



Investigation of Cold Stress Effects on Plants at Molecular Level

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Abstract

The responses of plants to biotic and abiotic stresses and the effects of these stresses on plant metabolism have led to the development of molecular approaches to determine these metabolic pathways. Plants produce both chloroplast and mitochondria ROS under normal conditions or when exposed to various stresses such as, drought, desiccation, radiation, flooding, herbicide applications, and pathogen attacks, cold and freezing. Restrictions in CO₂ fixation due to disruptions in the electron transport chain are the main cause of ROS formation in chloroplasts. In mitochondria, similarly, under stress conditions, disruptions in the electron transport chain are the main mechanism of ROS production. ROS causes peroxidation of membrane lipids, breaking of DNA chains and inactivation of enzymes. Enzymatic defense systems due to cold stress are composed of superoxide dismutase, ascorbate peroxidase, catalase, peroxidase, glutathione reductase and monodehydroascorbate reductase enzymes, while non-enzymatic defense systems are formed by ascorbate, glutathione, carotenoid, α -tocopherol, anthocyanin and flavonoids. In this context, the present review aims to provide an overview of the molecular mechanisms and expressions of related genes associated with cold stress in plants.

Keywords: Abiotic stress; Cold effect; Molecular pathway; ROS

Introduction

Many environmental stresses conditions, such as cold, break down the balance between absorbed light energy and utilized light energy. This causes the formation of singlet O₂ (¹O₂), that is, reactive oxygen species (ROS), instead of the reduction of O₂ [1]. The formation of ROS in light is called photooxidative stress. This can occur in two ways, such as the transfer of energy or electrons directly to oxygen as a result of photosynthetic activity and/or exposure of tissues to ultraviolet radiation. The formation of ROS during photosynthesis is minimized by many components and is eliminated by photooxidative stress regulatory mechanisms. ROS are rapidly destroyed by fast and efficient antioxidant systems [2]. Plants, like other photosynthetic organisms, grow in the presence of atmospheric oxygen; have developed metabolic pathways to use the great energy potential of oxygen and, at the same time, limit the destructive effects of this oxidant and active molecules derived from O₂ known as ROS [3]. Reactive oxygen species such as

superoxide molecule (O₂⁻), singlet oxygen (¹O₂), hydrogen peroxide (H₂O₂) and hydroxyl radicals (OH⁻) are normal cell metabolism products and are removed from the environment by defense mechanisms [4]. Under normal conditions or during exposure to various stresses such as cold and freeze, drought, desiccation, flooding, herbicide applications, pathogen attacks, and radiation), both chloroplast and mitochondria produce ROS (Figure 1). Restrictions in CO fixation due to disruptions in the electron transport chain are the main cause of ROS formation in chloroplasts. In mitochondria, similarly, under stress conditions, disruptions in the electron transport chain are the main mechanism of ROS production [5]. ROS causes peroxidation of membrane lipids, DNA chain breaks and inactivation of enzymes [6]. If the air temperature drops to values near to the freezing point during the growth period, the plant needs to develop a response to the cold stress. As a matter of fact, while the damage caused by cold stress in the tropical and the subtropical regions may cause

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significant losses, many species in the Polar Regions can tolerate extreme cold temperatures. Although there are no significant differences between the cell protoplasts of cold sensitive species compared with the resistant to extreme cold stress, the changes occur in the cell membrane systems and proteins associated with

this membrane system, as well as the formation of some osmoregulators against cold stress in the protoplasm of resistant ones. There are many studies on the molecular changes that occur in the membranes and metabolic activities of plants against cold stress (Table 1).

Table 1: The last five years studies on investigation of the cold stress effects in plants at the molecular level.

