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Can cold-resistant varieties of tropical fruits be developed through genetic engineering?

Mingyu Jiang

Hangzhou Dipont School of Arts and Science, Hangzhou, China

q13073678006@163.com

Abstract. Tropical fruits are highly sought after for their unique flavor and nutritional value, but their cultivation is limited by warm climatic conditions. With global climate change and frequent extreme weather events, they need to breed cold-resistant tropical fruit varieties that are becoming increasingly urgent. Gene editing technologies, especially the Clustered Regularly Interspaced Short Palindromic Repeats-CRISPR-associated protein 9 (CRISPR-Cas9) system, provide powerful tools for precise improvement of crop traits. However, due to challenges such as genome complexity and ecological impacts, few cases of gene editing in tropical plants have been grown in agriculture, and most of the studies are still in the laboratory or field trial stage. This topic investigates the mechanism of action during the response to low temperature stress and explores how these mechanisms can be utilized to better improve plant varieties, as well as how to accurately edit the genes related to cold tolerance discovered from the mechanisms. We are optimistic about the future development of new varieties that retain tropical characteristics and are cold-tolerant, and with the advancement of gene function analysis and editing tools, these new varieties are expected to expand their cultivation area and economic valuation.

Keywords: tropical fruits, cold-resistant, gene editing technologies

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1. Introduction

Tropical fruits such as mango, pineapple and banana are important cash crops and sources of nutrition for worldwide. However, these fruits are sensitive to low temperatures and are susceptible to cold damage, resulting in reduced yields and poor quality. Most of the plants originating from temperate regions can be domesticated at low temperatures to improve cold resistance and are cold-resisted crops, such as wheat and oilseed rape. Plants originating from tropical and subtropical regions lack the ability of cold domestication and are extremely sensitive to low temperatures, belonging to non-cold-domesticated crops, such as rice, maize and so on.

With the rapid development of molecular biology and biotechnology, as well as the model plant Arabidopsis thaliana and other crops cold resistance mechanism research in-depth, people on the cold resistance mechanism of plants, gradually by the physiological and biochemical level to the more microscopic molecular level. At the molecular level, plants sense cold stress through multiple mechanisms, including changes in cell membrane fluidity, calcium ion signalling, reactive oxygen species accumulation, cytoskeletal reorganisation, changes in protein conformation and changes in metabolite levels. These sensing mechanisms are transmitted through complex signalling networks such as Inducer of CBF Expression 1-C-repeat Binding Factor (ICE1-CBF) pathway, Mitogen-Activated Protein Kinase (MAPK) cascade response, which ultimately activate the expression of cold-resistant genes and help plants to adapt to the low-temperature environment.

With the further advancement of the identification of related genes and their functional studies, the understanding of the regulatory mechanisms of low-temperature tolerance in tropical fruits has been gradually deepened. Firstly, we need to know that plant resistance to low temperature is a complex process, because different species respond to low temperature by different mechanisms. A single indicator is difficult to comprehensively and accurately evaluate the strength of resistance, so it is necessary to increase the correlation between the physiological response indicators and cold tolerance in plants under low-temperature stress, and at the same time, for the growth of different species, physiological characteristics to select the appropriate indicators. At the same time, the selection of suitable indicators (transcription factor CBF; endogenous hormones such as dehydrin; osmoregulators such as proline and soluble sugar) for the growth and physiological characteristics of different species can effectively evaluate the cold hardiness of different species, and provide a basis for the selection and breeding of germplasm resources of cold-resistanted plants. Moreover, in improving the cold resistance of plants, the expression of related genes such as transcription factor CBF can also be altered by gene editing technology. Thus, changing the resistance ability of plants and providing a new way to breed cold-resistant tropical fruit varieties.

2. Literature review

2.1. Molecular responses to cold stress in model plants

Therefore, the CBF/DREB pathway is one of the low-temperature signalling pathways in cold stress conditions, and the model plant Arabidopsis thaliana activates CBF expression mainly through ICE1-CBF pathway, the MAPK cascade. The CBF gene is expressed when it is activated, further inducing the expression of the downstream Cold-Responsive (COR) genes, hence cultivating the freezing tolerance of plants. It was shown that ICE1 regulates around 40% of CBFs and 46% of cold-responsive transcription factors. ICE1 was proved to be important in adversity stresses for plant regulatory signaling.

