Journal of Medicinal Plants Studies
www.PlantsJournal.com

ISSN (E): 2320-3862
ISSN (P): 2394-0530
https://www.plantsjournal.com
JMPS 2023; 11(2): 91-96
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Received: 01-12-2022
Accepted: 05-01-2023

Sapna Chourdia
School of Biotechnology,
Gautam Buddha University,
Greater Noida, Uttar Pradesh,
India

Priya Raghuvanshi
School of Biotechnology,
Gautam Buddha University,
Greater Noida, Uttar Pradesh,
India

Sandeep Kumar
School of Biotechnology,
Gautam Buddha University,
Greater Noida, Uttar Pradesh,
India

Gunjan Garg
School of Biotechnology,
Gautam Buddha University,
Greater Noida, Uttar Pradesh,
India

Corresponding Author:
Sapna Chourdia
School of Biotechnology,
Gautam Buddha University,
Greater Noida, Uttar Pradesh,
India

Metabolic acclimation in a plant by cold stress induces carbohydrate accumulation

Sapna Chourdia, Priya Raghuvanshi, Sandeep Kumar and Gunjan Garg

Abstract

Plants cannot escape the numerous abiotic stresses such as drought, cold, heat, and salinity that prevent them from growing and developing under ideal conditions because they have a sessile lifestyle. One of the most damaging abiotic factors impacting temperate plants is low temperature. Plants that are exposed to low temperatures undergo a process called "cold acclimation," which ultimately increases their freezing tolerance. Abiotic stress factors depend on the production of particular genes and metabolites which are stress-related. To recognize cold stress, the cell membrane either modifies the fluidity of the membrane or uses sensory proteins. Plants also gather a variety of low-molecular-weight osmolytes during cold stress that helps the plant to survive, by maintaining proteins and membrane stability and aiding to control the osmotic pressure inside the cells. These osmoprotectants include sugar alcohols (sorbitol, inositol, and ribitol), low-molecular-weight nitrogenous compounds (i.e., proline and glycine betaine) and soluble sugars (saccharose, raffinose, trehalose, stachyose). Further research data showed that the plasma membrane is protected by additional solutes that are produced from the symplast against ice adherence and consequent cell damage in plant cells.

Keywords: Cold stress, cold acclimation, signal transduction, metabolites, transcription factors

1. Introduction

The organisms diversify their habitats due to the imbalance in the environmental and nutritional parameters. Plants are sessile in nature, so they are not able to escape from such unfavorable conditions. Due to their sessile nature, plants have adapted various types of mechanisms through which they can survive in disastrous environmental conditions. Plants may be able to use a variety of mechanisms to defend themselves against abiotic stressors including cold, heat, drought, salt, light, etc. There are three main forms of temperature stress that plants often endure [1]. Plants are quite sensitive to frost conditions and there is no freezing resistance capacity if plants fall into such stress conditions. Below the ideal temperature, the metabolic processes of these plants become dysfunctional. Plants with some tolerance to cold resistance fall into the second category. These plants can withstand varying degrees of exposure to temperatures below freezing. This group of plants exhibits a wide variety of freezing resistance, from annual summer plants with broad leaves to perennial grasses, which can withstand temperatures as low as 30 °C [2]. The third group consists of woody, temperate plant species that can withstand cold temperatures. After a period of cold acclimation, these plants can withstand extreme climatic conditions. The acclimation process comprises changes in many biochemical and metabolic properties, such as increased production and deposition of suitable solutes or osmolytes including soluble carbohydrates, proteins, and nitrogenous substances with low molecular weight. Plants that are suited to the cold have a slow growth rate and a very good capacity to store sugars/ carbohydrates in their underground tissue. The major factor that plays important role in cold acclimation is controlled carbohydrate metabolism, including their precursors, intermediates, and various products that function as osmoregulatory, cryoprotectants, and reactive oxygen species-scavengers (ROS-Sv). A series of cascading events known as signal transduction is used by plants to respond to these abiotic stresses. Abiotic stress factors depend on the production of particular genes and metabolites which are stress-related, on the initiation of a series of molecular channels involved in the detection of stress, and on signal transmission. However, a thorough understanding of the expression and role of the genes in cold signaling is urgently required to provide light on the pathways by which plants combat cold stress.

