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REVIEW ARTICLE

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# Can omics deliver temperature resilient ready-to-grow crops?

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**ABSTRACT**

Plants are extensively well-thought-out as the main source for nourishing natural life on earth. In the natural environment, plants have to face several stresses, mainly heat stress (HS), chilling stress (CS) and freezing stress (FS) due to adverse climate fluctuations. These stresses are considered as a major threat for sustainable agriculture by hindering plant growth and development, causing damage, ultimately leading to yield losses worldwide and counteracting to achieve the goal of "zero hunger" proposed by the Food and Agricultural Organization (FAO) of the United Nations. Notably, this is primarily because of the numerous inequities happening at the cellular, molecular and/or physiological levels, especially during plant developmental stages under temperature stress. Plants counter to temperature stress via a complex phenomenon including variations at different developmental stages that comprise modifications in physiological and biochemical processes, gene expression and differences in the levels of metabolites and proteins. During the last decade, omics approaches have revolutionized how plant biologists explore stress-responsive mechanisms and pathways, driven by current scientific developments. However, investigations are still required to explore numerous features of temperature stress responses in plants to create a complete idea in the arena of stress signaling. Therefore, this review highlights the recent advances in the utilization of omics approaches to understand stress adaptation and tolerance mechanisms. Additionally, how to overcome persisting knowledge gaps. Shortly, the combination of integrated omics, genome editing, and speed breeding can revolutionize modern agricultural production to feed millions worldwide in order to accomplish the goal of "zero hunger."

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## Introduction

Plants grow in atmospheres that execute a range of environmental stresses (biotic and abiotic) and variation in any of these stresses can hamper a series of morphological, physiological, and molecular changes at multiple stages; eventually, plant growth, and productivity get affected by these stresses [1–3]. Plants need to breed and grow further grow to sustain their existence in severe environmental conditions. Hence, there are several aids for maintaining an equilibrium among plant growth, development, and stress tolerance [3,4]. Some plants change their morphology to cope up with these changes while some of them change their physiology or show changes in gene expression, which alters their growing activities to withstand and tolerate such conditions [1,4,5]. Hence, plants have advanced mechanisms to play for the undesirable stressful environment by changing their developmental and physiological mechanism. Whereas, environmental stresses can affect and disrupt their underlying functioning mechanisms including amendments in gene expression, biosynthesis of distinct proteins and secondary metabolites, modifications in hormonal signaling, and the activities of antioxidant enzymes, etc. [4,6,7].

Over the past few years, due to drastic changes in climate, temperature fluctuations became a major limiting factor affecting plant growth, yield, and distribution, worldwide [1,4,8]. In the field environment, crops experience a variety of temperatures, that is, high

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temperature or heat stress (HT/HS; >25 °C), low temperature or chilling stress (LT/CS; 0–15 °C), and freezing temperature/stress (FT/FS) (<0 °C) throughout their growing periods and act as a major threat to agricultural food production [2,9]. Plant response to these temperature extremes has been explained in the next section. Nevertheless, knowledge about how plants adapt, respond and tolerate temperature fluctuations, is vital for the enhancement of plant productivity under changing climatic conditions. To reduce the adverse impact of temperature stress, reviewing how the plants have advanced stress tolerance, surviving mechanisms will deliver new visions and lead to innovative approaches in breeding for climate-resilient crops.

As a response to different external as well as internal stimuli, the growth and development of plants must be regulated on their own. Therefore, during the past few decades, among the modern biotechnological tools, omics approaches such as genomics, transcriptomics, proteomics, and metabolomics have emerged as the most promising and state-of-the-art approaches to provide a way forward for crop improvement and to secure world food security. These approaches have been widely used to enhance HS, CS, and FS tolerance in several plant species [10–13].

The accessibility of data developed from numerous omics tools offers the opportunity to respond to multifaceted queries in-plant investigations. Nevertheless, disadvantages can rise due to spaces in the data produced, and corresponding tools are vital to obtaining more wide-ranging datasets connecting to precise biological development, that is, responses to environmental stresses in plant systems. The introduction of high throughput next-generation sequencing (htNGS) technologies has transformed the transcriptomics arena via RNA-seq [14]. It has overwhelmed numerous restrictions postured by usually employed microarray techniques and not demanding previous data of the genome or sequence of interest, which permits genome-wide impartial discovery of known and new targeted data sets [14]. Since the start of the one-KP project, aiming to sequence 1000 plant transcriptomes, RNA-seq has been broadly used in transcriptome-base studies of different plant species. Additionally, a combination of RNA-seq with other omics tools has permitted the in-depth investigation of numerous aspects of the plant omics (transcriptome), including miRNA-seq, Chip-seq, Ribo-seq, and GRO-seq [15–18]. Among them, ChIP-seq is, to date, the finest method to explore the interaction between DNA and specific proteins due to its enhanced noise proportion and genomic sequence knowledge [19]. The considerable data produced by numerous high-throughput methods are frequently inclined to be piercing and comprise many undesirable alterations and technical objects. It is a test for the precise examination of extraordinary datasets to recognize accurate signals, syndicate adjustable data types, and comprehend their biological associations. While scheming integrated omics (RNA-seq, proteomics, and metabolomics) experimentations, RNA-seq can be carried out before ChIP-seq and Ribo-seq. Thus, the utmost enriched transcription factors in differentially expressed genes exposed by RNA-seq are measured as targets for ChIP-seq analysis [20]. Therefore, the integration of multi-omics data within the context of systems biology can provide deeper knowledge for future directions (molecular biology, genome editing, etc.). This review summarizes the recent advances in the omics approaches in order to understand the adaptation and tolerance mechanisms enhancing HS, CS, and FS tolerance in different crop plants.

## Plant responses to temperature stress: development and yield

Temperatures insistently above or below ideal for plant development may induce HS, CS, and FS, ultimately decreasing overall yield. At specific thresholds, plant growth and development will be interrupted and can significantly impact crop productivity. Therefore, extreme temperature fluctuations can be recorded in several ways. For instance, the supreme temperature level (intensity), how habitually the fluctuations occur (frequency), and how elongated they work (duration). Variations in morphological features are the outcome of changes in the physiological traits of plants under stressful environments. These variations are distinguished in several fundamental physiological aspects.

Numerous developing tissues have a different temperature threshold to tolerate across different crop plant species [21]. For example, in beans (*Phaseolus vulgaris* L.), pollen development per flower was decreased at >31/21 °C, pollen feasibility was theatrically decreased at >34/24 °C, and seed size was decreased >31/21 °C [22]. The HS (38/30 °C) decreased the pollen development (34%), germination (56%), and tube enlargement (33%) in soybean (*Glycine max* L.) [23]. Maize (*Zea mays* L.) has a threshold temperature (33–38 °C) for photosynthesis and pollen viability [24,25]. Rapeseed (*Brassica napus* L.) has a threshold temperature ∼30 °C for flowering [26]. In chickpeas (*Cicer arietinum* L.), the threshold temperature is 15–30 °C for growth and 25 °C for reproductive growth [27]. Olive (*Olea europaea* L.) exhibits a spring threshold

low and high temperature between 7–10 °C and 11–14 °C, respectively, for flowering and reproductive structure development [28].

Under HS, there is a substantial decrease in photosynthesis due to reduced chlorophyll (Chl) biosynthesis in plastids [29]. Further, HS, CS, and FS adversely affect DNA, proteins, and enzyme activities in plants [29]. This extreme temperature can also cause secondary water stress by damaging cellular structures and metabolic pathways [29]. Reproductive tissues are more sensitive to HS; for example, stress during the flowering stage lowers the grain yield [30]. Likewise, CS caused a wide range of changes at the physiological level as well as at the molecular level. Under CS, the plant enhances reactive oxygen species (ROS) production that increases lipid peroxidation resulting in higher membrane fluidity [9,31]. Furthermore, CS induces variations in proteins elaborated during carbohydrate metabolism, photosynthesis, stress-associated proteins between other progressions, protein folding, and dilapidation, as well as ROS scavenging and the production of companionable solutes [31,32]. Similarly, FS leads towards cellular desiccation, extracellular frost foundation, and inequities in the plasma membrane, leading to the development of overturned membrane structures, which disturbs the osmotic homeostasis [9,33]. It harms plants either directly or indirectly. Directly, it affects the plant's metabolic activities and indirectly by cold-induced osmotic stress (include freezing-induced cellular dehydration), and ROS generation [9]. If the FS period becomes prolonged, all water freezes and crystals are formed rupturing membrane, eventually leading to plant death [34,35]. To avoid its harmful effects, plants establish some pathways to prevent ice crystal formation; however, sugar accumulation can play an essential role in FS tolerance. Many plants can increase their degree of CS and FS tolerance by a phenomenon called cold acclimation [9,35]. This phenomenon can be defined as the exposure of plants to a low non-FS before the onset of freezing, enhancing cold tolerance [35].

