Omics Approaches for Cold Stress Tolerance in Plants

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Abbreviations

2D-DIGE	2D differential gel electrophoresis
ADH	Alcohol dehydrogenase
APETALA2	Ethylene responsive factor
AtCBF3	Arabidopsis thaliana C-repeat binding factors
CA	Cold acclimation
Cbf	C-repeat binding factors
CE-MS	Narrow electrophoresis coupled to mass spectrometry
Coda	Choline oxidase A
Cor	Cold-regulated genes
CT	Cold tolerant
DA	Deacclimation
DaCBF4	Deschampsia antarctica C-repeat binding factors
DHNs	Dehydrin proteins
DREB	Dehydration responsive element binding protein
ERD10	Early responsive to dehydration proteins

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FLAs	Fasciclin-like arabinogalactan protein
FT	Freezing tolerance
FT-ICR/MS	Fourier change particle cyclotron reverberation mass spectrometry
GABA	Gamma-aminobutyric acid (GABA)
GB	Glycine betaine
GB	Glycine betaine (GB)
GC-MS	Gas chromatography coupled to mass spectrometry
HSPs	Heat shock proteins
ICAT	Isotope-coded affinity tags
ILs	Introgression lines
iTRAQ	Isobaric tags for relative and absolute quantitation
LC-MS	Liquid chromatography coupled to mass spectrometry
LC-PDA/MS	Liquid chromatography- photodiode analysis coupled to mass spectrometry
LeCBF1	Lycopersicon esculentum C-repeat binding factors
LRR	Leucine-rich repeat protein kinase
MAS	Marker assisted selection
MDR1	Multidrug resistance 1
MS	Mass spectrometry
MS	Mass spectrometry
NMR	Nuclear magnetic resonance
OsiSAP1	Zinc finger protein of rice cultivars
PCR	Polymerase chain reaction
PDR8	Pleiotropic drug resistance 8
PEG	Polyethylene glycol
PLDδ	Phospholipase Dδ
PM	Plasma membrane
PSI, PSII	Photosystems I, II
PTMs	Post-translational modifications
QTL	Quantitative trait loci
RAP	Ethylene responsive factor
RG	Rootstock grafted
ROS	Reactive oxygen species
RuBisCO	Ribulose bisphosphate carboxylase/oxygenase
SILAC	Stable isotope labeling by amino acids in cell culture
SSR	Simple sequence repeats
STN7	Serine/threonine protein kinase
SYT1	Synaptotagmin
WCS120	Wheat cold stress responsive gene
WT	Wild type

1 Introduction

As plants are sessile, they are always exposed to various stresses, both biotic and abiotic, during their lifetime. Abiotic stresses, which mainly include drought, salt, temperature (low/high), flooding and nutritional deficiency/excess, are attributed to 50% crop losses (Ahmad et al. 2016). These abiotic stresses hamper crop growth and yield to a great extent (Bita and Gerats 2013). The ability to change is the key to adaptation of plants, which have developed highly sophisticated and effective mechanisms to counteract environmental cues and such adaptations also include changes in several proteins at pre- and post-transcriptional and translational levels (Lin et al. 2016). Cold temperature acts as a major constraint that affects growth, productivity and distribution of plants (Zaynab et al. 2017). Crops in temperate zones experience temperatures that range from 0 to 15 °C during their growing seasons (Megha et al. 2014). It is estimated that 42% of the total land area on the earth experiences periodic low temperatures (Gharechahi et al. 2016). Cold temperatures are very harmful to plants. They induce changes in proteins involved in carbohydrate metabolism, photosynthesis, stress-related proteins among other processes, protein folding and degradation, as well as reactive oxygen species (ROS) scavenging and biosynthesis of compatible solutes (Shi et al. 2014). Plants may experience chilling stress when they are exposed to temperatures from 0 to 15 °C (non-freezing temperatures) or freezing stress when exposed to temperatures below 0 °C (Miki et al. 2019). Chilling stress may hamper plant growth and development by reducing water absorption resulting in cellular desiccation, inducing alteration in metabolites leading to an oxidative stress, inhibiting cellular metabolism, perturbing gene transcription and finally causing cell death. Freezing temperatures, on the other hand, leads to cellular dehydration and extracellular ice formation and imbalances in plasma membranes, leading to the formation of inverted hexagonal-phase membrane structure (Shi et al. 2018). The changes in metabolic profile of crops help them to adapt and resist freezing stresses when air temperature decreases and day length shortens, which is called cold acclimation (Zhao et al. 2015). Various genes/gene products play important roles in inducing cold stress tolerance in crops, some of which are shown in Table 1. Comprehensive changes in photosynthesis and carbohydrate metabolism, protein biosynthesis, folding and degradation, as well as reactive oxygen species (ROS) scavenging and biosynthesis of compatible solutes and stress-related proteins, are aimed at minimizing the harmful effects of low temperatures and gaining a sufficient level of freezing tolerance (Preston and Sandve 2013).

2 Genomics Approaches for Resistance Against Cold-Induced Stress

One of the most common environmental stresses that affects growth and development of plant and reduces its productivity is cold temperature (Shinada et al. 2014). Within the temperature range of 0–10 °C, many plants of tropical or subtropical

Table 1 Genes/gene products involved in inducing cold stress tolerance in crops

Gene(s)/gene product	Cellular role	References
cor15a Cold-regulated gene	Promotes freezing tolerance	Sowemimo et al. (2019)
cbf1 CRT/DRE-binding factor	Transcription factor	Park et al. (2018)
dreb1 and dreb2 DRE-binding protein	Transcription factor	van Buer et al. (2019)
WCS120/COR39 CCGAC sequences like CRT/DREs in its promoter	Low-temperature-regulated gene	Cheng et al. (2019)
Coda Choline oxidase A	Glycine betaine biosynthesis	Zuther et al. (2018)
Coda Choline oxidase A	Glycine betaine biosynthesis	Cai et al. (2018)
DREB1A (CBF3) DRE-binding protein	Transcription factor	Zhang et al. (2018)
CBF3 DRE-binding protein	Transcription factor	Ma et al. (2018)
CBF1/DREB1b DRE-binding protein	Transcription factor	Zhang et al. (2019)
DREB1A (rd29A) DRE-binding protein	Stress-inducible promoter	Liu et al. (2018)
OSISAP1 Zinc-finger protein	Transcription factor	Barrero-Gil and Salinas (2018)

origin suffer damage (Goodstal et al. 2005). Susceptible crop species are affected by cold and they show different symptoms of chilling injury, such as chlorosis, necrosis and growth retardation, or are ultimately killed by non-freezing low temperatures. Plants from temperate regions are acclimatized to cold due to their constant exposure to cold and are considered to be chilling tolerant. Their constant exposure to chilling, non-freezing temperatures can also increase their freezing tolerance. Cold acclimation provides selective advantage to crop plants in temperate regions and positively influences their survival and distribution. However, plants in tropics and subtropics are not acclimatized to chilling stress. Cold tolerance is a very complex trait controlled by many genes and regulated by chill in atmosphere (Sanghera et al. 2011). It is, therefore, important to develop cold-tolerant varieties. As the underlying trait is complex in nature and regulated by many genes, integrated molecular approaches can assist in the development of cold-tolerant varieties.

