

Deciphering the myth of cold tolerance in soybean: An overview of molecular breeding applications

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Abstract

The soybean is a source of several dietary components, including milk, protein, and oil. Cold stress has significantly curtailed soybean growth and yield in large areas and caused a high risk to global food security. The main objective of soybean breeders is to improve soybean resistance to cold stress. Conventional breeding approaches have made significant progress in developing cold tolerance in soybean; however, the high cost and complex genetic mechanism of cold tolerance hindered the large scale of these techniques. Molecular tools like quantitative trait loci (QTL), genome-wide association studies (GWAS), transcription factors (TFs), genetic engineering, and transcriptome have been used to identify cold tolerant genes/QTL and to develop soybean cultivars tolerant to cold stress. Clustered, regularly interspaced short palindromic repeats (CRISPR/Cas9) is used to increase the abiotic stress tolerance in soybean; however, its use to edit the cold tolerance genes in soybean is limited. Mapping of QTL has accelerated the master-assisted selection (MAS) in soybean. This

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review presents a detailed overview of molecular techniques and their use in developing cold-tolerant soybean cultivars. Using CRISPR/Cas9 would increase the speed of molecular breeding for cold tolerance in soybean. This information will assist soybean researchers in uncovering the basis of cold stress tolerance in soybean and adopting the most suitable way to breed the cold tolerant cultivars which can thrive under the extreme pressure of cold stress.

Keywords: cold stress; CRISPR/Cas9; MAS; soybean; QTL; yield

Introduction

Expanding human population and increased threats of abiotic stresses have limited agricultural crop production, which is significant hindrance to food security. Today, safeguarding food security is one of the main challenges. The world population is overgrowing, and it can reach ten billion by the end of the year 2050, demanding a 60 to 100% increase in food production (FAOSTAT, 2021). Change in climatic circumstances is significant reason of crop growth deterioration (Bhat *et al.*, 2022). Soybean is one of the most valuable legume crops and an important source of oil and protein (Liu and Liu, 1997). Soybean has been cultivated worldwide in large areas, including high altitudes and latitudes (Jia *et al.*, 2014). Global demand for soybean is increasing as it provides essential nutrition (Amoanimaa-Dede *et al.*, 2022). These aspects led to the worldwide rise in soybean yield in the late 20th and early 21st centuries. In 2019 soybean acreage increased from 29.5 to 120.5 million hectares, and yield enhanced four times from 81 to 334 million tons (FAOSTAT, 2020). The soybean is a rich source of mineral salts (Bellaloui *et al.*, 2015). Soybean is used for consumption and fodder and has economic and ecological benefits (Bezdicsek *et al.*, 1978). Soybean has root nodules, fixes nitrogen, and improves soil fertility (Hungria *et al.*, 2005). Under favorable circumstances, soybean can absorb from 50 to 100 kg N ha⁻¹, allowing it to meet the nitrogen need by 30-60% (Bezdicsek *et al.*, 1978).

Soybean cold production areas are mostly dispersed in Russia, Canada, and China (Lu, 2011; Nedoluzhko and Dorokhov, 2007) (<http://www.soybeancouncil.ca>, accessed on 20-01-2022). Soybean is susceptible to stress, which halts growth and results in injuries during all stages of development (Nouri *et al.*, 2011; Ohnishi *et al.*, 2010). Despite these effects, soybean continued its expansion in cold climatic regions of the world (Funatsuki and Ohnishi, 2009). Cold stress is defined as a temperature in a low enough range to inhibit growth without ceasing cell function and induces multiple abnormalities at various levels of plant growth (Balestrasse *et al.*, 2010). Cold stress includes, (chilling stress, which occurs at temperatures between 0 to 15 °C and freezing at <0 °C). Chilling causes the destabilization of proteins, and damages photosynthesis, while freezing causes cell death due to ice formation (Pearce, 2001; Ruelland *et al.*, 2009). The severe cold stress negatively impacts soybean growth and geographical distribution (Tsegaw *et al.*, 2023) because of the lack of cold tolerance response in soybean genotypes (Zhang *et al.*, 2014). Cold stress is a central issue undermining soybean's geographical diversity and growth (Tsegaw *et al.*, 2023). Cold stress retards plant growth and yield and affects the distribution of a large area (Zhang *et al.*, 2012a). Cold stress leads to the production of reactive oxygen species (ROS) production (Figure 1) (Latef and Chaoxing, 2011). A study, led by Staniak *et al.* (2021) revealed that cold stress reduced the plant emergence rate by 5 to 10% (Staniak *et al.*, 2021).

The plant response to cold stress is linked with several factors (Levitt, 1980), and genetic variation or varietal variation is critical to improving soybean response to cold stress. Several molecular breeding tools have been effectively used to identify and improve cold tolerance in soybean. Understanding the genetic mechanism of cold tolerance will have immense value in breeding cold-tolerant soybean cultivars (Tsegaw *et al.*, 2023). QTL mapping and GWAS have been used to recognize the genomic areas for various traits in soybean, and these are reliable ways to clarify the complex genetic mechanism behind different characteristics (Chen *et al.*,

2021; Funatsuki *et al.*, 2005; Wu *et al.*, 2020). TFs, transcriptomes, and genetic engineering are novel tools for molecular breeding of tolerance against abiotic stresses, which helped researchers to identify and transfer the novel gene into susceptible cultivars (Liu *et al.*, 1998; S. Sanghera *et al.*, 2011; Song *et al.*, 2016). CRISPR/Cas9 is developed as the most influential tool and can potentially develop crop tolerance to abiotic stresses (Ahmad *et al.*, 2021). Genetic engineering and CRISPR/Cas9 use are not widely reported and demand further research. Many ways to enhance soybean cold tolerance include morphological and physiological tolerance indices. The second strategy is breeding for cold tolerance by introducing novel genes from tolerant genotypes. Many research studies deal with cold tolerance in soybean, but a comprehensive review on this aspect is rarely reported. In current review article, we have summarized the latest advancement in using molecular factors and molecular breeding tools to improve tolerance to cold stress in soybean. These above-mentioned studies would help soybean researchers to understand the genetic mechanism of cold tolerance to adopt a reliable breeding method.

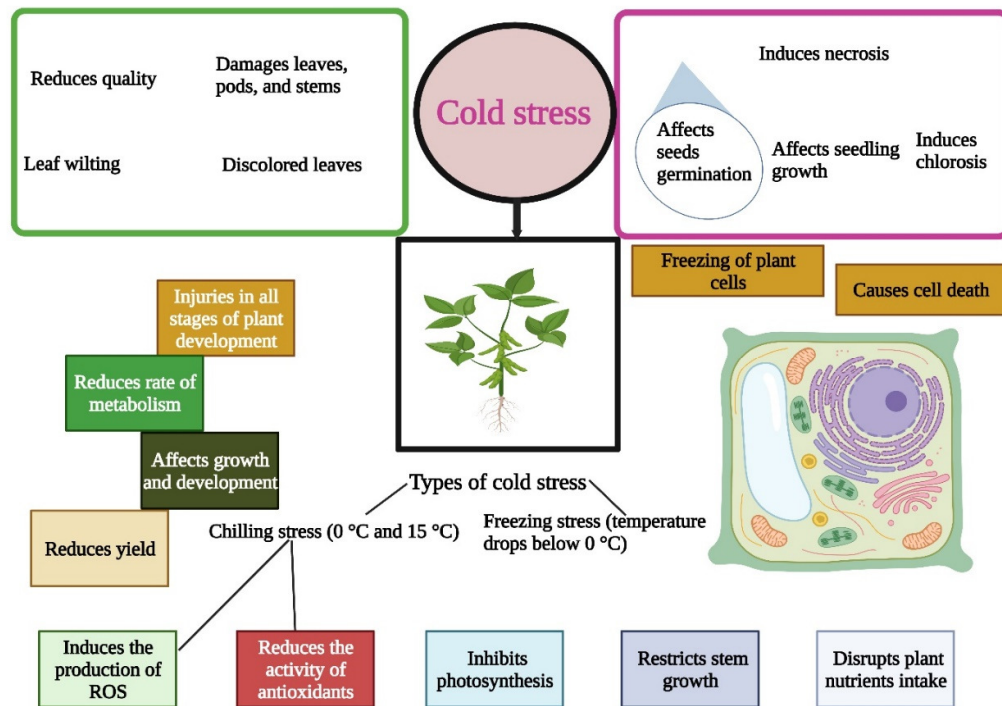


Figure 1. Cold stress influences germination of seeds, seedling growth, antioxidant activities like, SOD, POD, CAT and uptake of nutrients
Freezing stops the plant functions and causes death of plant cell. This Figure is created with [Biorender.com](https://www.biorender.com)

