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Signaling and protection systems in the adaptation of plants to cold

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ABSTRACT

Global climate change increases the urgency of the problem of plant resistance to stress temperatures. Due to temperature instability in winter, as well as the earlier onset of meteorological spring with frosts, the likelihood of low-temperature damage to plants increases. Adaptive strategies of the different plant species have their own specific features. The review uses mainly the examples of cultivated plants to analyze the current understanding of the mechanisms of cold stress signal perception and transmission to genetic apparatus. Emphasis is placed on the participation of new signal mediators (gasotransmitters) – nitric oxide and hydrogen sulfide – in the formation of plant defense reactions under hypothermia. Data on the role of antioxidant and osmoprotective systems as well as specific proteins in plant adaptation to cold are summarized. The issues of functional interaction between the components of stress-protective systems during cold adaptation are discussed. The opportunities are outlined for the practical use of gasotransmitter donors as agents for plant priming in order to increase their resistance to hypothermia. The possibilities of using indicators of the state of stress-protective systems to search for donors of resistance are also critically analyzed.

Keywords: cold and frost resistance, signal reception and transduction, calcium, ROS, gasotransmitters, antioxidant system, cold stress proteins

INTRODUCTION

Ambient temperature is one of the most important factors determining the growth, development, and distribution of plants in specific climatic zones. Despite the fact that in recent decades there has been an increase in the average annual temperature, for most European countries, the urgency of the problem of cold and frost resistance of plants has not only not diminished, but is even growing (Chervenkov and Slavov, 2022).

Winter thaws alternating with sudden frosts cause real damage to plants and result in lower yields of winter cereals. The earlier onset of meteorological spring increases the likelihood of damage to plants as a result of spring frosts. For example, the simulation of wheat ontogenesis under higher temperature conditions showed an earlier onset of flowering (by 1 week with a 1°C rise in temperature) (Sadras and Monzon, 2006).

In recent decades, the world has made progress in understanding the mechanisms of plant cell perception of cold, low-temperature signal transduction into the genetic apparatus (Markovskaya and Shibaeva, 2017; Guo et al., 2018; Manasa et al., 2022), and the formation of adaptive responses (Kazemi-Shahandashti and Maali-Amiri, 2018). According to current understanding, damage to plants due to low positive and negative temperatures begins with disturbances in membrane structure and functions, and cold and frost resistance properties of plants develop during a low-temperature adaptation at low hardening temperatures.

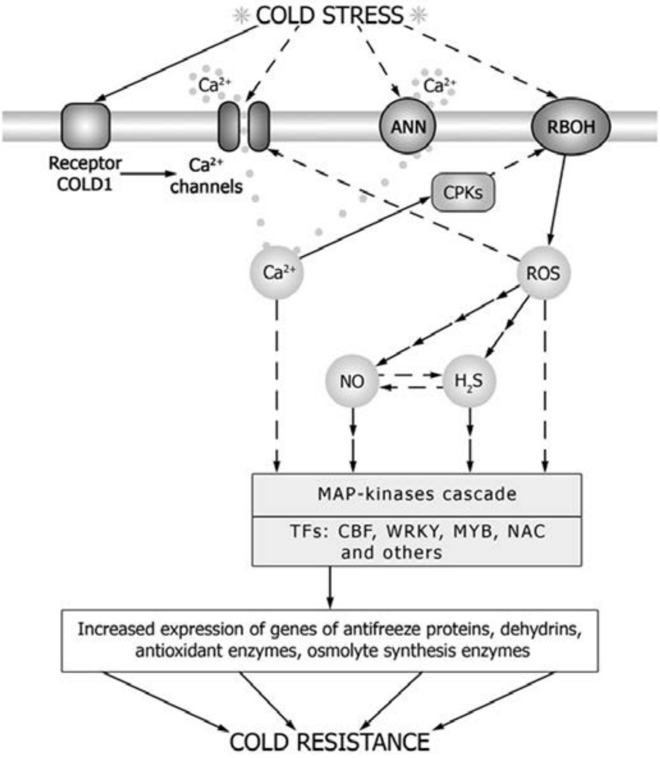
However, the regulation of plant adaptive responses to hypothermia is extremely complex, since it is carried out by a multicomponent network of signaling molecules and plant hormones (Guo et al., 2018; Hassan et al., 2021). The functional interaction between them remains poorly understood. Wherein the list of such signaling-regulatory molecules is constantly expanding. In recent years, in addition to such "canonical" components of signaling chains as calcium ions and reactive oxygen species (ROS), the role of gasotransmitters (in particular, nitric oxide NO and hydrogen sulfide H_2S) in cold adaptation has been considered (Fu et al., 2013; Fancy et al., 2017; Liu et al., 2019). The list of hormones (salicylic acid, brassinosteroids, and others) and hormone-like compounds (polyamines, melatonin, and etc.) that are important for plant adaptation to cold is also expanding (Nayyar and Chander, 2004; Vicente and Plasencia, 2011; Kosová et al., 2012; Bartwal and Arora, 2020; Zhang et al., 2021). These compounds or their donors and mimetics may find practical application as inducers of plant resistance to hypothermia.

