

Review

Are Heat Shock Proteins Important in Low-Temperature-Stressed Plants? A Minireview

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Abstract: Heat shock proteins (HSPs) are mainly known to play important roles in plants against high-temperature (HT) stress. Their main function is to act as molecular chaperones for other proteins. It has also been proven that HSPs have a protective effect during other environmental stresses including low temperature (LT). To the best of our knowledge, the expression and role of HSPs in plants that have been exposed to LT have not yet been sufficiently reviewed. The aims of this minireview were (1) to briefly describe the origin, classification, structure, localisation and functions of HSPs, (2) to present the current knowledge about the changes in the accumulation of HSPs in plants that have been exposed to LT, (3) to discuss some of the molecular changes that occur during LT action and that lead to the accumulation of HSPs in plants and (4) to discuss the potential role of HSPs in acquiring tolerance to cold and frost in plants including economically important crop species. Some directions of research on the role of HSPs in plants growing in LT conditions are proposed.

Keywords: molecular chaperones; cold; frost tolerance; stress response



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1. Introduction—Discovery, Classification and Structure of Heat Shock Proteins

Heat shock proteins (HSPs) are an important group of proteins that can be found in plants, fungi and animal cells [1,2]. They are known to play a protective role against many environmental stresses as well as in restoring cellular homeostasis [3,4]. The name of these proteins is associated with the fact that they were first discovered in *Drosophila melanogaster* that had been treated with high-temperature stress [5]. HSPs were first described in the work of Tissières et al. [6]. Later studies have shown that the expression of the *hsp* genes is not only induced by heat but also by other environmental stresses, among others, cold, UV light, water, salinity, osmotic and biotic stresses [2–4].

The main function of HSPs is to act as molecular chaperones and they are also responsible for the folding, assembly, translocation and degradation of proteins during normally occurring processes [2,3,7]. It is also known that HSPs play an important role in the stabilisation of proteins and membranes. Moreover, they can participate in protein refolding during stress [3]. It is also known that HSPs are taking part in plant growth and development under normal conditions, such as inflorescence induction or seeds and fruits set [8,9].

In plants, HSPs are divided into five main classes characterized by their activity as molecular chaperones, according to their molecular weight, i.e., HSP100, HSP90, HSP70, HSP60 (100 kDa, 90 kDa, 70 kDa and 60 kDa, respectively) and small heat shock proteins (sHSPs) (low-molecular-mass proteins; 12–40 kDa) [2–4,10,11]. In reference to Gupta et al. [12], classification for five HSP families (HSP100, HSP90, HSP70, HSP70 and sHSP) can be made according to their molecular weight, amino acid sequence homologies and functions.

Each HSP has some individual features, but generally, the structure of the HSPs in plants consists of an N- and a C-terminus, which are the nucleus-binding region -I and -II, and a middle one, which is characterised by an amino terminus followed by a carboxyl terminus [11]. Each of the HSP structure elements are playing an important role in understanding their functions. The role of those elements can be slightly different for

different HSP types. In the case of HSP100, it can contain five or one nucleotide-binding domains (NBDs) and it depends on the HSP100 class (I or II, respectively). HSP100 structure also includes middle and C-terminal domains [7,13]. NBDs and middle domains are characterised with a higher degree of conservation in HSP100 structure and it can be related to the highest importance of these regions with respect to their biological activity. The N- and C-terminal domains can have species-specific function [13]. In the case of HSP90, its structure consists of three domains: an N-terminal with an ATP-binding region, a middle domain and a C-terminal domain. The N-terminal domain together with the middle domain catalyses the ATP hydrolysis and the C-terminal domain is responsible for the formation of the HSP90 dimer, which is crucial for chaperone function [14–18]. The structure of HSP70 also consists of three domains: a 44 kDa nucleotide-binding domain (NBD), a 15 kDa substrate binding domain (SBD) and a 10 kDa carboxyl-terminal domain (CTD). The CTD forms a lid-like structure that helps to bind a substrate to the SBD [19–23]. ATP hydrolysis is crucial for HSP70 chaperone function. Because of low levels of intrinsic ATPase, co-chaperones (J-domain proteins (JDPs) and nucleotide exchange factors (NEFs)) are needed [3,23]. It is worth noting that HSP70 has a very high degree of structural homology among many species [3,21]. In plants, HSP70 genes are encoded by a highly conserved multigene family. In *Arabidopsis thaliana*, HSP70 is encoded by at least 18 genes. Meanwhile, at least 12 genes encoding HSP70 have been detected in the spinach genome [3,20]. It is thought that the HSP70 family is the most highly conserved HSP. They are considered to have about 50% identical residues between the *Escherichia coli* homolog DnaK and the eukaryotic HSP70 [3,20,24]. According to Waters and Vierling [25], the structure of both plant and bacterial sHSP includes an α -crystallin domain (ACD) at their C-terminus that consists of approx. 90 amino acids that build up a dense, seven-stranded β -sandwich that forms the structural core of all sHSPs. Other important features of the sHSP structure are a dimeric substructure of the oligomer and dimer–dimer interactions that are crucial for stabilising oligomers [25–31]. Oligomer formation is a common feature of sHSPs [31]. Unlike other HSPs, sHSPs do not require ATP to bind substrate proteins and have a very high ability to bind denatured substrates [25]. sHSPs operating in specific organelles or cellular compartments possess N-terminal transit, leader or signal sequences necessary for transfer of the sHSP to the appropriate organelle. To date, little is known about the higher levels of structural variation among plant sHSPs [32].

