 DOI: 10.19579/j.cnki.plant – d.p.2024.03.012

Advances in Molecular Regulatory Mechanisms of Fruit Trees under Low Temperature Stress

Huan LIU, Lijuan GAO, Minghui JI, Longfei LI, Jintao XU, Baofeng HAO *

Changli Institute of Pomology, Hebei Academy of Agriculture and Forestry Sciences, Changli 066600, China

Abstract The most recent research findings on the tolerance of fruit trees to cold stress are reviewed from a molecular perspective, including the perception and transduction of low temperature calcium signaling, CBF-dependent molecular regulatory mechanisms, non-CBF-dependent molecular regulatory mechanisms, and so forth. The objective is to provide a reference basis for further improving the cold resistance of fruit trees and cultivating new varieties of hardy plants.

Key words Low temperature stress; Fruit trees; Growth and development; Molecular mechanism; Research progress

1 Introduction

A variety of abiotic stresses affect the growth and development of fruit trees, including cold damage, drought, and salt stress, *etc.* Among these, low temperature stress represents a significant abiotic stressor, affecting the growth, development, yield, and fruit quality of fruit trees. Furthermore, it restricts the geographical distribution of numerous fruit tree resources[1]. Low temperature stress can be divided into two categories: chilling stress (0 – 16 ℃) and freezing stress (below 0 ℃). In general, the ability of plants to adapt to low temperatures above freezing is referred to as chilling resistance, while the ability to adapt to low temperatures below freezing is known as freezing resistance or cold resistance[2]. Fruit trees have evolved a multitude of intricate mechanisms to resist the detrimental effects of chilling stress. One such mechanism is cold acclimatization, a process by which plants adapt to low temperatures, gaining higher freezing tolerance after prior exposure to non-lethal low temperatures[3-4]. At the physiological level, low temperature not only destroys the structure of the plasma membrane of the fruit tree, reducing its ability of photosynthesis, but also accumulates reactive oxygen species (ROS) in excess, which causes the growth and development of the fruit tree to be slowed down or even stagnant[5-7]. At the molecular level, fruit trees have evolved a complex mechanism to resist chilling stress. Following the exposure of fruit trees to low temperatures, a low temperature signal is initially perceived by the cell membrane, subsequently transmitted downstream via the corresponding low temperature signal transduction pathway. This pathway combines with relevant transcription factors to initiate a cascade of transcriptional regulatory processes, ultimately regulating the expression of target genes, thereby enhancing the plants' chilling and cold stress tolerance[8].

The paper presents a summary of recent research on the molecular regulatory mechanisms of fruit trees in response to low temperature stress. The objective is to provide a theoretical basis for further research on the response mechanisms of fruit trees to low temperature stress and to establish a reference basis for the cultivation of new varieties of fruit trees with cold resistance.

2 Perception of cold signals

2.1 Perception of cold signals by cell membrane The reduction of cell membrane fluidity after low temperature stress is a well-documented mechanism of low temperature perception. It has been demonstrated that the expression of cold-responsive (*COR*) genes is induced by membrane hardening at 25 ℃, whereas at 4 ℃, it is inhibited by membrane fluidization. The fluidity of the plasma membrane is related to the proportion of desaturated fatty acids. Fatty acid desaturase 2 (FAD2) encodes an oleic acid desaturase that is essential for membrane fluidity. Mutations in FAD2 disrupt some of the physiological responses of plants to chilling stress, including the number of leaves and the length of hypocotyls[9].

2.2 Perception and transduction of low temperature calcium signaling Calcium (Ca^{2+}) is a crucial second messenger for plants to respond to environmental changes. Following low temperature treatment, the cytoplasm Ca^{2+} concentration in plants increases rapidly through Ca^{2+} channels. These Ca^{2+} signals then convert external signals into a variety of intracellular biochemical responses, which represents one of the earliest discoveries of cold signaling[10-11]. It is also the case that Ca^{2+} receptors exist in plants. The main Ca^{2+} receptors include calmodulin (CaMs), calcium-dependent protein kinases (CDPKs), calcineurin B-like proteins (CBLs), and reciprocal interaction protein kinases (CIPKs). Relay factors in Ca^{2+} receptors can bind Ca^{2+} and alter the conformation of different proteins, thereby regulating the expression of target genes. Conversely, response factors can transmit cold signals to downstream target genes, resulting in alterations in the expression of target genes[12-15]. Ca^{2+} transmits low tempera-

Received: January 20, 2024 Accepted: May 12, 2024

Supported by Basic Research Fund of Hebei Academy of Agriculture and Forestry Sciences (2024020202); "Three-Three-Three" Talent Project of Hebei Province (C20231157); Science and Technology Innovation Project of Hebei Academy of Agriculture and Forestry Sciences (2022KJCXZX-CGS-7); Hebei Agricultural Industry Research System (HBCT2024170406); Key Research and Development Program of Hebei Province (21326308D-1-2).