Another aspect that complicates the construction of a holistic understanding of the adaptation mechanisms to hypothermia is the presence of species and varietal differences in the functioning of key protective systems. Meanwhile, most of the fundamental knowledge in this field is based on the main model object – *Arabidopsis thaliana*. The synthesis of new knowledge on these aspects for cultivated plants, primarily cereal grains, was the main objective of this review.

RECEPTION AND TRANSDUCTION OF COLD STRESS SIGNAL INTO THE GENETIC APPARATUS

Temperature is a physical factor affecting molecules (proteins, nucleic acids, lipids) and supramolecular complexes through conventional thermodynamic effects. In this regard, each of the mentioned molecules (structures) can hypothetically act as a thermosensor (Markovskaya and Shibaeva, 2017).

A decrease in temperature causes a very rapid increase in cell membrane rigidity due to lipid bilayer phase transitions (Kazemi-Shahandashti and Maali-Amiri, 2018). This effect leads to the activation of many signaling pathways due to changes in the state of a number of membrane-bound proteins (Figure 1). In Japanese rice plants, a specific receptor *COLD1* (chilling tolerance divergence 1) involved in the cold stress recognition has been identified. The *COLD1* protein is localized in the plasma membrane and endoplasmic reticulum. It senses cold signals and triggers the formation of plant tolerance to cold stress (Manishankar and Kudla, 2015). Mutants with *cold1* knockout showed greater sensitivity to cold compared to wild-type plants.



ANN – annexin; CPKs – cold stress response protein kinases; RBOH – catalytic subunit of NADPH oxidase; TFs – transcription factors. Explanations are in the text

Figure 1. Reception and transduction of the cold stress signal in plants

One of the earliest reactions that occur after sensors' perception of a cold stress signal is the opening of calcium channels (Figure 1). Phenomena of a rapid increase in cytosol calcium content in response to low temperature action on various plant species have been recorded for quite a long time (Jian et al., 1997; 1999). It is assumed that activation of cold receptor *COLD1* may be at least one of the mechanisms for opening calcium channels during hypothermia. Thus, changes in calcium homeostasis triggered by cold stress were impaired in *cold1* mutants compared to wild-type plants (Yuan et al., 2018). The influx of $[Ca^{2+}]_{cyt}$ is thought to be regulated by this protein together with the rice G-protein α subunit (RGA1) (Ma et al., 2015). However, the mechanism of changes in calcium homeostasis involving the COLD1-RGA1 complex is not yet known (Chen et al., 2021).

Several different types of calcium channels appear to be involved in cold-induced changes in cytosolic calcium concentration. These include cyclic nucleotide-gated channels (CNGCs) and glutamate receptors (GLRs) (Chen et al., 2021). Recent studies have shown that in rice OsCNGC9 channels are activated by phosphorylation by the OsSAPK8 protein kinase (homologue of Arabidopsis OST1). This causes increased cytosolic calcium influx, activation of downstream cold-sensitive genes expression, and increased cold tolerance in plants (Chen et al., 2021).

Another mechanism of cold-induced increase in cytosolic calcium content has been studied in Arabidopsis and is associated with the participation of the Ca^{2+}-permeable transporter Annexin1 (AtANN1). Its transport activity under the action of cold is enhanced by OST1-mediated phosphorylation (Liu al., 2021).

It is well known that an increase in cytosolic calcium concentration causes changes in the functional activity of many proteins. The most important effects are considered to be the increased activity of Ca^{2+}- and calmodulin-dependent protein kinases involved in the phosphorylation of other proteins, including transcription factors (TF) (Ding et al., 2019).

It has long been established that Ca^{2+} signals induce enhanced CBF-COR (C-repeat binding factor) gene expression under cold stress conditions (Chinnusamy et al., 2007). EF-hand domain proteins such as CaMs, CML, CBL (Ca^{2+}-binding calcineurin B-like), and CDPK (Ca^{2+}-dependent protein kinase) also play an important role as Ca^{2+} sensors under cold stress (Guo et al., 2018).