2. Adaptation to cold stress

Plants have adapted to withstand unfavorable environmental conditions through natural selection. The plant growth pattern optimizes any heat released during the day time and lessens cooling during the night time because the temperature of the air is stabilized most efficiently near the surface of the soil [3]. The plant is affected by cooling in a variety of ways (Fig 1).

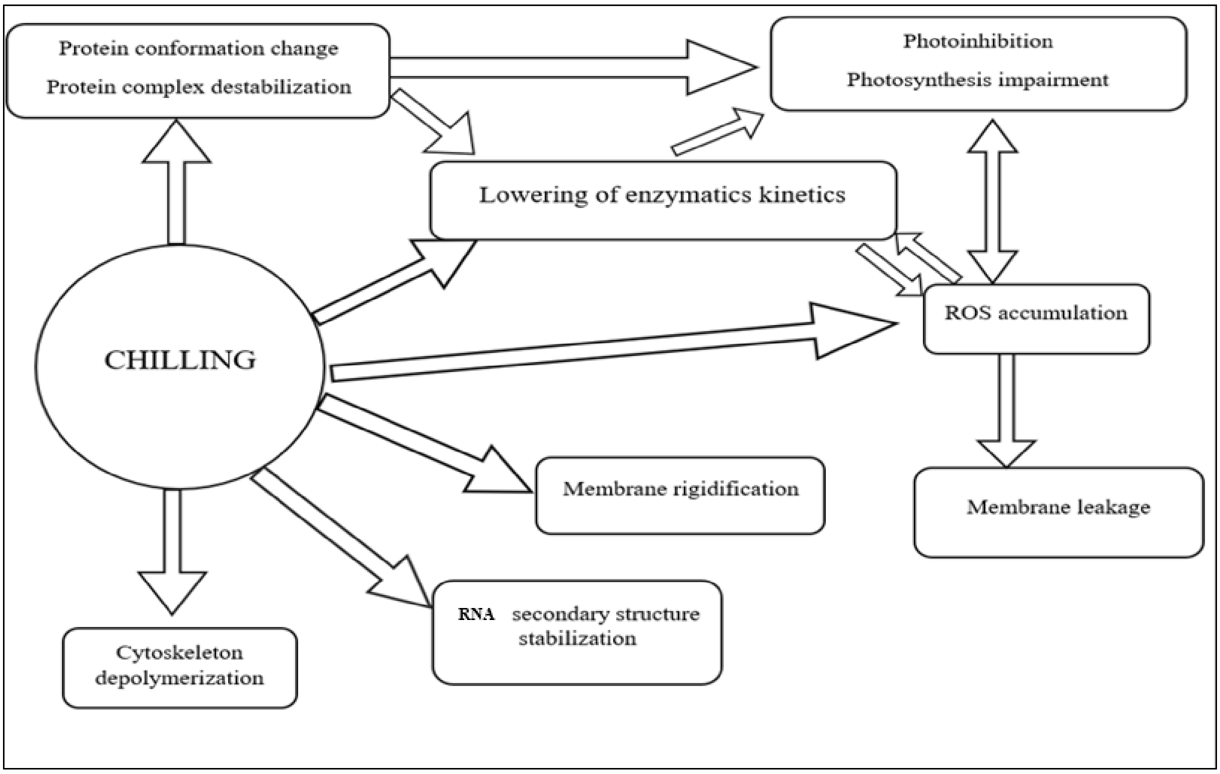


Fig 1: Chilling stress causes metabolic destabilization at the plant's physiological level