* Corresponding author. E-mail: hao1973@163.com

ture signaling through the activation of CAM, which in turn mediates a range of downstream pathways, including development, resistance to pathogens, and responses to abiotic stresses such as temperature, drought, salinity, and light. Calcium-related signal transduction is typically mediated by CDPKs.

3 CBF-dependent molecular regulatory mechanisms

C-repeat-binding factors (CBFs) are found in a wide range of herbaceous and woody plants[14]. In *Arabidopsis thaliana*, the *CBF* genes are distributed in tandem on chromosome 4, with *CBF1* (*DREB1B*), *CBF2* (*DREB1C*), and *CBF3* (*DREB1A*) forming a gene cluster arrangement. The CBF pathway represents the most critical regulatory pathway for plants to cope with low temperature stress. The ICE-CBF-COR signaling cascade pathway is the most dominant low temperature signaling pathway[15-16].

The ICE-CBF-COR pathway is induced by low temperature stress to activate the expression of downstream genes that encode osmoregulatory substances[17]. The upstream component of this cascade, ICE1, binds to the promoter region of the *CBF* gene, thereby activating its expression. The CBF protein, which is encoded by the upstream component of this cascade, binds to the *cis*-acting element CRT/DRE in the downstream promoter region. This binding facilitates the expression of cold resistance-related genes, which are known as *CORs*, thereby mediating plant cold resistance. The homologue of ICE1, ICE2, exhibits a high degree of sequence identity with ICE1, encoding the same bHLH structural domain sequence. Both ICE1 and ICE2 are significant regulators of stomatal formation and play pivotal roles in regulating cold response processes[18]. The most prominent downstream responsive gene in ICE-CBF-COR signaling is the *COR* gene[19]. The CBF, activated by the upstream *ICE* gene, activates the expression of the downstream *COR* gene and enhances the low temperature resistance of plants[20]. The *COR* gene, which is regulated by CBF, encodes a hydrophilic polypeptide that forms a secondary structure to stabilize the cellular lipid membrane. It was demonstrated that following the overexpression of VvCBF4 in grapevine, the transgenic plants exhibited not only enhanced cold tolerance but also a reduction in plant height[21]. The expression of the *CBF* gene under cold treatment conditions demonstrated that *PtCBF* could be induced to be expressed by low temperature stress. However, the response of *PtCBF* to low temperature differed in different tissues and organs. It is possible that there may be a difference in the transcriptional regulation level of *PtCBF* and navel orange *CsCBF* genes[22].

4 Non-CBF-dependent molecular regulatory mechanisms

It has been demonstrated that specific genes are not contingent upon the CBF pathway. The introduction or removal of their functionality does not influence the expression of *CBF* gene functionality; however, it can result in a modification of the freezing tolerance of the plant. For instance, the expression of *HOS9*, a constitutively expressed gene, induced by low temperatures, results in significantly higher transcript levels of *RD29A* and other *COR* genes than in the wild type. In contrast, in the mutant hos9-1, the expression of *CBF* genes is unaffected, yet they still exhibit sensitivity to freezing stress[23].

5 Interaction between two signaling pathways

It has been demonstrated that *COR* genes in *A. thaliana* can be induced by CBF-dependent and non-CBF-dependent signaling pathways, with the capacity to regulate the CBF-dependent pathway of cold stress through CBF/DREB1. Nevertheless, subsequent research has revealed that the non-CBF-dependent pathway is not entirely independent and that there is an interaction between them[24]. Previous studies have demonstrated that abscisis acid (ABA) also activates CRT-promoting factor *cis*-acting elements. ABA has been shown to induce the AtCBF4/DREB1D transcription factor in preference to low temperature stress[25]. Studies on *A. thaliana* have demonstrated that ABA treatment results in a sufficient increase in CBF protein expression to induce the expression of cold-regulated genes. This suggests that the accumulation of CBF1-3 transcript levels induced by ABA can trigger the expression of CRT components[25]. Nevertheless, the interaction of ABA-dependent and non-ABA-dependent signaling pathways remains to be elucidated.