By now, the CDPK role in the regulation of *COR* gene expression has been established (Chinnusamy et al., 2007; Manasa et al., 2022). Transformation of cultivated tomato plants with the calcium-dependent protein kinase gene *SpCPK33* from *Solanum pennellii* increased their resistance to cold stress, which was associated with modulation of the expression of several protective genes, including that controlling antioxidant system (Hu et al., 2022). The role of Ca^{2+}-dependent rice protein kinase OsCPK24 in response to cold stress was recently investigated. It was shown that OsCPK24 knockout rice plants showed increased sensitivity to cold compared to wild-type plants. Conversely, plants overexpressing OsCPK24 showed increased cold resistance (Liu et al., 2017). Such plants were characterized by increased accumulation of the osmoprotectant proline and the antioxidant glutathione. The authors concluded that OsCPK24 functions as a positive regulator of cold stress tolerance in rice (Liu et al., 2017). However, so far, specific downstream Ca^{2+}-dependent proteins involved in the cold signaling pathway in general remain poorly understood (Ding et al., 2019).

Along with calcium ions, reactive oxygen species (ROS) appear to be involved in cold stress signal transduction into the genetic apparatus. Thus, increased activity of NADPH oxidase and increased formation of hydrogen peroxide have been recorded as a response of maize plants to low positive temperatures (Piotrovskii et al., 2011). The role of ROS as mediators in cold-induced activation of the antioxidant system in rice has been shown (Zhang et al., 2016).

Accumulation of ROS due to cold stress is supposed to result in Ca^{2+} signals associated with the opening of ROS-sensitive calcium channels (Guo et al., 2018; Yuan et al., 2018). On the other hand, calcium ions are known to activate NADPH oxidase (Gautam et al., 2017).

Gasotransmitter nitric oxide (NO) is another signaling mediator involved in the formation of plant response to hypothermia. Nitric oxide is in a close functional relationship with hydrogen peroxide and other ROS (Yemets et al., 2019). However, it is unclear whether the increase in its content in plants in response to hypothermia is due to an increase in ROS generation. Under cold stress, an increase in the NO content was registered in organs of plants of several species (*Arabidopsis thaliana*, *Pisum sativum*, *Triticum aestivum*, *Citrus aurantium*) (Zhao et al., 2009; Puyaubert and Baudouin, 2014; Baudouin and Jeandroz, 2015; Fancy et al., 2016).

The main enzyme of NO synthesis induced by the action of hypothermia on plants is probably nitrate reductase. For example, double Arabidopsis mutants *nia1nia2* had almost no change in NO content under low positive temperatures, and their resistance to negative temperatures after cold exposure at 4 °C did not develop (Zhao et al., 2009). A significant increase in nitrate reductase activity and its gene transcripts has been shown in Arabidopsis and citruses when exposed to low positive temperatures (Puyaubert and Baudouin, 2014).

Specific adaptive responses that can be formed during cold stress with NO participation have been investigated. In particular, cold-induced expression of the Δ^1-pyrroline-5-carboxylate synthase gene and proline accumulation in Arabidopsis has been found to involve NO (Zhao et al., 2009). These effects were weakly manifested in the *nia1nia2* mutants and were suppressed by the NO scavenger 2-phenyl-4,4,5,5-tetramethylimidazoline-1-oxyl 3-oxide (PTIO). The dependence on the NO status of expression of specific cold-sensitive genes such as *CBF* and *LTI* genes families has also been shown (Puyaubert and Baudouin, 2014; Baudouin and Jeandroz, 2015). Enhanced expression of these genes was suppressed by PTIO and was weakly manifested in mutants defective in nitrate reductase genes.

The positive effect of NO on plant cold tolerance may be due to the S-nitrosylation of target proteins. Thus, in *Brassica juncea*, the effect of differential S-nitrosylation of 10 proteins was found, among which the antioxidant enzymes dehydroascorbate reductase and glutathione-S-transferase (Sehrauit and Deswal, 2014). An increase in superoxide dismutase (SOD) and ascorbate peroxidase activity due to S-nitrosylation has also been shown (Puyaubert and Baudouin, 2014).

The role of nitric oxide in plant adaptation to cold is also evidenced by the enhancement of their resistance with the help of exogenous NO. Thus, treatment of Bermuda grass plants with nitric oxide donor sodium nitroprusside (SNP) reduced cold-induced membrane damage (Fan et al., 2015). Increased photosynthetic intensity and chlorophyll content have been reported in plants of different species when treated with nitric oxide donors under cold stress conditions (Fan et al., 2015).

