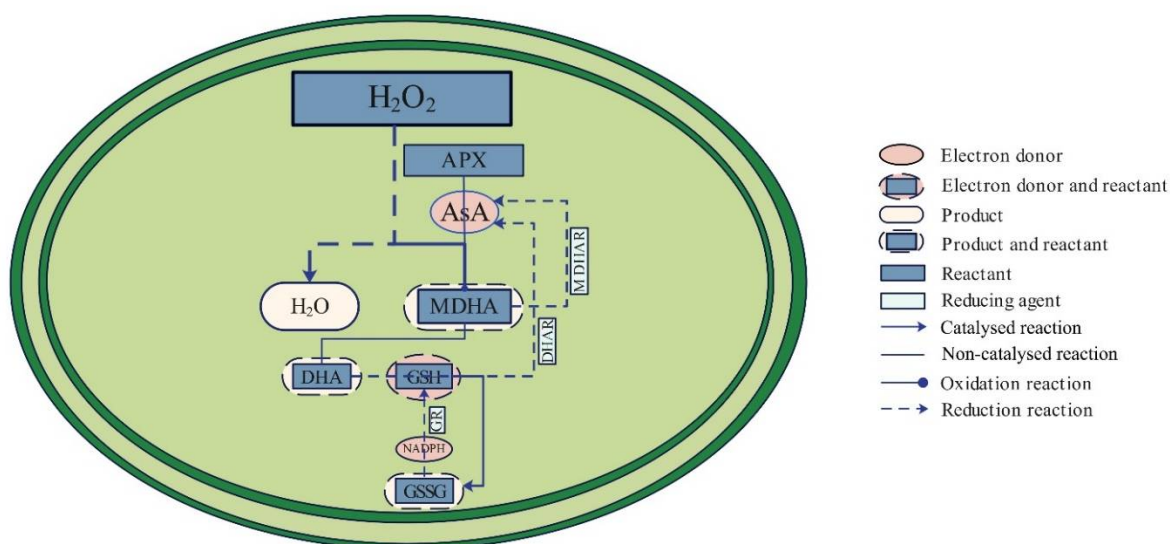


Figure 3. ROS scavenging mechanism based on AsA-GSH cycle in chloroplasts

In plants GST is directly involved in the scavenging of ROS and reduces oxidative damage. Under low-temperature stress, seven GSTs (*CmaGSTU3*, *CmaGSTU7*, *CmaGSTU8*, *CmaGSTU9*, *CmaGSTU11*, *CmaGSTU12* and *CmaGSTU14*) in *Cucurbita maxima* are highly expressed, indicating that the GST gene family plays an important role in plants' response to low-temperature stress [80]. Overexpression of the cytosolic redox protein thioredoxin h2 (*Trx-h2*) gene in *Arabidopsis* can activate cold-responsive (*COR*) genes. The transgenic lines show stronger cold tolerance, indicating that the *TRX* gene plays an important role in improving plant's cold tolerance [81]. GRX are common oxidoreductases in plants that utilise the reducing power of GSH to reduce disulfide bonds of substrate proteins and maintain cellular redox homeostasis. Studies have shown that expressing the *Arabidopsis AtGRXS17* gene in tomatoes improves the cold tolerance of their antioxidant enzyme activity, reduces H_2O_2 accumulation and enhances the cold tolerance of transgenic tomatoes [82].

Non-enzymatic ROS Scavenging Mechanisms

Under low-temperature stress, a large number of non-enzymatic antioxidants existing in plant cells will respond rapidly and work together with enzymatic antioxidants to scavenge excess ROS. Non-enzymatic ROS scavenging antioxidants include AsA, GSH, carotenoid, tocopherol/vitamin E, proline and flavonoids (Table 3).

Table 3. Non-enzymatic antioxidants scavenging ROS in plants

Non-enzymatic antioxidant	Location where ROS scavenging occurs	ROS type	Description of ROS scavenging process	Reference
AsA	Chloroplast, Cytosol, Mitochondria, Peroxisome, Apoplast	O_2^- , $\cdot OH$, H_2O_2	Under frost stress, exogenous proline, SA and AsA treatments significantly increase the antioxidant enzyme activity in grapes. AsA has the best effect in improving grape freeze tolerance.	[84]
GSH	Chloroplast, Cytosol, Mitochondria, Peroxisome, Apoplast	1O_2 , $\cdot OH$, H_2O_2	Exogenous GSH can activate AsA-GSH cycle in plants and enhance plant antioxidant capacity.	[85]
Carotenoids	Chloroplast	1O_2	Carotenoid content negatively correlates with plant cold tolerance.	[86]
Tocopherols /Vitamin E	Membranes	ROOH, O_2^1	A lack of tocopherol reduces the plant's cold tolerance.	[87]
Proline	Chloroplast	O_2^- , $\cdot OH$	Proline works together with other enzymatic and non-enzymatic antioxidants to scavenge ROS in plants.	[88]
Flavonoids	Cell wall	H_2O_2	Cold stress promotes accumulation of flavonoids in plants and enhances ROS scavenging ability.	[89]

AsA is a water-soluble antioxidant that can maximise the scavenging of excess ROS in plants. Under low-temperature stress, the AsA content in plants significantly increases, rapidly scavenging excess ROS. In tomatoes exogenous AsA effectively scavenges H_2O_2 by modulating the metabolism, osmolytes, antioxidants and the transcriptional regulation of catalase, and heat shocks proteins, reducing low-temperature stress damage and enhancing cold tolerance [83]. The AsA-GSH cycle shown in Figure 3 is an important mechanism for enzymatic and non-enzymatic antioxidants in scavenging ROS in plants.