Tomato is sensitive to both chilling and freezing temperatures, and low temperature (10 °C or below) inhibits tomato growth. Temperature below 6 °C causes irreparable damage to tomatoes. Several quantitative trait loci (QTLs) for shoot turgor maintenance (stm) under root chilling have been identified in an interspecific backcross population derived from crossing chilling-susceptible cultivated tomato (*Lycopersicon esculentum*) and chilling-tolerant wild *L. hirsutum*. Major QTL for

enhanced chilling tolerance is located on chromosome 9 (stm9) of the *Lycopersicon hirsutum*. Marker-assisted backcross breeding was used for introgression of the *L. hirsutum* allele at the QTL on chromosome 9 in cultivated tomato (*Lycopersicon esculentum*) (Goodstal et al. 2005). In another study conducted by Shinada et al. (2014), pyramiding of quantitative trait loci (QTLs) using marker-assisted selection (MAS) was carried out to improve cold tolerance at the fertilization stage (CTF) in the case of rice. Cold stress tolerance at the reproductive stage is an important parameter of spikelet fertility and thus stable yield per plant. CTF is a quantitatively inherited trait and three QTLs controlling CTF, namely, *qCTF7*, *qCTF8* and *qCTF12*, were identified using backcrossed inbred lines derived from a cross between rice cultivar Eikei88223 (vigorous CTF) and Suisei (very weak CTF). Using MAS pyramiding, QTLs controlling CTF levels were pyramided utilizing an F_3 population derived from a cross between Eikei and Suisei. These novel QTLs for CTF imparted cold tolerance in combinations between *qCTF7* and *qCTF12* and between *qCTF8* and *qCTF12*. In rice, cold damage (below 15 °C) at the seedling stage results in poor seedling establishment and greatly reduces yield. Advanced backcross between a japonica cultivar, Xiushui 09, and an indica breeding line, IR2061, revealed QTLs affecting cold tolerance (CT) at seedling stage. A total of four QTLs (*qSRS1*, *qSRS7*, *qSRS11a* and *qSRS11b*) for CT were identified on chromosomes 1, 7 and 11. Marker-assisted selection (MAS) holds a great potential for introgression of these QTLs imparting cold tolerance from resistant varieties to susceptible varieties (Li-rui et al. 2012). Liu et al. (2016) made a cross between a cold-sensitive cultivated *Solanum lycopersicum* L. XF98-7 and a cold-tolerant wild *Solanum pimpinellifolium* LA2184 and derived a RIL (recombinant inbred line) population. Genome mapping using simple sequence repeats (SSRs) helped in identification of QTLs conferring cold tolerance in tomato. The QTLs qCI-1-1, qCI-2-1, qCI-3-1 and qCI-9-1 were located on chromosomes 1, 2, 3 and 9, respectively. Marker-assisted selection serves as a means of indirectly selecting the trait of interest and promoting development of a new tomato variety tolerant to chilling stress. Booting stage of rice is more sensitive to cold stress than seedling stage. Cold stress at booting stage affects pollen survival, seed set, grain filling and ultimately yield. Therefore, identification of cold-tolerant QTLs for the booting stage is essential. Zhu et al. (2015) developed interconnected breeding (IB) populations using Huanghuazhan (HHZ) as the recurrent parent and eight diverse elite indica lines as donors to identify stably expressed QTLs for cold tolerance at the booting stage. Six QTLs for cold tolerance on the chromosomes 3, 4 and 12 were identified, among which QTL qCT-3-2 showed stable cold tolerance over years. Raharinivo et al. (2016) identified QTLs for cold tolerance at the seedling stage in rice from a breeding population derived from the cross between Chomrongdhan, a donor parent tolerant, and Vary borty, a susceptible parent. Four QTLs on chromosomes 2 and 10 were identified that conferred cold tolerance. Three QTLs *qSdGwth14-10-1*, *qSdGwth14-10-2* and *qLfGwth14-10-1* located on chromosome 10 conferred cold tolerances for seedling growth and leaf growth at 14 day after recovery and one QTL *qSdVig0-2-1* located on chromosome 2 was identified for seedling vigor after

recovery. Information and materials developed can be utilized for developing cold-tolerant rice cultivars by marker-assisted selection (MAS). In wheat, the major Freezing tolerance QTLs named Fr-1 and Fr-2 and the major vernalization gene *VRN-1* are located on the homologous group 5 chromosomes. A cluster of cold-responsive CBF transcriptional activators was mapped at Fr2 locus and several minor freezing tolerance (FT) QTLs have been identified on several other wheat chromosomes (2B, 4B, 4D, 6A, 7A) (Sutka 2001). Wainaina et al. (2018) identified two QTLs for cold tolerance at the booting stage on chromosome 8 (*qCTB-8*) and chromosome 10 (*qCTB-10*) and three QTLs for heading date (*qHD-4, 7 and 11*) in a rice cross of a Japanese tolerant variety, Hananomai, and a NERICA parent, WAB56-104. Identified QTLs can be introgressed in different cold-sensitive varieties through MAS to enhance their tolerance. Liang et al. (2018) identified 17 QTLs for cold tolerance (CT) in 84 cold-tolerant introgression lines (ILs) selected from five BC_2 populations. Among them, three were large-effect CT QTLs (*qCT4.6*, *qCT6.6* and *qCT11.5*) and the remaining were *qCT1.2* (RM532), *qCT2.4* (between RM29 and RM341), *qCT3.5* (between OSR13 and RM7), *qCT3.12* (RM85), *qCT4.2* (RM518), *qCT4.6* (between RM303 and RM317), *qCT6.6* (between RM3 and RM439), *qCT9.7* (between RM278 and RM160) and *qCT11.5* (between RM457 and RM21). Yu et al. (2018) identified one major QTL, *qSCT8*, and one QTL, *qSCT4.3*, on chromosomes 8 and 4 for cold tolerance at the seedling stage using an *Oryza sativa* × *O. rufipogon* backcross inbred line population. In the subpopulation, three QTLs, *qSCT4.1*, *qSCT4.2* and *qSCT12*, were detected on chromosomes 4 and 12.

3 Transcriptomic Approaches for Cold Stress Tolerance in Plants

3.1 Introduction

Transcriptomics is a prominent field of study related to functional genome of an organism. It deals with quantification of the total set of transcripts or a specific subset of it present in a particular cell type and transcript abundance in a specific developmental stage (Imadi et al. 2015). The main objective of transcriptomics is to catalogue all the transcripts to determine transcriptional status of genes, 5′ and 3′ end sites of genome, post-transcriptional modifications and splicing patterns. Moreover, it quantifies the modulations in gene expression levels during various stress conditions and developmental stages (Wang et al. 2009). Different technologies have been used to study transcriptome that include hybridization-based approaches, sequence-based approaches and RNA sequencing (Wang et al. 2009). RNA sequencing is the most recent approach to study transcriptome. It is a recently developed deep sequencing technology and does not have a reference genome to gain useful information about the transcripts (Strickler et al. 2012).

