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COLD STRESS: MOLECULAR EFFECTS ON MEDICINAL PLANTS

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ABSTRACT. Plants do not have the ability to migrate to another suitable environment like other living things, for this reason, they should adapt to the different environmental conditions or their reproduction, the development and the growth are negatively affected. All these negative situations that affect plants negatively are called stress that are divided into two groups biotic and abiotic. The cold stress has two main effects on the plant: low temperature and dehydration. Plants create a tolerance response to this stress by laying out the transcriptional levels of proteins with different functions and especially transcription factors and some genes. Molecules involved directly or indirectly here include cryoprotectant proteins, chaperones, transcription factors and kinases. In the current review article, it was aimed to examine the cellular responses of medicinal plants treated with the cold stress during the adaptation process at the molecular level.

Keywords: *CAMTA, CBF/DREB TFs, cold stress, Hsp Family, MYB TF.*

INTRODUCTION

Medicinal plants have antimicrobial properties thanks to the secondary metabolites they contain, which allows them to be used for traditional medicine from ancient civilizations to the present. Along with the ever-developing scientific medicine, medicinal plants are still widely used around the world. The presence of approximately 20,000 medicinal plants, which are also the active ingredients of drugs used in the healing of many diseases, and for this reason, the World Health Organization (WHO) informed the countries for the use of traditional medicine in 1977 [1] and a year later, research and training centers were established in order to examine the medicinal plants in detail. As a result of the call for the establishment of medicinal plants, more use was encouraged [2]. Again, accordingly the research of the WHO [3], 80% of the developing countries and 60% of the countries around the world perform their health care by using traditional medicine [4, 5, 6].

Medicinal plants are widely used in a many of industries in the world. Due to the over side effects of artificial drugs produced in the pharmaceutical sector today, people use plants again to find healing in natural ways [7, 8]. In addition to taking an important place in the pharmaceutical industry with the active substances obtained from the secondary metabolites of medicinal plants, they are also used in cosmetics, spices, food, paints, insecticides, resins, gums, etc. It is also used in other areas, and its essential oils with aromatic properties are also used in the perfume industry [1, 9].

The secondary metabolites of plant, unlike primary metabolites, are not essential for the plant viability and metabolism, but are small molecule metabolism products necessary for its secondary needs [10, 11]. Plants can synthesize secondary compounds including taxoids, polysaccharides, and flavones in addition to the basic nutrients they synthesize for their basic needs, such as protein, fat and carbohydrates, and in this way, the threats posed by bacteria, fungi and even other plants, as well as the biological stress they experience and environmental factors such as drought, temperature, humidity, UV rays and salinity. They can adapt to abiotic stresses caused by threats [12, 13]. At the same time, they communicate with other living things in which they are in harmony, and ensure the realization of events such as pollination and seed distribution, signaling, stimulating or inhibiting enzymes, acting as a cofactor for the catalytic activity of an enzyme [13, 14, 15]. Contrary to the sudden death of the plant in the absence of primary metabolites, in the absence or deficiency of secondary metabolites, adverse effects on the physical appearance of the plant, regression in reproduction and development are observed, while sometimes no change can be observed [16].

Plants are organisms that are in constant interaction with the external environment throughout their lives. As a result of changes in their environment, they do not have the ability to migrate to another suitable environment like other living things, and because they are sessile organisms, they either show the ability to adapt to the environmental condition changes or their reproduction, the development, the viability and the growth are negatively affected. All these negative situations that affect plants negatively are defined as stress [17, 18]. Some of these stresses are abiotic factors such as extreme cold, drought, excessive salinity, chemicals, UV radiation, while others are biotic factors created by microorganisms such as bacteria, fungi and predator animals [19]. Plant tolerance, however, refers to the continuation of the plant's potency to reproduce and survive by developing a physical, biochemical or molecular defense mechanism under these environmental and biological stress conditions [20]. In this review article, it was aimed to examine the cellular responses of medicinal plants treated with low teperature during the adaptation process at the molecular level. The present study is derived from a part of the related author's master's thesis.