Treatment with SNP at 0.1-0.5 mM concentrations increased the ability of seedlings of winter wheat and rye to cold hardening (6 days at 2-4 °C), resulting in a significant increase in their survival after freezing at -6 and -8 °C (Kolupaev et al., 2020b). Seed priming with NO donor increased the content of sugars, proline, and flavonoid compounds in these cereal seedlings. An increase in SOD and guaiacol peroxidase activity was also observed in them. Thus, exogenous NO can induce plant resistance to hypothermia. From a practical point of view, it is particularly promising to use NO donors or gaseous nitric oxide in the protected ground, as well as in the low-temperature storage of fruits and vegetables (Yemets et al., 2019).

Hydrogen sulfide, which, like nitric oxide, is one of the most important gasotransmitters, may be another participant in signaling processes responsible for the formation of plant cold resistance (Kolupaev et al., 2022). Thus, in Arabidopsis plants, under low temperatures, there was an increase in L/D-cysteine desulfhydrase gene expression and H_2S content in the leaves (Shi et al., 2015). An increase in hydrogen sulfide synthesis in grapes during cold adaptation has also been reported (Fu et al., 2013). A similar effect was found in cucumber leaves in response to 4 °C (Liu et al., 2019). One mechanism of hydrogen sulfide involvement in the Arabidopsis reaction to cold stress is associated with the persulfidation of at least one component of the MAP kinase signaling cascade (MAP4), leading to an increase in this enzyme activity (Du et al., 2021). Cucumber plants were also shown that endogenous and exogenous hydrogen sulfide induces a signaling chain involving plant hormone indole-3-acetic acid (IAA) and hydrogen peroxide (Zhang et al., 2021). In addition, the authors also suggest the possibility that H_2S affects H_2O_2 content without IAA involvement. One of the functions of the H_2S/IAA/H_2O_2 cascade under cold stress may be to induce the expression of the transcription factor *CBF1* gene and, as a consequence, the cold-sensitive *COR47* genes (Zhang et al., 2021). In general, hydrogen sulfide interacts with a complex system of hormonal regulation; in particular, it is involved in the signal transduction of important stress hormones such as abscisic acid and salicylic acid (Li et al., 2015; Shan et al., 2017).

In recent years, at various objects information has been obtained on an increase in plant resistance to low temperatures by exogenous hydrogen sulfide donors. For example, when treated with NaHS, the frost resistance of Bermuda grass was increased (Shi et al., 2014). Treatment with sodium hydrosulfide under normal conditions and against the background of cold hardening caused an increase in the survival of wheat and rye seedlings after freezing at damaging temperatures (Kolupaev et al., 2019a). Wherein it promoted an increase in guaiacol peroxidase and catalase activity, sugars and proline content. It was also found that under the action of hydrogen sulfide donor during cold hardening of wheat seedlings, the activity of phenylalanine ammonia - lyase, an enzyme that converts L-phenylalanine to trans-cinnamic acid, a precursor of most secondary metabolites, significantly increased (Kolupaev et al., 2018).

The downstream components of cold-induced signaling chains are transcription factors (TFs) that modulate the transcription of cold-sensitive genes (Figure 1). The TF family AP2/ERF, also known as CBF, is considered the most important (Ritonga et al., 2021). Some examples of the involvement of signaling mediators (particularly calcium, NO, H_2S) in the regulation of *CBF* gene expression have already been given above. A more detailed consideration of the involvement of these signaling mediators, as well as specific proteins, including various kinases, can be found in a number of recent reviews (Guo et al., 2018; Kazemi-Shahandashti and Maali-Amiri, 2018; Ritonga et al., 2021).

CBF TF together with a number of other TFs, in particular WRKY, bZIP, MYB, bHLH, C2H2, and NAC, regulate the expression of *COR* genes, leading to the formation of physiological responses to cold stress (Byun et al., 2015). Using tobacco plants as an example, bHLH TF has been shown to be a kind of transcription activator that functions by binding to the G-box/E-box motif in the *CBF* gene promoter, thereby controlling the expression of ROS removal-related genes and other genes involved in response to cold stress, and thus increases resistance (Sharma et al., 2020). A number of TFs belonging to bZIP groups are also known for they role in plant cold tolerance. An increase in their expression under cold stress was reported in various plant species such as Arabidopsis, soybean, and wheat. Arabidopsis transformation with the *TabZIP60* gene of wheat caused an increase in plant frost resistance (Sharma et al., 2020). In addition to these TFs, *COR* genes activation involves a cascade of protein kinases: MEKK1–MKK2–MPK4 (Ritonga et al., 2021).