Plant Species	Genes	References
Corn (<i>Zea mays</i> L.)	<i>Zea Mays chilling-tolerance divergence (ZmCOL1D1)</i>	[8]
Tomato (<i>Lycopersicon esculentum</i> Mill.)	<i>Lycopersicon esculentum cold-regulated 413 plasma membrane protein (LeCOR413PM2)</i>	[9]
Arabidopsis (<i>Arabidopsis thaliana</i> (L.) Heynh.)	<i>Arabidopsis thaliana leucine-rich repeat-only protein (AtLRRop2)</i>	[10]
Mulberry (<i>Broussonetia papyrifera</i> L.)	<i>Protein phosphatase (PP2C)</i>	[11]
Rice (<i>Oryza rufipogon</i> Griff.)	<i>Low temperature growth (LTG5)</i>	[12]
Grape (<i>Vitis amurensis</i> Rupr.)	<i>Vitis amurensis dehydrin (VamDHN3)</i>	[13]
Vervain (<i>Verbena bonariensis</i> L.)	<i>Verbena bonariensis WRKY transcription factor (VbWRKY32)</i>	[14]
Birch (<i>Betula platyphylla</i> Sukaczew)	<i>Betula platyphylla Ethylene-responsive transcription factor (BpERF13)</i>	[15]
Rice (<i>Oryza sativa</i> L.)	<i>Oryza sativa Dehydration-responsive element-binding protein (OsDREB1G)</i>	[16]
Tobacco (<i>Nicotiana tabacum</i> L.)	<i>Lepidium CBL-interacting serine/threonine-protein kinase (LlaCIPK)</i>	[17]
Plum (<i>Prunus mume</i> Siebold & Zucc)	<i>Prunus mume CBL-interacting serine/threonine-protein kinase (PmCIPK)</i>	[18]
Tomato (<i>Solanum lycopersicum</i> L.)	<i>Solanum lycopersicum cold-regulated 413 inner membrane protein (SICOR413IM1)</i>	[19]
Apple (<i>Malus sp.</i>)	<i>Cataract, congenital, cerulean type (CCA1), C-repeat/DRE binding factor (CBF1, CBF3), Cold-regulated (COR47)</i>	[20]
Arabidopsis (<i>Arabidopsis thaliana</i> (L.) Heynh.)	<i>Brassinosteroid signaling positive regulatory (BZR1), C-repeat/DRE binding factor (CBF1, CBF2)</i>	[21]
Arabidopsis (<i>Arabidopsis thaliana</i> (L.) Heynh.)	<i>MAP kinase (MPK4)</i>	[22]
Rice (<i>Oryza spp.</i>)	<i>Oryza NAC domain containing protein (ONAC095)</i>	[23]
Arabidopsis (<i>Arabidopsis thaliana</i> (L.) Heynh.)	<i>Ethylene-responsive transcription factor (CRF2, CRF3)</i>	[24]
Grape (<i>Vitis vinifera</i> L.)	<i>C-repeat/DRE binding factor (CBF1, CBF2, CBF3, CBF4)</i>	[25]
Barley (<i>Hordeum vulgare</i> L.)	<i>C-repeat/DRE binding factor (CBF6, CBF9, CBF12, CBF14), Cold responsive protein (COR14B), Dehydrin (DHN5)</i>	[26]

ROS are the most effective free radicals, which are formed endogenously in the photosynthesis reactions in chloroplasts, in plastids and peroxisomes, in the citric acid cycle in mitochondria, by the action of enzymes such as NADPH oxidase, cell wall peroxidases and amino oxidases [27,28]. They are formed by removing an electron from a non-radical atom or molecule or by adding an electron to an atom or molecule. Since they can donate or take electrons from other molecules, they act as reducing or oxidizing agents in the organism [29,30]. They are also synthesized during the normal development process of the plant, but they do

not cause harmful effects thanks to the balance between them and the detoxification mechanism [31]. The main known ROS in cells are singlet oxygen (1O_2), superoxide anion (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH^-), and their levels in the cell are constantly in equilibrium under normal conditions [30].