3.2 Transcriptomics: A Key to Understanding Cold Stress Responses in Plants

Low temperature stress is one of the major environmental stresses affecting plant yield, quality and distribution. A comprehensive understanding of molecular mechanisms through which plants respond to low temperature is of fundamental importance to plant biology. Transcriptomics can be better employed to study cold stress responses in plants. Nineteen microRNA genes of 11 microRNA families in *Arabidopsis thaliana* were identified that were upregulated in response to cold stress. A further analysis of their promoter sequence shows the prevalence of some stress regulatory *cis*-elements (Gupta et al. 2013). These cold-responsive microRNA genes directly or indirectly affect different signalling pathways during the period of stress. In order to understand the gene network controlling tolerance to cold stress, Lee et al. (2005) performed an *Arabidopsis thaliana* genome transcript expression profile using Affymetrix GeneChips that contained ~24,000 genes. A total of 939 cold-regulated genes with 655 upregulated and 284 downregulated were statistically determined. A large number of early cold-responsive genes encode transcription factors that likely control late-responsive genes, suggesting a multitude of transcriptional cascades. In addition, many genes involved in chromatin level and post-transcriptional regulation were also cold regulated, suggesting their involvement in cold-responsive gene regulation. A number of genes important for the biosynthesis or signalling of plant hormones are regulated by cold stress, which is of potential importance in coordinating cold tolerance with growth and development. They compared the cold-responsive transcriptomes of the wild type with the inducer of CBF expression 1 (ice1), a mutant defective in an upstream transcription factor required for chilling and freezing tolerance. The transcript levels of many cold-responsive genes were altered in the ice1 mutant not only during cold stress but also before cold treatments. This study provides a global picture of the *Arabidopsis* cold-responsive transcriptome and its control by ICE1 and will be valuable for understanding gene regulation under cold stress and molecular mechanisms of cold tolerance. Significant progress has been made in the past decade in elucidating the transcriptional networks regulating cold acclimation.

Cold stress induces the expression of APETALA2/ETHYLENE RESPONSE FACTOR family of transcription factors, that is, CBFs (C-repeat binding factors, also known as dehydration-responsive element-binding protein 1s or DREB1s), which can bind to *cis*-elements in the promoters of *COR* genes and activate their expression. Analyses of transgenic plants have shown that ectopic expression of CBFs is sufficient to activate the expression of *COR* genes and induce cold acclimation even at warm temperatures (Chinnusamy et al. 2007). Transgenic expression of *Arabidopsis* CBFs in different plant species was able to enhance chilling/freezing tolerance, and, conversely, the ectopic expression of *CBFs* from other plant species could enhance the freezing tolerance of transgenic *Arabidopsis* (Yamaguchi-Shinozaki and Shinozaki 2006). Microarray analyses of transgenic *Arabidopsis* plants ectopically expressing *CBFs* revealed a constitutive expression of downstream

cold-responsive transcription factor genes *Rap 2.1* and *Rap 2.6* that might control subregulons of the CBF regulon (Fowler and Thomashow 2002). Thus, CBFs play a pivotal role in gene regulation during cold acclimation in evolutionarily diverse plant species. However, CBF regulons from freezing-tolerant and sensitive plant species can differ as evident from microarray analysis of transgenic tomato and *Arabidopsis* plants overexpressing LeCBF1 and AtCBF3, respectively (Zhang et al. 2004). The reason why winter plants exhibit significant genotypic differences in constitutive freezing tolerance is poorly understood. Transcriptome and metabolome analyses in *Arabidopsis* accessions differing in constitutive freezing tolerance suggest that the CBF pathway might also have a crucial role in constitutive freezing tolerance (Hannah et al. 2006).

Byun et al. (2018) investigated the molecular mechanism of Antarctic adaptation of *Deschampsia antarctica* by identifying and characterizing *D. antarctica* C-repeat binding factor 4 (DaCBF4), which belongs to monocot CBF group IV. The transcript level of DaCBF4 in *D. antarctica* was markedly increased by cold and dehydration stress. To assess the roles of DaCBF4 in plants, a DaCBF4-overexpressing transgenic rice plant (Ubi:DaCBF4) was generated and its abiotic stress response was analysed. Ubi:DaCBF4 showed enhanced tolerance to cold stress without growth retardation under any condition compared to wild-type plants. The genes responsible for the improved cold tolerance in rice were screened by selecting differentially regulated genes in both transgenic rice lines because the cold-specific phenotype of Ubi:DaCBF4 was similar to that of Ubi:DaCBF7 (Byun et al. 2015). By comparative transcriptome analysis using RNA-seq, 9 and 15 genes were identified under normal and cold stress conditions, respectively, as putative downstream targets of the two *D. antarctica* CBFs. Hence, results suggested that Antarctic hairgrass *DaCBF4* mediates the cold-stress response of transgenic rice plants by adjusting the expression levels of a set of stress-responsive genes in transgenic rice plants. Thus, such Transcriptional factors which regulate expression of a set of selected downstream target genes will be useful for genetic engineering to enhance the cold tolerance of including rice.

4 Proteomic Changes in Response to Cold Stress

4.1 Introduction

Plants when exposed to cold temperatures experience myriad of changes at physiological, biochemical and molecular levels (Sasaki and Imai 2012). Cold-induced changes in expression of specific proteins have been observed in cold-tolerant plant species. Different cold-response proteins separated by one-dimensional SDS-PAGE and identified by specific antibodies or two-dimensional SDS-PAGE (2DE) combined with mass spectrometry (MS) help to identify differentially abundant proteins during cold treatment in several crops such as barley, soybean, *Arabidopsis thaliana*, rice, wheat and tobacco (Gharechahi et al. 2014; Lee et al. 2009; Gai

et al. 2011; Kosova et al. 2013; Hlavackova et al. 2013; Nakaminami et al. 2014; Yan et al. 2006).