In addition to a brief description of the discovery, classification and structure of HSPs presented above, the aim of the presented article is (1) to briefly describe HSPs localization and functions, (2) to present the current knowledge about the changes in the accumulation of HSPs in plants that have been exposed to low temperature (LT), (3) to discuss some of the molecular changes that occur during LT action and that lead to the accumulation of HSPs in plants and (4) to discuss the potential role of HSPs in acquiring tolerance to cold and frost in plants including economically important crop species. Moreover, some directions of further research on the role of HSPs in plants growing in LT conditions are proposed.

2. Heat Shock Proteins—Localisation and Function in Plants

HSPs can be found in cytoplasm as well as in other organelles. They were found in the nucleus of plants such as cereals including maize (*Zea mays* L.), wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.) and rice (*Oryza sativa* L.) [1,33,34]. Moreover, in plants such as *Arabidopsis thaliana* and soybean (*Glycine max* L.) [1,35], HSPs have been found in the nucleus. Other organelles in which HSPs occur are the mitochondria of plants such as cereals, including rice, maize, wheat and barley [34,36]. It is also known that HSPs are present in the mitochondria of plants such as tomatoes (*Solanum lycopersicum* L.), potatoes (*Solanum tuberosum* L.), tobacco (*Nicotiana tabacum* L.), *Arabidopsis thaliana* and soybean [1,35,37–39]. HSPs also occur in the chloroplasts of plants such as cereals including maize and wheat [1,28] as well as in creeping grass (*Holcus mollis* L.), tomatoes, *Arabidopsis thaliana* and soybean [1,35,40–45]. Finally, HSPs have also been found in the

endoplasmic reticulum (ER) of plants such as pea (*Pisum sativum* L.), mulberry (*Morus* L.), *Arabidopsis thaliana*, soybean [1,35,39,44,46,47] and cereals including rice and maize [1].

The localisation of specific HSP classes and their main functions are as follows: HSP100 is located in the cytosol, mitochondria and chloroplasts [48,49] and the major function of HSP100 is that it is responsible for protein disaggregation and/or degradation, which is very important for maintaining cellular homeostasis [48,49]. HSP90 can be found in the cytosol, chloroplasts, mitochondria and ER [1,14,16,50]. It is responsible for managing protein folding and is crucial in the signal-transduction networks, cell-cycle control, protein degradation and protein trafficking [1,14,16,50]. HSP70 is one of the best known HSPs. It is located in the cytosol, mitochondria, chloroplasts and ER [19–23,51]. The main functions of HSP70 include preventing protein aggregation, and assisting with protein refolding, protein import and translocation, signal transduction and transcriptional activation [19–23,51]. HSP60 (so-called chaperonins) are present in the cytosol, mitochondria and chloroplasts [52,53]. The main functions of chaperonins are protein folding, refolding, aggregation and transferring them to the chloroplasts and mitochondria [53].

Finally, the class of sHSP is the most common HSP class in plants. These proteins are located in the cytosol, mitochondria, chloroplasts, peroxisomes, ER and membranes [26–28,49,54]. They are known to prevent protein aggregation and they help to stabilise non-native proteins [26–28,49,54].