Singlet oxygen (1O_2)

Singlet oxygen (1O_2) may occur as a result of the O_2 molecule, which is in charge of the electron transport system, getting extra energy and shifting it to a different orbit in the opposite direction

of its rotation; It can also be formed as a result of the reaction of the $^1\text{O}_2$ radical with nitric oxide (NO) and the reaction of H_2O_2 with hypochlorite (ClO^-). Activation of oxygen by light absorption of P680, the reaction center of FSII in chloroplasts, leads to the formation of a highly reactive singlet oxygen $^1\text{O}_2$ radical [32]. $^1\text{O}_2$ can be rapidly detoxified with the help of β -carotene in the reaction center, as well as water, tocopherol, reduced plastoquinone, and flavonoids [33,34]. The production of $^1\text{O}_2$ may increase in plants, especially under abiotic stress conditions such as photooxidative stress [35]. As a result, oxidative damage occurs and programmed cell death is started [36].

Since $^1\text{O}_2$ has a similar quantum state with many biological molecules, it can easily react. It shows lipoxygenase properties like the OH- radical [30]. In particular, carbon-carbon double bonds are bonds with which singlet oxygen reacts. Cysteine, methionine, tryptophan, tyrosine and histidine are likely to undergo oxidation with $^1\text{O}_2$ due to the high electron density resulting from the double bonds or sulfur moieties [37].

Superoxide anion (O_2^-)

The main source of superoxide radicals formed in plant cells is believed to be electron leakage in electron transport systems in chloroplasts and mitochondria [38]. Oxygen (O_2) in the ground state can lose its spin restriction by gaining an electron. This electron is an electron that cannot be delivered to its real target during the electron transport reactions in plant cells or the reaction catalyzed by the enzyme nicotinamide adenine dinucleotide phosphate (NADPH) oxidase. When oxygen gains an electron in this way, a superoxide radical or superoxide anion (O_2^-) is formed [39].

The superoxide radical can participate in many reactions, but it primarily takes up an H^+ , causing the formation of the hydroperoxide radical (HO_2^-). This compound is both more reactive and more stable than the superoxide radical and can cross biological membranes. When two molecules of hydroperoxide react, O_2 and H_2O_2 are formed. This reaction is called "superoxide dismutation". In other words, as a result of the dismutation reaction, two structurally different products are formed from the same substance. The superoxide radical does not cause a chemical change in biological macromolecules, but is considered to be the main source of oxidative stress. It also reduces other active oxygen species, leading to the formation of stronger oxidizing molecules. Superoxide radical can reduce Fe^{+3} and Cu^{+2} ions to Fe^{+2} and Cu^+ forms. The reduced iron and copper ions formed can also react with H_2O_2 to form the hydroxyl radical. The hydroxyl radical also plays a central role in oxidative stress, as it causes structural changes in almost all macromolecules. In addition, superoxide and hydroperoxide radicals can react with nitric oxide to form extremely reactive peroxynitrite and alkyl peroxynitrite radicals [30].

Hydrogen peroxide (H_2O_2)

The regions where superoxide radicals are formed in plant cells are also accepted as the origin of hydrogen peroxide (H_2O_2) formation. Peroxidase group enzymes are held responsible for H_2O_2 formation in plants exposed to biotic stress factors [40]. Hydrogen peroxide is the most stable active oxygen species with important physiological functions in plant cells [41]. H_2O_2 is a weak acid compound with no unshared electrons. H_2O_2 is formed by the catalysis of superoxides by superoxide dismutase (SOD), an enzyme with a very high catalytic activity in aerobic organisms. H_2O_2 formation occurs with the catalysis of the superoxide dismutase (SOD) enzyme. The reason why H_2O_2 is known as an oxidizing species is that it acts as a precursor of the OH- radical in the presence of metal ions such as iron and copper. H_2O_2 reacts especially with the iron in the heme group in proteins and forms reactive iron forms with high oxidation levels. Iron in this form has very strong oxidizing properties and can initiate radical reactions such as lipid peroxidation in cell membranes. If H_2O_2 , which is formed in biological systems due to its oxidizing feature, is not removed from the environment, it may cause damage to the metabolic processes of the cell [42].