Plants have developed two strategies to combat the effects of cold stress: stress avoidance and tolerance. Preventing the freezing of sensitive tissues is included in stress avoidance. Some xerophytic plants can absorb heat during the daytime and disperse it slowly during the chilly night. Various annual herbs can exist in the dormant state of latent seed. Supercooling is a more advanced avoidance strategy that works by ceasing the formation of ice nucleators, which helps to inhibit endogenous nucleation of ice even at low temperatures such as 40°C. Even at 196 °C, particularly resistant species may create "liquid glass," which is a highly dense fluid that stops ice nucleation. By thermal, osmotic, and mechanical variables, such cells become less sensitive to the presence of exogenous ice. Resistant plants have developed the capacity to acclimate when the degree of stress is more progressive. Three main stages are depending on the plant's responsiveness to low-temperature stress. The first stage is cold acclimation which is also known as a pre-hardening phase that takes place at low temperatures only above 0°C. During the second stage i.e., hardening, exposure is required for the duration of sub-zero degree temperature to reach the full level of tolerance. In the last stage, after winter recovery of the plant takes place [4]. To completely develop cold tolerance, certain plants require both low temperatures and a short photoperiod. When the temperature of the plant is elevated above 0°C and the photoperiod is extended in these circumstances, tolerance may be lost [5].

3. Cold acclimation

Autumn is the time for overwintering temperate plants to adapt since their metabolism is now focused on producing chemicals that protect them from freezing, including soluble carbohydrates, alcohols (ribitol, inositol, sorbitol), sugars (raffinose, trehalose, saccharose, stachyose), and low-molecular-weight nitrogenous substances (glycine betaine, proline). Cold-regulated proteins (CORs), Heat-shock proteins (HSPs), and Dehydrin proteins (DHNs) work in combination with these to stabilize membrane proteins, phospholipids, and cytosolic proteins, scavenge the reactive oxygen species (ROS) and help in maintaining hydrophobic interactions [6].

4. Cold sensors and their perception in cold signaling

Cold acclimation is one of the chief methods for the plant to acclimatize the plant under cold stress through the activation of multiple cold responsive (COR) genes, which encoded cryo-proteins, which protect plant cells from cold-induced injury. Cold stress condition activates signaling pathways of important factor molecules like CBFs (C-repeat binding factor/ DREBS (dehydration responsive element binding factors), which work as sensory regulators in plant machinery under low-temperature stress. In the prokaryotic as well as eukaryotic system, cold-induced membrane rigidification is considered a chief cold sensor, which can trigger the auto-phosphorylation of the membrane histidine kinases (HKs) (an important key component of plant membrane) and activates Ca^{2+}ions channel. Further diacylglycerol kinase (DAGK) activity can also be used to examine the rigidification of the membrane. In addition, ADS2 (acyl-lipid desaturase2) acts as a vital enzyme in chilling and freezing tolerance in *Arabidopsis*. Potential cold sensors include receptors linked to G-proteins, Ca^{2+} influx channels, and two-component histidine kinase [7]. Following membrane rigidification, molecules/ specific cytoskeletal elements mentioned below alter the activity of the Calcium ion channel under stress, and help the plant in cold sensing.

4.1 Calcium channels

It is one of the most prominent cellular second messengers and mediates the connection of stimuli and responses to regulate physiological processes [8]. Plant's initial reaction to cold stress is cytoskeletal actin rearrangement which increases the Ca^{2+} levels. Plant plasma membranes include 3 Ca^{2+} gated ion channels. These include the depolarization-activated calcium channel (DACC), MCC, and hyperpolarization-activated Calcium channels (HACC) [9]. Cold stress causes a brief Ca^{2+} influx into the cytoplasm. As a result, calcium-permeable channels, which are in charge of this Ca^{2+} influx, are viewed as low-temperature sensors. Calcium channels are thought to open in response to cold due to changes in the physical constitution of cells (Fig 2). Calmodulins (CaM), calcineurin B-like proteins (CBLs), calcium-dependent protein kinases (CDPKs), Calmodulin-like proteins (CMLs) and their interacting kinases (CIPKs) which are prevalent calcium sensors, are found in gene families of plants and form complex signaling networks [10].