3. The Role of Heat Shock Proteins in High-Temperature (HT) Stressed Plants

Because of the discovery of HSPs as a result of HT exposure, the vast majority of research concerning HSPs in plants has been conducted in this aspect [55]. The data that were obtained in the mentioned studies show that HT mainly results in an increase in HSPs accumulation in plants. Especially nowadays, when we are dealing with climate change, HT is a serious problem for plants. This is of great importance in agriculture as HT can lead to problems with the survival, yield and quality of plants [49,56]. An increased HSPs accumulation is one of the mechanisms by which plants avoid and minimise the damaging effects of this stress. Briefly, it has been studied in many plant species such as soybean in which Altschuler and Mascarenhas [57] observed an increased HSP accumulation after plants had been exposed to a temperature of 40 °C for up to 2.5 h, after which a decreased HSPs level was observed (between 2.5 and 6 h). Moisyadi and Harrington [58] observed a higher accumulation of high-molecular-mass HSP in cultured sugarcane cells (*Saccharum officinarum* L.), which was caused by HT (32 °C or 34 °C), which lasted for as little as ten minutes. In the case of sHSP (18 kDa), its increased level was observed after heat shock at 36 °C for 30 min. Dupuis and Dumas [59] found that maize plants that were subjected to 40 °C for 4 h were characterised by an increased accumulation of HSP101, HSP70 and HSP17.6. Similarly, DeRocher et al. [60] found an increased accumulation of HSP18.1 and HSP17.9 transcripts and HSP18.1 protein in pea (*Pisum sativum* L.) plants that had been exposed to HT 38 °C for 4 h. Additionally, Hopf et al. [61] found that after maize was exposed to 40/45 °C for 2 h, the HSP accumulation increased in the vegetative tissues. Studies on spinach (*Spinacia oleracea* L.) and tomato have shown that the HSP expression increased after 1 h of heat shock (37 °C) and that it decreased to the level before stress after 2 h [62]. Dhaubhadel et al. [63] studied oilseed rape plants that were exposed to 45 °C and they observed an increased accumulation of HSP101, HSP90 and HSP70 after 4 h of HT treatment, compared to the control plants. In the case of potato plants, exposure to an HT (35 °C or 40 °C) caused an increased accumulation of sHSP compared to the control plants [64]. Pavli et al. [65] found that in sorghum (*Sorghum bicolor* (L.) Moench) there was an increase in the accumulation of the *hsp90* gene expression after plants were exposed to 47 °C for 30 or 60 min. However, the *hsp90* expression levels differed among the studied genotypes. Recently, Sun et al. [40] studied creeping bentgrass (*Agrostis stolonifera* L.) plants (cv. Penn-A4) that had been exposed to an HT of 37 °C for 4 h and they observed an increased expression of HSP26.8a in both the leaves and roots.

4. Changes in the Heat Shock Proteins in Plants That Had Been Exposed to Low-Temperature Stress

HSPs not only accumulate in plants growing under HT stress, but their expression also increases under the influence of other abiotic stresses including low temperature (LT) stress [66,67]; however, this aspect has been investigated much less. Therefore, as mentioned, one of the aims of this minireview is to present the current knowledge about what is known to date about the expression and a potential role of HSPs in the process of plant acclimation to LT.

In the beginning, it is important to say that in general, plants can be divided into two main groups. The first group includes species for which cold is a stress factor (tomato, cucumber) and which are generally unable to survive temperatures below zero. The second group includes plants (i.e., spring and winter cereals, oilseed rape) able to survive frost particularly after a process known as cold acclimation (cold hardening). The metabolic changes that occur during cold acclimation are quite well known [56,68]. These processes include a change in the lipid composition and membrane structure (an increase in the content of unsaturated fatty acids in the membrane lipids) and changes in the photosynthesis efficiency [69], content of soluble sugars [70] and the accumulation of proteins such as late embryogenesis abundant proteins (LEA), anti-freezing proteins (AFPs) and cold-shock proteins (CSPs), which increases during cold acclimation [71]. As was mentioned earlier, cold acclimation also results in an accumulation of HSPs [11,47,67,72]. As mentioned, cold acclimation allows many economically important species to acquire better frost tolerance.

Importantly, during cold acclimation, genes performing various functions are activated, including genes responsible for the transduction of the signal caused by LT. One of the most important ones is the gene of the transcription factor crucial for cold acclimation, i.e., CBF (C-repeat Binding Factor). The cold-induced or constitutive expression of CBF1 reduces the level of bioactive gibberellins, resulting in the accumulation of DELLA proteins. As a result of the accumulation of DELLA, plant growth slows down, while an increase in the level of active gibberellins in spring is responsible for their growth [73,74]. CBF also induces the expression of COR genes (Cold-Responsive Genes), which are important in the processes of plant acclimation to LT [75,76]. Both CBF and COR transcription factors help provide plants with frost tolerance by, i.a., the acceleration of osmolyte synthesis or reconstruction of cell membranes [76,77]. The effect of LT, like other abiotic stresses, contributes to the induction of the expression of the so-called Heat Shock Factor (HSF) gene family, which contribute to the induction of HSPs gene expression [78]. More details are presented in Section 5.

Generally, LT stress is an especially serious problem in horticulture and agriculture [56,79]. That is why mechanisms of the plant response to LT are extensively studied. The accumulation of HSPs has also been studied in many plant species including horticultural plants and agricultural plants but also in model plants.

4.1. Changes in HSP in Model Plants

In the case of the main model plants, HSP accumulation was already studied in *Arabidopsis thaliana* that had been subjected to LT. *A. thaliana* belongs to a group of plants capable of surviving frost especially after cold acclimation [80]. Bae et al. [81] found that *A. thaliana* plants that had been subjected to LT (4 °C) for 6 h had an increased accumulation of HSP70 transcripts compared to the non-acclimated plants (grown at 22 °C). Kawamura and Uemura [82] studied the plasma membrane proteins that were present in *A. thaliana* plants that had been subjected to 2 °C for 1, 2, 3 and 7 days and they found that after 1 day of cold acclimation, the accumulation of chaperonin 20 decreased and that it increased after 2 days compared to the non-cold-acclimated plants. Kagale et al. [83] observed an increased accumulation of the HSP90 transcript in *A. thaliana* seedlings that had been exposed to 2 °C for three days. HSP90 occurred after 1 h of LT exposure and its highest level was observed after 12 h of LT. After one and three days of LT, a similar high amount of the HSP90 level was observed. Kaplan et al. [84] studied *A. thaliana* plants that had been exposed to 4 °C

for up to 96 h and they observed an increased expression of *HSP70* after 12 h and especially after 24 h of LT exposure compared to the control plants. In the work by Sewelam et al. [85], *A. thaliana* plants that had been exposed to 0 °C for 3 h were characterised with a 4-fold higher up-regulation of *HSP17.4CI* expression, in comparison to control plants.