STRESS-PROTECTIVE PLANT SYSTEMS UNDER THE ACTION OF COLD

Antioxidant system

Back in the 1980s–1990s, experimental data were obtained indirectly pointed to the role of oxidative stress in the development of cold damage in plants. It was shown that low temperature stress as well as high concentrations of oxygen and ozone in the atmosphere and treatment of plants with oxidative stress inducer paraquat cause damage in cell membranes with similar symptoms: increased viscosity, formation of gel phase regions, degradation of phospholipids, and accumulation of free fatty acids (McKersie et al., 1988).

As an increase in viscosity of the lipid part of cell membranes is one of the most common consequences of hypothermia on plants, membrane-bound processes such as photosynthesis and respiration are more sensitive to temperature stress compared to processes occurring outside the membranes involving soluble enzymes. ROS formed as a result of electron-transport chains failures can subsequently initiate non-enzymatic reactions leading to an additional increase in their amount (Trchounian et al., 2016).

Mitochondria, like chloroplasts, contain a large number of electron carriers. The redox potential of those that form the initial and middle sections of the chain is often more negative than –0.3 V ($O_2/O_2^{\bullet -}$ potential). This means that the accidental interaction of these carriers with molecular oxygen can lead to a one-electron reduction of O_2 to $O_2^{\bullet -}$ (Cvetkovska and Vanlerberghe, 2013). Naturally, with hypothermia-induced disruption of membrane structure and properties the probability of such events increases considerably.

Exposure of plants to low damaging temperatures causes both a stochastic enhancement in ROS generation and an enzymatic increase associated with the NADPH oxidase activation (Awasthi et al., 2015).

In connection with the described prerequisites for enhancing ROS generation by plant cells under the cold action, the activation of the antioxidant system is crucial to avoid oxidative damage of biomacromolecules and to prevent disruption of redox regulation of cellular processes (Kolupaev et al., 2019b).

It should be noted that antioxidant system activation is important for plant resistance not only to low temperatures but also to other adverse factors of the winter period, in particular to fungal diseases. For example, one of the factors significantly affecting the survival of cultivated cereals in winter is their resistance to snow mold, a disease caused by the fungus *Microdochium nivale* (Tronsmo et al., 2001). The relationship between the resistance of winter wheat and triticale to both low temperatures and snow mold pathogens and resistance to oxidative stress has been shown (Ergon et al., 1998; Golebiowska et al., 2011). The increased tolerance of certain genotypes to both adverse winter factors, in particular, may be due to the high activity of certain forms of peroxidase (Gawronska and Gołebiowska-Pikania, 2016).

Antioxidant enzymes in cold adaptation

The antioxidant system is represented by enzymatic and low-molecular-weight antioxidants. Enzymatic systems catalyze mainly the detoxification of superoxide anion radical and peroxides (Alscher et al., 2002; Kolupaev et al., 2019b).

SOD is the only enzymatic antioxidant that neutralizes radical ROS (Alscher, 2002). SOD is represented by a significant number of molecular forms. Their active centers may contain metals such as Cu, Zn, Mn, and Fe. Cu/Zn-SOD is the most abundant form of this enzyme in plant cells. It is localized in the cytosol, chloroplasts, cell walls, and other compartments. Less common are Mn-SOD and Fe-SOD.

Many studies have shown an increase in the activity and gene expression of various SOD forms during cold adaptation. An increase in total SOD activity has been detected in plants of different taxonomic groups (Luo et al., 2011; Liu et al., 2013; Chen et al., 2014; Kolupaev et al., 2015).

Catalase is believed to be in close functional interaction with SOD and is involved in the decomposition of large amounts of hydrogen peroxide (Guan and Scandalios, 2000). An increase in the enzyme activity has been shown when plants of various species were exposed to low temperatures, including hardening (Janda et al., 2007; Kolupaev et al., 2015; 2020a; Ma et al., 2019). Rice plants transformed with the wheat catalase gene exhibited efficient hydrogen peroxide detoxification and higher cold tolerance compared to conventional plants (Matsumura et al., 2002).