Hydroxyl radical (OH-)

The hydroxyl radical (OH-) is one of the most reactive oxidants in the cell. Since OH- is not an enzyme system that can be used in the elimination of cells, it can easily react with all biological molecules, and when it is produced in excess, it causes the death of cells. Haber-Weiss (Cu^+ , Cu^{2+} , Fe^{2+} , Fe^{3+}) or fenton (Fe^{2+} and other transition metals; Cu, Zn, Mn, Cr, Co, Ni, Mo) reaction in the presence of metal ions of hydrogen peroxide (H_2O_2) and O_2^- anion, which are relatively less harmful occurs with [43].

Antioxidative responses to cold stress in plants

Symptoms of cold damage in plants vary depending on temperature, duration of exposure to cold, plant (genotype), plant developmental stage, tissue in contact with cold, wind, water, nutrients, and other environmental conditions such as light [44]. These symptoms generally are; It can be summarized as decrease in plant growth rate, decrease in leaf width, increase in cellular autolysis and senescence, programmed cell death, formation of chlorosis due to loss of chlorophyll as a result of photooxidation in light, deterioration of cell membrane structures and consequently deterioration of cellular integrity, minimization of protoplasmic flow and necrosis [45,46].

Plants have various antioxidants that provide control and detoxification of ROS in order to survive under the oxidative stress and to overcome the stress. Antioxidants are substances that can oxidize at low concentrations and reduce (with electron transfer) or

prevent the oxidation of another substrate, that is, act against the oxidation [43].

Antioxidants are divided into two categories: non-enzymatic antioxidants and enzymatic antioxidants. Non-enzymatic ones are ascorbic acid (vitamin C), tocopherols (vitamin E), carotenoids, glutathione and phenolic compounds. Enzymatic antioxidants are known as superoxide dismutase (SOD), ascorbate peroxidase (APX), glutathione peroxidase (GPX) and catalase (CAT). Enzymatic and non-enzymatic antioxidants differ according to their localization and role in the cell [47].

Non-enzymatic Antioxidants

Ascorbic Acid (vitamin C)

Ascorbic Acid affects many physiological processes that regulate the growth, differentiation and metabolism of plants [48] and an important role in this antioxidant plant defense system is to protect the metabolic processes of the cell against H_2O_2 and other toxic oxygen derivatives. Ascorbic Acid mainly acts as a reducing agent and scavenges many types of free radicals, which are active in the malondialdehyde (MDA) process. The electron donor of these antioxidants is usually NADPH and it is catalyzed by monodehydroascorbate reductase (MDAR) or ferredoxin in the water-water cycle state in chloroplasts. Ascorbic Acid provides membrane protection by directly scavenging 1O_2 , O_2^- and OH^- and recovering tocopherol from the tocopheroxyl radical [49].

Tocopherols (Vitamin E)

Tocopherols, which are known to play a role in the removing of lipid radicals and ROS, are concentrated in biological membranes, especially in the thylakoid membranes of chloroplasts. Among the four isomers found in plants (α -, β -, γ - and δ -), α tocopherols have the highest antioxidative activity because they contain three methyl groups in their molecular structures. α -tocopherols are synthesized from γ -tocopherol by the enzyme γ -tocopherol methyltransferase in chloroplasts. They are critical in maintaining membrane stability against types of ROS such as O_2^- [50,51].

Carotenoids

Carotenoids are mainly responsible for suppressing 1O_2 without disturbing the structure of β -carotene and have a conjugated double bond structure to delocalize unpaired electrons. β -carotene shows chemical reactivity with free radicals such as H_2O_2 , O_2^- and OH^- radicals. At high concentrations, carotenoids can protect lipids from peroxidative damage [52].