4.2. Changes in HSP in Horticultural Plants

Horticultural plants such as tomato, cucumber and maize belong to a group of plants that are sensitive to cold [86–89]. A few authors studied the HSPs accumulation in such plants that had been exposed to LT stress. Anderson et al. [90] studied spinach plants (cv. Bloomsdale) that had been cold-acclimated at 5 °C for 1, 2, 3, 4 and 7 days and they found an up-regulation of *HSP70* mRNA during cold acclimation compared to the control plants. Taylor et al. [91] studied pea plants that had been exposed to 4 °C for 36 h and they found an increased accumulation of *HSP90*, *HSP70* and *HSP22* compared to the control plants. Degand et al. [92] observed an increased *HSP70* accumulation in the roots of chicory (*Cichorium intybus* L.) that had been exposed to a minimum temperature below 5 °C for several days compared to the non-cold-exposed plants. Moreover, Dumont et al. [93] studied pea plants [cv. Champagne (cold-tolerant) and cv. Terese (cold-sensitive)] that had been cold-acclimated at 10/2 °C (day/night) for 11 days and they found an increased accumulation of *HSP70* in the pea stems of both of the studied cultivars compared to the non-acclimated plants. Research on three tomato genotypes by Kubienová et al. [94] showed a similar tendency, namely, the increase in *HSP70* protein accumulation was observed in only one genotype (*S. habrochaites*) that had been exposed to 4 °C for 1 h, 4 h and 24 h compared to the non-stressed plants. Growth at 10 °C caused a slight decrease in the *HSP70* protein accumulation in two genotypes (*S. habrochaites* and *S. lycopersicum*), while in the third (*S. chmielewskii*), no changes were observed compared to the control plants (grown at 20 °C) [94]. As for tomato, very interesting findings of practical importance were obtained by Sabeht et al. [95]. The authors observed that HT pretreatment of tomato fruits resulted in greater HSPs accumulation, which allowed them to be stored in LT for longer periods than normally possible. There are also studies that concern HSPs expression in cucumber fruits after storage at 4 °C [96]. Authors found that after 14 days of storage at 4 °C, cucumber fruits were characterised with a significantly higher relative expression level of *HSP70* and *HSP40.9* than control fruits [96].

4.3. Changes in HSP in Agricultural Plants

Winter plants—cereals and oilseed rape—acclimate to frost during the autumn months via a process of cold acclimation. Spring cultivars also have the ability to acclimate to the cold, which is especially useful during periods of temperature anomalies, e.g., frost in the early spring. Frost, especially with insufficient snow cover, can cause significant yield losses, thereby generating economic problems. That is why the mechanisms of cold acclimation and plant frost tolerance have been the subject of studies for many years. There have also been studies concerning the HSPs accumulation in LT-exposed agricultural plants. Cabané et al. [97] studied soybean plants (cv. Verdon and Maple Ardon) that had been cold-acclimated at 14/8 °C (d/n) for five days and they observed an increased accumulation of *HSP70* compared to the control plants. Krishna et al. [98] studied *HSP90* accumulation in spring oilseed rape (*Brassica napus* L., cv. Westar) plants that had been exposed to 5 °C for seven days. The authors found that the LT-exposed plants were characterised by a significantly higher accumulation of the *HSP90* transcripts and proteins than the control plants (grown at 20 °C) [98]. Pareek et al. [99] observed an increased accumulation of *HSP90* in the shoots of rice seedlings that had been cold-stressed at 5 ± 2 °C for 96 h compared to the control plants that had been grown at 28 °C. Cui et al. [100] found that rice seedlings that had been exposed to LT of 15, 10 and 5 °C (24 h for each exposure) were characterised by a greater than two-fold up-regulation of *HSP70* compared to the control plants. The highest level of *HSP70* was observed in the plants that had been exposed to 5 °C. Similar results were observed by Yan et al. [66] in rice plants that had been exposed to LT 6 °C

for 24 h and they noticed an increased accumulation of HSP70 and HSP26 compared to the control plants. Moreover, Lee et al. [101] observed that after chilling stress of rice at 10 °C for 24 h and 72 h, there was an up-regulation of the HSP70 proteins in the rice roots especially after 72 h of chilling stress compared to the control plants that had been grown at 28 °C. Renaut et al. [102] studied two potato cultivars (PS3 and Desiree) that had been exposed to 4 °C for 21 days and they found an increase in the amount of the HSP70 protein in both cultivars compared to the control plants. The cold acclimation (6 °C, 12 weeks) of winter wheat (*Triticum aestivum* L.) caused an increase in the accumulation of HSP70 (after 3, 7 and 21 days of cold acclimation) and a decrease in HSP90 (after 21 and 84 days of cold acclimation) in a crude extract of the crowns [103]. Hlaváčková et al. [104] studied winter barley (cv. Luxor) and they found that LT exposure (3/2 °C (d/n) for three weeks) caused an increased accumulation of HSP70 protein in the crowns compared to the control plants (18/13 °C (d/n)).