Proteomic approaches help in unraveling stress-inducible proteins and thus aid in dissection and understanding of pathways associated with crop physiological and stress responses (Zhang et al. 2017). Understanding of such stress pathways can be implemented into biotechnological applications for improving stress tolerance in plants. The 'omics' technologies, which essentially include metabolomics, proteomics and genomics, are being put to use to dissect key proteins, metabolites and novel genes involved in stress signalling (Cramer et al. 2011) (Fig. 1). Proteomics is a science that focuses on the study of proteins: their roles, structures, localization, interactions, expression profile, post-translational modifications (PTMs), and other factors under stress and non-stress conditions (Yates et al. 2009). In the wake of cold stress, notable changes in protein expression levels have been observed with differential abundance and expression (Koehler et al. 2012; Grimaud et al. 2013; Xu et al. 2013; Chen et al. 2015; Zhang et al. 2017). Proteomic studies of different organs as well as subcellular compartments under stress are conducted to infer the responses of plant cells to abiotic stresses in different organs. Plants respond to cold

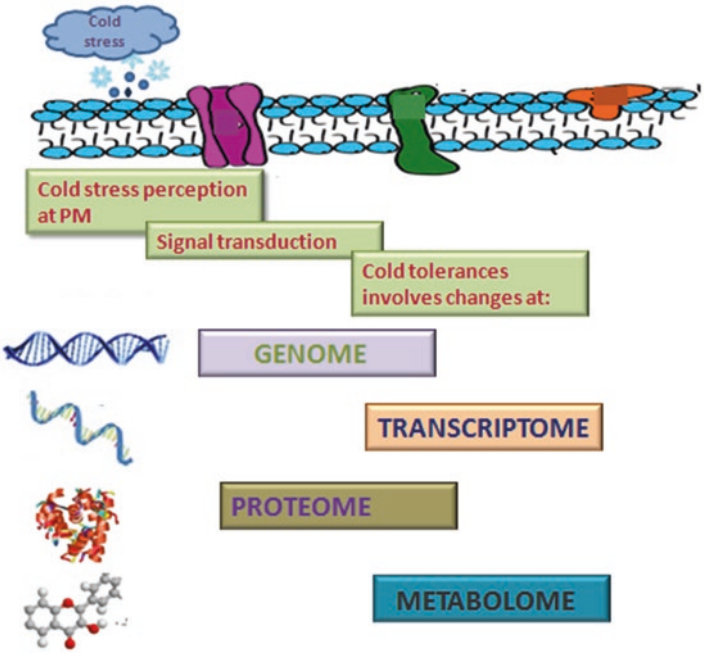

Fig. 1 Cold signals are perceived primarily at the plasma membrane, which, through a series of secondary signalling molecules, transduces the signal to the nucleus. Transcription of cold-responsive genes and transcriptional factors may confer cold tolerance to plants. Transcripts and proteins sometimes may undergo different pre- and post-transcriptional changes or post-translational changes that result in altered transcriptome, proteome, or metabolome associated with cold tolerance in crop plants

by bringing about structural and compositional modifications of compatible solutes in various subcellular compartments via changes in the transcriptome and metabolome (Gharechahi et al. 2016).

Proteomics acts as a central link between gene expression and metabolism as the proteins encoded by transcripts undergo different modifications, such as acetylation, biotinylation and phosphorylation, that may regulate their cellular function and distribution differently. Protein profiling can be done through 'high-throughput mapping', which involves separation of all proteins by 2D electrophoresis followed by their identification, 'differential comparison' or 'protein interaction mapping'. Protein profiling can be achieved via gel-based techniques such as 2D gel electrophoresis, 2D differential gel electrophoresis (2D-DIGE) and gel-free approaches, include isotope-coded affinity tags (ICAT), isobaric tags for relative and absolute quantitation (iTRAQ), stable isotope labeling by amino acids in cell culture (SILAC), and so on (Yates et al. 2009). Protein isolation by TCA/acetone method or phenol/SDS method is generally carried out and sometimes a polyethylene glycol (PEG) mediated pre-fractionation method is used to remove RuBisCO and their derivatives (Damerval et al. 1986; Hurkman and Tanaka 1986). Comparative proteomic approaches have already been applied to analyse changes in cold-sensitive proteins in different cold-tolerant cultivars, such as meadow fescue (leaf), pea (leaf and chloroplast), perennial ryegrass (leaf), strawberry (crown) and winter wheat (leaf) (Kosmala et al. 2009; Bocian et al. 2011; Dumont et al. 2011; Koehler et al. 2012; Grimaud et al. 2013; Xu et al. 2013; Chen et al. 2015). Proteins involved in energy metabolism, photosynthesis, reactive oxygen species (ROS) scavenging, storage, protection from stress, regulation of the cell cycle and plant development in wheat and barley showed differential abundance between stress-tolerant and stress-sensitive genotypes (Kosova et al. 2014; Chen et al. 2015).

4.2 Plasma Membrane Proteomics

Plasma membrane (PM) acts as the primary site of freezing injury. Protein profiles and specifically lipid raft composition of PM act as an important determinant of freezing tolerance in crop plants (Ruelland et al. 2009). Increased activity of certain proteins like P-type ATPase, disassembly of microtubules and accumulation of several dehydrin family proteins occur on the plasma membrane (Ishikawa and Yoshida 1985; Abdrakhamanova et al. 2003; Kosová et al. 2007). These have been also confirmed by semi-proteomic analysis such as 2D electrophoresis. Comparative proteomic analysis revealed that several PM proteins play an important role in cold acclimatization such as overexpression of phospholipase Dδ (PLDδ) or deficiency in PLDα. Several dehydrin families mitigate freezing injuries in *Arabdiopsis*. Synaptotagmin-1 (SYT1) reseals PMs disrupted by extracellular ice crystals (Yamazaki et al. 2008; Takahashi et al. 2013a, b). PM lipid profile changes significantly in response to cold in cold-tolerant varieties. Specific lipid classes and increase in levels of unsaturated phospholipids enhance the cryostability of the PM.

Comprehensiveness of the proteomic approaches with the help of rapid advance in analytical techniques using mass spectrometry, advanced shotgun proteomics, nano-LC using a LTQ Orbitrap XL mass spectrometer has enhanced our knowledge regarding PM proteome (Li et al. 2012; Takahashi et al. 2013a, b, 2016; Abdallah et al. 2012).