Nonetheless, HS, CS, and FS exert adverse effects on crop physiology (reduced photosynthesis and enhanced respiration rate), plant growth, and production [36]. Notably, temperature replies towards photosynthesis vary between different temperature regimes within the same species. Moreover, growth at different temperature regimes also affects the maximum photosynthesis without changing the temperature response curve in C3, C4, and CAM plants [37]. Likewise, the respiration rate varies with changing temperature and even a slight increase in ambient temperature increases the $CO_2$ flux from leaves to the atmosphere [38,39]. Respiration is a more sensitive process under HS as compared to photosynthetic reactions [40]. Unlike photosynthetic adjustment, respiration adaption may occur rapidly [40], by changing existing enzymes' activities and altering the composition of mitochondrial proteins [41]. It has also been reported that the respiration rate poses as a tolerance mechanism to adapt FS [42]. Another adverse effect of temperature fluctuations is the reduction in water use efficiency [43,44]. Similarly, HS, CS, and FS cause harmful effects on the plant root–shoot system, which offers strength, water, and nutrient uptake, and transportation to other above-ground parts [45,46], resulting in interrupted pollination, flowering, root progress, and root development phases [47,48]. Moreover, temperature variation also interrupts the cell membrane integrity, under post-harvest environments. The loss of cell membrane integrity, directly linked with the ROS production, upsurges the membrane penetrability, damages cell structure, and disturbs the plasma membrane variability in plants [49,50].

Likewise, plant growth and production, seed germination, shoot length, and grain yield are greatly influenced by HS, CS, and FS. For example, CS disturbs respiratory metabolism and photosynthetic efficiency, which eventually hampers plant growth, while FS forms intracellular ice crystals resulting in plant death or mechanical injury [51]. CS at the seedling stage causes a severe negative impact on plant growth, physiology, and morphology by causing cellular damage and diminishing trees' survival chances [52]. There is a linear curve in germination percentage; however, seed germination may occur between the maximal and minimum temperature, while the highest germination rate corresponds to the optimal temperature [53,54]. In this consistency, the tomato (*Solanum lycopersicum* L.) seed germination rate was evaluated under different temperature ranges [55]. A 95.3, 93.3, and 10% seed germination rates were observed at 40, 10, and 25 °C, respectively.

Extreme HS harms root length, plant height, grain quality, and biomass production amongst most field crops. For instance, the rice (*Oryza sativa* L.) plants are grown under HS (39 °C), showing a 16.67% reduction in the shoot length [56]. Similarly, the shoot lengths of maize (*Zea mays* L.) plants were condensed under CS (15/12 °C) [57], and HS (40 °C) [58]. Under HS (45 °C), 80 to 90% of seedling mortality was observed in wheat (*Triticum aestivum* L.) [59]. HS decreased the panicle length and relative water content of rice leaves [60]. A reduction in grain yield by 58% and 1000-grain weight by 83% in wheat grown under HS (30/25 °C) was observed [61]. Conversely, 33.9% yield reduction was

reported under HS (23 °C) in wheat [62]. It has been well documented that CS reduces yield percentage in different plants. For example, 40% wheat yield reduction was observed at 10/5 4 °C [63], and 21.87% in maize at 13/8 °C [64]. Under FS, in *Bombax ceiba* plants, a grain yield reduction of 3.3 and 8.4% at −14 and −17 °C, respectively, were reported [65].

## The interplay of omics approaches to reveal novel genes, proteins, and metabolites

Plant response towards HS, CS, and FS depends on the regulation of genes (up-regulation or down-regulation). In this context, integrated omics research has been widely used to understand the plant's biological networking and molecular mechanism against HS, CS, and FS (Tables 1–4). Despite tremendous progress in genomics, there is a need to study other omics levels, including transcriptomics, proteomics, and metabolic profiling for a comprehensive understanding at the molecular level (Figure 1). All these approaches have aimed to identify key genes, their regulation, interactions, or changes developed at various metabolic pathways when exposed to HS, CS, and FS in plants. For instance, the integrated transcriptome and metabolome analysis of rapeseed (*Brassica napus*) revealed numerous specific genes and metabolites in response to CS (4 °C) [83]. The joint data show that: abscisic acid (ABA), lipid, secondary metabolism, signal transduction, and several transcription factors (bHLH, ERF, MYB, and WRKY) were involved in the composite regulation of both spring and winter rapeseed genotypes. Accumulated metabolites belonged to organic acids, amino acids, and sugars. Suggesting that differences in gene expression and metabolite accumulation levels under CS played a substantial role in CS tolerance with rapeseed [83]. Under FS (−2, −4, and −6 °C), the integrated metabolome and proteome analysis were carried out in three gum trees (*Eucalyptus*) species [105]. Biochemical and molecular analysis revealed that *Eucalyptus benthamii* Maiden Cambage (*Eb*) displayed higher tolerance compared to *Eucalyptus grandis* Hill ex Maiden (*Eg*), and *Eucalyptus dunnii* Maiden (*Ed*). This higher tolerance was due to the higher accumulation of phenolics, soluble sugars, anthocyanins, osmoprotectants, and antioxidants. Metabolic and proteome profiling supports the biochemical and molecular analysis results by identifying: photosynthesis, osmoprotectants, antioxidant-related compounds, and proteins under FS. Further, the integrated analysis also revealed differences in tolerance mechanisms among the three species [105]. Similarly, many integrated omics-based experiments have been performed under temperature stress in different plant species, such as transcriptome and metabolome of pepper (*Capsicum annuum* L.) under HS [106], proteomics and metabolome profiling of avocados (*Persea americana*) under HS [107], transcriptome and metabolome analysis of tomato under CS [108]. All these studies have revealed complex regulatory mechanisms for temperature stress tolerance. Scientific research and present knowledge derived using omics approaches, targets signaling pathways, key regulators, and integrated mechanisms to enhance HS, CS, and FS tolerance for crop improvement. Some of the vital examples of individual omics tools have been explained in the subsequent sections with different plant species.

### *Genomics: helps to reveal the stress-responsive mechanisms*

Genomics covers the genome of an organism providing adequate information about the chemical, physiological, biological processes and structure of genes, gene sequences, and their functional annotation [109,110]. The evolutionary history of genomics started in the 1970s (first generation) and continued as next-generation sequencing (NGS) and currently made swift developments in genome sequencing technology by third-generation sequencing [111]. Functional genomics aids in identifying genes and their functions involved in stress stimuli [112]. The knowledge of gene expression and regulation with complex stress-responsive traits at a genome-wide level and contributes to generating climate-resilient crops [109]. Genomics and online genome data provide a platform for further research on plants through approaches like transcriptomics, proteomics, metabolomics together with genome engineering (CRISPR/Cas) system [113,114].

### *The contribution of QTL mapping*

A set of mapping approaches including quantitative trait loci (QTL)-seq analysis, conventional QTL mapping, and RNA-seq has been introduced to replace the fine-mapping process as it can identify candidate genes within major QTLs in no time. For example, five major QTLs (*qHll-1-1*, *qHll-1-2*, *qHll-1-3*, *qHll-2-1*, and *qCC-1-5)* have been detected on chromosome 1 under HS in the tomato genome [115]. These QTLs were detected by phenotype, heat injury, and measuring physiology for three major indexes Chl content, maximum photochemical quantum efficiency ($F_v/F_m$) of photosystem II (PSII), and relative electrical conductivity. Four genes (*SlCathB2, SlGST, SlUBC5,* and *SlARG1*) standing under