Genetic mechanisms and screening of cold tolerance in soybean

Plants have evolved complex mechanisms and use several strategies to cope with changing environmental conditions (Figure 2). Plants tolerate cold stress after prolonged exposure to low temperatures, called cold acclimatization (Guy, 1990; Hughes and Dunn, 1996). Several physiological, biochemical, and molecular actions are involved in cold tolerance (Guo *et al.*, 2018). Many soluble sugars, proline, and cold-tolerant proteins are activated during cold tolerance (Kaplan *et al.*, 2007). At the molecular level of cold tolerance, cold stress signals are perceived by the fluidity of the cell membrane, Ca^{2+} channels, and electrophysiology, which cause the rigidity of the plasma membrane and the role of the Ca^{2+} channel, leading to an influx of Ca^{2+} into the cell. For example, in *Arabidopsis*, cold activates a receptor-like cytoplasmic kinase insensitive responsive

protein kinase 1 (CRPK1), in which CPRK1 phosphorylates and promotes the accumulation of 14-3-3 proteins in nucleus. 14-3-3 proteins promote the plant response to abiotic stress (Ormanecy *et al.*, 2017). Phosphorylated 14-3-3 proteins induce the degradation of CBFs *via* 26S proteasome (Liu *et al.*, 2017). Evaluating soybean cultivars under different temperature regimes may help select cold-tolerant cultivars based on other morphological, physiological, and molecular traits. Alsajri *et al.* (2019) evaluated 64 soybean genotypes under cold stress during the seedling stage. The genotype GS47R216 was designated as highly tolerant to low temperature (Alsajri *et al.*, 2019). This data would be beneficial for selecting cold-tolerant soybean genotypes (Alsajri *et al.*, 2019). Hence, studies on extensive-scale screening of cultivated and wild-type soybeans must be conducted to choose the tolerant genotypes to accelerate cold-tolerant breeding.

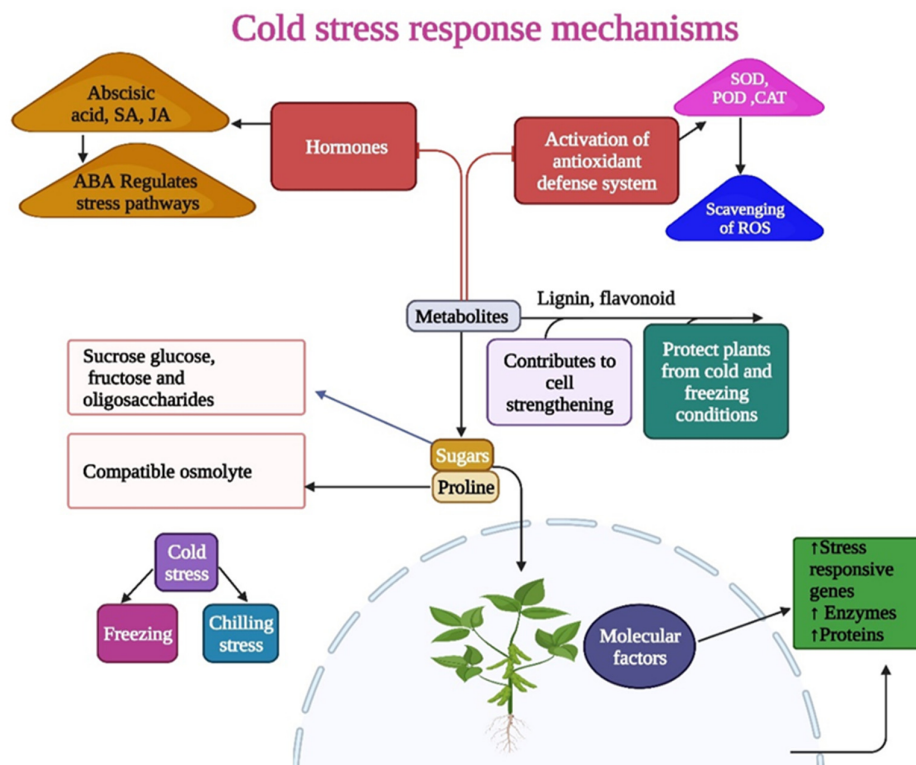


Figure 2. The plant uses several strategies to reduce the harmful consequences of cold stress. These strategies include antioxidant enzymes, hormones, metabolites, sugars, proline, genes and enzymes. This Figure is created with [Biorender.com](https://www.biorender.com). SA, salicylic acid; JA, jasmonic acid; ABA, abscisic acid; ROS, reactive oxygen species; SOD, superoxide dismutase; POD, peroxidase; CAT, catalase.

QTL/genes linked with cold tolerance

QTL mapping and gene identification are reliable ways to enhance soybean tolerance to cold stress. Earlier and recent studies showed that different QTL and genes are directly involved in cold tolerance during different growth stages in soybean. If the temperature is low, it can affect seedling growth and germination and causes yield loss. Backcross introgression lines (BILs) were exposed to cold stress to map the cold tolerance QTL in soybean. Two methods detected two QTL, *Satt237* and *SOYPRP1*, which should be the main effect QTL and more believable. These QTL were linked with cold tolerance. Hence, low-temperature tolerant regions at the germination stage can be further exploited to breed tolerant cultivars (Jiang *et al.*, 2009). Zhang *et al.* (2012a) evaluated advanced backcross introgression lines (BILs) under low-temperature conditions. They have

identified 25 QTL at the germination stage and 13 at the seedling phase. 10 overlapped QTL showed greater soybean cold stress tolerance (Zhang *et al.*, 2012a). A total of 23 low temperature tolerance QTL were detected in BC₂F₃ lines of soybean. The overlap QTL, Satt253, Satt513, and Satt255 were recognized by at least one drought and cold stress method. These QTL were significant for drought and cold tolerance and can play a role in MAS for the breeding drought or cold-tolerant soybean cultivars (Zhang *et al.*, 2012b). A chromosome segment substitution lines (CSSL) soybean population was developed by wild soybean ZYD00006, and cultivated soybean SN14 and used to map three QTL for cold tolerance. Five candidate genes were recognized by gene annotation, GO enrichment analysis and protein function prediction. *Glyma.09G162700* may be a candidate gene to improve cold temperature tolerance at the germination stage (Zheng *et al.*, 2023).