In many plant species, an increase in the nonspecific peroxidase activity was registered in response to cold. This effect was detected in oat plants under moderate frosts (Liu et al., 2013). The electrophoretic spectrum of peroxidase expanded in wheat plants under the action of hardening low temperature (Diachenko et al., 2007). Cold hardening caused a significant (2-3-fold) increase in guaiacol peroxidase activity in green plants and etiolated seedlings of rye (Streb et al., 1999; Kolupaev et al., 2015).

During the cold hardening of plants of various species, an increase in ascorbate peroxidase activity occurs. Such effects, in particular, are typical for winter wheat, triticale, and other cereals (Janda, 2003; Janda et al., 2007; Golebiowska et al., 2011).

Glutathione metabolism enzymes are also involved in plant response to hypothermia. Thus, in response to moderate low temperatures, an increase in glutathione reductase activity was recorded in wheat (Janda et al., 2007), barley (Radyuk et al., 2009), and Scots pine plants (Wingsle et al., 1999).

Alternative oxidase as a component of antioxidant system

Alternative oxidase catalyzes ubiquinol oxidation with simultaneous reduction of molecular oxygen to water. This prevents $O_2^{\bullet-}$ formation due to electron leakage from mitochondria complex III (Moller, 2001). Thus, mitochondrial alternative oxidase can be regarded as a component of the antioxidant system.

The expression level of alternative oxidase gene in plants has been shown to increase under the influence of adverse factors of various natures, including extreme temperatures (Searle et al., 2011).

In wheat, two genes encoding an alternative oxidase, *WAOX1a* and *WAOX1c*, have been identified which number of transcripts increases during cold hardening (Takumi et al., 2002; Mizuno et al., 2008). Along with the accumulation of transcripts, the ability of the enzyme to transport electrons increases and largely in more frost-resistant winter wheat compared to spring one (Mizuno et al., 2008). The results of Arabidopsis plants transformation with the wheat *WAOX1a* gene support the hypothesis of the antioxidant function of alternative oxidase at low temperatures (Sugie et al., 2006).

Low-molecular-weight antioxidants

Ascorbate is the most common low-molecular-weight antioxidant in plants (Kaur and Nayyar, 2014). There is evidence of an increase in its content in plants during cold adaptation (hardening). Thus, an increase in the amount of reduced ascorbate was noted in Scots pine (WIngsle et al., 1999), rye (Galiba, 2013), and barley (Radyuk et al., 2009). However, a positive relationship between ascorbate content and plant resistance to cold is not always evident. For example, a more cold-resistant strawberry variety had lower ascorbate content than a weakly resistant one (Luo et al., 2011). Apparently, despite the universality of ascorbate as the most common antioxidant, species and varietal features of its participation in adaptation processes are possible.

Glutathione (L-γ-glutamyl-L-cysteinyl-glycine) as an antioxidant containing sulfhydryl group can directly interact with hydrogen peroxide and also be involved in dehydroascorbate reduction (Foyer and Noctor, 2009). The permanent removal of hydrogen peroxide requires that the level of reduced ascorbic acid and glutathione are sufficiently high. To do this, several enzymes work together in the so-called ascorbate-glutathione cycle, which provides hydrogen peroxide neutralization. The cycle includes interconnected redox reactions involving ascorbate, glutathione, and NADPH (Asada, 1999). There is evidence of an increase in reduced glutathione content during cold adaptation of Scots pine (Wingsle et al,. 1999), strawberry (Luo et al., 2011); (Radyuk et al., 2009), and plants of many other species. GSH and ascorbate content values are usually found to be interdependent (Galiba, 2013).

Flavonoids are polyphenolic antioxidants widely distributed in the plant world (Khlestkina, 2013). All of them, to one degree or another, are involved in the antioxidant protection of cells. According to the generally accepted viewpoint, the antioxidant properties of flavonoids are due to their ability to serve as traps for free radicals as well as to chelate metal ions involved in radical processes (Es-Safi et al., 2007).

Cold and other stressors have a significant impact on the flavonoid content in plant tissues. For example, in corn plants when exposed to cold (10 °C), there was an increase in gene expression for phenylalanine ammonia-lyase and other enzymes involved in the synthesis of flavonoids (Christie et al., 1994). Etiolated rye seedlings characterized by a certain level of constitutive frost resistance contained a significantly higher amount of anthocyanins compared to non-resistant wheat seedlings (Kolupaev et al., 2016). During cold hardening of a frost-resistant wheat variety, the flavonoid content increased 3-fold, while that of a non-resistant variety increased 1.5-fold (Olenichenko et al., 2008). In seedlings of different triticale varieties, a relationship was found between frost resistance (against the background of hardening) and the content of anthocyanins (Kolupaev et al., 2020a).