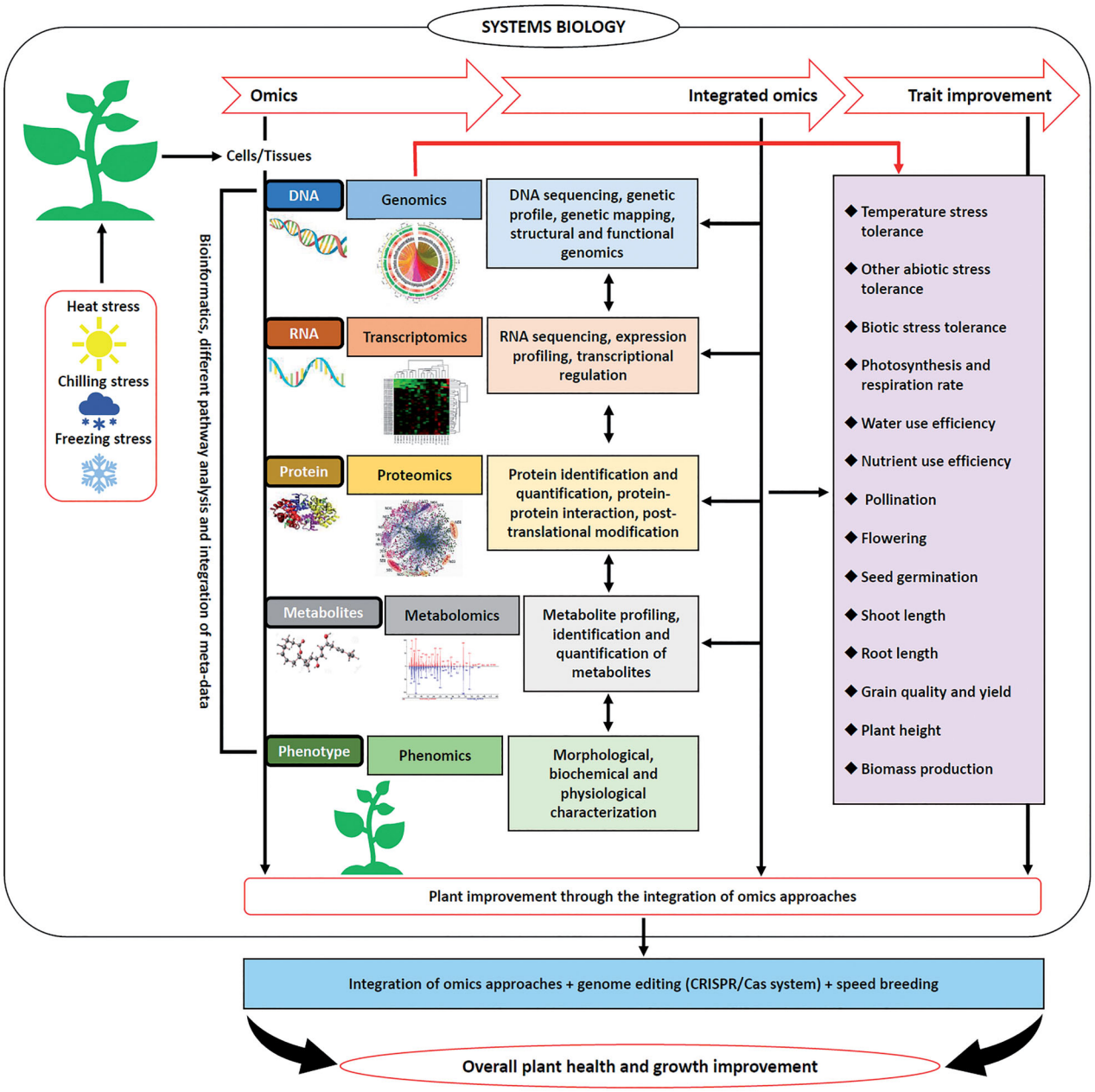


**Figure 1.** Overview of omics approaches in the context of systems biology. The central dogma of molecular biology covers the ongoing functionalization of the genotype to the phenotype. The omics approaches (mainly genomics alone or the integration analysis of combine multi-omics tools) improved several plant traits through the biological system. Integrated omics analysis can be performed by combining two, three or multi omics approaches in one project with the same stress and tissue to obtain a comprehensive omics data set. Conversely, the utilization of omics approaches, genome editing using CRISPR/Cas system, and the speed breeding on a large scale can improve the overall plant health and feed the billions worldwide to achieve a goal of "zero hunger."

HS identified between major QTLs and can be used further to develop an HS tolerant variety of tomato [115]. Another approach named "QTL-seq" pointed out genomic regions linked with spikelet fertility in rice and has identified three QTLs (*qSF1, qSF2,* and *qSF3*) on chromosome 1, 2, and 3, respectively, under HS [116]. This region proposed three candidate genes influencing another dehiscence and pollen development when exposed to HS and can be helpful for further study on molecular mechanisms for spikelet fertility under HS [116]. To recognize the genetics of leaf photosynthesis under HS, RILs of rice cv. Improved White Ponni (IWP) introgressed with two QTLs (*qHTSF 1.1* and *qHTSF 4.1*) directing spikelet fertility were grown under HS [117]. Notably, the introgression lines (ILs) showed: improved photosynthetic rates, PSII efficiency, stomatal closure, and reduced transpiration rate.

Based on physiological responses, introgressed QTLs can be used for the development of HS-tolerant rice cultivars [117]. Similarly, fine mapping of the introgressed QTL (*qHTB1-1QTL*) at the booting phase confers the HS tolerance using ILs in rice [118].

Recent studies in maize using the MAGIC population as an efficient tool identified many QTLs under CS. These QTLs are mostly located in specific regions having an interaction with CS tolerance related traits, that is, the maximum quantum efficiency of PS II ($F_v/F_m$) and a most common Chl content open gateways for genomic selection (GS) to boost the CS tolerance in maize [119]. Another study in rice reported the main effect QTLs under CS using 230 ILs in $BC_1F_7$. Data revealed a total of 27 QTLs localized on 12 chromosomes, explaining 10% phenotypic variance [120]. Furthermore, mapping five major QTLs on chromosomes 1, 5, and 7 identified genes associated with low-temperature germination index traits explaining 16 to 23.3% phenotypic variance. Identification of 16 candidate genes in major QTLs could help find functional markers for multiple traits to produce CS tolerant rice cultivars [120].

For CS, two important QTLs, *qCTB-8* and *qCTB-10* on chromosome 8 and 10, respectively, have been identified at the booting stage in rice. Three QTLs (*qHD-4, 7,* and *11*) identified for heading date in a Japanese tolerant variety along with the previously identified QTLs could be used further in cold-sensitive varieties to enhance their tolerance against CS via marker-assisted selection [121]. A backcross inbred line population for *O. sativa* × *Oryza rufipogon* elucidated two loci for CS tolerance during the seedling phase, namely, *qSCT8* and *qSCT4.3* on chromosome 8 and 4, respectively [122]. Another study on RILs of rice at the seedling stage identified other QTLs for CS (*qCG12-1, qGl12-1, qGV9-1, qMLIT12-1, qPV6-1, qMDG12-1, qLDWcold10-1,* and *qLFWcold10-1*), via multiple interval mapping methods [123]. Using these QTLs, researchers identified many potential genes in plants to survive under temperature stress according to the environment.

### The contribution of GWAS

A genome-wide association study (GWAS) analysis was performed on a collection of 207 cultivars for 19 phenotypic traits in wheat, identified 125 marker-trait associations (MTAs) under HS during the grain filling stage [124]. These MTAs prevailing in 16 chromosomes at a total of 63 single nucleotide polymorphism (SNP) loci revealed phenotypic variation ($R^2$) of 3.0–21.4%. Four major QTLs for HS tolerance identified impact starch accumulation in grain, grain filling, and grain flour related traits, that is, QTL on 2B significantly affects grain weight and flour pasting properties [124]. Besides, six HS responsive traits were considered to conduct a GWAS analysis in 135 accessions of pea (*Pisum sativum* L.) plants under three different environments [(genotype (G), environment (E), and G × E interaction)] [125]. Notably, 32 MTAs were determined by using a total of 16,877 SNPs. These MTAs were associated with stress-resistant traits, that is, canopy temperature, Chl concentration, and photochemical reflectance index. Moreover, 48 candidate genes were identified within this region, having the potential for developing HS-resistant pea cultivars [125]. Recently, 272 chickpeas (*Cicer arietinum* L.) genotypes were used to perform GWAS analysis to identify markers associated with HS and key agronomic traits. The study identified a total of 262 MTAs with 203 unique SNPs. Furthermore, SNP annotation identified 48 SNPs present in 47 unique genes with known function. These findings can further be used for the development of heat-tolerant chickpea cultivars [126].

Recently, a GWAS experiment was conducted using 257 rice accessions worldwide to examine genetic behavior during germination under CS [127]. Interestingly, 51 QTLs were identified, and of these 17 QTLs were identified at different chilling points. A subset of QTLs was identified at the loci of identified genes. In contrast, the japonica and indica subset has identified 10 and 1 potentially novel QTLs, respectively, providing a molecular basis for crop improvement under CS [127]. Also, CS tolerance at 10 and 4 °C was measured with GWAS analysis in rice. The QTL (*qLTSS4-1)* region identified a gene encoding the UDP-glycosyltransferase enzyme UGT90A1, which exhibited CS tolerance by maintaining membrane integrity and reduced ROS levels. It also affected phytohormonal activity but resumes the growth and development of plants under stress recovery [128]. Likewise, CS (4–16 °C) was set to observe tolerance among 354 rice cultivars using GWAS mapping approaches. This study screened 178 unique QTLs, while 48 were identified by multiple traits using Rice Diversity Panel (RDP1). Candidate genes identified were involved in pathways deliberating CS tolerance enriched by transmembrane transport, signal transduction, and stress response [129].

In wheat, GWAS was conducted using 543 accessions against CS and FS (4 to −5 °C) tolerance. A total of 76 SNPs scattered over 18 chromosomes and 361 candidate genes related to CS and FS were screened, out of which 85 were differentially expressed. These candidate genes would contribute to the breeding of FS tolerance in wheat [130]. Frost tolerance was observed in the faba bean (*Vicia faba* L.) by a GWAS study using 101 inbred lines (biparental population) and 189 genotypes

(single seed descent) at FS (−16, −18, and −19 °C) [131]. A total of 59 SNP markers were identified against both genetic backgrounds, out of which five SNPs were significantly associated with frost tolerance. The marker, VF_Mt3g086600 associated with winter hardiness was reported, and such markers would be useful to improve frost tolerance, leading to high crop yields [131].

### *The contribution of the CRISPR/Cas system: the most promising future*

CRISPR/Cas9 is a novel and efficient genome editing tool worldwide due to its specificity, efficiency, ease of use, less time is taken, and a wide range of applications. The CRISPR/Cas9 technology revolutionized applied research in plant breeding and was successfully adapted to improve major crops by editing targeted genes. The CRISPR/Cas9 technology is making knock-in/out, deletion and insertion mutations, targeted regulatory genes influenced by temperature stress, hence, improved different crops by enhancing their scavenging capability [132]. This system has been widely used to enhance temperature tolerance in different plant species (Table 1).