Cold stress also curtails the reproduction stage in soybean. QTL mapping for cold tolerance at the reproductive phase can enhance the breeding of cold-tolerant soybean genotypes. Results showed that three QTL were mapped for cold tolerance in seed-yielding ability. QTL, *qCTTSW1*, and *qCTTSW2* (Table 1) were identified near QTL for flowering time, and the latter had an epistatic interaction with a marker locus positioned near another QTL for flowering time, where there was no novel QTL for cold tolerance (Funatsuki *et al.*, 2005). Another study by Githiri *et al.* (2007) identified five QTL under cold stress for flowering date, fd1, fd2, fd3, and fd4. fd2 and fd4 perhaps correspond to E1 and E3 based on relative position and markers (Githiri *et al.*, 2007). Recently, Jähne *et al.* (2019) screened the 103 RILs and 35 early maturing cultivars of soybean for their cold tolerance assessment at the flowering phase. A cold tolerance QTL was mapped on chromosome 11. They concluded that genomic selection is an effective tool for cold tolerance (Jähne *et al.*, 2019). Low temperature also affected the seed development stage of soybean. If it is not managed, cold stress can cause severe loss in soybean yield. A tolerant QTL with a significant effect associated with seed-yielding ability was identified under cold stress. This genomic region was located near marker (Sat_162) on linkage group A2. Previously, no QTL was detected in this region. This novel genomic locus influences seed development during cold stress (Ikeda *et al.*, 2009). Cold stress also induces seed discoloration and affects seed quality. Two major QTL linked with seed discoloration were identified in soybean. The main QTL was positioned in the proximal area of the I locus, and the structural difference of this locus can be used as a useful DNA marker, called the Ic marker. Results suggested that MAS selection of the Ic allele helps enhance the seed discoloration tolerance in soybean (Yamaguchi *et al.*, 2019). Cold stress results in cracked seeds (CSs) appearing in soybean cultivars. QTL, *qCS8-1*, and *qCS11-1* were recognized by QTL mapping method using the cracked-seed index (CSI), the arcsine transformed CS ratio. Near isogenic lines (NIL) were employed to verify the recognized QTL. Results exhibited that *qCS8-1* and *qCS11-1* could be used in MAS to breed the cold-tolerant soybean cultivars (Yamaguchi *et al.*, 2015). These studies indicated that QTL mapping for germination stage and reproductive stage should be further analyzed for comprehensive understanding of genetic mechanism of cold tolerance in soybean. However, studies on QTL for physiological and biochemical traits are limited which emphasizes to conduct further studies in future. These identified potential genomic regions could be used for MAS to accelerate the cold tolerant breeding in soybean and to combat the rising threats of changing environment.

Table 1. QTL for cold tolerance in soybean

Genotypes	QTL	Trait	References
104 RILs	<i>qCTTSW1</i> and <i>qCTTSW2</i>	Total seed weight	(Funatsuki <i>et al.</i> , 2005)
41 BILs	<i>Satt237</i> and <i>SOYPRP1</i>	Germination stage	(Jiang <i>et al.</i> , 2009)
Kitakomachi and Koganejiro and their 58 F ₂ plants	E1 and E3	Flowering date	(Githiri <i>et al.</i> , 2007)
103 RILs and 35 early-maturing soybean cultivars	<i>qPD11</i>	Pod number	(Jähne <i>et al.</i> , 2019)
BC ₂ F ₃ lines	Satt253, Satt513, Satt693, Satt240, Satt323, and Satt255	Cold tolerance at the germination stage	(Zhang <i>et al.</i> , 2012b)

QTL, quantitative trait loci; PD, pod number; RIL, recombinant inbred lines; BIL, backcross inbred lines.

Potential TFs for soybean cold tolerance

The soybean is a cold-sensitive crop; however, it can adapt to cold climates, but its response to cold stress is low compared to other crop species (Robison *et al.*, 2017). Previous studies have reported many TFs and their complex networks involved in cold tolerance (Kidokoro *et al.*, 2022). The cis-acting dehydration-responsive element (DRE) is imperative in inducing gene expression against abiotic stresses. Kidokoro *et al.* (2015) identified 14 DREB1-type TFs (*GmDREB1s*) in the soybean genomic database. A DREB TF, *GmDREB1s*, was recognized, and cold and other stresses triggered its expression. The TF, *GmDREB1B;1*, (Table 2) activates the expression of *GmPYL21* and improves the ABRE-mediated gene expression in an ABA-independent manner. These outcomes showed that *GmDREB1* proteins increase the expression of various genes linked to responses against many abiotic stresses (Kidokoro *et al.*, 2015). A DREB TF, *GmDREB3*, belongs to the A-5 subgroup of the DREB subfamily and was obtained from soybean using rapid amplification of cDNA ends (RACE). In addition, overexpression of *GmDREB3* improved tolerance to cold in transgenic *Arabidopsis* (Chen *et al.*, 2009). Usually, soybean plants respond to cold stress by activating stress-responsive genes. Genome sequencing has led to the identification of novel TFs in many crops. A homologous gene of AtTCF1 in soybean (named *GmTCF1a*), which facilitates plant tolerance to low temperatures was identified by Dong *et al.* (2021). *GmTCF1a* comprises five RCC1 domains and located in the nucleus. *GmTCF1a* is specifically induced by cold stress. *GmTCF1a* positively controls cold tolerance in soybean and may offer novel understandings of the genetic development of freezing tolerance in crops (Dong *et al.*, 2021). CBF/DREB1 transcript levels increase rapidly during cold stress; however, predictable downstream targets seem unresponsive. The ethylene signaling pathway, which is improved in the cold, can negatively control the soybean CBF/DREB1 cold-responsive pathway, thus, contributing to the comparatively poor cold tolerance. Inhibition of the ethylene signaling pathway caused a significant surge in *GmDREB1A;1* and *GmDREB1A;2* transcripts, while stimulation led to reduced *GmDREB1A;1* and *GmDREB1B;1* transcript (Robison *et al.*, 2019).

NAC is an essential family of TFs and linked with multiple developmental processes, including leaf senescence (Guo and Gan, 2006) and tolerance to abiotic and biotic stresses (Nuruzzaman *et al.*, 2013). A NAC gene, *GmNAC109*, increased the cold tolerance in soybean, and its expression was induced by cold stress. The *GmNAC109* overexpressing transgenic plants of *Arabidopsis* showed the upregulation of the COR15A gene (COLD REGULATED 15A) (Yang *et al.*, 2019). Another family of TFs, WAX INDUCER1 (WIN1), plays a crucial role in the biosynthesis of cutin in plants. Six *GmWIN1* genes were identified from soybean,

with *GmWIN1-5* having high expression under cold stress. *GmWIN1-5* overexpression (*GmWIN1-5-OE*) increased oil content and phospholipids level in cell membrane formation under cold stress (Cai *et al.*, 2022). A WUSCHEL-related homeobox (WOX) is an essential family of TFs unique to plants and is categorized by the existence of a homeodomain. WOX TFs are essential in plant growth, development, and response to abiotic stresses. Hao *et al.* (2019) identified a WOX family in soybean by bioinformatics analysis. Expression profile and quantitative real-time polymerase chain reaction (qRT-PCR) showed that most of the gmWOX genes were involved in response to cold and drought stress. Further analysis of WOX genes will enhance the understanding about the role of WOX genes in soybean growth and response to abiotic stress (Hao *et al.*, 2019). The C2H2-type zinc-finger proteins (ZFPs) were crucial in plant development and stress responses. A ZFP gene *GsZFP1* was recognized and then cloned from *Glycine soja*. Overexpression of the *GsZFP1* gene increased the cold tolerance in *Arabidopsis*. Hence, *GsZFP1* is crucial in plant response to cold stress (Luo *et al.*, 2012). Maruyama *et al.* (2012) performed a microarray analysis of the soybean genome and identified the cold-inducible genes. Diverse transcriptional regulatory pathways were identified in soybean and indicated the role of gene in cold tolerance in soybean (Maruyama *et al.*, 2012). TFs and their role in cold tolerance should be further analysed to identify the new genes which can possibly contribute to cold tolerance in soybean. TFs families, WRKY, EF, and NF are not often described in soybean and therefore, further studies must be pursued to recognize and clone the novel TFs to breed the cold tolerant cultivars of soybean.