The Abiotic Stress In Plants

Recently, as a result of climatic changes such as global warming, which tends to increase, and anthropogenic activities such as improper fertilization, sudden increases and decreases in temperatures, drought, excessive salinity, excess or insufficient amount of soil nutrients, changes in the amount of light and abiotic reactions such as UV rays. Stress factors, either alone or in combination, disrupt plant homeostasis, and this adversely affects plant growth, development and reproduction. Plants, like all other living things, suppress their developmental germination, reduce growth and reproduction against abiotic stresses in order to survive and continue their generation; physiologically, decrease in water intake, change in transmission rate, decrease in photosynthesis and nitrogen assimilation, change in respiration, accumulation of growth inhibitors; molecularly, it responds in the form of the gene expression regulation, synthesis of macromolecules, reduction of the activities of crucial enzymes, the synthesis reduction of protein and irregularity of membrane structure [21]. Depending on the severity and duration of any abiotic factors, the plant tolerance with its responses to this negative situation changes, and if the plant is subjected to excessive stress for a long time, it may not be able to tolerate this situation and result in death.

Among abiotic factors, cold stress is one of the most restrictive stresses for plant metabolism, development and growth. Cold stress has two main impacts on the plant: low temperature and dehydration. The cold effect occurs when the plant is exposed to temperatures below 15˚C, which is called chilling temperature, or below 0˚C, which is called freezing temperature (Fig. 1). At temperatures below 15˚C, some limitations are observed in the enzyme activation included in the plant's respiration and photosynthesis processes [22, 23, 24] and biological events such as flowering, but the temperature level When it returns to an optimal level for the plant or when adaptation is in question, the plant continues its metabolic activities. However, at freezing temperatures under the 0˚C, formation of ice begins in the extracellular area of plant cells and the water content in the soil, and this prevents water flow, causing dehydration and osmotic stress, and puts the plant on an irreversible path [21, 25, 26]. When dehydration occurs in the plant depending on the freezing temperature, ice formation begins in the intracellular area. This causes negative turgor pressure to occur and the protoplast volume to decrease. In addition, it can cause the formation of radicals that cause oxidative stress, denaturation of proteins, and changes in membrane potentials. As a result of intracellular and extracellular ice formation, it is the breakdown of plant tissues and membrane bilayer layer by freezing dehydration that will cause the worst damage to the plant [21, 25, 27, 28, 29].

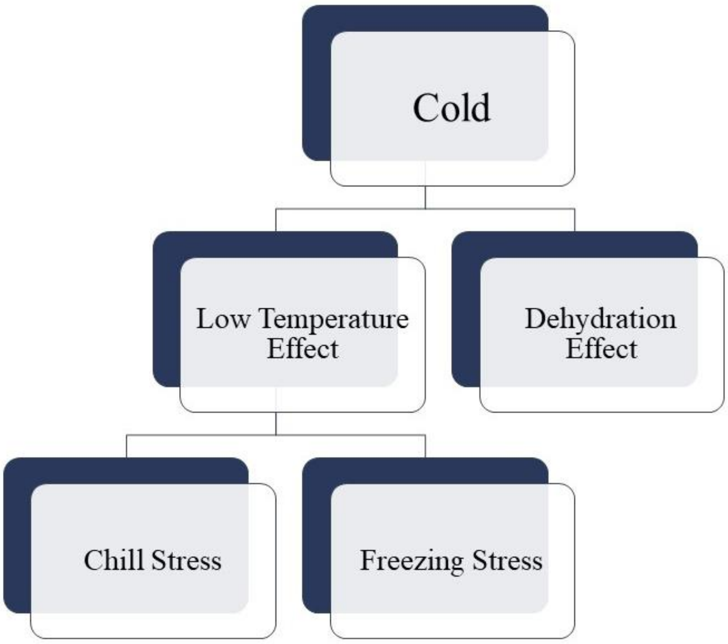

Fig. 1. *Effects of cold stress on plants* [25].

Different species show very different tolerances to cold temperatures to cope with cold stress. For example, plants that are sensitive to low temperature in tropical regions are damaged irreversibly as a result of deterioration of metabolism, alteration of protein and membrane structure, and inhibition of some enzymatic reactions even at cold temperatures, while plants that are tolerant to low temperatures and sensitive to freezing temperatures are susceptible to temperatures slightly below freezing. They can even survive, but they can get very risky damage as a result of their tissues freezing. In addition, plants that are tolerant to freezing temperatures can survive at these temperatures depending on the cold treatment time and the freezing severity [30]. As a result, the extent of the damage that low temperatures will cause on the plant;

- the tissue type,
- the stage of growth and development,
- to freezing time
- and depending on the cooling rate.