Polyfunctional low-molecular-weight compounds

Soluble carbohydrates are a group of low-molecular-weight organic compounds whose contribution to frost and cold resistance has been studied for many decades. Moderate cold action, which usually causes a hardening effect, leads to the accumulation of sugars in plants of various species (Kocsy et al., 2011).

The most abundant plant carbohydrate is the disaccharide sucrose and the dominant water-soluble compounds accumulating in the tiller nodes of wheat during cold hardening are fructans (Yoshida and Kavakami, 2013). The effect of accumulation of fructose, sucrose, and fructans was noticeably manifested in wheat plants after 3 days of hardening (Vagujfalvi et al., 1999). In rye plants, a fairly rapid increase in sucrose and raffinose content was observed during cold adaptation (Koster and Linch, 1992). The accumulation of sugars in rapeseed during acclimation at 4 °C was shown to be observed after 7 and reached a maximum after 14 days. However, species differences in the rate of sugar accumulation in response to low hardening temperatures can be quite significant. Thus, in the model object *Arabidopsis thaliana*, a significant accumulation of sucrose, glucose, and fructose was noted already after 1-4 hours of incubation at low positive temperatures (Kaplan et al., 2007). Wherein soluble carbohydrates accumulation correlated with the development of plant resistance to cold.

Sugars are currently regarded as polyfunctional protective compounds in plants. They are membrane, protein, and lipid structure stabilizers (Deryabin and Trunova, 2021). The phase transition temperature and fluid properties of biological membranes depend on the content of sugars in the surrounding solution (Quinn, 1989). Sugars are thought to stabilize the structure and fluidity of cell membranes under hypothermic conditions by interacting with membrane lipids and proteins (Sum et al., 2003).

Another very important function of soluble carbohydrates under stress is the ROS neutralization, especially their radical forms. At present, sugars are considered to be the full-fledged components of the non-enzymatic antioxidant system of a plant cell (Gangola et al., 2018).

Along with sugars, proline is a very important stress metabolite involved in plant adaptation to hypothermia. This imino acid functions as an osmoprotectant, membrane-protecting compound, and antioxidant (Liang et al., 2013). In addition to data on the proline's ability to bind free radicals, there is evidence of its antioxidant activity in experiments *in vivo*. For example, sugarcane plants transformed with the Δ^1-pyrroline-5-carboxylate synthase gene and accumulating a large amount of proline were shown to differ from normal plants in their high resistance to the oxidative stress agent paraquat (Molinari et al., 2007).

An increase in proline content during cold hardening was recorded in many plant species: rye (Koster and Linch, 1992), rice (Aghaee, 2011), oats (Liu et al., 2013), Bermuda grass (Zhang et al., 2010), cabbage (Klima et al., 2012), tobacco (Konstantinova et al., 2002), chrysanthemum (Chen et al., 2014), *Jatropha curcas* (Ao et al., 2013), etc. For plants of some species (rye, clover), an increase in proline content has been shown under damaging, incl. negative temperatures (Svenning et al., 1997; Kolupaev et al., 2015). A report by Tantau et al. (2004) showed a fairly close relationship between proline accumulation and frost resistance of barley lines grown *in vitro*. Higher proline content was noted during cold adaptation in the more cold-resistant strawberry genotype, which was combined with a lower manifestation of oxidative damage (Luo et al., 2011).

Some cold stress proteins

One of the important mechanisms of plant adaptation to negative temperatures is the accumulation of specific proteins. Antifreeze proteins play a role in reducing damage associated with ice formation. Thus, in winter rye, six antifreeze proteins with molecular weights from 16 to 35 kDa were found, all of which are able to bind to the ice surface and inhibit its growth *in vitro* (Hon et al., 1994). It has been established that during cold acclimatization, the accumulation of these proteins correlates with development of frost resistance of rye, wheat, and barley varieties (Marentes et al., 1993; Antikainen and Griffith, 1997). Two genes encoding antifreeze IRI proteins, *LpIRI-a* and *LpIRI-b*, were found in cold-resistant ryegrass plants (Zhang et al., 2010). It was shown that already after 1 hour of cold acclimatization, the *LpIRI-a* transcripts level increased by about 40-fold, and *LpIRI-b* by about 7-fold. The involvement of signaling mediators in the cold-induced synthesis of antifreeze proteins remains largely unexplored.