Miki et al. (2019) successfully identified 873 PM proteins responsive to cold acclimation and de-acclimation treatment by conducting shotgun proteomics with label-free semiquantification on plasma membrane fractions of *Arabidopsis* leaves during cold acclimation (CA) and de-acclimation (DA). A comprehensive understanding of PM protein profile in response to rising temperature was gained. This study revealed that global cold-acclimation-responsive proteins return to non-acclimation levels following de-acclimation. This change in protein profile following de-acclimation tends to allow plants to restart normal growth and development. However, levels of certain representative cold-acclimation-responsive proteins were maintained following de-acclimation, which may suggest their role in guarding against the threat of sudden temperature drop. Significant changes were observed in PM proteome during CA in *Arabidopsis* along with decrease in the proportions of transporters and alterations in the activities of several transporters such as ATPases and aquaporins. The study found a number of low temperature-induced proteins such as low-temperature-induced 29 (LTI29, At1g20450.1), cold-regulated 78 (COR78, At5g52310.1), temperature-induced lipocalin (TIL, At5g58070.1), proteins LTI29 and COR78 (dehydrin family proteins) and LTI29 (membrane- and protein-protective hydrophilic protein), COR78, TIL, GPI-anchored lipid transfer protein (LTPG1, At1g27950.1), blue copper-binding protein (BCB, At5g20230.1), SVL2 (At1g66970.1), AIR12 (At3g07390.1), β-1,3-glucanase putative plasmodesmata-associated protein (BG_PPAP, At5g42100.1), a glycoprotein (At5g19240.1) and FLAs (At2g45470.1, At5g44130.1) and SVL2, AIR12 and FLA8 (cell structure-related proteins). A protein, PIP1D, that act as negative regulator and whose abundance decreases during cold acclimation was also identified. PIP1D belongs to a group of proteins known as PIPs, which are freezing-related proteins that function as water transporters and might be involved in rehydration kinetics during freezing recovery processes. Cytoskeletal proteins such as tubulins and actins acted as CA-downregulated proteins. Some stable proteins showing a CA response but no significant changes in abundance during the CA and DA processes are ERD4 (At1g30360.1), SYT1 (At2g20990.1), SHV3 (At4g26690.1), SVL1 (At5g55480.1) and SKU5 (At4g12420.1), PM-localized ATP-binding cassette (ABC)-type transporters, two patellins (PATLs) PATL1 and PATL2 (sec14-like proteins that bind to phosphoinositides and play a role in cell plate formation), ten leucine-rich repeat protein kinase (LRR) family proteins (cell-surface receptor kinases) and two ABC transporters, that is, pleiotropic drug resistance 8 (PDR8, At1g59870.1) (associated with hypersensitive-like cell death) and multidrug resistance 1 (MDR1, At3g28860.1). These proteins can be used as control markers for studies of CA and DA mechanisms.

Chen et al. (2015) conducted studies on freezing-tolerant and freezing-sensitive cultivars of alfalfa to reveal the difference between them at proteomic levels.

Results revealed that proteins to chilling were related to photosynthesis, protein metabolism, energy metabolism, stress and redox, and other proteins were mobilized in an adaptation to chilling stress. The relative abundance of the cytochrome b6-f complex iron-sulfur subunit, oxygen-evolving enhancer protein, chlorophyll A/B binding protein was altered in cold-resistant genotype. In yet another study, levels of 38 plasma membrane proteins were altered in *Arabidopsis* 3 days after cold acclimation. These proteins include early responsive to dehydration proteins (ERD10 and ERD14) (Kosová et al. 2007). Another novel protein plant synaptotagmin-1 (SYT1) that is believed to be involved in resealing the freeze-fractured membranes imparts freeze tolerance (Reddy et al. 2001). Clathrins and dynamin-related proteins accumulate in the microdomain during cold acclimation. They are associated with the clathrin-dependent endocytosis pathway. Certain proteins such as dehydrin proteins (DHNs), cold-regulated proteins (CORs) and heat-shock proteins (HSPs) act in conjunction with symplastic and apoplastic soluble osmolytes, such as glucose, sucrose, fructose, trehalose, raffinose, to stabilize both membrane phospholipids and proteins and cytoplasmic proteins (Livingston et al. 2006). These metabolites maintain hydrophobic interactions and ion homeostasis, scavenge reactive oxygen species (ROS) and protect the adhesion of ice to plasma membrane, thus preventing cell disruption (Janska et al. 2010).

4.3 Differential Proteome Profile in Response to Cold Stress

Tian et al. (2015) compared proteome of seedling leaves of cold-tolerant and cold-sensitive soybean varieties and found 57 proteins significantly changed in abundance and they were identified by MALDI-TOF/TOF MS. Proteins identified were found to be involved in 13 metabolic pathways and cellular processes, including 15 differentially expressing proteins involved in photosynthesis, protein folding and assembly, cell rescue and defense, cytoskeletal proteins, transcription and translation regulation, amino acid and nitrogen metabolism, protein degradation, storage proteins, signal transduction, carbohydrate metabolism, lipid metabolism, energy metabolism and unknown. The proteins associated with photosynthesis were implicated in plastid division and heme and chlorophyll biosynthesis: protein coproporphyrinogen III oxidase and cell division protein ftsZ homolog 1, photosystems I (PSI) and II (PSII), ribulose bisphosphate carboxylase/oxygenase (RuBisCO) proteins and interconversion of CO_2 and HCO_3. Differentially expressed proteins identified were coproporphyrinogen III oxidase, cell division protein ftsZ homolog 1, chlorophyll *a*/*b*-binding protein type II, chlorophyll *a*/*b*-binding protein, light-harvesting complex I type II, ferredoxin-NADP reductase, chloroplastic cytochrome b_6/f complex iron-sulfur subunit, photosystem II stability/assembly factor HCF136, NDH-dependent cyclic electron flow, ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit, carbonic anhydrase, chloroplastic protein folding and assembly proteins that include protein disulfide isomerase, peptidyl-prolyl *cis–trans* isomerase CYP37, chloroplastic-like 92; protein degradation proteins; cysteine proteinase, cysteine proteinase RD21a-like, cysteine proteinase inhibitor, proteasome

subunit beta type-3-A, proteasome subunit beta type, putative 20S proteasome beta subunit PBC2, ubiquitin fusion degradation 1, aminopeptidase N-like; cell rescue and defense proteins; thiol methyltransferase, nucleotide-binding site-containing resistance-like protein, ferritin; cytoskeletal proteins, such as tubulin/FtsZ family protein, transcription and translation regulation, eukaryotic translation initiation factor, elongation factor 1-delta-like cytidine/deoxycytidylate deaminase-like protein, 60S acidic ribosomal protein; amino acid and nitrogen metabolism proteins; arginase, O-methylthioadenosine/S-adenosylhomocysteine nucleosidase 1, putative pterin-4-alpha-carbinolamine dehydratase 1-like; Storage proteins glycinin A-2-B-1a subunit precursor; carbohydrate metabolism: chloroplast ribose-5-phosphate isomerase, phosphoglycerate kinase; ipid metabolism: beta-hydroxyacyl-ACP dehydratase, Allene oxide cyclase 3; energy metabolism: ATP synthase delta chain, electron transfer flavoprotein subunit alpha, mitochondrial-like stem-specific protein TSJT1. Proteomic analysis of a spring wheat cultivar in response to prolonged cold stress showed relative abundance of proteins involved in ascorbate recycling (dehydroascorbate reductase, ascorbate peroxidase), protein processing (proteasome subunit, cysteine proteinase) and enzyme involved in tetrapyrrole resynthesis (glutamate semialdehyde aminomutase). Downregulation of proteins involved in Krebs cycle; enzymes (isocitrate dehydrogenase, malate dehydrogenase), photosynthesis-related proteins (oxygen-evolving complex proteins, ATP synthase subunits, ferredoxin-NADPH oxidoreductase and some Calvin cycle enzymes) after cold stress was observed (Rinalducci et al. 2011). Zhang et al. (2017) used iTRAQ for comparative protein profiling of cold-tolerant and susceptible wheat varieties and identified 140 proteins that showed decreased protein abundance. These proteins were components of the following main groups: protein metabolism, stress/defense, carbohydrate metabolism, lipid metabolism, sulphur metabolism, nitrogen metabolism, RNA metabolism, energy production, cell wall metabolism, membrane transport and signal transduction. They also identified three novel proteins that play a vital role in conferring cold tolerance in bread wheat; the proteins are Hsp90, BBI and REP14. Comparatively low abundance of two fasciclin-like arabinogalactan proteins (FLAs), fasciclin-like arabinogalactan protein 11-like (M8BBJ1) and fasciclin-like arabinogalactan protein 7-like (A0A0A9EJ37), was reported in bread wheat. These FLAs belong to cell wall glycoprotein family arabinogalactan proteins (AGPs) and are implicated in cell wall biosynthesis, cell wall remodelling and signalling. Earlier FLAs were reported to express differentially in response to salt stress; this study provided first evidence of their degradation in response to salt. Shi et al. (2019) used an isobaric tag for relative and absolute quantification (iTRAQ)-based quantitative proteomic approach to compare the protein profile of self-grafted (SG) and pumpkin rootstock-grafted (RG) watermelon seedlings in response to cold. Root grafting improved cold tolerance in watermelons. A total of 752 proteins were accumulated in grafted watermelon seedling leaves post cold stress; root-grafted watermelon was more cold tolerant than self-grafted watermelon. RG watermelon had improved the scavenging capacity of ROS and arginine biosynthesis, besides producing more energy through photosynthesis, carbon metabolism and oxidative phosphorylation. Activity of the certain antioxidant enzymes such as superoxide dismutase, glutathione peroxidase, glutathione reductase, ascorbate