When plants are faced to sudden variations in their environmental conditions, they try to adapt to the environment with some arrangements at the molecular level, despite the deterioration of their physiological and metabolic intracellular balances. When most plants are exposed to chilling temperatures, they increase the molecules accumulation such as osmolytes and cryoprotectants, which contain soluble sugars, and also change the cellular membrane composition. This process, called cold acclimation, helps plants tolerate frost stress [24, 31, 32]. When the plant experiences cold-induced stress at lower temperatures, it senses this by conformational changes in membrane stability and proteins as a result of the plasma membrane changing from a liquid crystal state to a solid gel state. This perception results in the transfer of the signal into the cell by causing the release of Ca2+ ions, which are the secondary messengers, from the cell surface via Ca+2 channels, and from within the cell from some organelles (mitochondria, endoplasmic reticulum) to the cytosol [33, 34, 35, 36, 37]. This triggers protein kinases and a number of transcription factor cascades, thereby activating various signal transduction pathways. Thus, the activation and/or inhibition of proteins such as cold-induced Hsp, LEA, COR, ICE is achieved, and eventually a molecular level response to stress occurs in the nucleus (Fig. 2).

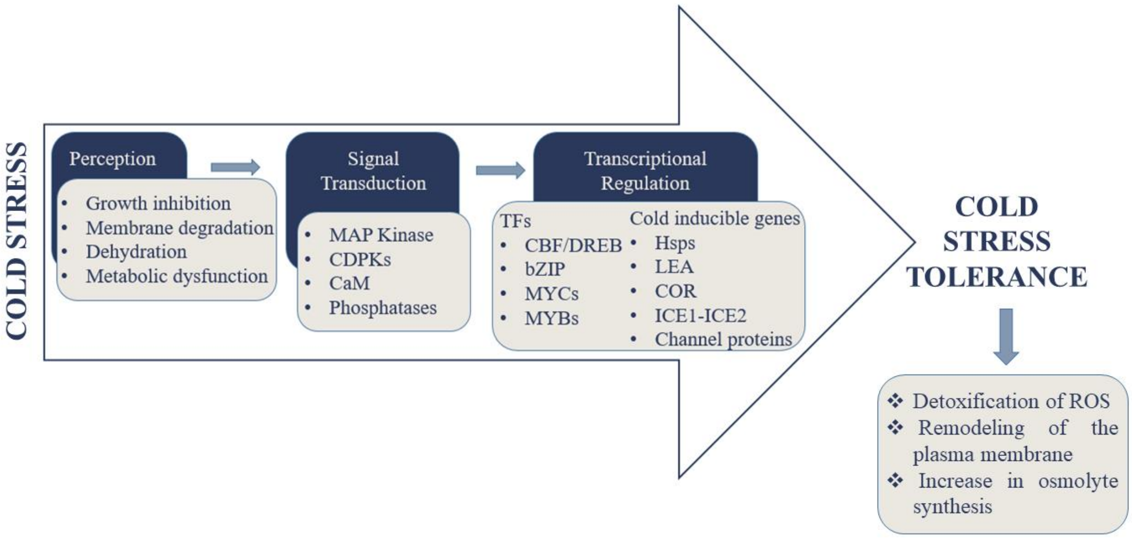

Fig. 2. *A general molecular response to cold stress*

Cold Stress Genes

Low temperatures are one of the most significant environmental stresses affecting plant productivity, growth, and development as well as their geological distribution [69]. Plants create a tolerance response to this factor by arranging the transcriptional levels of proteins with different functions and especially transcription factors and some genes. Molecules involved directly or indirectly here include cryoprotectant proteins, chaperones, transcription factors and kinases [31, 37].

C-Repeat/Dehydration Responsive Element-Binding Factor

CBFs, also known as dehydration sensitive element binding factors (DREB), which are members of the AP2/ERF (APETALA2/Ethylene Sensitive Factor) transcription factor superfamily, play a role in the expression of cold-induced *COR* genes [24, 37, 38, 39]. For the protein expressions in charge of the cold stress response, CBF/DREB TFs bind to specific sequences (C-repeat: TGGCCCGAC) containing highly conserved CCGAC bases, also known as DRE/CRT, in the promoter site of cold and dehydration sensitive genes to positively control the expression of genes of interest [37, 40, 41, 42]. It has been reported by studies that proteins synthesized by CBF/DREB TFs in many plants are effective in the formation of cold tolerance by affecting the increase of the accumulation of proline, unsaturated fatty acids, and soluble sugars. There are some studies showing increased cold tolerance in some plant species of CBF TFs and in transgenic plants developed to overexpress *CBF/DREB* genes [43]. As seen in Table 1, CBF signal transduction pathway is very important for many plants in tolerating low temperature stress.