Another group of protective proteins is dehydrins. Dehydrin genes belong to the set of cold-sensitive genes (Hassan et al., 2021). They are so-called late embryogenesis abundant (LEA) proteins, which accumulation is primarily linked to plant adaptation to dehydration (Hara, 2010). Dehydrin synthesis is induced by the effect of low temperatures, drought, salinity, and heavy metal ions. Calcium, together with CDPK, plays an important role in the regulation of stress-induced dehydrins synthesis (Sun et al., 2021). It is thought that the increased calcium influx into the cytosol and increased CDPK activity due to increased membrane rigidity lead to the activation of key *CBF* family transcription factors involved in cold adaptation. The expression of *DREB1A/CBF3* or *DREB1C/CBF2* is regulated at the transcription level by INDUCER OF CBF EXPRESSION 1 (ICE1) or calmodulin-binding transcription activator (CAMTA), respectively (Hirayama and Shinozaki, 2010). ICE1 is a MYC-type transcription factor that regulates many plant responses to abiotic stresses (Hirayama and Shinozaki, 2010). CAMTA transcription factors recognize sequences of conservative motifs that provide a link to Ca^{2+} signaling, which is activated under abiotic stresses, including exposure to low temperatures (Doherty et al., 2009). Activation of *CBF* family transcription factors in turn induces the cold response gene families *COR* and *LEA* (Chang et al., 2021). Notably, the enhancement of cold-sensitive *VvICE1* and *VvCBF3* gene expression in grape (*Vitis vinifera*) plants was affected by the exogenous hydrogen sulfide donor NaHS, while the increase of plant frost resistance was observed and exposure to low positive temperatures resulted in a transient increase of H_2S synthesis. Thus, it appears that hydrogen sulfide, along with calcium, may be involved as a signaling mediator in the regulation of cold stress response genes (Fu et al., 2013).

Dehydrins are supposed to perform not only chaperone, cryoprotective, and antifreeze, but also antioxidant and ion-binding functions, as revealed in experiments *in vitro* (Kosová et al., 2010). One of the functions of dehydrins under cold stress may be related to the fact that they can interact with plasmalemma under freezing-induced dehydration and prevent its fusion with intracellular organelle membranes (Takahashi et al., 2018).

A special property of dehydrins is their ability to bind calcium. It has been suggested that so-called acidic dehydrins may function as ionic buffers or calcium-dependent protein chaperones, which may be important for maintaining cellular homeostasis under stress conditions (Hara, 2010). In addition, dehydrins are involved in maintaining the redox balance as they have the ability to bind superoxide radicals, hydroxyl radicals, and other ROS, as well as metal ions that catalyze the non-enzymatic formation of ROS (Sharma, 2022).

At transcript and protein contents, there has been repeatedly reported a correlation between dehydrin accumulation and plant acquired frost resistance. It is assumed that the relative accumulation of transcripts of dehydrins and WCS120 proteins can be considered a marker of frost resistance of common wheat under controlled conditions (Kosová et al., 2014).

CONCLUSION

Mechanisms of plant perception of cold stress signal and its transduction into the genetic apparatus are still far from being fully understood. It is assumed that the primary signal in temperature stress perception by plants is a change in biological membrane fluidity. There is still no unambiguous understanding of what may be the key sensor for these signals. The sequence of events in the plant cell during cold stress signal transduction into the genetic apparatus also remains unclear. Nevertheless, the critically important role of such universal signaling mediators as calcium ions and ROS in these processes is beyond doubt. In recent years, knowledge has been dynamically accumulating on the participation of gasotransmitters nitric oxide and hydrogen sulfide in cold signaling and the formation of adaptive plant responses. The availability of inexpensive, low-toxic, and sufficiently stable donors of gasotransmitters opens up new opportunities for increasing plant resistance to low temperatures by priming them with these compounds.

Among the plant defense systems important for survival at extremely low temperatures, the antioxidant system occupies a special place. Its activation during hardening and moderate cold stress has been shown by numerous studies performed on plants of different taxonomic affiliation. At the same time, the antioxidant system is multicomponent. Its components are in functional interaction, the peculiarities of which began to be actively studied only in recent years.

A certain contribution to the maintenance of pro-/antioxidant balance is probably also made by dehydrin proteins. In this regard, it seems tempting to quantify the contribution of these agents to antioxidant defense and low-temperature resistance of plants. At the same time, the role of certain defense systems can differ significantly in plants of different species. Thus, effective screening of resistance donors for the needs of breeding is possible only by taking into account the specific features of antioxidant and osmoprotective systems.

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