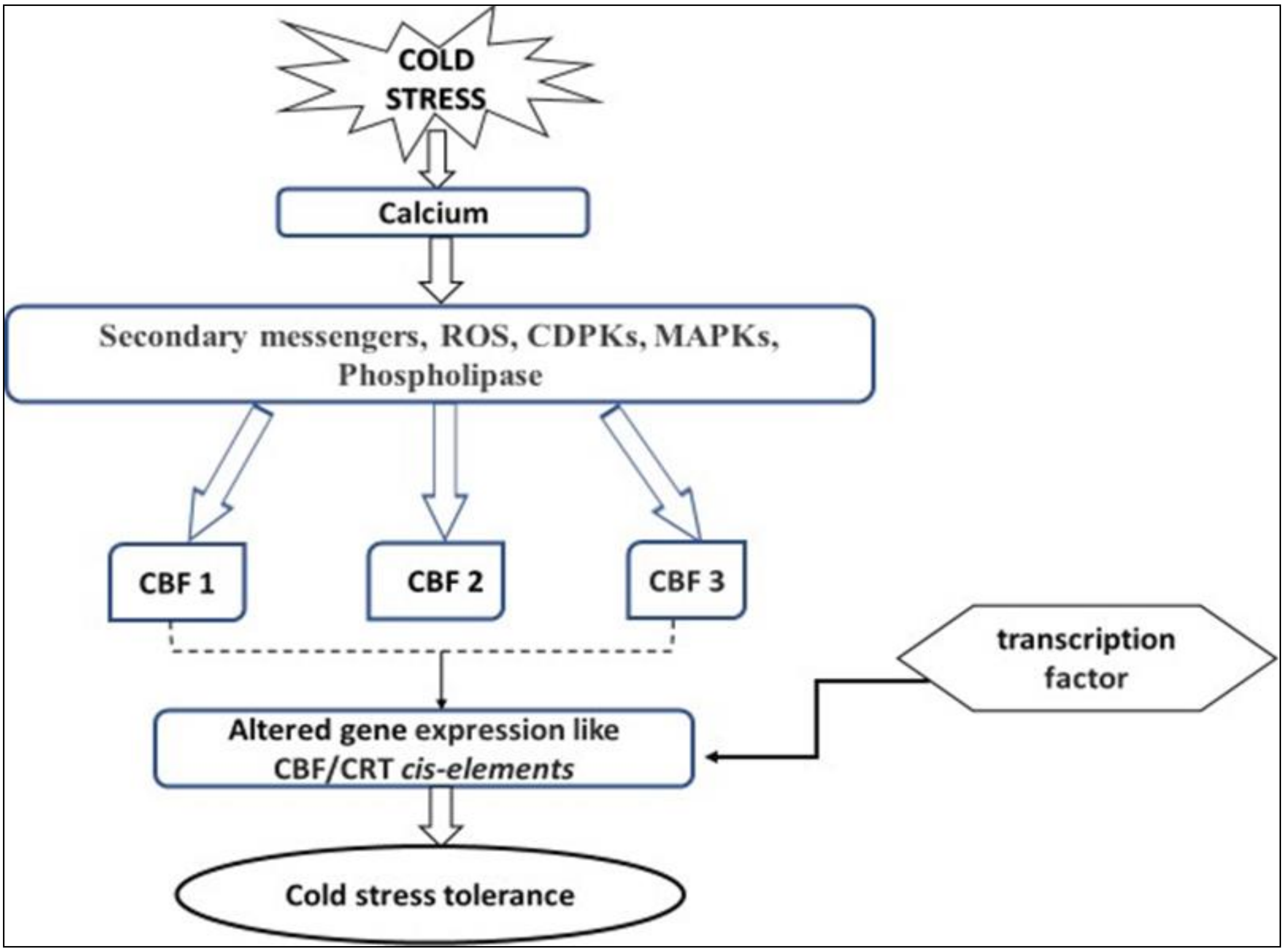


Fig 2: Perceptiveness of cold sensors in cold signaling

4.2 Histidine kinases

The lowest temperature sensors in plants are the two-component histidine kinase. The histidine kinases DesK and Hik33 from the cyanobacterium and *Bacillus subtilis*, are temperature sensors that regulate the expression of the desaturase gene in response to low temperatures. Numerous putative two-component histidine kinases have been identified in *Arabidopsis*. Low temperature, high salinity, and dehydration increase AtHK1 [11][12]. The AtHK1 protein recognizes the stress condition and transmits the signal through a phosphorylation pathway to the nucleus.

4.3 Receptor Kinase

Receptor-like kinases, (RLKs) are additional possible low-temperature sensors. RLKs are important for the development and growth of the plant and involve in the plant stress condition. RLKs have membrane-spanning regions