Glutathione

Glutathione is a tripeptide (γ -glutamylcysteinyl glycine) detected in almost all cell parts such as the cytosol, chloroplasts, endoplasmic reticulum, vacuoles and mitochondria. Glutathione is

the main source of non-protein thiol in most plant cells. The chemical reactivity of the thiol group of glutathione makes it particularly suitable to serve a wide range of biochemical functions in all organisms. The nucleophilic nature of the thiol group is important in forming mercaptid bonds with metals and reacting with selected electrophiles. Glutathione is an antioxidant that acts in a variety of ways. In the ascorbate-glutathione cycle, GSH is used to reduce both enzymatic and non-enzymatic DHA (dehydroascorbate) and is itself oxidized to GSSG (oxidized glutathione). The regeneration from GSSG to GSH is catalyzed by glutathione reductase and NADPH is used as the reducing power. A central nucleophilic cysteine residue is responsible for the high reduction potential of GSH. This central role of GSH in antioxidative defense is due to its ability to regenerate another potent antioxidant, ascorbic acid, via the ascorbate-glutathione cycle [53].

Phenolic Compounds

Phenolic compounds, one of the most important secondary metabolite groups in plants and they have a very crucial antioxidant function. The phenylpropanoid metabolism and the amount of phenolic compounds increase under different environmental factors and stress conditions [54]. These compounds has been determined that the synthesis of isoflavones, which is one of the flavonoids, and some other flavonoids increase when the plant is infected, injured, under low temperatures and low nutrient conditions [55]. It is also known that plants accumulate UV-absorbing flavonoids in the vacuoles of epidermal cells in order to be protected from UV-B effects [56].

Enzymatic Antioxidants

Superoxide Dismutase (SOD)

Superoxide dismutase belongs to a class of metalloproteins that catalyze the splitting of superoxide (O_2^-) into molecular oxygen (O_2) and H_2O_2 . SODs are metalloenzymes that convert possible O_2^- to H_2O_2 after stress and it has been suggested that increases in the expression of SODs have important roles in coping with oxidative stress due to biotic and abiotic stress and contributing to the survival of plants under stress conditions [57]. SODs, which have the role of converting O_2^- to H_2O_2 , have three isoenzymes according to the metal ions located in their active centers. These are Cu/Zn SOD containing copper and zinc, Mn SOD containing manganese and Fe SOD containing iron [58].

Ascorbate Peroxidase (APX)

Ascorbate peroxidase (APX), another enzyme whose expression increases under stress conditions, is one of the enzymatic antioxidants thought to have an important role in the defense against ROS in many organisms, especially in advanced plants

[59]. APX exists in 4 different forms in the cell: soluble (sAPX), bound to thylacoid (tAPX), cytosolic (cAPX) and bound to the glyoxisome membrane (gmAPX) in the chloroplast stroma [60]. APX is involved in the ascorbate-glutathione cycle and reduces hydrogen peroxide using ascorbic acid as an electron donor [61].

Glutathione Peroxidase (GPX)

Glutathione peroxidase (GPX) is a large family of various isozymes that use glutathione to reduce the amount of H₂O₂, organic and lipid hydroperoxides, and are responsible for protecting plants against oxidative stress [62].

Catalase (CAT)

Catalase (CAT) is one of the most important enzymatic antioxidants responsible for protecting cells against stress by directly converting the harmful H₂O₂ formed under stress conditions to H₂O and O₂ [63].

Conclusion

Plants encounter many similar factors such as salinity, drought, pollution, heat and cold throughout their lives, and if they do not have the metabolism to tolerate this stress factors, their normal growth and development will be adversely affected. Changes in these conditions in plants are defined as stress. Due to the changing climatic conditions due to global warming, it has become very important to reduce the loss of species and varieties due to stress in the future. For this purpose, the technological developments; Understanding the defense mechanisms, especially in plant species that are resistant to cold and freeze stress, it will be a very important step in decreasing crop losses. In this context, in the studies carried out so far in which the molecular responses of plants to stress were evaluated, genes that may be related to stress were tried to be determined, and the expression levels of genes thought to be related under different plant and stress conditions were examined. Following the determination of stress-related target genes and their behavior against stress, studies are carried out to develop stress-resistant biotechnological products through special molecular methods such as gene transfer or gene silencing.

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