Sadura et al. [105] studied the HSPs accumulation in barley that had been acclimated at 5 °C for 21 days. The authors found that while the cultivar Delisa accumulated significantly more HSP70 in the cell membrane fraction compared to the non-acclimated plants, the second studied cultivar, Bowman, was characterised by a notably lower accumulation of this protein in the cold-acclimated plants than in the non-acclimated plants. The accumulation of the HSP90 protein was lower for both of the studied cultivars that had been cold-acclimated than for the non-acclimated plants [105]. Similarly, in the work of Stachurska et al. [106], an increased accumulation of HSPs (HSP70 cytoplasmic, HSP70 chloroplastic and HSP90) was observed in three out of four of the tested cultivars of oilseed rape plants (cv. Bojan, Feliks and President), which had been cold-acclimated at 4 °C for three weeks compared to the control plants. In the case of the cultivar Rokas, the opposite effect was observed for the HSP70 cytoplasmic and HSP90 types, namely, the accumulation of these proteins was lower for the cold-acclimated plants than for the control plants [106]. The reviewed studies are summarised in Table 1.

4.4. Are HSPs Required for Acquiring Frost Tolerance?

According to Thomashow [75], the HSP70 and HSP90 genes can be associated with the acquisition of frost tolerance by plants. Other authors investigated whether the changed HSPs content in cold-acclimated plants translated into a changed frost tolerance in these plants. In the work by Kawamura and Uemura [82], an increased accumulation of chaperonin 20 in the plasma membrane of *A. thaliana* plants that had been cold-acclimated at 2 °C corresponded to increased frost tolerance in the plants. Vítámvás et al. [103] found that the highest level of winter wheat tolerance to frost was observed after 21 days of cold acclimation at 6 °C and that at the same time, the HSP70 accumulation was higher compared to the control plants. However, the HSP90 content decreased after 21 and 84 days of cold exposure. The results that were obtained by Sadura et al. [105], which indicated an increased accumulation of HSP70 in the barley cultivar Delisa and its decreased accumulation in the cultivar Bowman, did not correspond with values of frost tolerance that were obtained for these plants. Lower values of frost tolerance were obtained for cultivar Delisa than for Bowman [107]. Stachurska et al. [106] studied the HSPs accumulation in four cultivars of oilseed rape (Bojan, President, Rokas and Feliks) that had been cold-acclimated at 4 °C for three weeks and they found that for three of the four cultivars, an increased HSP90 and HSP70 accumulation was accompanied by an increase in their frost tolerance. The exception was cultivar Rokas, which was characterised by an increased frost tolerance and a decrease in the accumulation of HSPs, although interestingly in non-acclimated plants of this cultivar, relatively high basal accumulations of HSP90 and cytoplasmic HSP70 were noted, in comparison to other cultivars.

Table 1. The HSP accumulation in plants that had been exposed to a low temperature.