When plants perceive temperature stress signals, they will launch complex cold stress-specific signal transduction pathways. Low temperatures cause cell membrane lipids to change from liquid to gas state. That will lead the fluidity changing of cell membrane. Specific changes in cell membrane fluidity are perceived by membrane-localized proteins. Then, it causes changes in their conformation and activity, and mediate the low-temperature stress response by that.

Chilling Tolerance Divergence 1 (COLD1) is a protein that is found in plants exposed to low temperatures within the cellular membranes. It functions as a cell membrane receptor and can form a complex with RICE G-PROTEIN α SUBUNIT 1 (RGA1) [1]. For the cold stress situation, the COLD1-RGA1 dyad will bind a complex.

It may also be largely responsible for the cold-triggered inward current of intracellular Ca^{2+} entry. Furthermore, Ca^{2+} might control the activity of cold- and desiccation-inducible Chilling-Responsive Receptor-Like Kinase (CRLK). CRLK is a type of plasma membrane receptor-like kinase involved in Ca^{2+}/calmodulin signaling processes. It can be up-regulated by its activity and thus in turn affect the COR and cold adaptation processes positively. CRLK1 and CRLK2 cooperate with Mitogen-Activated Protein Kinase Kinase Kinase 1 (MEKK1) cascade, preferably in the upstream of Mitogen-Activated Protein Kinase Kinase 2-Mitogen-Activated Protein Kinase 4 (MKK2-MAPK4) pathway. They are also positively regulated during cold adaptation by CBF through enhancing the expression of CBF [2].

Mitogen-Activated Protein Kinase (MAPK) pathway is involved in this signaling. It is composed of MAPK Kinase (MAPKK), MEKK1, and MPK4 which are also members of this pathway. The ICE1-MEK1-MPK4 cascade increases the level of CBF and CBF-mediated freezing tolerance by antagonizing the MKK4/5-MAPK3 pathways. MPK3 will bind ICE1 as the latter gets phosphorylated at the conserved Ser/Thr residues. This cascade of phosphorylation will therefore result in fast degradation of ICE1 and compromised transcriptional activity of ICE1 as well. Hence, from one of its functions, it may negatively regulate the CBF expression and freezing tolerance.

ICE, a family of proteins, has a basic Helix-Loop-Helix (bHLH) motif in all of them. This mechanism may be through regulation of gene expression controlling the regulator genes through enhancers [3]. The possible ICE target in the upstream is referred to as the ICE response. They fall within the category C-terminal bHLH binding domains. In the ICE protein, the CANNTG CBF3 is recognized as the canonical MYC cis-elements within its promoter [4]. Thereafter, the initiation of the signaling cascade that leads to the activation of CBF regulator may be observed. Adding on, ICE gene also have two homologous genes termed ICE1 and ICE2. ICE1 generally displays the processes that control ICE1 at the post-translational level of these proteins through Post-Translational Modifications (PTMs). The presence of different kinds of PTMs therefore distinguishes the ICE1 behavior during cold stress conditions. The CBF may be induced via the ICE signaling pathway, which furthermore may contribute to the control of CBF activity. CBF is one of the Dehydration-Responsive Elements (DRE). They are regarded as the key transcription factor involved in the cold acclimation through regulating gene expression of down-stream cold-responsive gene. The CBF gene is quickly activated by cold stress, which in turn prepares the pathway to activate the expression of downstream Cold-Responsive Genes (COR) and enhances freezing tolerance in plants.

The genes, which C-repeat Binding Factor (CBF) binding enhances their transcription, feature at least one C-repeat (CRT)/Dehydration-Responsive Element (DRE) cis-element in the promoters of these genes that is denoted with CCGAC (COR genes). The best results of this part suggest that regulating ICE-CBF-COR pathway can increase the cold resistance [5]. If most factors are supportive and helpful for the improvement of plant tolerance against cold then we will try to enrich CBF pathway as an example of it, the more positive adaptations should be obtained [6].

High Expression of Osmotically Responsive Genes 1, or HOS1, is an E3 ubiquitin ligase that is involved in protein degradation. It has an RNG-finger motif. Data on the interaction of HOS1 with other protein partners can be used to support functional research in Arabidopsis thaliana in which HOS1 is over-expressed, which reduces the level of GFP-ICE1 protein. In other words, we can conclude that its target gene is ICE1. Well, on the contrary, both of the genes related to responding stress appeared to be more expressed with the HOS1 phenotype mutant. From these findings we can conclude that HOS1 is involved not only with the degradation of ICE1 but also in negatively modulating the ICE1 target genes in a way.