As shown in Fig. 4, transcription of *CBF* genes is regulated by some cold signaling molecules such as ICE, CAMTA, HOS, CCA, BZR and MYB [44, 45]. ICE, CAMTA, BZR and CCA regulate the positive gene expression by connecting to the promoter site of the *CBF* gene, while HOS1 negatively regulates the CBFs expression indirectly through 26S proteosome-mediated degradation of ICEs.

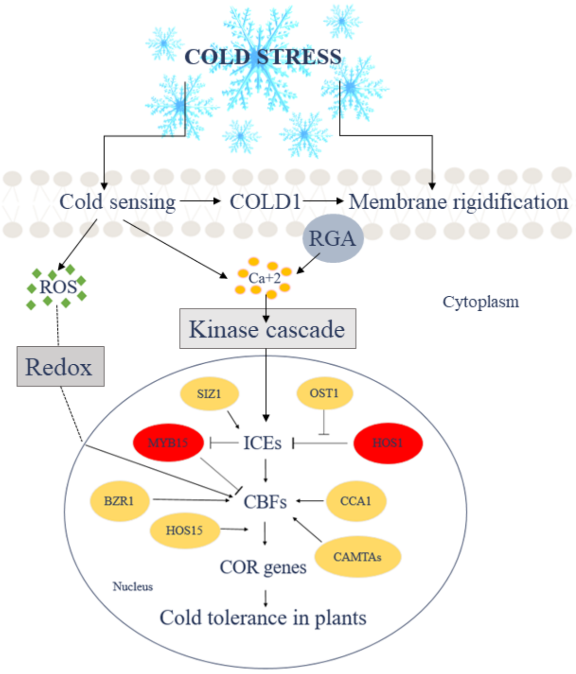

Fig. 3. *The role of CBF transcription factors in the acquisition of cold stress tolerance [24].*

MYB Transcription Factors

Plants manage to survive by arranging various signal networks with their wide range of TFs to adapt to the alteration of the environmental factors in their environment. MYBs are also one of the largest TFs families, accounting for approximately 9% of total TFs in plants. MYB proteins have MYB domains consisting of different repeats such as R1, R2, R3, and R4 at their N-terminal ends and are basically divided into four subgroups according to these repeats. 1R-MYBs are classified as MYB-associated proteins, 2R-MYBs are R2R3-type MYB proteins, 3R-MYBs are R1R2R3-MYB proteins, and 4R-MYBs are classified as 4R-like MYB proteins [46, 47]. It has been stated in the works that *MYB* genes positively regulate the expression of many proteins included in the formation of the response to abiotic factors (Table 1). When studies on transgenic *A. thaliana* and *O. sativa* plants, which were developed to overexpress *MYB* genes, are examined, it is seen that *OsMYB3R-2* gene is effective in gaining tolerance against freezing, drought and salt stresses in *A. thaliana* [48]. It has provided tolerance to freezing and cold stress [49]. In *A. thaliana*, the *AtMYB15* gene was found to be effective in frost tolerance [50], and the *GmMYBJ1* gene in acquiring cold and drought tolerance [51], while the *OsMYB30* gene in *O. sativa* increased frost sensitivity [52] has been reported to be effective [37]. In another study, it was shown that PbrMYB5, an *R2R3-type MYB* gene in *Pyrus betulifolia*, positively modulated AsA synthesis, which is effective in cold stress tolerance, by binding to the promoter of the *PbrDHAR2* gene as a transcriptional activator [47].

MYBs are TFs recognized to be effective in the cold stress response. MYBs also activate the relevant signal transduction pathways, as in other TFs, and also positively regulate the protein expression by connecting to the cis-elements located in the promoter site of target proteins (Fig. 4) [53].