peroxidase and catalase also increased in response to cold (Chen and Li 2002). Shi et al. (2014) conducted comparative proteomic analysis to identify proteins involved in calcium-mediated cold response mechanisms in the Bermuda grass. Differentially expressing proteins mainly were involved in redox, tricarboxylic acid cycle, glycolysis, photosystem and amino acid metabolism. Study also confirmed that $CaCl_2$ plays a vital role in ROS detoxification, which might have contributed to enhanced freezing tolerance. Identification of a cold-responsive novel protein 1-FFT, the enzyme involved in inulin synthesis in the roots of biannual crop chicory that has to adapt to freezing temperatures, points towards possibilities of identification of novel proteins involved in freeze tolerance in different organs of different crops. In addition to expected proteins (e.g. related to metabolism, energy, protein synthesis or cell structure), proteins related to folding and stability, proteolysis and stress response were also observed (Degand et al. 2009). Comparative phosphoproteomics in response to cold in banana revealed three unique phosphoproteins MKK2, HY5 and STN7. The findings of the study suggest that phosphorylation happens at the early stage of cold stress as a primary response to cold stress. A conserved MKK2 network associated with the regulation of cellular functions is responsible for the high cold tolerance in the cold-tolerant banana variety. Transcription factor HY5 is a bZIP transcription factor involved in processes such as light signalling and photomorphogenesis and mediates plant responses to UV-B and different hormones, such as ABA, gibberellin, cytokinin and auxin. Serine/threonine-protein kinase STN7, chloroplastic (STN7) plays a role in assembly of the photosynthetic machinery and control of redox balance in the electron transfer chain (Gao et al. 2017).

4.4 LEA Proteins/Dehydrins

These are a group of heat stable, glycine-rich LEA proteins that impart membrane stabilization and protect proteins under cold-induced dehydrating conditions. Some important dehydrins that play an important role in cold acclimation include COR15am (protectant preventing protein aggregation) (Nakayama et al. 2008), ERD10 (early response to dehydration) and ERD14 (chaperones) (Kovacs et al. 2008). COR41an integral chloroplast inner envelope protein. The SFR2 protein outer envelope membrane (Fourrier et al. 2008).

4.5 HSP Proteins

Induced in response to cold, these proteins confer a strong cryoprotective effect by refolding denatured proteins, preventing their aggregation and imparting membrane protection. Expression of HSP90, HSP70, several small HSPs and chaperonins 60 and 20 in response to cold increases fold times in plants (Timperio et al. 2008).

4.6 PR Proteins

Some PR proteins such as PR-2 (β-1,3-glucanase); PR-3, PR-4, PR-5 (thaumatin-like proteins); PR-11 (chitinases) (apoplastic antifreeze proteins), PR-8, PR-10 (Bet v-1 homologues); and PR-14 (lipid transfer proteins) are also responsive to cold (Wisniewski et al. 1999; Griffith and Yaish 2004; Renaut et al. 2006; Janska et al. 2010).

5 Metabolomics in Response to Cold Stress in Plants

5.1 Introduction

Exposure to freezing situations results in genuine harm to the plant cell owing to ice formation and dysfunction of cell membranes. Many plant species increment freezing resilience amid presentation to non-freezing low temperature by a procedure known as cold acclimation (Ghatak et al. 2018). The molecular premise of this procedure has been broadly considered and the commitment of specific metabolites including compatible solutes and the transcriptional regulatory system is elucidated (Zuther et al. 2018). Metabolomics is a generally new methodology for improved comprehension of metabolic systems and the resulting biochemical organization of plants and other biological organisms. It relates to non-biased identification and quantification of all metabolites in an organic framework, and thus the selectivity and affectability of the explanatory strategy must be high (Du et al. 2018). Analytical tools inside metabolomics, including mass spectrometry (MS) and nuclear magnetic resonance (NMR) spectroscopy, can decide the effect of time, stress, wholesome status and ecological challenges on several metabolites all the while, bringing about enormous, complex informational indexes. The most commonly utilized strategies for plant metabolite examination are gas chromatography coupled to mass spectrometry (GC-MS) and fluid chromatography coupled to mass spectrometry (LC-MS), and further vital expository systems incorporate fluid chromatography (photodiode exhibit discovery) coupled to mass spectrometry (LC-PDA/MS), narrow electrophoresis coupled to mass spectrometry (CE-MS), Fourier change particle cyclotron reverberation mass spectroscopy (FT-ICR/MS) and NMR spectroscopy (Rinschen et al. 2018). Metabolic profiling can likewise be utilized for identification and quantification of a selected number of pre-characterized metabolites, for the most part identified with a particular metabolic pathway(s). Metabolic fingerprinting is additionally utilized for global analysis of samples to give sample order, in which quantification and metabolic identification are usually not utilized; however, such screening allows to segregate between samples of different biological status or origins (VanWallendael et al. 2019).