A CRISPR-mediated study identified a gene, *OsNTL3,* involved in HS tolerance in rice. The gene *OsNTL3* encodes an NAC TF and mediates a regulatory circuit among plasma membrane, endoplasmic reticulum, and nucleus, soon after binding with *OsbZIP74,* as *OsNTL3* regulates the expression of *OsbZIP74* under HS, while *OsbZIP74* helps *OsNTL3* in up-regulation by HS [67]. In rice, CRISPR-Cas9 induced mutant studies have been conducted to identify the function of *ONAC127* and *ONAC129* during caryopsis development under HS at the rice filling stage. Incomplete filling and shrunken caryopsis were observed in CRISPR-induced mutants. In short, *ONAC127* and *ONAC129,* along with multiple pathways (sugar transportation), regulate caryopsis filling, including monosaccharide transporter *OsMST6*, sugar transporter *OsSWEET4*, calmodulin-like protein *OsMSR2*, AP2/ERF, *OsEATB*, cell wall construction, and nutrient transport under HS [66]. In tomato, CRISPR-mediated *Simapk3* mutant unveiled improved tolerance to HS, less cell damage and wilting, lower ROS contents, increasing antioxidant activity, and the higher expression of genes encoding heat shock factors/proteins (HSFs and HSPs) that mainly regulates HS [133]. In a different study, brassinazole resistant (BZR1) like protein available in tomato is involved in HS tolerance. CRISPR mutation induces *RESPIRATORY BURST OXIDASE HOMOLOG1* (*RBOH1*) and enhances HS tolerance. Production of hydrogen peroxide ($H_2O_2$) as ROS signaling through RBOH-1 is enhanced by *FER2* and *FER3* in CRISPR mutant [70].

Expression and regulation of a gene *OsAnn5* were observed by knocking out the gene via CRISPR/Cas9 in rice at the seedling stage. The gene happened to positively regulate during CS tolerance as mutants resulted in chilling treatment sensitivity when the gene was knocked out [71]. Multiplex genome editing technique has been recently used in rice by excising the following genes: *OsPIN5b*, *GS3,* and *OsMYB30*, simultaneously. Developed mutants exhibited a higher yield and improved chilling tolerant traits. This study evaluated that gene-editing techniques (CRISPR/Cas9 system) execute generating new rice varieties with a higher yield, improved agronomic traits, and enhanced stress resistance [72]. In tomato, a vital gene (*SlCBF1*) for cold tolerance was investigated by CRISPR-Cas9-mediated genome editing. Knock-out of this protein sequence showed higher chilling injury in the *slcbf1* mutant with higher $H_2O_2$ contents, activities of antioxidant enzymes, electrolyte leakage, and malondialdehyde (MDA) levels. Mutants have a lower protein, proline content, and decreased hormone contents which were further verified by downregulation of the CBF-related genes [73]. Scientists are trying to develop CS-tolerant maize using the CRISPR system. They have knocked out six key genes involved in CS tolerance in *Arabidopsis*, which are homologs of potential candidate genes in maize. Successful mutants of *Arabidopsis* have been developed that can easily distinguish phenotypic traits. Further investigation on these proposed DNA fragments of the maize controlling CS tolerance is required [69].

In *Arabidopsis*, researchers identified 10 genes stimulated by *CBF2,* and thus regulates starch metabolism, sugar biosynthesis, cell membrane structure, and some transcriptional level. All these genes and LOF-CBF2 (lose-of-function) lines exhibited major FS tolerance between two different ecotypes [75]. Another study investigated FS tolerance in *Arabidopsis* by knocking out MYB15-a CBF transcriptional repressor that acts as a negative regulator of cold signaling. Degradation of MYB15 promoted plant FS tolerance due to enhancing the expression of PUB25 and PUB26 [134]. PHYTOCHROME-INTERACTING FACTOR 3 *(PIF3)* is an undesirable gene in regulating the CBF pathway during freezing temperatures in *Arabidopsis,* has the potential to generate FS tolerant plants using CRISPR technology [135].

From the above studies, it has been demonstrated that genomics tools play a vital role in identifying key stress-related mechanisms and their functionality under temperature stress. The identified mechanisms can be engineered to enhance stress tolerance in crops.

**Table 1.** Applications of the CRISPR/Cas9 system under temperature stress in some crops.

| Species | Stress condition | Targeted gene | Modification | Key role/function | References |
|---|---|---|---|---|---|
| *Oryza sativa* | HS, 35 °C | *ONAC127, ONAC129* | Gene knock-out (GKO) | Key role in starch accumulation during rice caryopsis filling. Transcriptional regulatory network via TFs to modulate caryopsis under HS. | [66] |
| *Oryza sativa* | HS, 29 °C, and 45 °C | *OsNTL3* | Loss-of-function mutation | Encodes a NAC transcriptional factor. Loss-of-function mutation of gene increase heat sensitivity. Regulatory circuit mediates between OsbZIP74 and OsNTL3 under HS. | [67] |
| *Arabidopsis thaliana* | HS, 37 °C | *HSP90, YODA* | Insertion | HSP90 collaborating with YODA cascade, regulate stomata formation. Affects cellular polarization and regulate phosphorylation by activation of MPK and SPH | [68] |
| *Arabidopsis thaliana* | HS, 23 °C | *ERD14, ARK2, PLL5, DWF5, SDP, AT1PS2,* and *GA2OX8* | GKO | Knock out *Arabidopsis* genes homologs of maize responsible for HS tolerance | [69] |
| *Solanum lycopersicum* | HS, 42 °C | *BZR1* | Gene silencing | *BZR1* induces *RBOH1* and is thermosensitive by regulating *(FER).* Reduced growth and production of $H_2O_2$ were found in the CRISPR-*bzr1* mutant. | [70] |
| *Oryza sativa* | CS, 4–6 °C | *OsAnn5* | GKO | *OsAnn5* acts as a positive regulator for CS tolerance. | [71] |
| *Oryza sativa* | CS, 4 °C | *OsPIN5b*, *GS3*, and *OsMYB30* | Multiplex genome editing | Mutants with higher yield (enlarged grain size and increased panicle length) and CS tolerance. Different agronomic traits have improved by editing three genes simultaneously. | [72] |
| *Solanum lycopersicum* | CS, 4 ± 0.5 °C | *SlCBF1* | GKO | Higher levels of electrolyte leakage and $H_2O_2$, low level of protein and proline contents. Reduced levels of methyl jasmonate, abscisic acid, and zeatin riboside contents, however, increased indole acetic acid content in the mutant under CS. | [73] |
| *Oryza sativa* | CS, 4–6 °C | *OsAnn3* | GKO | Shows tolerance against CS by reducing electrical conductivity. Annexins regulate ATPase and $Ca^{+2}$ dependent activities. | [74] |
| *Arabidopsis thaliana* | CS, 4 °C; FS, −2 °C and −7 °C | *CBF2* | Deletion | Identified 10 genes regulated by *CBF2*. All these genes and *CBF2* (lose-of-function) lines exhibited major FS tolerance between two different ecotypes. | [75] |
| *Arabidopsis thaliana* | CS, 4 °C; and FS, 0 °C | *CBF1* | GKO | Generated double and triple mutants CBFs that showed high sensitivity towards FS. | [76] |

HS: heat stress; CS: chilling stress; FS: freezing stress.

## *Transcriptomics: what is happening at the transcript level?*

Transcriptomics includes the functional genome of living organisms dealing with the total number of transcripts, their abundance in a specific cell, and post-transcriptional modifications [90,136]. Transcriptomic studies have been conducted by several technologies, including RNA sequencing, a hybridization-based approach, and other sequencing applications (Table 2).

Transcriptome analysis of hybrid rice was conducted under HS (42 °C) and revealed 4016, 3073, and 3596 deferentially expressed genes (DEGs) that are involved in metabolic and TF activities, regulate signal transduction and photosynthesis [90]. Clusters of genes were exhibited according to their expression, which mainly involved thermotolerance against HS. Some genes were found to participate in acetylation and methylation in anthers at HS [90]. In another study, transcriptome analysis was conducted between a susceptible and resistant rice genotype under HS for its impact on panicle development [78]. A total of 4070 DEGs were identified and categorized into three groups, such as heat-