6 Conclusions and prospects

It is evident that the low temperature environment impedes the growth and development of fruit trees. The study of the molecular regulatory mechanisms of fruit trees in response to low temperature stress is of significant theoretical and practical importance for the future selection and breeding of excellent hardy varieties. It has been demonstrated that substantial alterations in cell membrane permeability, osmotic pressure regulating substances, and antioxidant systems occur in fruit trees when subjected to low temperature stress. The molecular regulatory mechanisms are divided into two categories: CBF-dependent and non-CBF-dependent regulatory pathways. Among the cold signaling pathways, the ICE1-CBF-COR signaling pathway is the most widely studied and considered the most critical regulatory pathway. Nevertheless, there is a paucity of research investigating the interactions between physiological, biochemical, and molecular mechanisms and regulatory mechanisms of fruit trees under low temperature stress. Consequently, future research will prioritize comprehensive and systematic studies, such as the integration of transcriptomics, proteomics, and metabolomics to elucidate the intricate response mechanisms of low temperature stress in fruit trees. This approach promises to provide a more comprehensive understanding of the response to low temperature stress in fruit trees.

References

[1] WANG F, WANG Q, ZHAO XY. Research progress of phenotype and

physiological response mechanism of plants under low temperature stress[J]. Molecular Plant Breeding, 2019, 17(15): 5144 – 5153. (in Chinese).

[2] LYONS JM. Chilling injury in plants[J]. Annual Review of Plant Physiology, 1973, 24(1): 445 – 466.

[3] GUY CL. Cold acclimation and freezing stress tolerance: Role of protein metabolism[J]. Annual Review of Plant Physiology and Plant Molecular Biology, 1990(41): 187 – 223.

[4] THOMASHOW MF. Plant cold acclimation: Freezing tolerance genes and regulatory mechanisms[J]. Annual Review of Plant Physiology and Plant Molecular Biology, 1999(50): 571 – 599.

[5] AGURLA S, GAHIR S, MUNEMASA S, *et al.* Mechanism of stomatal closure in plants exposed to drought and cold stress[J]. Advances in Experimental Medicine and Biology, 2018(1081): 215 – 232.

[6] SONG Y, FENG L, ALYAFEI M, *et al.* Function of chloroplasts in plant stress responses[J]. Insternational Journal of Molecular Sciences, 2021, 22(24): 13464.

[7] YOU J, CHAN ZL. ROS regulation during abioticstess responses in crop plants[J]. Frontiers in Plant Science, 2015(6): 1092.

[8] WANG F, LI YX, JIANG LP, *et al.* Research advances in molecular response mechanisms of plants under low temperature stress[J]. World Forestry Research, 2020, 33(6): 15 – 21. (in Chinese).

[9] MARTINIÈRE A, SHVEDUNOVA M, THOMSON AJ, *et al.* Homeostasis of plasma membrane viscosity in fluctuating temperatures[J]. New Phytologist, 2011, 192(2): 328 – 337.

[10] KNIGHT MR, KNIGHT H. Low-temperature perception leading to gene expression and cold tolerance in higher plants[J]. New Phytologist, 2012(195): 737 – 751.

[11] LIU Q, DING Y, SHI Y, *et al.* The calcium transporter ANNEXIN1 mediates coldinduced calcium signaling and freezing tolerance in plants[J]. The EMBO Journal, 2021, 40(2): e104559.

[12] KLIMECKA M, GRAZYNA M. Structure and functions of plant calcium-dependent protein kinases[J]. Acta Biochimica Polonica, 2007, 54(2): 219 – 233.

[13] REDDY VS, REDDY ASN. Proteomics of calcium-signaling components in plants[J]. Phytochemistry, 2004, 65(12): 1745 – 1776.

[14] GUAN Y, LIU S, WU W, *et al.* Genome-wide identiffcation and cold stress-induced expression analysis of the *CBF* gene family in *Liriodendron chinense*[J]. Journal of Forest Research, 2021(32): 2531 – 2543.

[15] ZHOU MQ, SHEN C, WU LH, *et al.* CBF-dependentsingaling pathway: A key responder to low temperature stress in plants[J]. Critical Reviews in Biotechnology, 2011, 31(2): 186 – 192.

[16] WANG XP, SONG QP, LIU Y, *et al.* Correction to: The network centered on ICES play roles in plant cold tolerance, growth and development[J]. Planta, 2022, 255(4): 89.

[17] ZHANG F, JIANG Y, BAI LP, *et al.* The ICE-CBF-COR pathway in cold acclimation and AFPs in plants[J]. Middle East Journal of Scientific Research, 2011(8): 493 – 498.

[18] KURBIDAEVA A, EZHOVA T, NOVOKRESHENOVA M. *Arabidopsis thaliana ICE2* gene: Phylogeny, structural evolution and functional diversification from ICE1[J]. Plant Science, 2014(229): 10 – 22.

[19] HWARARI D, GUAN Y, AHMAD B, *et al.* ICE-CBF-COR signaling cascade and its regulation in plants responding to cold stress[J]. International Journal of Molecular Sciences, 2022, 23(3): 1549.