which transmit signals to intracellular target molecules from external sources. In *Arabidopsis*, it has been shown that RPK1, a member of this family, is activated by dehydration and cold [13].

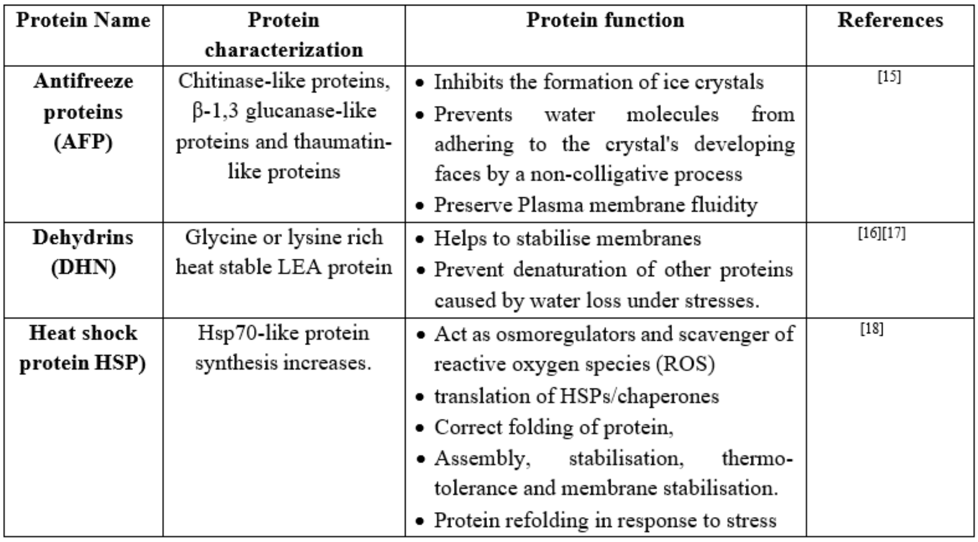
4.4 Phospholipases

Phospholipase C, Phospholipase D, and Phospholipase A are three different phospholipase types that are activated by cold stress. Alterations in the metabolism of membrane phospholipids have been connected to signaling in the cold response. Phospholipase C gets accumulated after it receives cold therapy. It promotes the synthesis of phosphatidic acid (PtdOH), which is a membrane-based secondary messenger molecule. The microtubules are connected to the "plasma membrane" by phospholipase D, and the shape of the cytoskeleton changes when it is activated. Additionally, it results in a rearrangement of the actin filaments, perhaps activating the Calcium ion channels [14].