Table 2. TFs for cold tolerance in soybean

TFs family	Gene	Role	References
DREB	<i>GmDREB1B;1</i>	Activates the expression of <i>GmPYL21</i>	(Kidokoro <i>et al.</i> , 2015)
DREB	<i>GmDREB3</i>	Improved cold tolerance	(Chen <i>et al.</i> , 2009)
ABI3	<i>GmABI3</i>	Regulated the cold tolerance	(Manan and Zhao, 2020)
CBF	<i>GmTCF1a</i>	Upregulated <i>COR15a</i> levels	(Dong <i>et al.</i> , 2021)
CBF/DREB1	<i>GmDREB1A;1</i> and <i>GmDREB1A;2</i>	Inhibited ethylene pathway	(Robison <i>et al.</i> , 2019)
NACs	<i>GmNAC109</i>	Increased cold tolerance in transgenic lines	(Yang <i>et al.</i> , 2019)
WIN1	<i>GmWIN1-5-OE</i>	Enhanced oil contents under cold stress	(Cai <i>et al.</i> , 2022)
ZFP	<i>GsZFP1</i>	Increased cold tolerance	(Luo <i>et al.</i> , 2012)

Cold tolerant genes

Cold-tolerant genes are potential genetic resources to breed the cold-tolerant varieties in soybean. In earlier study, 105 genes, including 22 pentatricopeptide repeat (PPR) genes and 4 growth-related genes, were expected to be the targeted genes of soybean miRNA miR1508a (*gma-miR1508a*). Cold stress induced the expression of *gma-miR1508a* gene. Soybean plants with overexpression of *gma-miR1508a* showed dwarfing and thick cell compared to their wild types. Transgenic plants exhibited cold tolerance at germination and seedling phases. Meanwhile, the soluble sugar contents were higher than the wild type after cold stress treatment (Sun *et al.*, 2020). In another study, to identify the role of microRNAs (miRNAs) and their targeted genes in the cold tolerance of four soybean cultivars, Augusta, Fiskeby V, Toyomusume,, and *Glycine soja* with variable degrees of susceptibility to cold stress, 72 small RNA libraries and 24 degradome libraries for high-

throughput sequencing were created. In this study, 321 miRNAs were recognized, and 348 new miRNAs were projected in three examined tissues. Furthermore, under stressful circumstances, the varied expression of 162 miRNAs, including well-conserved, legume- and soybean-specific miRNAs and 18 novel miRNAs, was found in the four cultivars. They were related to abiotic stress response mechanisms like ROS scavenging, flavonoid biosynthesis, and osmotic potential regulation based on GO and KEGG annotations (Kuczyński *et al.*, 2022).

Circular RNAs (circRNAs) are a newly known type of noncoding-RNA and have a crucial role in microRNA (miRNA) and transcriptional control function. They play a key role in soybean response to low-temperature stress. A GWAS was conducted to identify the soybean circRNAs under cold stress conditions *via* deep sequencing. Gene ontology (GO) analysis showed that circRNAs participated in low-temperature responses. A new circRNA-miRNA-mRNA network was identified, which is crucial in low-temperature tolerance (Wang *et al.*, 2020). Earlier studies showed that omega-3 desaturase genes were crucial in soybean response to cold stress. Under cold stress conditions (5 °C), an increase in *GmFAD3A* transcripts was found in soybean cells and leaves, whereas no change in *GmFAD3B* or *GmFAD3C* expression levels was detected. The regulatory mechanism of omega-3 fatty acid desaturase in soybean affects the specific isoforms in plastid and endosperm to maintain the level of linoleic acid under low-temperature stress (Román *et al.*, 2012). Kao *et al.* (2022) proposed an advanced systems biology framework of feature engineering to identify the genes from omics and non-omics soybean data. A total of 44, 143, and 45 cold tolerant genes (CT genes) were recognized in short-, mid-, and long-term cruel treatment, correspondingly, from the corresponding gene pool. These CT genes outperformed the remaining genes, the random genes, and the other candidate genes recognized by other methods in an independent RNA-seq database. This proposes that the CT genes recognized from our projected systematic framework can efficiently differentiate cold-resistant and cold-sensitive lines (Kao *et al.*, 2022). These studies indicated the role of potential genes in soybean response to cold stress; however, the data on this aspect is still insufficient to unfold the genetic mechanisms of cold tolerance and to accelerate the breeding programs. More studies are needed to identify the novel gene for soybean cold tolerance.

Proteomics analysis for cold tolerance

Apart from the gens and TFs, cold stress triggers the expression of several stress-responsive proteins in soybean. Comparative proteomics analysis of cold-tolerant and sensitive soybean genotypes has suggested the comprehensive mechanism of cold tolerance. Proteomic analyses have also discovered that cold-tolerant soybeans produce more protective substances (Tian *et al.*, 2015). In an earlier study, 57 proteins were identified and significantly changed in abundance and identified by MALDI-TOF/TOF MS. These proteins were linked with 13 metabolic pathways, amino acid and nitrogen metabolism, photosynthesis, cell rescue and defense, protein degradation, lipid metabolism, and energy metabolism (Tian *et al.*, 2015). Swigonska and Weidner (2013) employed a proteomics technique to analyze the effect of cold stress on germinating seeds of the soybean. Proteome maps were made for control samples and stress-treated samples. Identified proteins were related to carbohydrate metabolism, protein synthesis, signal transduction, and cellular transport.

Analysis of differences in expression patterns of soybean gives insights into the soybean response to long-term abiotic stress (Swigonska and Weidner, 2013). Many important molecular mechanisms and plant responses are yet to be discovered. In another study, 40 proteins were found in response to low-temperature stress in soybean. These proteins were part of plant metabolites like cell defense, energy protein, transcription, and transport. These results give valuable insights about soybean seed response to low temperature stress (Cheng *et al.*, 2010). Studies showed different soybean responses to cold stress and identified various proteins involved in stress-related pathways. Earlier, Kim *et al.* (2004) isolated the three ribosomal-related protein genes from soybean induced by cold stress. Expression of ribosomal protein genes started to increase 3 days after treatment, while cold stress protein *src1* was significantly induced from the first day of treatment. The introduction of ribosomal protein genes might improve the translation process during low-temperature

conditions (Kim *et al.*, 2004). The proteomics technique plays a key role in mining the cold tolerant proteins in soybean, so the studies on this aspect should be enhanced. The published date is not sufficient to fully uncover the protein-based tolerance mechanism in soybean. More studies should be conducted to compare the proteomics profile of different soybean species under cold stress conditions.

Genetic engineering and CRISPR/Cas9 for cold tolerance in soybean

While traditional breeding has been practiced for soybean improvement, with new biotechnological tools, breeders have engineered the soybean for various traits like resistance to insects, pathogens, and herbicides (Rahman *et al.*, 2022). Genetic engineering (GE) methods such as transgenesis and gene silencing help to curtail the risks and enhance the resilience of soybean (Rahman *et al.*, 2022). There is a dire need to improve genetic research to increase soybean production. One of the big reasons for genetic research on soybean is to increase its resistance to various stresses (Naveed *et al.*, 2022). Some bZIP genes in soybean were responsive to cold stress and improved the cold tolerance in transgenic *Arabidopsis*. The transgenic plants were more tolerant to freezing stress than normal plants (Liao *et al.*, 2008). Likewise, transgenic *Arabidopsis* lines with overexpression of soybean genes, *GmMYBJ1* showed tolerance to cold stress compared to wild-type plants (Su *et al.*, 2014).