[20] LIU Y, DANG P, LIU L, *et al.* Cold acclimation by the CBF-COR pathway in a changing climate: Lessons from *Arabidopsis thaliana*[J]. Plant Cell Reports, 2019(5): 511 – 519.

[21] TILLETT RL, WHEATLEY MD, TATTERSALL EAR, *et al.* The *Vitis vinifera* C-repeat binding protein 4 (VvCBF4) transcriptional factor enhances freezing tolerance in wine grape[J]. Plant Biotechnology Journal, 2012, 10(1): 105 – 124.

[22] HE LG, JIANG YC, XU M, *et al.* Construction of expression vector of trifoliate orange driven by CBF promoter with partial deletion[J]. Hubei Agricultural Sciences, 2013, 52(21): 5228 – 5232. (in Chinese).

[23] ZHU JH, SHI HZ, LEE BH, *et al.* An arobidopsis homeodomain transcription factor gene, *HOS9*, mediates cold tolerance through a CBF-independent pathway[J]. Proceedings of the National Academy of Sciences, 101(26): 9873 – 9878.

[24] ZHU Q, ZHANG J, GAO X, *et al.* The *Arabidopsis* AP2/ERF transcription factor RAP2.6 participates in ABA, salt and osmotic stress responses[J]. Gene, 2010, 457(1 – 2): 1 – 12.

[25] KNIGHT H, ZARKA DG, OKAMOTO H, *et al.* Abscisic acid induces *CBF* gene transcription and subsequent induction of cold-regulated genes via the CRT promoter element[J]. Plant Physiology, 2004(3): 1710 – 1717.

(From page 11)

[21] HU XX. Traceability, integrated fragment genetic diversity and transcriptome analysis of sugarcane baculoviruses[D]. Nanning: Guangxi University, 2020. (in Chinese).

[22] KARUPPAIAH R, VISWANATHAN R, KUMAR VG. Genetic diversity of sugarcane bacilliform virus isolates infecting *Saccharum* spp. in India[J]. Virus Genes, 2013, 46(3): 505 – 516.

[23] HAREGU S, KIDANEMARIAM D, ABRAHAM A. Molecular identification and characterization of badnaviruses infecting sugarcane in Ethiopia[J]. Acta Virologica, 2022, 66(1): 3 – 10.

[24] KRISHNA GV, KUMAR VM, VARMA PK, *et al.* Detection of sugarcane bacilliform virus in diseased sugarcane plants in Andhra Pradesh, India by serological and molecular methods[J]. Brazilian Journal of Microbiology, 2023, 54(3): 1399 – 1409.

[25] LI WF, HUANG YK, JIANG DM, *et al.* Detection of sugarcane bacilliform virus isolate and its influence on yield and quality of cane in Yunnan[J]. Acta Phytopathologica Sinica, 2010, 40(6): 651 – 654. (in Chinese).

[26] COMSTOCK J C, LOCKHART BEL. Effect of sugarcane bacilliform virus on biomass production of three sugarcane cultivars[J]. Sugar Cane, 1996: 12 – 15.

[27] VAMSI KG, MANOJ KV, KISHORE VP, *et al.* Identification of resistance to sugarcane mosaic virus, sugarcane streak mosaic virus, and sugarcane bacilliform virus in new elite sugarcane accessions in India[J]. Frontiers in Microbiology. 2023(14): 1276932.

[28] WU NN. Detection and genotype identification of sugarcane bacilliform virus in sugarcane regions of China[D]. Nanjing: Nanjing Agricultural University, 2022. (in Chinese).

[29] XIA BS. Occurrence of sugarcane baculoviruses in sugarcane areas of China and establishment and application of qPCR and RCA detection methods[D]. Haikou: Hainan University, 2023. (in Chinese).

[30] HEMA M, SAVITHRI HS, SREENIVASULU P. Comparison of direct binding polymerase chain reaction with recombinant coat protein antibody based dot-blot immunobinding assay and immunocapture-reverse transcription-polymerase chain reaction for the detection of sugarcane streak mosaic virus causing mosaic disease of sugarcane in India[J]. Current Science: A Fortnightly Journal of Research, 2003, 85(12): 1774 – 1777.

[31] SINGH D, TEWARI AK, RAO GP, *et al.* RT-PCR/PCR analysis detected mixed infection of DNA and RNA viruses infecting sugarcane crops in different states of India[J]. Sugar Tech, 2009, 11(4): 373 – 380.

[32] SUN SR. Molecular genetic variation and evolution mechanism of sugarcane bacilliform virus populations[D]. Fuzhou: Fujian Agriculture and Forestry University, 2016. (in Chinese).