For the last step, the COR gene expression is producing varieties of products which repair damaged membranes and proteins and create better freezing tolerance: COR15A and COR15B, two proteins that prevent cells from crystallizing in the cold by forming a lipid barrier around the membrane and protecting the protein from denaturation. Late Embryogenesis Abundant (LEA) proteins such as LEA14, which inhibits cellular dehydration. Seedling protection through the action of enzymes antioxidants like Superoxide Dismutase (SOD) and Catalase (CAT), which heal oxidative injures. Highly osmoregulatory hormones like proline and soluble sugar synthase, which generate osmoregulatory substances through the process after the accumulation of these hormones.

These molecular studies in model plants such as Arabidopsis thaliana have laid a solid foundation for understanding the core pathways involved in cold stress responses, particularly through well-characterised mechanisms like the ICE-CBF-COR regulatory cascade. However, despite the mechanistic insights provided by these studies, their direct applicability to tropical fruit species remains limited due to genetic, physiological, and ecological differences. Therefore, it is essential to investigate whether similar or alternative molecular strategies operate in tropical fruit trees, which face unique challenges under low-temperature conditions. This necessity provides the rationale for shifting the focus to cold response research in tropical species, as discussed in the following text.

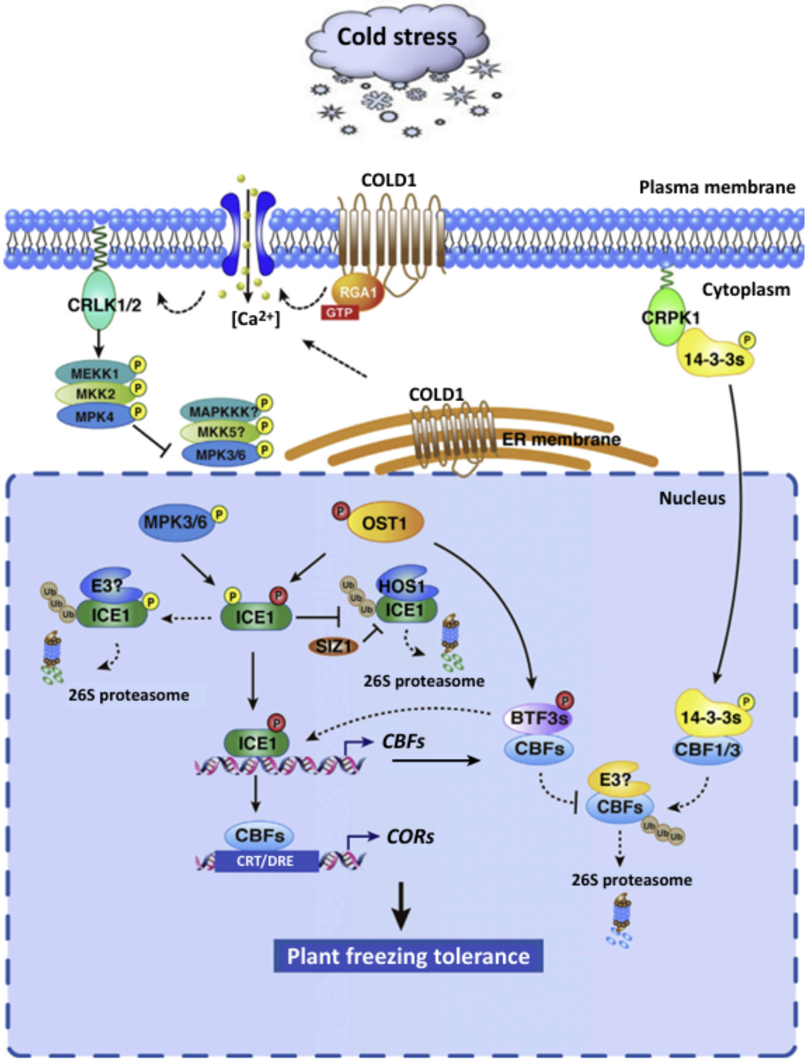

Figure 1. Schematic diagram of the molecular regulatory mechanism of plant cold stress response

As shown in Figure 1, the nucleus is seen as the beginning of the cold signal transmission, from which the plasma membrane is considered the primary target. The plasma and Endoplasmic Reticulum (ER) localizing cold sensor COL1 (COLD1) are involved in the Rice G Protein A Subunit 1 (RGA1)-mediated activation of the Ca^{2+} signaling pathway, which induces cold stress response in rice plants through the upregulation of cold-responsive genes, such as C-repeat Binding Factor/Dehydration-Responsive Element Binding Protein (CBF/DREB) genes. A family of plasma membrane (Ca^{2+}-regulated) receptor-like kinases

(Ca/K MAR1/2 or CRLK1/2) are post-translationally phosphorylated by the MEKK1-MKK2-MPK4 cascade, and promote cold-induced gene expression.