5.2 Metabolomics: Role in Cold Acclimation

The first metabolomic investigations of cold acclimation were performed by two groups (Le Signor et al. 2018). Cook et al. (2004) investigated metabolomic changes amid cold acclimation in two ecotypes of *Arabidopsis thaliana*, Wassilewskija-2 (Ws-2) and Cape Verde Islands-1 (Cvi-1), which are moderately freezing tolerant and sensitive individually. The metabolome of Ws-2 plants was widely modified because of low temperature. Seventy-five percent of metabolites observed were found to increment in cold-acclimated plants, including metabolites known to increment in *Arabidopsis* plants upon introduction to low temperature, for example, the amino corrosive proline and sugars glucose, fructose, inositol, galactinol, raffinose and sucrose. They likewise discovered novel changes, particularly the expansion of trehalose, ascorbate, putrescine, citrulline and some TCA cycle intermediates. There was extensive overlap in the metabolite changes that happened in the two ecotypes in light of low temperature. Nonetheless, quantitative contrasts were apparent (Nounjan et al. 2018). Kaplan et al. (2007) led metabolome examination of *Arabidopsis* over the time course following the shift to cold and heat conditions. Shockingly, most of heat shock reactions were shared with cold shock, including the expansion of pool sizes of amino acids from pyruvate and oxaloacetate, polyamine precursors and compatible solutes. The after-effects of this study were analysed together with following transcript profiling information by a similar group and it was found that the regulation of gamma-aminobutyric acid (GABA) shunt and proline accumulation under cold conditions are accomplished by transcriptional and post-transcriptional habits separately. Gray and Heath (2005) analysed the impacts of cold acclimation on the *Arabidopsis* metabolome utilizing a non-targeted approach on metabolic fingerprinting. It uncovered global reprogramming of metabolism just as differential responses between the leaves that moved to and those that developed wide open to cold (Bor and Ozdemir 2018). Hannah et al. (2006) exploited the genetic variation of *Arabidopsis* to elucidate the capacity of metabolomics in cold acclimation. In spite of the fact that there is no clear connection between global metabolite changes and contrasts in acclimation capacity or contrasts between the acclimated freezing resistance, the plausible significance of carbohydrate metabolism is shown by the identification of glucose, fructose and sucrose among metabolites emphatically relating to freezing resilience (Schwacke et al. 2019). Kaplan et al. (2007) analysed the impact of diurnal gene/metabolite guideline amid cold acclimation by methods for metabolomics and transcriptomics. Roughly 30% of every single analysed metabolite demonstrated circadian motions in their pool size and low temperature influenced the cyclic pattern of metabolite wealth. These outcomes showed that the collaborations seen among circadian and cold guideline are likely significant components of cold acclimation (Walia et al. 2018).

Metabolomics was likewise used to reveal the functions of some specific genes in cold acclimation. In the above investigation, Cook et al. (2004) additionally

explored plants overexpressing CBF3, which is one of the C-repeat/dehydration element binding factor (CBF) transcriptional activators instigated quickly under low-temperature conditions. The metabolite profiles of non-acclimated CBF3 overexpressing lines were like those of the cold-acclimated Ws-2 ecotype, suggesting a conspicuous role for the CBF cold response pathway in designing the low-temperature metabolome of *Arabidopsis* (Aghcheh and Braus 2018). Maruyama et al. (2009) investigated metabolic and transcript changes in *Arabidopsis* plants overexpressing CBF3/dehydration-responsive element-binding proteins (DREB1A and DREB2A) and observed a minor impact on metabolic profile of CBF3-overexpressing plants. The eskimo1 mutants of Arabidopsis were as such disregarded as freezing tolerant without past acclimation, yet the capacity of this gene was obscure (Tardieu et al. 2018). Lugan et al. (2009) attempted to illustrate the premise of the freezing resilience of esk1 by performing metabolomic analysis under different natural conditions, in particular cold, salinity and dehydration. At that point, the most explicit metabolic response to cold acclimation was not phenocopied by esk1 transformation. Be that as it may, esk1 amassed lower measure of Na in leaves than the wild type and its metabolic profile and osmotic potential were somewhat affected under dehydration stress (D'Amelia et al. 2018). These findings suggest that ESK1 could rather be associated with water homeostasis and all things considered featured the significance of cellular water status in stress resistance.

5.3 Metabolite Profiling in Response to Cold Stress

Global metabolite profiling investigation holds the guarantee to allow synchronous observation of precursors, intermediates and results of metabolic pathways. It is a discovery tool that can recognize and screen unidentified mass spectral tags (MSTs) just as distinguished metabolites that assume critical roles in metabolism and physiology and stress resistance (Zhou et al. 2019). In one investigation, metabolic profiling analysis was performed to decide metabolite temporal dynamics related to the acceptance of procured thermotolerance in light of heat shock and acquired freezing resistance because of cold shock. Low-Mt Polar metabolite investigations were performed utilizing gas chromatography–mass spectrometry. Eighty-one recognized metabolites and 416 unidentified mass spectral tags portrayed by retention time indices and specific mass fragments were checked. Cold shock affected metabolism more significantly than heat shock. The steady-state pool sizes of 143 and 311 metabolites or mass spectral tags were changed because of heat and cold shock, individually. Correlation of heat and cold shock response designs revealed that most of heat shock responses were shared with cold shock responses, a formerly obscure relationship. Arrange increments in the pool sizes of amino acids obtained from pyruvate and oxaloacetate, polyamine precursors and compatible solutes were observed amid both heat and cold shock. Also, a large number of the metabolites

that indicated increment in light of both heat and cold shock in this analysis were earlier not linked to temperature stress (Longo et al. 2018).

Alcohol dehydrogenase (ADH) plays an important role in the metabolism of alcohols and aldehydes, and it is a key catalyst in anaerobic fermentation. ADH1 responds to plant development and natural pressure. Nevertheless, the capacity of ADH1 in response to short-term freezing stress stays obscure (Dar et al. 2017). Utilizing real-time quantitative fluorescence polymerase chain reaction (PCR), the quantitative expression of ADH1 was investigated at low temperature (4 °C). The lethal temperature was determined by the electrolyte spillage tests for both ADH1 deletion mutants (adh1) and wild-type (WT) plants. To additionally examine the connection among ADH1 and cold resistance in plants, low-Mr polar metabolite analysis of *Arabidopsis* adh1 and WT were performed at cold temperatures utilizing gas chromatography–mass spectrometry. This analysis concentrated on freezing medications (cold acclimation group: −6 °C for 2 h with earlier 4 °C for 7 days; cold shock group: −6 °C for 2 h without cold acclimation) and recovery (23 °C for 24 h) for seedling development at an ideal temperature. The exploratory outcomes uncovered a critical increment in ADH1 expression amid low-temperature treatment (4 °C) and at a higher lethal temperature in adh1 contrasted with that in the WT. Retention time indices and explicit mass fragments were utilized to screen 263 factors and elucidate 78 distinguished metabolites. From these investigations, contrasts in the level of metabolite accumulation among adh1 and WT were distinguished, including soluble sugars (e.g. sucrose) and amino acids (e.g. asparagine). Likewise, the correlation-based analysis system featured a few metabolites (e.g. melibiose, fumaric acid, succinic acid, glycolic acid and xylose) that upgraded connectedness in adh1 arrange under cold shock. At the point when considered aggregately, the outcomes demonstrated that adh1 had a metabolic response to freezing stress and ADH1 assumed a critical role in the cold stress response of a plant (Wani et al. 2018).