CRISPR/Cas9 has evolved as an influential tool that allows us to edit the desired gene in many crops, including soybeans (Figure 3). These advanced biotechnology tools are broadly utilized to increase crop production, quality, and yield, develop disease resistance, and are eco-friendly (Naveed *et al.*, 2022). To increase the speed of molecular breeding, it is essential to exploit the gene pool of soybean genotypes. The first SoyBase was established in 1980 (United States Department of Agriculture (USDA) Soybean Genetics Database), which collected genetic resources for soybean, including genetic maps and information on Mendelian genetics (www.soybase.org (accessed on 22 January 2022)). With efforts from numerous groups and investigators, the first soybean reference genome (Williams 82) was published in 2010, marking a new era in soybean research (Schmutz *et al.*, 2010).

Molecular breeding for cold tolerance in soybean

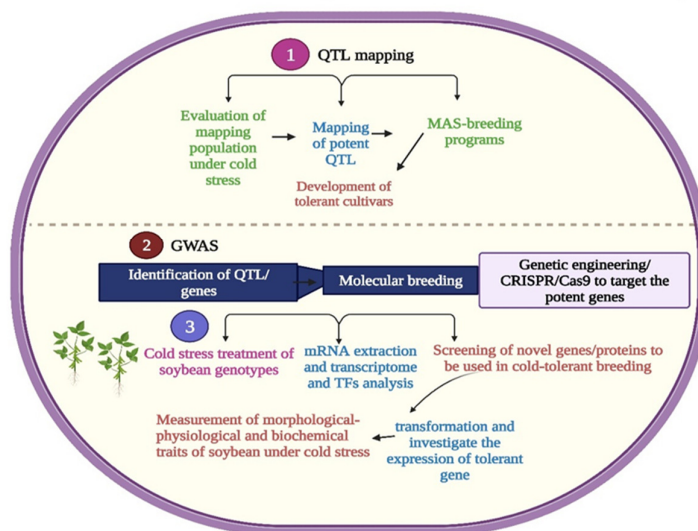


Figure 3. The use of molecular breeding tools has successfully developed the cold tolerant cultivars in soybean. QTL mapping and GWAS have successfully identified the novel QTL/genes to develop susceptible cultivars. Identifying genes/TFs under cold stress conditions would be highly useful for improving molecular breeding programs. This Figure is created with [Biorender.com](https://biorender.com).

Genetic engineering has made significant progress in past years, and this tool is becoming more powerful, creating numerous benefits (Siddique, 2022). Through the novel capability to alter the genome of living organisms, genome manipulation tool has changed genetic and biological investigation. Genetic manipulation techniques first seemed in the 1990s, and different approaches for gene alterations were developed. CRISPR/Cas9 is a valuable gene editing technique based on the complementarity of the guide RNA (gRNA) to a precise sequence and the endonuclease action of the Cas9 (Siddique, 2022). This RNA-guided genome alteration technique has produced differences in plant biology fields. CRISPR technology is frequently improving, permitting more genetic alterations such as generating knockouts, specific changes, and targeted gene activation and repression (Siddique, 2022). The genome editing tools like transcription activator-like effector nucleases (TALENs) and zinc finger nucleases (ZFN) have many benefits but also have some disadvantages too (Hsu *et al.*, 2014).

Earlier studies reported the use of CRISPR/Cas9 for the development of soybean cultivars tolerant to drought (Zhong *et al.*, 2022), pathogen (Fan *et al.*, 2022), salinity stress (Wang *et al.*, 2021) and heat stress (Huang *et al.*, 2019), etc. Earlier, Zhong *et al.* (2022) used CRISPR/Cas9 to edit the soybean's drought tolerance gene, *GmHdz4n*. *GmHdz4* expression was induced by drought stress. *GmHdz4* gene in transgenic lines increased the growth in above-ground parts and root system architecture compared to non-transgenic lines (Zhong *et al.*, 2022). Unfortunately, the use of CRISPR/Cas9 to enhance cold tolerance in soybean is rarely reported, and more studies are needed to make significant changes in the soybean genome. The large-scale use of CRISPR/Cas9 is highly recommended to develop cold-tolerant soybean cultivars, which can thrive under low-temperature stress to sustain growth and yield. The above published studies are significant examples of CRISPR/Cas9 use in soybean.

Conclusions

Cold stress is one of the most devastating abiotic stresses that curtail plant growth and development in large areas. The genetic mechanism of cold tolerance is complex and poses hindrances to expanding conventional breeding methods. Despite all of the efforts, cold stress is continuously deteriorating the soybean yield and quality because sufficient work has not been done to tackle the ongoing issue. Improving the genetic makeup of soybean is a powerful way to combat the rising threats of cold stress. We mentioned in this review that QTL mapping is one of the most robust ways to identify the potential genomic regions for cold tolerance in soybean. There are still many studies on this aspect. Future researchers should focus on developing novel mapping populations for QTL mapping and try to solve the challenges in QTL pyramiding. GWAS has been described as a powerful way to detect the traits marker association for abiotic stress tolerance in soybean. The genes mentioned above, QTL and SNP, should be used in the molecular breeding of soybean against cold stress. In the same way, genetic engineering helps to develop transgenic lines resistant to cold stress in soybean. TFs and transcriptome analyses have identified many genes and protein families involved in cold tolerance in soybean. Many of these genes are significantly associated with cold tolerance and must be used in a molecular breeding program.

Genome editing technique like CRISPR/Cas9 has been used in many crops, but their applications to edit cold-tolerant genes are rarely reported. In the same way, transgenic breeding has led to the development of cold-tolerant lines, and its use at a broad level would bring more success in molecular breeding. The use of new gene editing systems like, base editing (BE) and prime editing (PE) should be exploited to improve the efficiency of gene editing in soybean. The development of cold-tolerant soybean mutants will provide an in-depth overview of the genetic improvement of the soybean genome; as this tool can edit the gene without any biological barrier. We can target the diverse genomes of species to bring any desired change in soybean. The exposure of soybean to different levels of cold stress and sequencing of the genome will be helpful in the characterization of cold-tolerant genomic regions and enhance their breeding applications. It will be more

beneficial to exploit the cold tolerance potential of wild soybean relatives as they thrive under harsh climatic conditions and contain novel stress-tolerant genes, which will serve as valuable genetic material for future breeding plans. Upcoming studies should also emphasize on improving soybean quality traits to maintain the existing nutritious values and increase its market significance. Soybean breeders can get significant clues and ideas from this review paper.