2.2. Breeding strategies for cold-tolerant crops

The molecular insights derived from model plants not only deepen our understanding of how plants respond to cold stress but also provide a theoretical and technical basis for crop improvement. In particular, discoveries related to the ICE-CBF-COR pathway and stress-related transcription factors (e.g. DREB, NAC, MYB) have informed various breeding strategies, ranging from marker-assisted selection to transgenic approaches. These advancements make it possible to manipulate specific genetic components that regulate cold response, especially in species with established transformation systems.

Conventional breeding remains a fundamental approach in developing cold-tolerant varieties. It involves selecting individuals with desirable phenotypes under chilling or freezing conditions. While effective in some temperate crops, this method faces significant limitations in tropical fruit trees, where cold-tolerant germplasm is scarce, and long generation cycles hinder selection efficiency. Furthermore, phenotypic traits associated with cold tolerance are often complex and influenced by multiple genes and environmental interactions, making traditional selection both time-consuming and imprecise.

2.2.1. Genetic engineering

Overexpression of CBF genes, MAPK genes, and transcription factor ICE1 genes through trans-genic technology can enhance plant cold tolerance. For instance, Arabidopsis thaliana's CBF1, CBF2, and CBF3 genes have been successfully utilized to improve cold resistance in various crops. The process involves cloning CBF genes, constructing expression vectors, transforming Arabidop-sis, and conducting screening and identification to achieve CBF gene overexpression, thereby strengthening Arabidopsis' cold tolerance.

Gene editing is the one of the most revolutionary technologies in life sciences in recent years. It already become a transformative force in both medicine and identifying of complex biological processes. In plant breeding, gene editing is also a crucial tool. Fundamentally, gene editing technology acts as a biological mutagen that induces targeted genetic mutations in plants. Compared to conventional mutagens like radiation and chemical agents used in traditional mutagenesis breeding, it has superior precision, simplicity, and speed in creating required genetic modifications for crop improvement. This will help the development of crops with enhanced stress resistance, improved quality, higher yields, and reducing relying on pesticides and chemical fertilizers.

As an environmentally friendly production tool, gene editing is becoming an important technique in molecular breeding. CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats), originally found in bacteria. It represents an ancient immune mechanism of bacteria protection from viral infections. The Cas9 protein, a CRISPR-associated protein, utilizes RNA mole-cules as guides to precisely identify and cleave target DNA sequences, enabling directed genetic modifications. The advent of CRISPR/Cas9 technology marks our achievement of molecular-level precision in genetic code editing. With continuous technological advancements and optimizations, the CRISPR system's capabilities are becoming increasingly powerful, foreshadowing its promis-ing future applications in biological breeding and related fields [7]. For instance, knockout of cold sensitivity-related repressors or targeted activation of stress-responsive genes could enhance cold tolerance while minimising unintended phenotypic changes. Although still in the experimental phase for most tropical species, CRISPR holds substantial promise for future application, especially as transformation protocols become more efficient.

2.2.2. Molecular marker-assisted breeding

Molecular Marker-Assisted Selection (MAS) plays an indispensable role in modern crop breeding. The core of this technology lies in utilizing DNA molecular markers closely linked to target traits for early selection and evaluation of breeding materials at the genomic level. By employing molecular markers to screen plants with high CBF expression capacity, breeders can accelerate the development of cold-tolerant varieties. With rapid advances in molecular biology, multiple marker systems including RFLP, SSR, and SNP have been successively applied to plant breeding practices [8]. However, in tropical fruit trees, MAS is still underutilised due to the lack of high-density genetic maps and reference genomes, although ongoing sequencing projects (e.g., papaya, mango) may change this landscape in the near future.