**Table 2.** Summary of some transcriptomic studies under temperature stress in different plants.

| Species | Stress condition | Specific tissue | Approach | Functional annotation method | Key findings | References |
|---|---|---|---|---|---|---|
| *Oryza sativa* | HS, 38 °C/28 °C day/night | Anther | RNA-Seq | FPKM, GO, KEGG, Swiss-Prot, Nr, Pfam, KOG/COG | 131 DEGs are regulated across all time points.<br>Increased expression of metabolic process, cellular process, catalytic activity and biological regulation.<br>Identified the *OsACT* gene as a thermotolerance.<br>Involved in RNA biosynthesis and metabolism. | [77] |
| *Oryza sativa* | HS, 40 °C | Tillers | RNA-Seq | FPKM, DESeq, GO, KEGG | 4070 DEGs identified.<br>DEGs involved in starch and sucrose metabolism under HS inhibit panicle development.<br>Improved signal transduction by endogenous hormone. | [78] |
| *Triticum aestivum* | HS, 40 °C | Flag leaf and root | RNA-Seq | KEGG, KOG, GO, FPKM, Uni-Prot, Nr, Pfam, | 50 DEGs identified.<br>Genes involved in metabolic and cellular processes, catalytic activity, photosynthesis transport, stress and cell cycle.<br>Biosynthesis of secondary metabolite, protein processing in ER, starch and sucrose metabolism. | [79] |
| *Camellia sinensis* | HS, 38 °C | Leaves | RNA-Seq | GO, KEGG, COG, KOG | 923 DEGs identified as 299 up-regulated and 624-down-regulated.<br>DEGs are related to signal transduction, transcriptional regulation, and post-translation modification.<br>Exogeneous $Ca^{+2}$ enhances thermotolerance, proline and soluble sugars, and Chl contents. | [80] |
| *Capsicum annuum* | HS, 40 °C; CS, 10 °C | Leaves | RNA-Seq | GO | 12,494 DEGs for different abiotic stresses (heat, cold, salinity, and osmotic).<br>Identified DEGs to provide various stimuli for developing cold-resistant cultivars. | [81] |
| *Solanum brevicaule* | CS, 4 °C | Tubers | RNA Seq | GO, FPKM, CuffDiff analysis | 52 DEGs were selected for analysis.<br>Increase chilling induced stress resistance, cell wall strengthening, and phospholipases.<br>Chilling induced DNA damage repair. | [10] |
| *Saccharum spontaneum* | CS, 10 °C | Roots | RNA-Seq | GO, KEGG | 4425 DEGs were identified.<br>Identification of CS responsive genes and metabolic pathways, inducing CS tolerance.<br>Phenylpropanoid and galactose pathways were significantly up-regulated, thus stimulate the synthesis of sugars antioxidants and phytohormones. | [82] |
| *Brassica napus* | CS, 4 °C | Leaves | RNA-Seq | GO, KEGG | 25,460/28,512 DEGs for spring/winter oilseed ecotype.<br>Lipid, ABA, signal transduction, TFs respond towards CS tolerance. | [83] |
| *Secale cereale* | CS, 4 °C | Leaves | RNA-Seq | Nr, Nt, GO, KOG, KEGG, Swiss-Prot, and InterPro | 419 out of 29,874 DEGs have been identified under six groups.<br>*MNS1* and *MNS3* genes were identified to resist CS.<br>Identifying regulation of cutin, suberin, wax synthesis, and biological pathways. | [84] |

(*continued*)

**Table 2.** Continued.

| Species | Stress condition | Specific tissue | Approach | Functional annotation method | Key findings | References |
|---|---|---|---|---|---|---|
| *Ziziphus jujuba* Mill | CS, 4 °C; FS −10, −20, −30, and −40 °C | Branch | RNA-Seq | GO, KEGG | 1831, 2030, 1993, 1845, and 2137 DEGs under five different treatments.<br>Upregulation of galactose metabolism under FS.<br>Genes identified regulating ROS, plant hormones, and anti-freeze proteins. | [11] |
| *Brassica napus* | FS, −2 °C | Seedling | RNA-Seq | Nr, Swiss-Prot, GO, COG, KOG, KEGG, eggNOG, and Pfam | 3905 DEGs identified as 2312 DEGs are upregulated, and 1593 were down-regulated.<br>DEGs involved in carbohydrates and energy metabolism, signal transduction, amino acid metabolism and translation.<br>Content of MDA, proline, soluble protein soluble sugars, and relative electrolyte leakage was increased under FS. | [85] |
| *Malus domestica* | FS, 16 °C | Leaves | RNA-Seq | GO, KEGG | 21,192 DEGs at different time points.<br>3 MYB TFs having several CBF elements were induced.<br>Anthocyanin accumulation increased in apple leaves under CS. | [86] |
| *Triticum aestivum* | FS, −5 °C | Crown of seedlings | RNA-Seq | GO, KEGG | 29,066 DEGs after cold acclimation/ 745 genes were upregulated following FS.<br>FS regulates ABA/JA, phytohormones signaling and proline biosynthesis | [87] |

DEGs, differentially expressed genes; HS, heat stress; CS, chilling stress; FS, freezing stress.

resistant-cultivar-related genes having 1688 DEGs, heat-susceptible-cultivar-related genes – 707 DEGs, and common heat stress-responsive genes – 1675 DEGs. Endogenous hormones exhibited enhanced signal transduction and promoted HS tolerance. However, weak metabolism of starch and sucrose suppresses developing a young panicle under HS [78]. Exogenous $Ca^{+2}$ enhances heat resistance when applied to tea (*Camellia sinensis* L.) plants under HS. Transcriptome profiling revealed 923 DEGs expressing signal transduction, transcriptional regulation, and post-translational modifications. Notably, $Ca^{+2}$ pretreatment, together with HS, adversely affects the photosynthetic apparatus. HS accumulates starch granules and abolishes stroma lamella in plants, and helps to withstand HS [80]. In wheat, the response of HS was observed between susceptible and tolerant genotypes by transcriptome study using four different databases. The identified common DEGs expressed under HS were involved in various biological processes, metabolic pathways, starch, and sucrose metabolism, and photosynthetic transport. Insights into new pathways were reported for an understanding and developing HS tolerant wheat varieties [79].

Cold-induced sweetening (CIS) was observed in tubers when exposed to CS (2–4 °C). Transcriptome sequencing was conducted in eight potatoes (*Solanum tuberosum* L.) cultivars to observe biological processes and gene expression correlated to glucose before and after exposure to CS [10]. Some potato cultivars have CS resistance genes that replicate DNA and its damage repair, thus expressing an invertase inhibitor gene resulting in low glucose levels and increased resistance against CIS. Production of glucose is highly affected by genetic variation in chilling injury as it is directly related to CIS resistance or cold acclimation [10]. Transcript profiling of Kans grass (*Saccharum spontaneum*) roots was conducted under low CS to identify stress-responsive genes [82]. Several key gene pathways and some indices regulating CS (i.e. calcium-dependent kinase, G-coupled proteins, histidine kinase, and contents of proline, MDA) and activating signal transduction were identified upregulating CS responsive genes, thus, increasing CS tolerance. Notably, some metabolic pathways were identified as CS responsive and sugar metabolism for the synthesis of sucrose, fructose, galactose, antioxidants, phytohormones, and some secondary metabolites for transcriptional regulation [82].

A comparative transcriptome analysis between two cultivars of Chinese jujube (*Ziziphus jujuba* Mill.) was accomplished at CS (4 °C) and FS (−10, −20, −30, and −40 °C). Some of the highlighted DEGs contributed to the $Ca^{2+}$ signaling pathway, sucrose metabolism, while others were involved in ROS regulation, plant hormones, and antifreeze proteins. Strong FS was observed responsible for catalytic activity, activation of some significant TFs like (WRKY, AP2/ERF, NAC, and bZIP) and metabolic pathway [11]. Root transcriptome analysis has been carried out in five different alfalfa (*Medicago sativa* L.) varieties to identify their molecular evolution and gene expression [137]. A total of 12,455 orthologs have been identified, among them some unigenes related to FS tolerance, calcium-binding, and some antioxidant enzymes (catalase, ascorbate) exhibiting themselves in all given varieties. These genes are mainly involved in signal transduction, transcriptome regulation, and metabolism [137].

## Proteomics: can proteins make it happen?

Proteomics deals with proteins' role, structure, function, localization, interactions with other proteins, and their execution in stress response or normal circumstances. Knowledge about stress signaling in plants, key proteins, and their metabolic pathways executed into biotechnological tools lead to expanding stress tolerance [93,138]. Table 3 documented some recently conducted

**Table 3.** Summary of some proteomic studies under temperature stress in plants.

| Species | Stress condition | Specific tissue | Extraction protocol | Analytical approach | Key findings | References |
|---|---|---|---|---|---|---|
| *Nicotiana tabacum* | HS, 42 °C | Leaves | Acetone | iTRAQ, LC-ESI-MS/MS, HPLC, GO, KEGG, and COG | 2034 DAPs identified.<br>Expressed proteins involved in post-translational modification, energy production, sugar and energy related metabolic biological processes, and glycolysis pathway.<br>HS down-regulates the photosynthesis pathway and accelerates leaf senescence to regulate cell homeostasis/viability. | [12] |
| *Brassica juncea* | HS, 30 °C | Sprout | Acetonitrile | LC-MS/MS, UPLC, UNIPROT, and KEGG | 172 DAPs identified.<br>Increased expression of genes/proteins related to melatonin, electrolyte leakage, GSH and POD.<br>Increased defense pressure, protein biosynthesis, signal transduction and transcription under HS.<br>Involved in protein transport, carbohydrate metabolism. | [88] |
| *Musa acuminata* | HS, 30 °C | Banana peel | SDS-PAGE | PLS-DA, OPLS-DA, HPLC, 2D PAGE, and MS/MS | 66 DAPs identified.<br>Proteins involved in stress response, photosynthesis, energy metabolism, signaling.<br>Increase expression of proteins encoding cell wall degrading enzymes.<br>Hormonal signaling (auxin, GA, ethylene) is affected by HS.<br>A decrease in activity of several antioxidant enzymes. | [89] |
| *Oryza sativa* | HS, 42 °C | Flag leaves | Acetone | 2D PAGE, and MS/MS | 58 DAPs identified.<br>bHLH and HSF–HSP protein involve regulating higher photosynthesis, and antioxidant defense system. | [90] |
| *Arachis hypogaea* | CS, 1 °C | Bud | Tricarboxylic acid (TCA)/Acetone | iTRAQ, and LC-MS/MS | 333 DAPs identified.<br>DAPs involved in cellular and metabolic processes, initiating and regulating the translation.<br>Participation in protein | [91] |