Plant Species	Temperature	Duration of Low-Temperature Exposure	HSP Transcripts HSP Proteins	HSP Accumulation Compared to the Control Conditions	Reference
Soybean (<i>Glycine max.</i> L.) cv. Verdon, Maple Arrow	14/8 °C (d/n)	5 days	HSP70	increase	[97]
Spinach (<i>Spinacia oleracea</i> L.) cv. Bloomsdale	5 °C	1, 2, 3, 4 and 7 days	HSP70	increase	[90]
Oilseed rape (<i>Brassica napus</i> L.) cv. Westar	5 °C	7 days	HSP90 HSP90	increase	[98]
Rice (<i>Oryza sativa</i> L.)	5 ± 2 °C	96 h	HSP90	increase	[99]
<i>Arabidopsis thaliana</i>	4 °C	6 h	HSP70	increase	[81]
<i>Arabidopsis thaliana</i>	2 °C	1, 2, 3 and 7 days	Chaperonin 20	1 d decrease 2 d increase	[82]
<i>Arabidopsis thaliana</i>	0 °C	3, 6, 12 h	HSP17.4CI	3, 6, 12 h increase	[85]
Pea (<i>Pisum sativum</i> L.)	4 °C	36 h	HSP90, HSP70, HSP22	increase	[91]
Rice (<i>Oryza sativa</i> L.)	15, 10 and 5 °C	24 h for each exposure	HSP70	gradual increase with decreasing temperature	[100]
Rice (<i>Oryza sativa</i> L.) cv. Nipponbare	6 °C	24 h	HSP70 HSP26	increase	[66]
<i>Arabidopsis thaliana</i>	2 °C	3 days	HSP90	increase	[83]
Chicory roots (<i>Cichorium intybus</i> L.)	≤ 5 °C	several days	HSP70	increase	[92]
<i>Arabidopsis thaliana</i>	4 °C	up to 96 h	HSP70	increase after 12 h and especially 24 h of LT exposure	[84]
Potato (<i>Solanum tuberosum</i> L.) cv. PS3, Desiree	4 °C	21 days	HSP70	increase	[102]
Rice (<i>Oryza sativa</i> L.)	10 °C	24 and 72 h	HSP70	gradual increase with time of cold acclimation	[101]
Pea (<i>Pisum sativum</i> L.) cv. Champagne, Terese	10/2 °C (d/n)	11 days	HSP70	increase	[93]
Winter wheat (<i>Triticum aestivum</i> L.)	6 °C	12 weeks	HSP70 HSP90	HSP70—3 d, 21 d and 84 d increase HSP90—21 d and 84 d decrease	[103]
Winter barley (<i>Hordeum vulgare</i> L.) cv. Luxor	3/2 °C (d/n)	21 days	HSP70	increase	[104]
Tomato (<i>Solanum lycopersicum</i> L., <i>Solanum habrochaites</i> L., <i>Solanum chmielewskii</i> L.)	10 and 4 °C	1, 4 and 24 h	HSP70	4 °C (1 h, 4 h, 24 h)—increase (<i>S. habrochaites</i>); 10 °C (1 h, 4 h, 24 h)—slight decrease (<i>S. lycopersicum</i> , <i>S. habrochaites</i>)	[94]
Spring barley (<i>Hordeum vulgare</i> L.) cv. Delisa, Bowman	5 °C	21 days	HSP70 HSP90 HSP70 HSP90	HSP70 cv. Delisa—increase cv. Bowman—decrease HSP90 cv. Delisa and Bowman—decrease HSP70 cv. Delisa—NC cv. Bowman—increase HSP90 cv. Delisa—increase cv. Bowman—increase	[105]
Oilseed rape (<i>Brassica napus</i> L.) cv. Bojan, President, Feliks, Rokas	4 °C	3 weeks	HSP70 cytoplasmic, HSP70 chloroplasmic, HSP90	cv. Bojan, President, Feliks—increase cv. Rokas—decrease	[106]

NC—no change

Therefore, it can be stated that the increased accumulation of HSPs in plants that have been exposed to LT is often accompanied by their increased frost tolerance, although a detailed regulatory mechanism of this phenomenon requires further research. In this context, particularly interesting studies were completed by Hlaváčková et al. [104]. The authors studied winter barley (cultivar Luxor) that had been exposed to a frost temperature ($-3\text{ }^{\circ}\text{C}$) after three weeks of cold acclimation ($3/2\text{ }^{\circ}\text{C}$ (d/n)) and they observed a seven-fold increase in the HSP70 level in frost-treated plants compared to plants that had been exposed to cold. As shown, for example, by Sadura et al. [105], in some cases, i.e., in cereals, the accumulation of HSPs transcripts during cold acclimation is high. However, this is not accompanied by a high HSP protein content (Figure 1—Bowman plants and their NILs). Therefore, the question arises whether the increased transcript abundance is not a ‘preliminary’ mechanism during cold acclimation and whether the increase in the protein level does not occur/enhance as a result of stronger stress like frost in cereals?