CB-1 and K326 are closely related tobacco cultivars. However, their cold resistance limits are unique. K326 is significantly more cold tolerant than CB-1 (Masoodi et al. 2016). In an investigation, transcriptomes and metabolomes of CB-1 and K326 leaf samples treated with cold stress uncovered about 14,590 differentially expressed genes (DEGs) in CB-1 and 14,605 DEGs in K326. There were additionally 200 differentially expressed metabolites in CB-1 and 194 in K326. In addition, there were many overlapping genes (around half) that were cold responsive in both plant cultivars in spite of the fact that there were additionally numerous distinctions in the cold-responsive genes between the two cultivars. Significantly, for a large proportion of the covering cold-responsive genes, the degree of the adjustments in expression was regularly considerably more expressed in K326 than in CB-1, which may help elucidate the unrivaled cold resilience of K326 (Zhou et al. 2019). Comparable outcomes were found in the metabolome analysis, especially in the analysis of essential metabolites, including amino acids, organic acids and sugars. A substantial number of specific responsive genes and metabolites feature in the complex regulatory mechanisms related with cold stress in tobacco.

5.4 Glycine Betaine Against Cold Stress in Plants

Both the exogenous application of glycine betaine (GB) and the genetically engineered biosynthesis of GB build the resistance of plants to cold stress and they can upgrade ensuing development and yield (Showkat et al. 2017). Reactive oxygen species (ROS) are delivered persistently as a result of different metabolic pathways even when plants are subjected under non-stress conditions. These ROS are scavenged by an assortment of antioxidant defense frameworks that keep ROS from achieving lethal levels (Rastogi et al. 2019). All types of abiotic stress, including salinity, cold, freezing and drought, cause an oxidative burst in plant cells. The utilization of hydroxyl radicals (OH∗) in *Arabidopsis* roots brought about a massive, dose-dependent efflux of K^+ particles from epidermal cells into the elongation zone. Notwithstanding, the nearness of GB at 5 mM in the incubation medium essentially decreased this efflux of K^+ particles. Besides, in tomato plants, exogenously applied GB altogether diminished the chilling-induced generation of H_2O_2. Since GB does not scavenge ROS specifically, GB must relieve the damaging impacts of oxidative stress in different ways, for instance by enacting or settling ROS searching proteins and additionally stifling the creation of ROS by an obscure system (Tada et al. 2019). Two important factors that impact the resilience of plants to cold stress are concentration and localization of GB in the cell. In numerous investigations of the engineered accumulation of GB in plants, GB-biosynthetic enzymes have been targeted to chloroplasts, while in others the catalysts have been targeted to cytosol or mitochondria or to both the cytosol and the chloroplasts at the same time. Three kinds of transgenic tomato plants were produced by utilizing a *codA* gene that was targeted to chloroplasts (Chl-codA plants), cytosol (Cyt-codA plants), or chloroplasts and cytosol at the same time (ChlCytcodA plants). Cyt-codA and ChlCyt-codA plants accumulated up to 5.0- and 6.6-fold, individually, larger amounts of GB in their leaves than did Chl-codA plants (0.3 mmol g^1 FW). Each one of the three kinds of transgenic plants showed more noteworthy cold resistance than wild-type plants (Dumont and Rivoal 2019). In Chl-codA plants, the stress resistance of photosystem II (PSII) and the recurrence of seed germination were like those in the other two kinds of transgenic plants. In any case, the stress resistance amid the development of seedlings of Chl-codA plants was higher than that of transgenic plants, despite the fact that the dimension of GB was much reduced in the former. Subsequently, the amassing of GB in chloroplasts is more viable than the collection of GB in the cytosol for the protection of plants against cold stress (Schwachtje et al. 2019).

5.5 Differential Metabolic Response to Low Temperature

Zoysia grass local to high latitude may have advanced higher cold resistance than the ones local to low latitude. A study was conducted to explore the cold stress response in *Zoysia* grass local to different latitudes at phenotypic, physiological and

metabolic dimensions (Kudo et al. 2019). Two zoysia grass (*Z. japonica*) genotypes, Latitude-40 (higher scope) and Latitude-22 (lower scope), were exposed to four temperature medications (ideal, 30/25 °C, day/night; suboptimum, 18/12 °C; chilling, 8/2 °C; freezing, 2/−4 °C) dynamically in growth chambers. Low temperature (chilling and freezing) expanded leaf electrolyte spillage (EL) and reduced plant development, turf quality, chlorophyll (Chl) content, photochemical productivity (F_v/F_m) and photosynthesis (P_n, net photosynthetic rate; g_s, stomatal conductance; intercellular CO_2; Tr, transpiration rate) in two genotypes, with increasingly quick changes in Latitude-22. Leaf carbohydrate content (glucose, fructose, sucrose, trehalose, fructan, starch) expanded with the decrease in temperature to an extraordinary reach in Latitude-40. Leaf abscisic acid (ABA), salicylic acid (SA) and jasmonic acid (JA) content expanded, while indole-3-acetic acid (IAA), gibberellic acid (GA_3) and *trans*-zeatin riboside (t-ZR) content diminished with the decrease of temperature, with higher content in Latitude-40 than in Latitude-22. *Zoysia* grass local to higher latitude showing higher freezing resistance might be credited to the higher carbohydrates and stress protectants that balance out cellular membranes (Takahashi et al. 2019).

The impact of short-term cold stress on the metabolism of non-structural carbohydrates in polar grasses has been researched. Flowering plants of the family Poaceae growing in the Arctic and Antarctic were researched (Zhu et al. 2019). Their response to cold stress were analysed under research centre conditions. Samples were collected after 24 and 48 h of cold treatment. Quantitative and qualitative changes of sugars were found among various species; however, they could vary within a genus of the family Poaceae. The estimations of the examined parameters in *Poa annua* contrasted extensively depending to the biogeographic origin of plants. At the start of the trial, plants of the Antarctic were acclimatized in nursery portrayed by essentially higher content of sugars, including storage reserves, sucrose and starch, however lower all out protein content. After 24 h of introduction to cold stress, small changes in the analysed parameters were noted in Antarctic plants than in locally grown samples. Absolute sugar content and sucrose, starch and glucose levels were about steady in *P. annua*; however, they changed fundamentally. These progressions are responsible for the high adaptability of *P. annua* to survive and develop in exceedingly unsupportive environments and colonize new regions (Fàbregas and Fernie 2019).

6 Conclusion

Cold tolerance is a complex trait resulting from multiplicative molecular interaction at genome, transcriptome, proteome and metabolomics levels in an organism. Tolerance to cold is developmental stage specific and can manifest as a mechanism of cell stability in response to stimuli. Genomic loci governing cold stress tolerance in plants share a certain degree of homology across species, yet the relative expression and localization of protein products may vary with systems across, which may

change altogether, the context defining 'cold response'. Eventually, in order to understand cold stress tolerance in plant species, the role of omics technologies is of immense value and of direct solicitation in drawing out new pathways underlying such a mechanism.

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