(*continued*)

**Table 3.** Continued.

| Species | Stress condition | Specific tissue | Extraction protocol | Analytical approach | Key findings | References |
|---|---|---|---|---|---|---|
| | | | | | synthesis, nucleotide metabolism and RNA transport pathway. | |
| *Cocos nucifera* | CS, 8 °C | Leaf | SDS-PAGE | iTRAQ, and LC-MS/MS | 2468 DAPs identified.<br>DAPs involved in abiotic stress stimuli (heat shock, hormonal stress).<br>Increased expression of proteins involved in secondary metabolites biosynthesis, energy production, and posttranslational modifications. | [92] |
| *Prunus persica* | CS, 1 °C | Fruit | TCA/Acetone | iTRAQ, HPLC, LC-MS/MS, and Qpcr | 325 DAPs identified.<br>Proteins expressed mainly regulate carbohydrate, energy, lipid and amino acid metabolism, antioxidant ability, and overwhelm $Ca^{2+}$ transduction.<br>Involves in stress response and defense, cell wall and membrane degradation. | [93] |
| *Arabidopsis thaliana* | CS, 2 °C | Cell | TTC/TTA, acetonitrile | LC-MS/MS | Proteins respond to hormone stimulus, organic substance, and flower development enhanced significantly.<br>Expressed proteins regulate cell growth and transmembrane receptor protein tyrosine kinase signaling pathway.<br>ABA-dependent and -independent pathways involved in the cold acclimation process, especially in the PM proteome. | [94] |
| *V. amurensis and V. vinifera* | CS, 15, 11 and 9 °C; FS, −3 °C | Bud | TCA/Acetone | iTRAQ, LC-MS/MS, HPLC, and RT-PCR | 472 and 713 DAPs-235 shared ones.<br>Phenylpropanoid biosynthesis-related proteins are highly expressed.<br>Pathways regulated by DAPs are endocytosis, protein processing in the endoplasmic reticulum, oxidative phosphorylation, chaperones, and folding catalysts.<br>Proteins enhanced ribosome biogenesis, carbon fixation, mitochondrial biogenesis, and glutathione metabolism pathway. | [95] |
| *Solanum tuberosum* | CS and FS, 15, 4 and 0 °C | Tuber | Acetone | iTRAQ, LC-MS/MS, and qPCR | 51 DAPs identified.<br>15 HSPs induced to act to prevent cellular damage as defense and maintain homeostasis.<br>Increased expression of soluble sugars such as sucrose, fructose and glucose.<br>Enzymes regulating sugars expressed significantly. | [96] |

DAPs, differentially accumulated proteins; HS, heat stress; CS, chilling stress; FS, freezing stress; HSPs, heat shock proteins.

experiments for temperature stress tolerance using several proteomic platforms.

By using an iTRAQ technology, proteome analysis of tobacco (*Nicotiana tabacum*) leaves under HS identified 2034 differentially accumulated proteins (DAPs) [12]. These DAPs are mainly involved in sugar production, energy-making metabolic pathways, and then its conversion, post-translational modification. Interestingly, HS down-regulated the photosynthesis pathway, deteriorates cellular components to maintain cell viability, and accelerated leaf senescence [12]. In the banana (*Musa acuminate*), proteomic analysis was conducted to identify DAPs to find out molecular mechanisms under HS [89]. The proteins made under HS are mainly involved in stress response, photosynthesis, energy metabolism, and signaling. Proteins related to Chl metabolism and hormonal signals such as auxins, gibberellin, ethylene were affected by HS. An increased expression level of proteins or genes was observed by encoding cell wall degrading enzymes, thus causing loss of firmness in the banana peel [89].

Rice root proteins have been studied using iTRAQ technology under CS [139]. The study reveals a total of 433 DAPs. Results revealed that *Lsi1* (low silicon rice 1) and overexpressing (Lsi1-OX) identified enhanced ability of the antioxidant system and signaling pathway at the physiological level. Besides, improving rice capability to regulate interconnected signals stimulating biochemical processes under CS [139]. Cold stress regulation networks acknowledged under proteomic study using iTRAQ in peanut (*Arachis hypogaea* L.) buds. Peanut buds' response to CS is associated with the RNA transport pathway, proteins initiating and regulating translation [91]. In coconut (*Cocos nucifera* L.), major proteins among DAPs were involved in metabolic and biological pathways by iTRAQ proteomic study under CS [92]. DAPs found in two different varieties were also related to photosynthesis and respiration. CS accumulates stress-responsive proteins in the Hainan Tall variety, also increasing the scavenging power of ROS under CS [92]. Peach (*Prunus persica* L.) fruit ripening is prone to CS and studied at the proteome level [93]. A total of 325 DAPs were found to regulate: signal transduction, defense in stress response, carbohydrate and lipid metabolism, energy, and amino acid metabolism. CS storage regulates antioxidant ability, avoids $Ca^{2+}$ signal transduction, thus enhancing the shelf life of peach fruit. However, long-term CS storage may degrade cell walls due to oxidative stress, consequently losing flavor [93].

Two different species of Amur grape, *Vitis amurensis* (cv. Zuoshan-1) and *V. vinifera* (cv. Jingzaojing), were studied to observe their response towards cold acclimation [95]. Buds of grapes were subjected to iTRAQ based proteome technology at two different time points at CS and FS. Hence, shared 235 DAPs that are involved in metabolic pathways, predominantly carbohydrate metabolism and protein chaperone. In response to cold acclimation, the phenylpropanoid biosynthesis pathway was also observed [95]. Low-temperature stress (CS and FS) in potato tubers analyzed by proteome analysis revealed 15 heat shock proteins that prevent cellular impairment, thus maintaining homeostasis. CS and FS during storage that causes physiological and biochemical changes in tubers. Many soluble sugars like glucose, sucrose, and fructose were increased by the activity of starch synthase1, beta-amylase, and invertase inhibitor [96].

## *Metabolomics: are metabolites responsible for phenotypic effects?*

Metabolomic emphases on the comprehensive profiling of the low molecular weight (<1000 Da) molecules or metabolites that are the end products of absorptions in any tissues [5,140]. For preserving metabolic homeostasis, plants need to accomplish the precise amount of carbon, osmolarity, energy, and membrane stability. A metabolic profile is one of those aspects that determine gene expression, protein accumulation, or the functional pathway of the gene as well as the phenotypic effects of the response [141] along with transcriptome and proteome study. Metabolomics is also widely used to generate novel insights into plant responses by detecting alterations within the metabolic pattern or proteins (Table 4) [5,13].

HS influencing rice seeds was observed by metabolic profiling [13]. Masses of sugars (sucrose, glucose, fructose), tricarboxylic acid (TCA) cycle, and starch biosynthesis were strongly linked with HS tolerance. In another cluster of genes, the physical deterioration of starch granules, modification of the mature seed, and aspartate accumulation under nighttime HS were observed [13]. Notably, 19 out of 57 metabolites were differentially expressed between HS and control treatment [13]. Under HS, the metabolome profile of the *Arabidopsis* plant responded differently towards every level of HS, that is, control, prolong warming, and heat shock [97]. Decreased stomatal conductance and suppressed TCA cycle was observed under prolonged warming. While heat shock enhanced transpiration and the glycolysis pathway but limited the formation of acetyl coenzyme A. Both levels of HS elevated the activities of antioxidant enzymes and the accumulation of ROS,