5. Proteins and osmolytes involved in plants under cold stress

Many diverse protein types are expressed under the cold stress state, including dehydrins (DHN) antifreeze proteins (AFPs), chaperonins, pathogenesis-related proteins (PR), and heat shock proteins (HSPs) that are involved in signal transduction and signaling pathway (Table 1). The main proteins which are involved during plant cold stress conditions are as follows:

Table 1: List of cold stress proteins involved in signal transduction pathway in plant



Protein Name	Protein characterization	Protein function	References
Antifreeze proteins (AFP)	Chitinase-like proteins, β-1,3 glucanase-like proteins and thaumatin-like proteins	• Inhibits the formation of ice crystals • Prevents water molecules from adhering to the crystal's developing faces by a non-colligative process • Preserve Plasma membrane fluidity	[15]
Dehydrins (DHN)	Glycine or lysine rich heat stable LEA protein	• Helps to stabilise membranes • Prevent denaturation of other proteins caused by water loss under stresses.	[16][17]
Heat shock protein HSP)	Hsp70-like protein synthesis increases.	• Act as osmoregulators and scavenger of reactive oxygen species (ROS) • translation of HSPs/chaperones • Correct folding of protein, • Assembly, stabilisation, thermo-tolerance and membrane stabilisation. • Protein refolding in response to stress	[18]

The osmoprotectants or compatible solutes are undersized organic compounds having a neutral charge and negligible toxicity at elevated concentrations. They operate as osmolytes and assist in the survival of organisms under rigorous osmotic stress. These osmolytes comprise soluble sugars such as sucrose, glucose, and fructose. Under various abiotic stresses, these osmoprotectants play a vital function via increasing the osmotic strain in the cytoplasm and help in the regulation of photosynthesis, separation of carbohydrates, lipid metabolism, osmotic alteration, proteins stabilization, and eventually regulate the overall growth of the plant. Such sugar moieties improved plant tolerance under harsh environmental stresses, such as drought, heat/ cold, and salinity [19].

Earlier studies proved that higher accumulation of sugars such as sucrose, fructose, and glucose help in extenuating damages caused by temperature stress in plants. The major function of these sugars as osmoprotectants during stress is the defense of plant membranes and hunting of free radicals and regulating the osmotic balance. To improve their tolerance capability to stress, the plants have to synthesize a huge quantity of osmoprotectants for quenching the toxic ROS [20]. These osmolytes further function as a source of both energy and nitrogen. Osmoregulation is the best strategy for the tolerance of abiotic stress when osmoregulatory genes get activated. These osmoprotectants include sugar alcohols (sorbitol, inositol, and ribitol), low-molecular-weight nitrogenous compounds (i.e., proline and glycine betaine) and soluble sugars (saccharose, raffinose, trehalose, stachyose). The plasma membrane is protected by additional solutes that are produced from the symplast against ice adherence and consequent cell damage in plant cells [21, 22].

6. Dynamics of Carbohydrates in Plant Cold Acclimation

Carbohydrates, the primary by-product of photosynthesis, are essential for growth, energy metabolism, temperature regulation, and stress signaling. To assist in the maintenance of membrane stability under freezing conditions, carbohydrate links with the membrane interface directly alter cell membrane stability. Sugars have critical functions in membrane integrity, osmoprotectant, and preventing protein desiccation. When subjected to cold stress, sugars such, as raffinose, trehalose, starch, maltose, and fructans help to retain membrane integrity [23].

6.1 Trehalose

Trehalose sugar has the unique ability to maintain cell membrane, which loses fluidity as temperature decreases. Trehalose can preserve the fluidity of cell membranes, which disappears when temperature shifts. After cold stress, trehalose concentration and trehalose-6-phosphate phosphatase activity suddenly get elevated which shows early cooling stress response in rice involves brief activation of synthesis of trehalose [24].

6.2 Fructans

An enzyme called fructosyl transferase is used in the synthesis of fructans from their precursor, sucrose. Fructans serve as osmoprotectants, which are essential for stabilizing membranes because they attach to the choline and phosphate groups of membrane lipids. This stabilizing procedure stops water evaporation via the desiccated membrane.

6.3 Raffinose

Raffinose family oligosaccharides (RFOs) can shield membranes from the effects of cold stress and increase freezing resistance. To safeguard thylakoid membranes, raffinose is synthesized in the cytosol and transferred into the plastids, helping to maintain PSII integrity and perhaps scavenging ROS.

6.4 Maltose

When the starch breaks down, maltose is created. By preventing the dehydration of stromal proteins, maltose, a by-product of starch hydrolysis, may act as a direct osmoprotectant in chloroplasts.