Authors' Contributions

WJ, QQ, GY, RQ, and AR conceptualized and prepared the manuscript. GJ, ZQ, ZC, WY, and LC, participated in the literature search. NM, SHQ, AAA, and HAW reviewed the manuscript. XZ, and WX supervised the study. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

References

- Ahmad A, Ashraf S, Munawar N, Jamil A, Ghaffar A, Shahbaz M (2021). CRISPR/Cas-mediated abiotic stress tolerance in crops. *CRISPR Crops: The Future of Food Security* 177-211. https://doi.org/10.1007/978-981-15-7142-8_6
- Alsajri FA, Singh B, Wijewardana C, Irby JT, Gao W, Reddy KR (2019). Evaluating soybean cultivars for low-and high-temperature tolerance during the seedling growth stage. *Agronomy* 9:13. <https://doi.org/10.3390/agronomy9010013>
- Amoanimaa-Dede H, Su C, Yeboah A, Zhou H, Zheng D, Zhu H (2022). Growth regulators promote soybean productivity: a review. *PeerJ* 10:e12556. <https://doi.org/10.7717/peerj.12556>
- Balestrasse KB, Tomaro ML, Batlle A, Noriega GO (2010). The role of 5-aminolevulinic acid in the response to cold stress in soybean plants. *Phytochemistry* 71:2038-2045. <https://doi.org/10.1016/j.phytochem.2010.07.012>
- Bellaloui N, Bruns HA, Abbas HK, Mengistu A, Fisher DK, Reddy KN (2015). Agricultural practices altered soybean seed protein, oil, fatty acids, sugars, and minerals in the Midsouth USA. *Frontiers in Plant Science* 6:31. <https://doi.org/10.3389/fpls.2015.00031>
- Bezdicke D, Evans D, Abede EB, Witters R (1978). Evaluation of peat and granular inoculum for soybean yield and N fixation under irrigation 1. *Agronomy Journal* 70:865-868.

- Bhat KA, Mahajan R, Pakhtoon MM, Urwat U, Bashir Z, Shah AA, Agrawal A, Bhat B, Sofi PA, Masi A (2022). Low temperature stress tolerance: An insight into the omics approaches for legume crops. *Frontiers in Plant Science* 13. <https://doi.org/10.3389/fpls.2022.888710>
- Cai G, Liu B, Zhou Y, Gao H, Xue J, Ji C, Zhang L, Jia X, Li R (2022). Functional characterization of transcription factor WIN1 genes associated with lipid biosynthesis and stress tolerance in soybean (*Glycine max*). *Environmental and Experimental Botany* 200:104916. <https://doi.org/10.1016/j.envexpbot.2022.104916>
- Chen H, Pan X, Wang F, Liu C, Wang X, Li Y, Zhang Q (2021). Novel QTL and Meta-QTL mapping for major quality traits in soybean. *Frontiers in Plant Science* 12:774270. <https://doi.org/10.3389/fpls.2021.774270>
- Chen M, Xu Z, Xia L, Li L, Cheng X, Dong J, Wang Q, Ma Y (2009). Cold-induced modulation and functional analyses of the DRE-binding transcription factor gene, *GmDREB3*, in soybean (*Glycine max* L.). *Journal of Experimental Botany* 60:121-135. <https://doi.org/10.1093/jxb/ern269>
- Cheng L, Gao X, Li S, Shi M, Javeed H, Jing X, Yang G, He G (2010). Proteomic analysis of soybean [*Glycine max* (L.) Meer.] seeds during imbibition at chilling temperature. *Molecular Breeding* 26:1-17. <https://doi.org/10.1007/s11032-009-9371-y>
- Dong Z, Wang H, Li X, Ji H (2021). Enhancement of plant cold tolerance by soybean RCC1 family gene *GmTCF1a*. *BMC Plant Biology* 21:1-16. <https://doi.org/10.1186/s12870-021-03157-5>
- Fan S, Zhang Z, Song Y, Zhang J, Wang P (2022). CRISPR/Cas9-mediated targeted mutagenesis of *GmTCP19L* increasing susceptibility to *Phytophthora sojae* in soybean. *Plos One* 17:e0267502. <https://doi.org/10.1371/journal.pone.0267502>
- FAOSTAT (2020). Available online. Retrieved 2021 January 12 from: <http://www.fao.org/faostat/en/-data/QC>
- FAOSTAT (2021). Available online. Retrieved 2021 March 21 from: <http://www.fao.org/faostat/en/#data/QC2017>
- Funatsuki H, Kawaguchi K, Matsuba S, Sato Y, Ishimoto M (2005). Mapping of QTL associated with chilling tolerance during reproductive growth in soybean. *Theoretical and Applied Genetics* 111:851-861. <https://doi.org/10.1007/s00122-005-0007-2>
- Funatsuki H, Ohnishi S (2009). Recent advances in physiological and genetic studies on chilling tolerance in soybean. *Japan Agricultural Research Quarterly: JARQ* 43:95-101. <https://doi.org/10.6090/jarq.43.95>
- Githiri SM, Yang D, Khan NA, Xu D, Komatsuda T, Takahashi R (2007). QTL analysis of low temperature-induced browning in soybean seed coats. *Journal of Heredity* 98:360-366. <https://doi.org/10.1093/jhered/esm042>
- Guo X, Liu D, Chong K (2018). Cold signaling in plants: Insights into mechanisms and regulation. *Journal of Integrative Plant Biology* 60:745-756. <https://doi.org/10.1111/jipb.12706>
- Guo Y, Gan S (2006). *AtNAP*, a NAC family transcription factor, has an important role in leaf senescence. *The Plant Journal* 46:601-612. <https://doi.org/10.1111/j.1365-313X.2006.02723.x>
- Guy CL (1990). Cold acclimation and freezing stress tolerance: role of protein metabolism. *Annual review of Plant Physiology and Plant Molecular Biology* 41:187-223. <https://doi.org/10.1146/annurev.pp.41.060190.001155>
- Hao Q, Zhang L, Yang Y, Shan Z, Zhou X-a (2019). Genome-wide analysis of the WOX gene family and function exploration of *GmWOX18* in soybean. *Plants* 8:215. <https://doi.org/10.3390/plants8070215>
- Hsu PD, Lander ES, Zhang F (2014). Development and applications of CRISPR-Cas9 for genome engineering. *Cell* 157:1262-1278. <https://doi.org/10.1016/j.cell.2014.05.010>
- Huang Y, Xuan H, Yang C, Guo N, Wang H, Zhao J, Xing H (2019). *GmHsp90A2* is involved in soybean heat stress as a positive regulator. *Plant Science* 285:26-33. <https://doi.org/10.1016/j.plantsci.2019.04.016>
- Hughes MA, Dunn MA (1996). The molecular biology of plant acclimation to low temperature. *Journal of Experimental Botany* 47:291-305. <https://doi.org/10.1093/jxb/47.3.291>
- Hungria M, Franchini J, Campo R, Graham P (2005). The importance of nitrogen fixation to soybean cropping in South America. *Nitrogen Fixation in Agriculture, Forestry, Ecology, and the Environment* 25-42. https://doi.org/10.1007/1-4020-3544-6_3
- Ikeda T, Ohnishi S, Senda M, Miyoshi T, Ishimoto M, Kitamura K, Funatsuki H (2009). A novel major quantitative trait locus controlling seed development at low temperature in soybean (*Glycine max*). *Theoretical and Applied Genetics* 118:1477-1488. <https://doi.org/10.1007/s00122-009-0996-3>