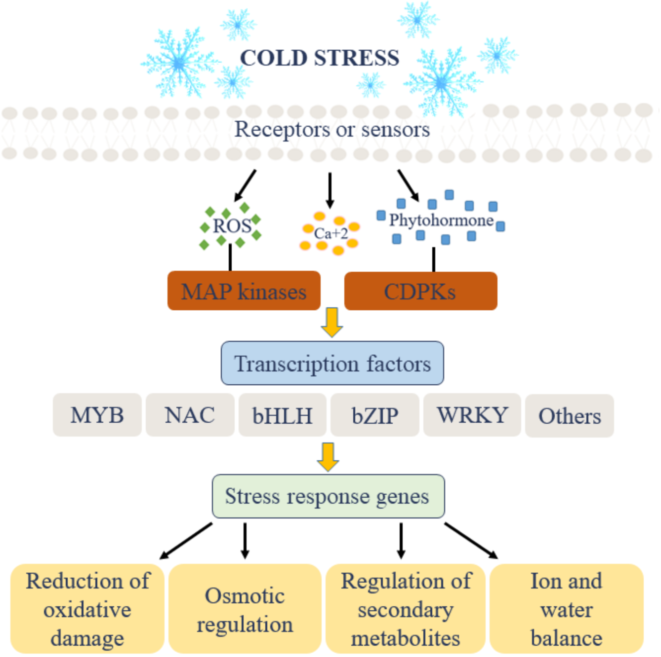

Fig. 4. *The role of myb transcription factors in the acquisition of cold stress tolerance [53].*

Calmodulin-Binding Transcription Activator

The calmodulin binding factors that contain the IQ domain for calmodulin binding belong to the CAMTA family. The N-terminal end of CAMTA proteins has the CG-1 domain, which shows properties of specific DNA binding. This domain binds to the vCGCGb sequence, also known as the CG-1 element, located 1 kb upstream from the start codon of the *CBF2* gene [45]. This interaction positively affects the expression of the CBF2 transcription factor, which is known to play an active role in the cold stress response. As a result, the plant gains cold stress tolerance (Fig. 5).

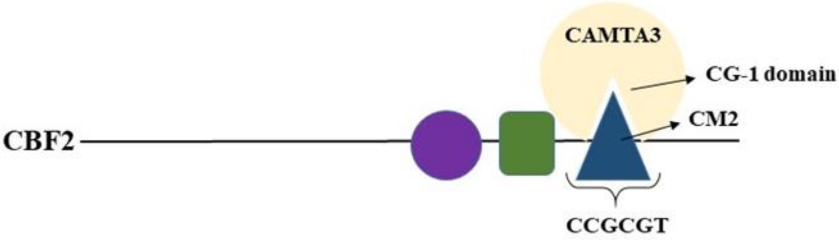

Fig. 5. *The role of the CAMTA3 molecule in the acquisition of cold stress tolerance*

Heat-Shock Protein Family

Abiotic stress factors such as cold and drought cause the proteins to deteriorate structurally and thus to lose their functions. Heat shock proteins, also known as molecular chaperones, are responsible the membrane integrity and protein structures of plant cells in the absence of any stressor, thereby maintaining cellular homeostasis [54]. Under stress, the expression levels of Hsps are controlled by transcription factors called Hsfs. Hsp molecules, whose expression level changes with heat stress, contribute to the establishment of stress tolerance by performing events such as protein folding or degradation, and determination of protein localization [55]. For this reason, Hsps have a very important role against environmental stresses (water scarcity, heavy metals, cold, heat) experienced by plants. Among the heat shock proteins have been relatively well defined [54].

Table 1. *Cold stress tolerance studies and observed effects on some natural and transgenic plants in the last ten years*

Gene	**Plant**	**Effect**	***Reference***
AnCBFs	*Ammopiptanthus nanus*	Increased cold tolerance	[55]
GthCBF4	*Gossypium herbaceum*	Increased cold tolerance	[56]
JcCBF2	*Nicotiana benthamiana*	Enhanced drought tolerance	[57]
VvCBF2, VvCBF3, VvCBF4, VvCBF6	*Vitis vinifera* *V. riparia*	Cold tolerance	[58]
AtDREB1A	*Solanum lycopersicum*	Frost tolerance	[59]

KcCBF3	*Kandelia candel*	Cold tolerance	[60]
MeCBF1	*A. thaliana* *Manihot esculenta*	Increased cold tolerance	[61]
GmDREB2A	*Solanum melongena*	Increased cold tolerance	[62]
PmhCBFc	*A. thaliana*	Increased freezing and oxidative stress tolerance	[63]
GhCBF3	*A. thaliana*	Increased drought and salt tolerance	[64]
DaCBF7	*O. sativa*	Enhanced cold tolerance	[65]
GmDREB1	*A. thaliana*	Cold, temperature and drought tolerance	[66]
CbCBF	*N. tabacum*	Frost tolerance	[67]
NtDREB1A *NtERD10B* *NtERD10C*	*Malus domestica 'Gala'*	Increased cold tolerance	[68]
VaCBF1	*N. tabacum*	Increased cold tolerance	[69]
VaCBF4	*A. thaliana*	Cold, salinity and drought tolerance	[70]
ZmCBF3	*O. sativa*	Cold, salinity and drought tolerance	[71]
ZmDBP4	*A. thaliana*	Enhanced cold and drought tolerance	[72]
PpCBF1v	*Apple (Malus × domestica)*	Increased cold tolerance	[73]