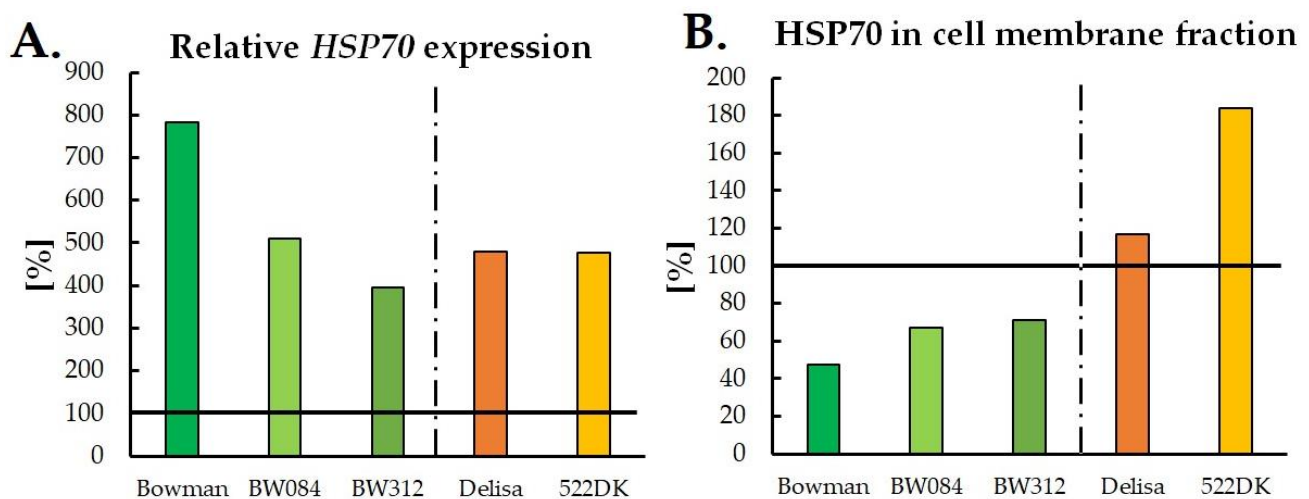


Figure 1. Relative percentage changes in the HSP70 transcript accumulation (A) and HSP70 protein accumulation in the cell membrane fraction (B) in the barley plants that had been acclimated at $5\text{ }^{\circ}\text{C}$ relative to the plants that had been grown at $20\text{ }^{\circ}\text{C}$. The results that were obtained for the plants that had been grown at $20\text{ }^{\circ}\text{C}$ were considered to be 100% and are indicated by the horizontal black line. The original data of HSP70 expression are available in Sadura et al. [106]. Bowman—a reference cultivar for two NILs: BW084—plants with disturbances in the early stage of the BR biosynthetic pathway and BW312—plants with a BRI1 receptor defect. Delisa—the reference cultivar for the 522DK mutant with disturbances in the late stage of the BR biosynthesis pathway.

5. Possible Molecular Mechanisms of HSPs Action in Plants under LT Conditions

As mentioned above, a better understanding of the role of HSPs in LT-exposed plants requires a deeper study of the molecular mechanisms that are involved in the actions of HSPs. According to Horváth et al. [54], HSPs are responsible for thermal sensing via the membranes. sHSPs play especially important roles in membranes as under stressful conditions, they become membrane-associated, and they can modify the lipid order and phase state of membranes.

According to Usman et al. [22], after plants have been exposed to stressful conditions (such as HT or LT), specific and highly regulated signalling cascades are initiated, and the outer membrane proteins that are present in the cytosol send signals to the inner membrane proteins, which causes the up-regulation of the heat shock transcription factors (HSFs). As a result, the synthesis of HSPs is triggered [8,22,54,108]. The promoter regions of the HSF bind to the so-called Heat Shock Elements (HSEs), after which there is an increased amount of HSPs. There are three types of HSFs: HSFA, B and C [8,109]. The studies of Kotak et al. [49] and Scharf et al. [109] indicated that the increased expression of HSPs and other stress-responsive genes in conjunction with HSFs is an important factor in modifying

the physiological and biochemical processes that are associated with the development of a tolerance to stresses.

The general model of the molecular mechanism of HSPs biosynthesis in plants exposed to various stresses, which can also be applied to LT stress, is presented in Figure 2.

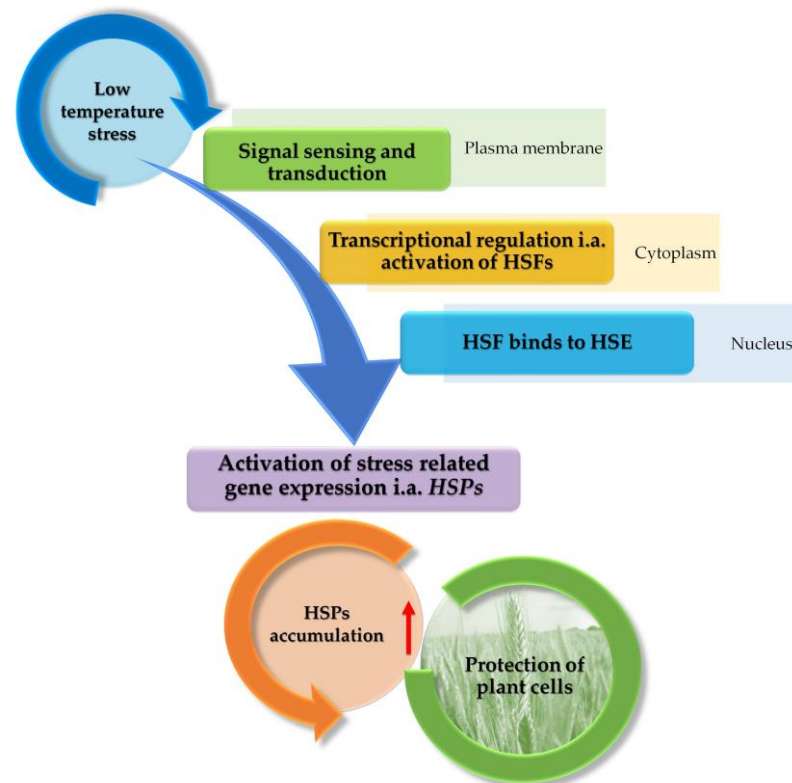


Figure 2. The proposed model of the molecular mechanism of HSPs biosynthesis in plants exposed to LT stress (based on [8,22,54,108]). Briefly, LT is a signal that is received in the cell membrane and transmitted to the cytoplasm, where heat shock transcription factors (HSFs) are activated. In the nucleus, HSFs are associated with HSEs (Heat Shock Elements). *HSPs* gene expression is then activated, which may contribute to HSPs accumulation.