- Jähne F, Balko C, Hahn V, Würschum T, Leiser WL (2019). Cold stress tolerance of soybeans during flowering: QTL mapping and efficient selection strategies under controlled conditions. *Plant Breeding* 138:708-720. <https://doi.org/10.1111/pbr.12734>
- Jia H, Jiang B, Wu C, Lu W, Hou W, Sun S, Yan H, Han T (2014). Maturity group classification and maturity locus genotyping of early-maturing soybean varieties from high-latitude cold regions. *PLoS One* 9:e94139. <https://doi.org/10.1371/journal.pone.0094139>
- Jiang H, Li C, Liu C, Zhang W, Qiu P, Li W, Gao Y, Hu G, Chen Q (2009). Genotype analysis and QTL mapping for tolerance to low temperature in germination by introgression lines in soybean. *Acta Agronomica Sinica* 35:1268-1273. <https://doi.org/10.3724/SP.J.1006.2009.01268>
- Kao P-H, Baiya S, Lai Z-Y, Huang C-M, Jhan L-H, Lin C-J, Lai Y-S, Kao C-F (2022). An advanced systems biology framework of feature engineering for cold tolerance genes discovery from integrated omics and non-omics data in soybean. *Frontiers in Plant Science* 13:1019709. <https://doi.org/10.3389/fpls.2022.1019709>
- Kaplan F, Kopka J, Sung DY, Zhao W, Popp M, Porat R, Guy CL (2007). Transcript and metabolite profiling during cold acclimation of *Arabidopsis* reveals an intricate relationship of cold-regulated gene expression with modifications in metabolite content. *The Plant Journal* 50:967-981. <https://doi.org/10.1111/j.1365-3113X.2007.03100.x>
- Kidokoro S, Shinozaki K, Yamaguchi-Shinozaki K (2022). Transcriptional regulatory network of plant cold-stress responses. *Trends in Plant Science*. 27:922-935. <https://doi.org/10.1016/j.tplants.2022.01.008>
- Kidokoro S, Watanabe K, Ohori T, Moriwaki T, Maruyama K, Mizoi J, ... Shinozaki K (2015). Soybean DREB1/CBF-type transcription factors function in heat and drought as well as cold stress-responsive gene expression. *The Plant Journal* 81:505-518. <https://doi.org/10.1111/tpj.12746>
- Kim KY, Park SW, Chung YS, Chung CH, Kim JI, Lee JH (2004). Molecular cloning of low-temperature-inducible ribosomal proteins from soybean. *Journal of Experimental Botany* 55:1153-1155. <https://doi.org/10.1093/jxb/erb125>
- Kuczyński J, Gracz-Bernaciak J, Twardowski T, Karłowski WM, Tyczewska A (2022). Cold stress-induced miRNA and degradome changes in four soybean varieties differing in chilling resistance. *Journal of Agronomy and Crop Science* 208:777-794. <https://doi.org/10.1111/jac.12557>
- Latef AAHA, Chaoxing H (2011). Arbuscular mycorrhizal influence on growth, photosynthetic pigments, osmotic adjustment and oxidative stress in tomato plants subjected to low temperature stress. *Acta Physiologiae Plantarum* 33:1217-1225. <https://doi.org/10.1007/s11738-010-0650-3>
- Levitt J (1980). Responses of Plants to Environmental Stress. Volume 1: Chilling, Freezing, and High Temperature Stresses. Academic Press.
- Liao Y, Zou H-F, Wei W, Hao Y-J, Tian A-G, Huang J, Liu Y-F, Zhang J-S, Chen S-Y (2008). Soybean *GmbZIP44*, *GmbZIP62* and *GmbZIP78* genes function as negative regulator of ABA signaling and confer salt and freezing tolerance in transgenic *Arabidopsis*. *Planta* 228:225-240. <https://doi.org/10.1007/s00425-008-0731-3>
- Liu K, Liu K (1997). Chemistry and nutritional value of soybean components. *Soybeans: Chemistry, Technology, and Utilization* 25-113. https://doi.org/10.1007/978-1-4615-1763-4_2
- Liu Q, Kasuga M, Sakuma Y, Abe H, Miura S, Yamaguchi-Shinozaki K, Shinozaki K (1998). Two transcription factors, *DREB1* and *DREB2*, with an EREBP/AP2 DNA binding domain separate two cellular signal transduction pathways in drought- and low-temperature-responsive gene expression, respectively, in *Arabidopsis*. *The Plant Cell* 10:1391-1406. <https://doi.org/10.1105/tpc.10.8.1391>
- Liu Z, Jia Y, Ding Y, Shi Y, Li Z, Guo Y, Gong Z, Yang S (2017). Plasma membrane CRPK1-mediated phosphorylation of 14-3-3 proteins induces their nuclear import to fine-tune CBF signaling during cold response. *Molecular Cell* 66:117-128. <https://doi.org/10.1016/j.molcel.2017.02.016>
- Lu W (2011). The soybean cultivate technique in northern area of Heilongjiang province. *China Seed Industry* 59-60.
- Luo X, Bai X, Zhu D, Li Y, Ji W, Cai H, Wu J, Liu B, Zhu Y (2012). *GsZFPI*, a new Cys2/His2-type zinc-finger protein, is a positive regulator of plant tolerance to cold and drought stress. *Planta* 235:1141-1155. <https://doi.org/10.1007/s00425-011-1563-0>
- Manan S, Zhao J (2020). Role of Glycine max ABSCISIC ACID INSENSITIVE 3 (*GmABI3*) in lipid biosynthesis and stress tolerance in soybean. *Functional Plant Biology* 48:171-179. <https://doi.org/10.1071/FP19260>