**Table 4.** Summary of some metabolomics studies under temperature stress in plants.

| Species | Stress condition | Specific tissue | Metabolomics analysis approach | Data analysis | Key findings | References |
|---|---|---|---|---|---|---|
| *Arabidopsis thaliana* | HS, 28 and 38 °C | Leaves | GC/TOF-MS analysis | OPLS-DA, PCA, KEGG, GO | 34 out of 181 SDMs were narrowed down between prolonging warming and heat shock stress.<br>Increase in production of ROS, rate of transpiration, and photosynthesis by a heat shock.<br>Increase the concentration of $H_2O_2$ and activities of antioxidant enzymes.<br>Upregulation of bZIPs, DREB and HSFA. | [97] |
| *Oryza sativa* | HS, 28 ± 1 °C | Immature and mature seed | GC-MS analysis | PCA, HCA | 19 out of 57 SDMs.<br>Alteration in mature seed, structural deterioration of starch granules, accumulation of aspartate under High Nighttime (HNT).<br>Decreased proline, lysine, citric acid, xylose, and glycolic acid during seed development, increased protein content, and sugar metabolism. | [13] |
| *Populus tomentosa* | HS, 45 °C | Leaves | GC/TOF-MS analysis | KEGG | Increase antioxidant enzyme activities and concentration of $H_2O_2$, proline, raffinose, and melibiose.<br>Transformed metabolites enhanced glycolysis.<br>Significant increase in Va, Leu, Arg, and proline metabolic pathways by HS. | [98] |
| *Musa acuminate* | CS, 15 and 6 °C | Banana fruit fingers | NMR, HPLC | PCA, KEGG, MetaboAnalyst | 33 SDMs identified.<br>Chilling causes an increase in glycolysis.<br>Higher sucrose contents, linolenic acid, malic acid, oleic acid, linoleic acid, proline, acetic acid, leucine, and isoleucine observed by induced SA chilling. | [99] |
| *Brassica napus* | CS, 4 °C | Leaves | UPLC, LC-MS/MS | GO, KEGG, PCA, PLS-DA | 41/47 SDMs for spring/ winter oilseed ecotype.<br>81 SDMs were direct to primary metabolites.<br>Secondary metabolism (flavonoid biosynthesis, phenylpropanoid biosynthesis, flavone and flavonol biosynthesis, and carotenoid biosynthesis) respond towards CS. | [83] |
| *Oryza sativa* | CS, 15 °C | Seed | LC-MS/MS, HPLC | PCA, KEGG, PLS-DA | 35 out of 730 metabolites identified as SDMs.<br>SDMs involves in the biosynthesis of amino acids and phenylpropanoids.<br>Regulate the metabolism of glutathione and inositol phosphate. | [100] |
| *Nicotiana tabacum* | CS, 15 and 4 °C | Seedling | LC-MS, UHPLC | KEGG, OPLS-DA, PCA | 35 SDMs identified.<br>Metabolites involved in TCA cycle and phenylpropanoid biosynthesis.<br>CS cause electrolyte leakage and an increase in MDA content. | [101] |

(*continued*)

**Table 4.** Continued.

| Species | Stress condition | Specific tissue | Metabolomics analysis approach | Data analysis | Key findings | References |
|---|---|---|---|---|---|---|
| *Vitis amurensis* and *Vitis vinifera* | CS, 4 °C | Leaf | GC-TOF-MS, | PCA | 12 SDMs identified.<br>CS causes an increase in proline contents and organic acid, the sugar level of glucose, maltose, lactose, and psicose.<br>Upregulation of amino acids like pyroglutamate and glutamine, and glycerol and phosphate remarkably upregulated in both species under CS. | [102] |
| *Hordeum distichon* | CS and FS, 24, 12, 5, 0, −5, and −8 °C | Seedling | UPLC-QTRAP-MS/MS | PCA, PLS-DA, KEGG, HCA | 8 out of 770 SDMs identified as potential biomarkers.<br>CS and FS alter metabolic responses, especially organic acids and derivatives, flavonoids, lipids, polyphenols.<br>Upregulates glutathione disulphide and level of glutathione, glutamic acid, ascorbate, and putrescine were downregulated. | [103] |
| *Triticum aestivum* | CS and FS, 21 to −3 °C and −3 °C | Young microspore | GC-QqQ-MS analysis, HPLC | PCA, PLS-DA, KEGG | Stress causes a decrease in amino acids and amines, including alanine, glycine, methionine, phenylalanine, proline, and valine.<br>13 lipids increased significantly under CS and FS. | [104] |

SDMs: significantly different metabolites; HS: heat stress; CS: chilling stress; FS: freezing stress.

especially $H_2O_2$. Heat shock factors (HSFA1s), DREBs, and bZIs were observed to be upregulated under all stress levels [97].

Salicylic acid, improving CS tolerance in bananas, was observed with a metabolome study using nuclear magnetic resonance (NMR) [99]. Some metabolic differences and the accumulation of glucose were observed at normal ripening temperatures. However, CS damaged important metabolites, that is, glutamine, serine, and glucose. Synthesis of unsaturated fatty acids and amino acids, some critical biological changes, including sugar metabolism, have enhanced the energy charge and CS tolerance [99]. Exposure of tomato fruit to CS caused the chilling injury (CI) affecting a regulatory network was detected by comparative transcriptomics and metabolomics. The study highlighted a series of metabolites (27 organic acids, 16 sugars, 7 amino acids, and 22 other compounds), while 1735 DEGs were observed for transcriptomics data. Increased gene expression of ATP-citrate synthase (ACS) and isocitrate dehydrogenase (IDH) under CS may have raised the synthesis of citrate, *cis*-aconitate [108]. Metabolic profiling under normal and CS was conducted in two different rice varieties. A total of 730 metabolites were identified, among which 35 responded differently under CS. Seven key metabolites among 35 were involved in combinations of amino acids and phenylpropanoid, which also regulates the metabolism of glutathione and inositol phosphate [100].

Recently, in alfalfa, FS tolerance has been improved by working on the water deficit treatment strategy [142]. Treatments resulted in increased soluble sugars, total amino acids, especially proline, and higher lipids content, including fatty acids, unsaturated fatty acids, and glycerophospholipids. The enhanced FS tolerance (at 0 to −6 °C) is mainly contributed by improved metabolic pathways and some abundant flavonoids and carbohydrates [142]. Similarly, FS (−6.5 °C) tolerance can be enhanced by using a strategy of exogenous application of enhanced antioxidant capacity and is reported in spinach (*Spinacia oleracea* L.) [143]. Metabolite profiling of spinach leaves was conducted before and after exogenous antioxidant application, that is, ascorbic acid (AsA). This exogenous application may have increased ROS under oxidative stress, antioxidant enzymatic activities, enhanced cell wall/lignin augmentation, secondary metabolism, and branched amino acids, which ultimately enhanced FS tolerance [143]. Various levels of FS tolerance were observed in different species of *Eucalyptus*, among which *E. benthamii* Maiden Cambage (*Eb*) showed higher tolerance as compared to *E. grandis* Hill ex Maiden (*Eg*), *E. dunnii* Maiden (*Ed*) [105]. This

higher frost tolerance might result from the higher accumulation of: phenolics, soluble sugars, anthocyanins, osmoprotectants, and antioxidants. Metabolic profiling of these species under FS (−2, −4, and −6 °C) revealed the differences in tolerance mechanisms among species [105].

### *Phenomics: what has occurred so far?*

The connection between genotypes and phenotypes is key for breeding programs. Plant phenomics is considered as the phenotype of a plant or genotypic expression in a specific stress condition [144]. A plant's phenotype includes several parameters that can be evaluated through direct investigation or by applying frequent analytical techniques and can also be defined by the connection between environmental stress and the plant genotype [145]. Phenotyping is still a massive task under stressful conditions due to the occurrence of complex biosynthesis processes that govern various abiotic stress tolerances in plants [146]. The significance of phenotyping has become apparent in the post-genomic era because of the approaches used for crop improvements such as genomic selection (GS), GWAS, MAS, and QTL mapping heavily reliant on the high-throughput phenotyping (HTP) in crops [147]. Nonetheless, HTP technologies have been obtaining tremendous advancement. The strategies for data mining, interpretation, and storage are automated, precise, accurate, and economical. Genetic dissection of various characteristics and detailed investigation of plant structure and function permits the study of plant phenotypic expression [144].

The integration of holistic phenotyping tools can elucidate the functional gene polymorphism and explain the complex mechanisms responsible for stress tolerance in crop plants. For instance, the nondestructive phenomics approach was used for the evaluation of HS (35 °C) tolerance at anthesis in *Brassica* species [148]. Results show that flower volume was the key phenomic character for HS tolerance. Besides, whole-plant measurements were extremely associated with fresh weight variations, signifying that the entire plant imaging might be a valuable replacement for the fresh weight in upcoming investigations [148]. In another study, a mechanized non-invasive phenomics scheme was used to evaluate the day-to-day variations in plant morphology and photosynthetic routine after the experience to HS (45 °C) in *Arabidopsis* [149]. Notably, HS reduced the quantum yield of PSII and enhanced the leaf angle. It also hampered morphology and plant growth during prolonged HS. By investigating the association between the traits, results depict early variations in the photochemical quenching paralleled with the rosette extent at future steps, which advocates quenching to complete HS tolerance [149].

## The crucial role of bioinformatics within the context of omics platforms

Bioinformatics is an interdisciplinary field comprising: biology, mathematics, statistics, computer science, and information engineering to understand and analyze complex biological data. It helps interpret biological queries using computational software. Integrated omics approaches provide a basic understanding of the molecular aspects involved in abiotic stresses. However, bioinformatics consolidates with these omics approaches favoring enhancing the scientific knowledge of biological functionalities and also provide new perspectives for increasing tolerance in crops against biotic and abiotic stress [150]. Bioinformatics involves data production (data mining and organization) supporting omics technologies. These high throughput technologies provide a considerable amount of information about the functional system of genes, filtered and interpreted by bioinformatics tools. Bioinformatics provide maintained accessible resources to the scientific community, mainly available on the web as open-access programs hence a major key to the success of this research field [151].