It is known that after plants have been exposed to HT stress, the gene expression of HSPs is initiated very quickly, in as little as a couple of seconds of the HT, and the maximal amount of *HSPs* can be observed after 2 h of HT stress [77]. A similar effect can be observed in plants that are exposed to LT. Increased *HSPs* transcript accumulation was observed after 1 h of LT stress [94]. To the best of our knowledge, changes in transcript accumulation after less than 1 h have not been studied. The biosynthesis of HSP proteins is under both transcriptional and post-transcriptional control [63,110], and therefore the levels of the HSP transcripts and proteins can differ during the exposure of plants to temperature stress. This was observed, for example, in the work on barley by Sadura et al. [105]. The authors observed that despite a higher *HSP90* transcript level after cold acclimation (4 °C) relative to the control (20 °C), the *HSP90* protein level in membranes was consistently lower in both of the studied cultivars, especially in the barley Bowman cultivar. Moreover, the several-times higher level of the *HSP70* transcript in both cultivars (Bowman and Delisa) in cold conditions compared to the control at 20 °C did not have such a clear impact on the accumulation of *HSP70* in the membranes and cytoplasm (Figure 1). In addition, in the work of Kubienová et al. [94] on tomato plants (*Solanum lycopersicum* L., cultivar Amateur), which had been cold-exposed at 4 °C, the *HSP70* transcript accumulation was higher compared to the control plants. After the 1st h of exposure, there was an eleven-fold increase in the *HSP70* gene expression and after the 4th and 24th h, a further increase was observed. However, low levels of the *HSP70* proteins were observed for this cultivar. Other

studied cultivars have also differed in the levels of HSP70 gene expression and HSP70 protein accumulation [94]. Therefore, the mechanism of the post-transcriptional regulation of HSPs biosynthesis may play an important role in LT-exposed plants and requires deeper studies.

6. Hormonal Control of HSPs Biosynthesis in LT-Exposed Plants

Some studies indicate that the phytohormone level, e.g., brassinosteroids (BR), might have an impact on the accumulation of HSPs in LT-exposed plants. Kagale et al. [83] studied *A. thaliana* plants that had been exposed to 2 °C for up to three days and then treated with 1 µM 24-epibrassinolide (EBR). However, the authors did not observe any differences in the HSP90 transcript accumulation between the EBR-treated and the untreated plants. On the other hand, Sadura et al. [105] studied the accumulation of HSPs in cold-acclimated barley mutants with a BR deficiency (BW084, 522DK) and impaired BR-signalling (BW312) (5 °C, 21 days) and the authors observed differences in HSPs accumulation in studied plants. Mutants with a BR deficit (with few exceptions) and a BRI1 receptor defect were most often characterised by lower levels of the accumulation of the transcripts of all of the HSPs compared to their wild types (cv. Bowman, Delisa). This could indicate a role for BR as positive regulators of HSPs transcription in barley. As for HSP protein accumulation, the BRI1 receptor defect was associated with a decrease in the accumulation of the HSP70 and HSP90 proteins after cold acclimation. This could also indicate the involvement of BR in the regulation of the biosynthesis of the HSP proteins via the BRI1 receptor and the associated signal transduction pathway. However, a BR deficit in plant tissues was not always associated with changes in the HSPs biosynthesis [105]. The relative percentage changes in the HSP70 expression in the barley BR mutants and their wild types that had been acclimated at 5 °C relative to the control plants that had been grown at 20 °C are presented in Figure 1.

Finally, Stachurska et al. [106] found that an increased accumulation of HSPs in cold-acclimated (4 °C, three weeks) oilseed rape plants was accompanied by an increased accumulation of abscisic acid (ABA) and a decreased accumulation of growth-promoting hormones. However, a direct connection between HSPs biosynthesis and phytohormones' level was not proven in this work.

Hormonal mechanisms of HSPs biosynthesis regulation still require further research.

7. Conclusions and Further Perspectives

Despite the fact that the discovery of HSPs was as cell protective factors at HT, these proteins may also play important roles in counteracting the negative effects of LT stress in plants. The results that are discussed in this minireview indicate that the accumulation of HSPs occurs in plants that have been exposed to LT and that this often depends on the plant species, the genotype, the duration of LT exposure and the plants sensitivity to LT. A high accumulation of transcripts is not always reflected in the final content of the corresponding proteins. Therefore, further research is necessary, for example, on the mechanisms of the post-transcriptional regulation of HSPs biosynthesis. Further research on the hormonal regulation of HSPs proteins in plants exposed to LT seems to be equally important. According to some studies, a higher content of HSPs may translate into greater frost tolerance in plants. This is an issue worth further investigation as it would allow to determine whether the modification of the HSPs content in plants would be useful for breeders to breed more frost-tolerant plants. The same could also concern cold-sensitive species and possibilities of obtaining more cold-tolerant plants. An increase in the HSPs content may also have other practical significance, namely in cold storage of fruits. In the case of tomato fruit, for example, it has been proven that higher HSP content can extend the duration of its cold storage.

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