- Maruyama K, Todaka D, Mizoi J, Yoshida T, Kidokoro S, Matsukura S, Takasaki H, Sakurai T, Yamamoto YY, Yoshiwara K (2012). Identification of cis-acting promoter elements in cold-and dehydration-induced transcriptional pathways in *Arabidopsis*, rice, and soybean. *DNA Research* 19:37-49. <https://doi.org/10.1093/dnares/dsr040>
- Naveed M, Javed J, Waheed U, Sajid M, Ijaz M, Sehar A, Attique M, Javed S (2022). The role of CRISPR/Cas9 for genetic advancement of soybean: a review. *Asian Journal of Biotechnology and Genetic Engineering* 5:75-88.
- Nedoluzhko A, Dorokhov D (2007). Study of the biosafety of genetically modified soybean in the center of its origin and diversity in the Far East of the Russian Federation. *Cytology and Genetics* 41:190-198. <https://doi.org/10.3103/S0095452707030097>
- Nouri M-Z, Toorchi M, Komatsu S (2011). Proteomics approach for identifying abiotic stress responsive proteins in soybean. *Soybean-Molecular Aspects of Breeding* 187-214. <https://doi.org/10.5772/15518>
- Nuruzzaman M, Sharoni AM, Kikuchi S (2013). Roles of NAC transcription factors in the regulation of biotic and abiotic stress responses in plants. *Frontiers in Microbiology* 4:248. <https://doi.org/10.3389/fmicb.2013.00248>
- Ohnishi S, Miyoshi T, Shirai S (2010). Low temperature stress at different flower developmental stages affects pollen development, pollination, and pod set in soybean. *Environmental and Experimental Botany* 69:56-62. <https://doi.org/10.1016/j.envexpbot.2010.02.007>
- Ormancey M, Thuleau P, Mazars C, Cotelle V (2017). CDPKs and 14-3-3 proteins: emerging duo in signaling. *Trends in Plant Science* 22:263-272. <https://doi.org/10.1016/j.tplants.2016.11.007>
- Pearce RS (2001). Plant freezing and damage. *Annals of Botany* 87:417-424. <https://doi.org/10.1006/anbo.2000.1352>
- Rahman SU, McCoy E, Raza G, Ali Z, Mansoor S, Amin I (2022). Improvement of soybean; A way forward transition from genetic engineering to new plant breeding technologies. *Molecular Biotechnology* 65:162-180. <https://doi.org/10.1007/s12033-022-00456-6>
- Robison J, Arora N, Yamasaki Y, Saito M, Boone J, Blacklock B, Randall S (2017). *Glycine max* and *Glycine soja* are capable of cold acclimation. *Journal of Agronomy and Crop Science* 203:553-561. <https://doi.org/10.1111/jac.12219>
- Robison JD, Yamasaki Y, Randall SK (2019). The ethylene signaling pathway negatively impacts CBF/DREB-regulated cold response in soybean (*Glycine max*). *Frontiers in Plant Science* 10:121. <https://doi.org/10.3389/fpls.2019.00121>
- Román Á, Andreu V, Hernández ML, Lagunas B, Picorel R, Martínez-Rivas JM, Alfonso M (2012). Contribution of the different omega-3 fatty acid desaturase genes to the cold response in soybean. *Journal of Experimental Botany* 63:4973-4982. <https://doi.org/10.1093/jxb/ers174>
- Ruelland E, Vaultier M-N, Zachowski A, Hury V (2009). Cold signalling and cold acclimation in plants. *Advances in Botanical Research* 49:35-150. [https://doi.org/10.1016/S0065-2296\(08\)00602-2](https://doi.org/10.1016/S0065-2296(08)00602-2)
- Sanghera SG, Wani HS, Hussain W, Singh BN (2011). Engineering cold stress tolerance in crop plants. *Current Genomics* 12(1):30. <https://doi.org/10.2174/138920211794520178>
- Schmutz J, Cannon SB, Schlueter J, Ma J, Mitros T, Nelson W, Hyten DL, Song Q, Thelen JJ, Cheng J (2010). Genome sequence of the palaeopolyploid soybean. *Nature* 463:178-183. <https://doi.org/10.1038/nature08670>
- Siddique S (2022). Role of CRISPR/Cas9 in soybean (*Glycine max* L.) quality improvement. *Recent Advances in Research and Applications*. <https://doi.org/10.5772/intechopen.102812>
- Song L, Prince S, Valliyodan B, Joshi T, Maldonado dos Santos JV, Wang J, Lin L, Wan J, Wang Y, Xu D (2016). Genome-wide transcriptome analysis of soybean primary root under varying water-deficit conditions. *BMC Genomics* 17:1-17. <https://doi.org/10.1186/s12864-016-2378-y>
- Staniak M, Stępień-Warda A, Czopek K, Kocira A, Baca E (2021). Seeds quality and quantity of soybean [*Glycine max* (L.) merr.] cultivars in response to cold stress. *Agronomy* 11:520. <https://doi.org/10.3390/agronomy11030520>
- Su L-T, Li J-W, Liu D-Q, Zhai Y, Zhang H-J, Li X-W, Zhang Q-L, Wang Y, Wang Q-Y (2014). A novel MYB transcription factor, *GmMYB1*, from soybean confers drought and cold tolerance in *Arabidopsis thaliana*. *Gene* 538:46-55. <https://doi.org/10.1016/j.gene.2014.01.024>
- Sun M, Jing Y, Wang X, Zhang Y, Zhang Y, Ai J, Li J, Jin L, Li W, Li Y (2020). *Gma-miR1508a* confers dwarfing, cold tolerance, and drought sensitivity in soybean. *Molecular Breeding* 40:1-13. <https://doi.org/10.1007/s11032-020-01116-w>
- Swigonska S, Weidner S (2013). Proteomic analysis of response to long-term continuous stress in roots of germinating soybean seeds. *Journal of Plant Physiology* 170:470-479. <https://doi.org/10.1016/j.jplph.2012.11.020>

- Tian X, Liu Y, Huang Z, Duan H, Tong J, He X, Gu W, Ma H, Xiao L (2015). Comparative proteomic analysis of seedling leaves of cold-tolerant and-sensitive spring soybean cultivars. *Molecular Biology Reports* 42:581-601. <https://doi.org/10.1007/s11033-014-3803-4>
- Tsegaw M, Zegeye WA, Jiang B, Sun S, Yuan S, Han T, Wu T (2023). Progress and prospects of the molecular basis of soybean cold tolerance. *Plants* 12:459. <https://doi.org/10.3390/plants12030459>
- Wang T, Xun H, Wang W, Ding X, Tian H, Hussain S, Dong Q, Li Y, Cheng Y, Wang C (2021). Mutation of *GmAITR* genes by CRISPR/Cas9 genome editing results in enhanced salinity stress tolerance in soybean. *Frontiers in Plant Science* 2752. <https://doi.org/10.3389/fpls.2021.779598>
- Wang X, Chang X, Jing Y, Zhao J, Fang Q, Sun M, Zhang Y, Li W, Li Y (2020). Identification and functional prediction of soybean CircRNAs involved in low-temperature responses. *Journal of Plant Physiology* 250:153188. <https://doi.org/10.1016/j.jplph.2020.153188>
- Wu C, Mozzoni LA, Moseley D, Hummer W, Ye H, Chen P, Shannon G, Nguyen H (2020). Genome-wide association mapping of flooding tolerance in soybean. *Molecular Breeding* 40:1-14. <https://doi.org/10.1007/s11032-019-1086-0>
- Yamaguchi N, Hagihara S, Hirai D (2019). Field assessment of a major QTL associated with tolerance to cold-induced seed coat discoloration in soybean. *Breeding Science* 69:521-528. <https://doi.org/10.1270/jsbbs.19024>
- Yamaguchi N, Taguchi-Shiobara F, Sayama T, Miyoshi T, Kawasaki M, Ishimoto M, Senda M (2015). Quantitative trait loci associated with tolerance to seed cracking under chilling temperatures in soybean. *Crop Science* 55:2100-2107. <https://doi.org/10.2135/cropsci2015.02.0081>
- Yang X, Kim MY, Ha J, Lee S-H (2019). Overexpression of the soybean NAC gene *GmNAC109* increases lateral root formation and abiotic stress tolerance in transgenic *Arabidopsis* plants. *Frontiers in Plant Science* 10:1036. <https://doi.org/10.3389/fpls.2019.01036>
- Zhang D, Li Z, Li J-F (2016). Targeted gene manipulation in plants using the CRISPR/Cas technology. *Journal of Genetics and Genomics* 43:251-262. <https://doi.org/10.1016/j.jgg.2016.03.001>
- Zhang S, Wang Y, Li K, Zou Y, Chen L, Li X (2014). Identification of cold-responsive miRNAs and their target genes in nitrogen-fixing nodules of soybean. *International Journal of Molecular Sciences* 15:13596-13614. <https://doi.org/10.3390/ijms150813596>
- Zhang W-B, Jiang H-w, Qiu P-C, Liu C-Y, Chen F-L, Xin D-W, Li C-D, Hu G-H, Chen Q-S (2012a). Genetic overlap of QTL associated with low-temperature tolerance at germination and seedling stage using BILs in soybean. *Canadian Journal of Plant Science* 92:1381-1388.
- Zhang WB, Qiu PC, Jiang HW, Liu CY, Xin DW, Li CD, Hu GH, Chen QS (2012b). Dissection of genetic overlap of drought and low-temperature tolerance QTLs at the germination stage using backcross introgression lines in soybean. *Molecular Biology Reports* 39:6087-6094. <https://doi.org/10.1007/s11033-011-1423-9>
- Zheng L, Xie J, Sun X, Zheng Y, Meng F, Fan X, Li G, Zhang Y, Wang M, Zhou R (2023). QTL mapping and candidate gene analysis of low-temperature tolerance at the germination stage of soybean. *Europe PMC*. <https://doi.org/10.21203/rs.3.rs-2480293/v1>
- Zhong X, Hong W, Shu Y, Li J, Liu L, Chen X, Islam F, Zhou W, Tang G (2022). CRISPR/Cas9 mediated gene-editing of *GmHdz4* transcription factor enhances drought tolerance in soybean (*Glycine max* [L.] Merr.). *Frontiers in Plant Science* 13:988505. <https://doi.org/10.3389/fpls.2022.988505>



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