TaDREB2 TaDREB3	*Triticum aestivum* *Hordeum vulgare*	Cold and drought tolerance	[74]
CsR2R3-MYB	*Camellia sinensis*	Increased cold tolerance	[75]
GmMYB81	*Glycine max*	Increased cold tolerance	[76]
EgMY18,19,20	*Elaeis guineensis*	Increased cold tolerance	[77]
MdMYB108L	*Malus communis*	Increased cold tolerance	[78]
PbrMYB5	*Pyrus betulaefolia*	Increased cold tolerance	[47]
MdMYB124	*Malus domestica*	Increased cold tolerance	[79]
MdMYB88	*A. thaliana*	Soğuğa dayanıklılığının artması	[79]
OsMYBR1	*O. sativa*	Increased cold tolerance	[80]
FtMYB9	*Fagopyrum tataricum*	Increased cold tolerance	[81]
MYB96	*A. thaliana*	Increased cold tolerance	[82]
MdSIMYB1	*M. domestica 'Gala'*	Increased cold tolerance	[68]
TaMYB56-D	*A. thaliana*	Increased cold tolerance	[83]
TaMYB56-B	*A. thaliana*	Increased cold tolerance	[83]
R2R3 MYB	*Cephala f. tricolor*	Increased cold tolerance	[83]
OsMYB2	*O. Sativa*	Increased cold tolerance	[84]
CAMTA	*A. thaliana*	Increased cold tolerance	[85]

TaCAMTA1-A, 1-D, 3-A ve 3-D	*Triticum aestivum L.*	Increased cold tolerance	[86]
CAMTA3 CAMTA5	*A. thaliana*	Increased cold tolerance	[87]
Camta3	*A.thaliana*	Increased cold tolerance	[88]
ZmCAMTA4a *ZmCAMTA7a* *ZmCAMTA7b*	*Zea mays* L.	Increased cold tolerance	[89]
Camta2	*A.thaliana*	Increased cold tolerance	[90]
Camta1	*A.thaliana*	Increased cold tolerance	[90]
CAMTA3	*A. thaliana*	Increased cold tolerance	[45]
HbHsfA4a *HbHsfA4d,* *HbHsfA9b,* *HbHsfC1a* *HbHsfC1b*	*Hevea brasiliensis*	Increased cold tolerance	[91]
RcHSP70	*Rosa hybrida* L.	Increased cold tolerance	[92]
QBS00812_HSP70	*Lens culinaris*	Increased cold tolerance	[93]
RcHSP70	*R. hybrida* L.	Increased cold tolerance	[92]
SlHSP17.7	*S. lycopersicum* L.	Increased cold tolerance	[94]
LpHSFC1	*Lolium perenne L.*	Increased cold tolerance	[95]
LpHSFC1b	*L. perenne* L.	Increased cold tolerance	[95]
HbHsfA4a	*H. brasiliensis*	Increased cold tolerance	[96]
HbHsfA1 *HbHsfB1*	*H. brasiliensis*	Increased cold tolerance	[97]

KoHsp70	*G. hirsutum*	Increased cold tolerance	[98]

CONCLUSIONS AND FUTURE PERSPECTIVES

Adaptation to the cold enables the low temperature resistant medicinal plants to gain frost resistance and to spend the winter months without being damaged by frost. The survival of these types of plants through the winter depends on their adaptability to the cold. Considering that the lowest average annual temperature is around 0 °C in most of the world and -10 °C in nearly half of it, the importance of cold adaptation and frost resistance on the yield to be obtained from agricultural activities in such environments will be better understood. Knowing the metabolic and molecular changes that occur in cold-resistant plant species and genotypes during cold adaptation will be beneficial both in terms of breeding studies and in terms of giving an idea about the species and genotype to be grown. Meeting the nutritional needs of the ever-increasing world population depends on the prevention of losses caused by low temperature stress. For this reason, breeding studies are carried out to obtain genotypes that are more tolerant to low temperatures, more adaptable to cold, and higher frost resistance.

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