6.5 Starch

Starch is a carbon-storage molecule and a direct by-product of photosynthesis. During abiotic stress, starch production and degradation are strictly controlled (Fig 3). Redox regulation is used to control enzymes essential for the metabolism of starch. In *Arabidopsis*, starch metabolism is an important factor in determining the fitness of plants under abiotic stress and exhibits tremendous flexibility when responding to variations in growth conditions during cold stress. The early response is starch degradation because starch metabolism can reduce the impacts of product inhibition on enzymes connected to the Calvin cycle and may even enable Pi release in chloroplasts during cold conditions. Increased beta-amylase activity helps in the supplementation of maltose when exposed to cold. Additionally, several *Arabidopsis* wild accessions mobilize starch in different ways, which might affect how well those plants can withstand low temperatures. The starch breaks into sugars, producing osmo-protective carbohydrates and fast sources of energy, which is a critical step in the adaptation of plants to cold stress.

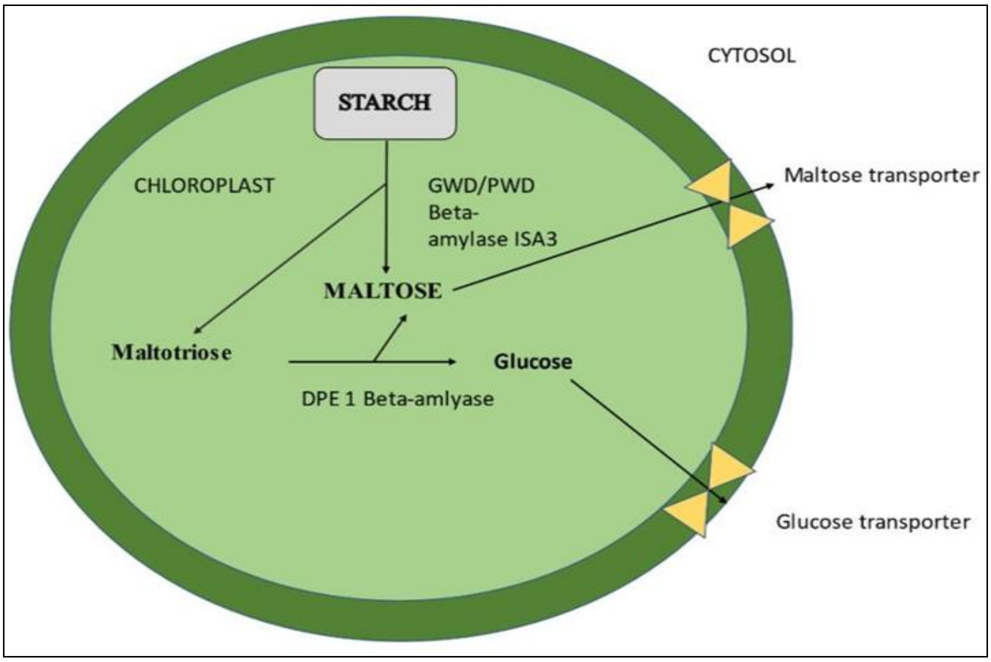


Fig 3: Impact of cold stress on Starch degradation

7. C-binding factors (CBF)/ DREBs in the cold signaling process

Various transcription factors called C binding factors (CBF) or DREBs are found in *Arabidopsis* and aid in the cold signaling process. The *Arabidopsis* CBF cold-response pathway was discovered via the study of cold-regulated gene expression. These genes were given a variety of names, including COR (cold-responsive), LTI (low-temperature-induced), KIN (cold-induced), and RD (responsive to dehydration) [25]. When plants were moved to low temperatures, their gene expression increased; if they were kept at low temperatures, it remained raised; and when the plants were moved to high temperatures, their expression of these genes decreased. CBF1, CBF2, and CBF3 are members of a small family of low-temperature-sensitive transcriptional activators that are also known as DREB1a, DREB1b, and DREB1c in *Arabidopsis*. Low temperature induces the expression of the CBF regulon of CRT/DRE-regulated genes, which also causes the initiation of