Multiple genome databases and bioinformatics tools have been used for various omics approaches. Integration of computational modeling, biological network, and broad omics approaches would be a pivotal combination to improve plant systems [152]. Computational modeling and simulation in network analysis are mainly driven for systems biology by integrating bioinformatics and multiple omics [153]. Moreover, bioinformatics tools include: genomic databases, metabolic pathway databases, sequence alignment programs, and protein structure predictions that have come up with raw data of genome assembly, gene sequences, functional annotations, and experimentally proved data related to plant stress response. Some important stress-based databases include Plant Stress Gene Database (http://ccbb.jnu.ac.in/stressgenes/frontpage.html), PlantPReS Database (http://www.proteome.ir/), Plant Stress Protein Database (http://www.bioclues.org/pspdb/), STIFDB2 (http://caps.ncbs.res.in/stifdb2/). Any change caused as a stress response at the proteome level or during the metabolic pathway can be detected with the help of bioinformatics (*in silico* analysis). All stress-responsive genes can be analyzed

by genome sequences and QTLs via bioinformatics. New computational facilities and algorithms urged the use of bioinformatics to predict stress response in plants. The huge ocean of knowledge acquired by bioinformatics tools along with omics approaches can be used to develop stress-tolerant varieties against unfavorable environmental conditions [154].

## Concluding remarks and future directions

To warrant the food security of upcoming generations and achieve the goal of "zero hunger" anticipated by the FAO, crop productivity must be doubled to feed the increasing population expected to surpass 9.7 billion by 2050. However, due to the increased temperature and other environmental effects of the global climate, the agricultural output is dramatically decreasing and counteracting the goal of "zero hunger." Climate change gives rise to several abiotic stresses, among which temperature extremes (HS, CS, and FS) are considered major growth and yield-limiting factors worldwide. These temperature extremes disrupt metabolic homeostasis due to which plants need extensive modifications to adjust to these environments. Although substantial events have been accomplished in detaching the plant flexibility to temperature stresses, due to the multi-layered and quantifiable nature of these flexible characters, very few accomplishments have been attained over the conventional plant breeding methods. Biotechnological platforms offer the prodigious possibility to enhance crop production for a persistent rise in the worldwide population. Several innovative omics approaches have been developed during the previous few decades to precisely explore the variations at the: genome, transcriptome, proteome, and metabolome levels, which are occurring due to many changes in plants' response to altering stress environments. Under temperature stress, plants modify themselves to adjust within the current stress by modulating genes, proteins, and metabolites regulation. It is vital to clarify the roles of recently acknowledged stress-responsive genes to know plants' stress responses.

In short, in response to questions raised in Sections "Transcriptomics: what is happening at the transcript level?","Proteomics: can proteins make it happen?","Metabolomics: are metabolites responsible for phenotypic effects?",and "Phenomics: what has occurred so far?", several genes, metabolites, and proteins have been clearly described in their respective section and the table regulating tolerance to HS, FS, and CS in different plant species. Among them, *Arabidopsis*, rice, and wheat are the major and widely evaluated crops employing various omics platforms under different temperature environments. These crops can be a great source to obtain large omics data sets to translate into other major crops for developing temperature-resilient ready-to-grow crop plants.

Nevertheless, recent developments in high-throughput sequence platforms and functional genomics have helped us identify several novel genes or stress regulators conferring temperature stress and their genome-wide expression profiling. Furthermore, transgenic approaches possess great potential for transferring temperature tolerant genes across different plant species. Subsequently, plant responses to stresses are habitually very definite. Thus, omics approaches should be targeted to separate cell types and tissues at diverse developmental stages. In this contrast, the combination of fast-evolving omics approaches and bioinformatics into systems biology at the single-cell level could provide new ways to enhance plant stress tolerance. These investigations are important to recognize the molecular system of interrelating proteins that consults temperature tolerance in plants. The accessibility of data developed from integrated-omics tools offers the opportunity to respond to multifaceted queries in-plant investigations under stressful environments. Thus, shortly, more integrated omics-based research should be planned on different tissues/cell types on a large scale to obtain insight into the complex regulatory mechanisms responsible for temperature stress tolerance in several plant species. Additionally, the difficulty of the phenotyping of complex traits can be overwhelmed by trying genotypes harboring temperature under stressed and non-stressed conditions along with multi-location testing. Thus, HTP tools advance new avenues for the measurement of component traits accompanying temperature tolerance. In the current modern biotechnological era, it is presently problematic to predict whether bioinformatics and computational biology can keep the bound with the exponential advancement of omics tools. Attentive statistics can fill this gap and account at the experimental strategy point of the integrated-omics experiment. After that, there is a chance to develop demanding systems-near statistical models that entirely benefit from the codependent works of biological systems. Apart from the above-mentioned gaps and new directions, there are some persisting bottlenecks in utilizing omics approaches for the identification of novel stress-related genes and regulators. Thus, there is an urgent need to solve such bottlenecks (Figure 2).

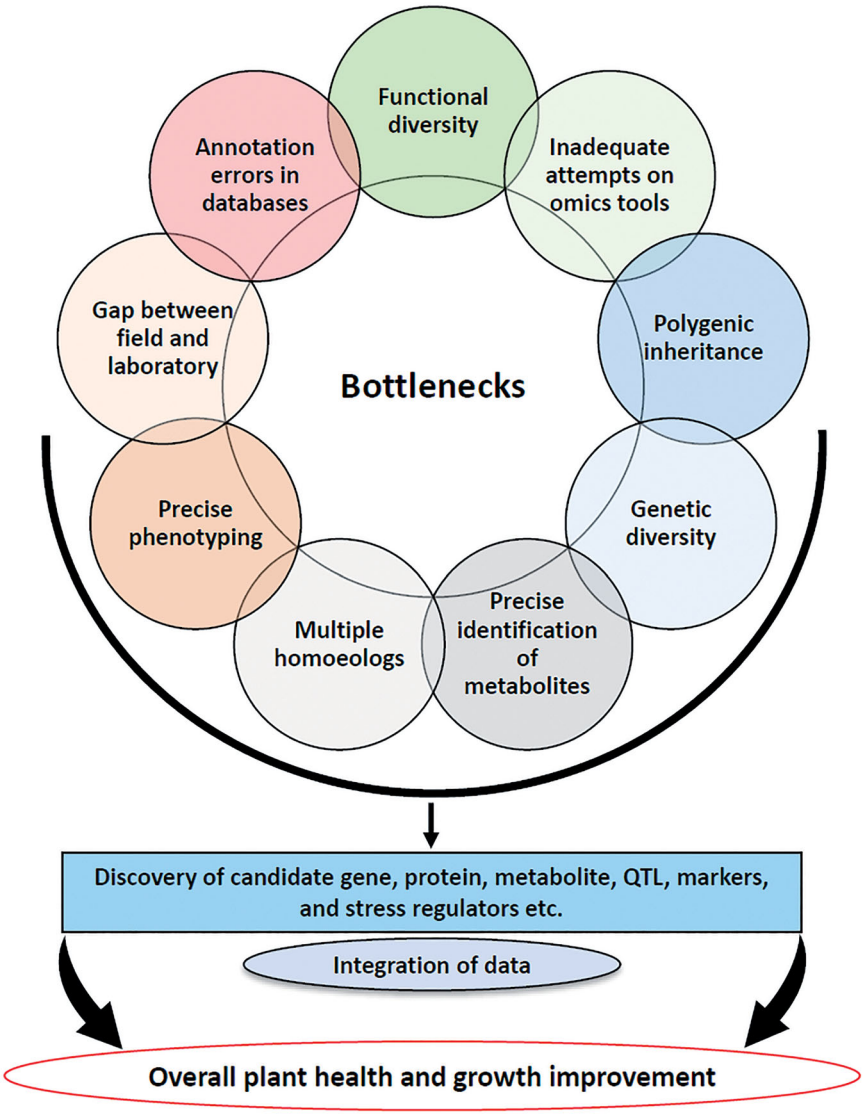


**Figure 2.** Persisting bottlenecks in the development of temperature-resilient plants. Even though outstanding innovation has been attained in the biotechnological era, numerous questions and bottlenecks currently restrict the application of omics approaches for stress tolerance investigations with crop plants. Thus, the release of these bottlenecks employing molecular tools will help us exploit the new manifesto to develop temperature tolerance (adapted and modified from Raza et al. [155]).

Moreover, genome editing using the CRISPR/Cas system and engineering stress-related genes could be one of the most promising futures to enhance stress tolerance. Similarly, the engineering of metabolic pathways could open new windows to develop climate-resilient ready-to-grow plants. Recently, speed breeding has emerged as the most powerful tool to enhance plant growth and the production of a specific environment. Thus, the combination of omics, genome editing, and speed breeding can be surprising ways for sustainable agriculture and feed the billions worldwide.

Lastly, the significant step to crop advancement is to endorse crystal clear discussion among molecular biologists and plant physiologists on one pointer and growers, breeding corporations, and the community to resolve the financial, sociological, lawful, and ethical steeplechases mutually.

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## Author contributions

AR conceived the idea. All authors (AR, JT, HK, and RKV) contributed to writing the manuscript. RKV, HK, and AR finalized the manuscript. All authors have read and approved the final version of the manuscript.

## Disclosure statement

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