Carotenoids are plant pigments that can scavenge 1O_2 and other harmful free radicals generated during photosynthesis and act as antioxidants. In low-temperature treated mangoes, carotenoid content decreases with decreasing temperature. When the carotenoid content is too high, it may lead to a decrease in antioxidant capacity in the plant, promote oxidation reactions, and be unfavourable for the plant to resist low-temperature stress [90].

Tocopherol/vitamin E is a lipid-soluble antioxidant and the major antioxidant in biological membranes [86]. Under low-temperature stress, tocopherol-deficient *Arabidopsis thaliana* mutants exhibit significant physiological changes compared with the wild type. Among them, in *vte1* and *vte2* mutants, anthocyanin content, growth, fruiting ability and photosynthetic electron transport rate decrease while photoassimilate export, soluble sugar level and starch content increase, indicating that tocopherol plays an important role in plant responses to low-temperature stress [91].

Proline is an indispensable non-enzymatic antioxidant that reduces ROS-induced damage by effectively scavenging $\cdot OH$ [89]. Another study shows that proline-rich proteins play an important role in plant resistance to low-temperature stress. *OsPRPI* gene knockout rice mutants are more sensitive to low temperature. Under low-temperature stress, the activities of antioxidants in the mutants are lower, and the contents of proline, chlorophyll, ABA and AsA are also lower than those in wild-type rice. The cold tolerance of the mutant rice is decreased, indicating that the *OsPRPI*

enhances the cold tolerance of plants by regulating changes in antioxidants [92].

Flavonoids are a class of secondary metabolites widely present in plants, usually found in plant vacuoles. They are common antioxidants that scavenge ROS in plants. They can localise and neutralise ROS before excess ROS damage cells. Studies have shown that low-temperature stress enhances the biosynthesis of flavonoids as well as that of anthocyanins and related metabolic pathways in *Citrus sinensis*, thereby enhancing the plant's cold tolerance [93]. In *Camellia sinensis* down-regulation of the *CsUGT78A14* gene results in decreased flavonols accumulation and ROS scavenging ability. Up-regulation of the same gene increases flavonols accumulation and enhances ROS scavenging ability and cold tolerance in the plant, indicating that flavonoid antioxidants play an important role in scavenging ROS [94].

Other ROS Scavenging Mechanisms

Soluble sugars directly influence the generation rate of ROS by regulating ROS production metabolic pathways such as mitochondrial respiration and photosynthesis [95]. Photorespiration produces H₂O₂ involved in signal transduction and gene expression in plants. At the same time, it is also considered an important part of the plant stress response to prevent ROS accumulation, which can alleviate oxidative stress under cold and other abiotic stress conditions [96]. Uncoupled mitochondrial respiration participates in the scavenging of ROS and NO, regulating the balance of ROS in plants. The energy and reducing equivalents produced by mitochondrial respiration provide energy for antioxidant enzymes and antioxidant substances to scavenge excess ROS and maintain the dynamic balance of ROS [97].

DISCUSSION AND FUTURE OBJECTIVES

Low temperature is a limiting factor for the normal growth and development of many plants. The ROS scavenging system is an important mechanism for plants to resist low-temperature stress and enhance cold tolerance. Rapidly scavenging excess ROS in plants can effectively reduce the impact of low temperature on plants and enhance plant cold tolerance [21]. Therefore, it is necessary to clarify the production pathways, functions and scavenging mechanisms of ROS so as to enhance the ability of plants to resist low temperature.

Under low-temperature stress, ROS has complex and diverse production pathways. The production mechanisms and interconnection between different ROS production pathways are still unclear and can be further studied in the future. In addition, ROS can serve as signalling molecules, transcription factors, antioxidants and regulators in plants. The signal transduction mechanisms of different ROS, the molecular mechanisms of transcription factor functions, the biological function differences of antioxidants and regulators, and their interrelationships also need to be further studied.

Enzymatic and non-enzymatic antioxidants play an important role in scavenging excess ROS in plants. As research deepens, some enzymatic reactions and scavenging mechanisms have gradually become clearer. However, the differences in ROS scavenging ability and variation patterns of different ROS scavenging enzymes and non-enzymatic antioxidants are still unclear. In addition, the process of ROS scavenging in plants often involves the synergistic action of multiple antioxidant enzymes and antioxidants. The signal transduction mechanisms between different antioxidant enzymes and antioxidants are unclear. In addition to enzymatic and non-enzymatic antioxidants, increasing evidence shows that soluble sugars including disaccharides, cotton

oligosaccharides and fructans have dual roles in scavenging ROS [95]. In summary, the mechanism of ROS scavenging in plants under low-temperature stress is a complex process that requires the synergistic action of multiple ROS scavenging mechanisms and related signalling pathways. In the future, we should focus on the study of the interrelationships and signal transduction mechanisms of enzymatic, non-enzymatic and other ROS scavenging processes. A deeper understanding of these mechanisms will help us not only to better understand the mechanism of plant responses to environmental stresses, but also to provide new ideas for maintaining the health and efficient growth of plants.

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