the CBF 1, CBF 2, and CBF 3 genes. Both ICE1, which is a positive regulator of CBF expression, and HOS1, which is a negative regulator of ICE1, are located upstream of CBF. The HOS1 product is a RING E3 ligase that targets the proteasome for the degradation of ICE1 (Fig 4). A DNA regulatory component known as the CRT (C- repeat)/DRE (dehydration responsive element) was present in the promoter regions of these genes and it responded to both low temperature and dehydration stress. CRT/DRE elements, which have a conserved five base pair (bp) core sequence CCGA, are found in the promoter regions of several LT- and dehydration-responsive genes, including those identified as cold-regulated (COR) genes in *Arabidopsis*. Similar to other plants, *Arabidopsis* has a similar expression pattern. New methods and strategies need to be developed to learn more about the CBF-dependent pathway.

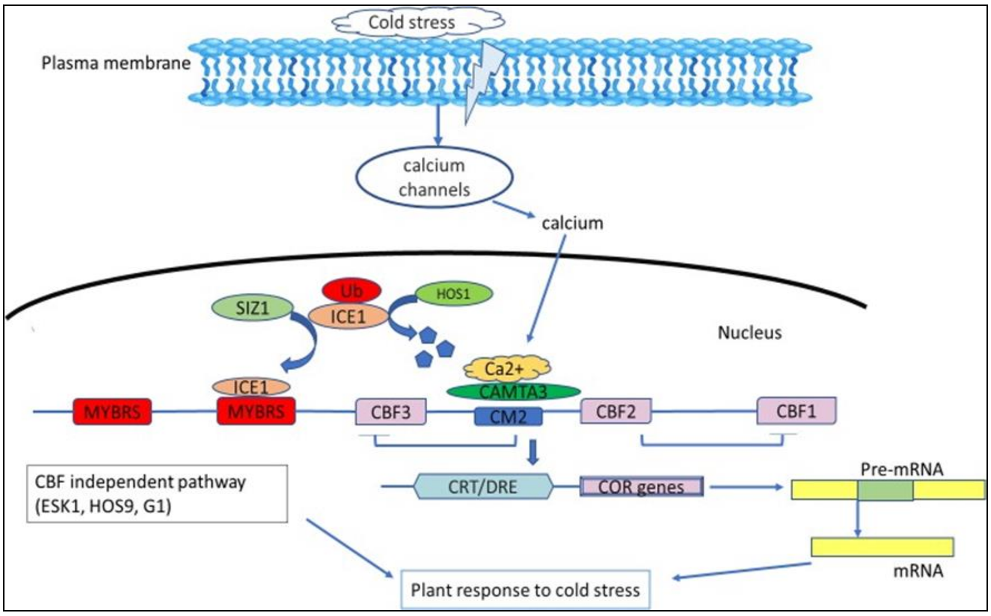


Fig 4: C-binding factors (CBF)/ DREBs pathway in the cold signaling

8. Conclusion

With the current global scenario, climatic changes have led to various abiotic stress on the plant. Cold stress is the major stress through which plant suffers. Due to the cold stress, crop productivity and yield have decreased. A sudden change in the environment highly affects plant growth and development. This review covers the mechanism of signal transduction, plant sensors, various proteins and carbohydrates involved during cold stress, and how the plant develops tolerance against cold stress conditions. To recognize cold stress, the cell membrane either modifies the fluidity of the membrane or uses sensory proteins. To start the transcriptional process, these signals are then sent to the nucleus. To find a new way of analysis, a multidisciplinary strategy that combines physiological and biochemical investigations with platforms based on genomics and proteomics can be applied. With the advancement and the technologies, more research regarding signaling pathways can be established. Genetic engineering can be used to see the function of various genes, and how genes play a function when they are under abiotic stress using the gene knockout method.

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