2.2.3. Exogenous substance treatment

In addition to cultivating good varieties with relatively strong cold tolerance, researchers have tried to treat them with appropriate exogenous substances to improve the adaptability of seedlings to low temperatures, so that the plants can grow normally under low temperatures and enrich the variety of vegetables in the winter market [9]. Abscisic acid is an important regulator of plants in the external adversity environment, in the many unfavourable environmental conditions for the growth of plants, the body of the plant will increase the synthesis of abscisic acid, with the regulation of the metabolic level of the plant, for the poor growth of the environment to improve the role of resistance [10]. Salicylic acid is a class of small molecule phenolic compounds prevalent in plant tissues and plays a wide range of roles in plants. Salicylic acid provides a protective effect on cell membranes and some photosynthetic functions during low-temperature stress, which may increase plant resistance to adversity [11]. There are many types of gibberellins, one of the most diverse of the plant hormones, and some of the active gibberellins are not only able to regulate the normal growth and development of plants but are also associated with cold resistance. Treatment with appropriate doses of abscisic acid, salicylic acid and gibberellin exogenous substances can effectively reduce the damage of low temperature stress on plants [12].

2.3. Research advances in cold stress resistance of tropical fruits

Although research on model plants has provided valuable insights into the molecular mechanisms of cold tolerance, the application of these findings to tropical fruit crops still faces many challenges. This section will focus on the molecular mechanisms of cold tolerance in tropical fruits, reviewing recent research progress and discussing the main challenges and future research directions.

Mango is one of the most important fruits in the world. It has grown in more than 100 countries. The nutritional value of mango is high, containing protein, fat, carbohydrates, dietary fiber, calcium and many types of beneficial substances in it, so it's known as the "King of Tropical Fruits". However, it is a tropical and seasonal fruit, which means it can only grow in high temperature regions. The growth rate of mango will decrease under 18 degrees and stop growth under 10 degrees. That is the biggest problem for the large demand of global market. If we want to increase the amount of mango production, we need to research on the mechanism of low temperatures responses.

So far, many publications on the transcriptional reactions of plants subjected to low-temperature stress exist, including those about tropical crops somehow related to mango, pineapple, banana, and others. These studies have determined multiple genes associated with cold conditions, which are implicated in low-temperature adaptation. Scientists have revealed that the molecular processes involve gene expression, redox status, and multilayered signal transduction pathways featuring Ethylene Responsive Factor (ERF), which, in turn, regulates the biotic and abiotic stresses.

For the research of cold tolerance in mango, the metabolome and transcriptome were combined to analyze the leaves of three different mango cultivars with different cold stress tolerances: Jinhuang (J), Tainung (T), and Guiremang (G) ones being susceptible after 24 (LF), 48 (MF), and 72 (HF) hours of exposure to the cold. This study encompassed the detection of 1,323 metabolites from 12 different compound classes. During this time period, not only the concentration of amino acids and their derivatives, nucleotides and their derivatives, but also lipids, which were gradually increased, were observed in the three cultivars. As for Jinhuang leaves, there were higher levels of flavonoids, terpenoids, lignans, coumarins, and alkaloids, and the increasing trend in these compounds specifically was shown with the exposure time. Regarding the phytohormones, the jasmonic acid and the abscisic acid levels were reduced, while the levels of N6-isopentenyladenine were increased with the prolonged cold stress. The transcriptome analysis showed that there were 22,526 Differentially Expressed Genes (DEGs) with varying expression levels. Particularly, the level of genes associated with the chloroplast thylakoids, light-harvesting proteins, and biosynthesis of secondary metabolites like flavonoids, terpenoids, and alkaloids were upregulated in the Jihuang leaves. Moreover, the evidence of the differential expression of the genes controlling for phytohormones, MAPK (like calcium and H_2O_2) and ICE-CBF-COR-specific pathway indicates their role in the defense of plants from cold stress.

Thus, it was concluded that the biosynthesis-pathway regulation of primary and secondary metabolites is in mango leaves and is directly related to the cold stress tolerance. In the experiment, jasmonic acid, abscisic acid, and cytokinins were mentioned as possible participating factors in the response to the cold stress in mango leaves. One possible way for us in the future is to discover the key point of cold tolerance for supplying the exceedingly large demands of the global market. That is the main goal of future research. That might be the most important fortuitous event in mango development ever known [13].

Overall, although omics technologies have made significant progress in studying the cold tolerance mechanisms in tropical fruits, achieving breakthroughs in cold tolerance breeding for tropical crops still faces numerous challenges. The following section will focus on the potential of developing cold-resistant tropical fruit varieties through genetic engineering and provide new molecular insights into cold tolerance breeding for tropical fruits.

3. Discussion

3.1. Possibility of developing cold-resistant tropical fruit varieties through genetic engineering

The feasibility of developing cold-resistant tropical fruit varieties through genetic engineering hinges on the precise regulation of the ICE-CBF-COR pathway, while addressing challenges such as polygenic coordination, technical transformation efficiency, and biosafety. This section will systematically evaluate its potential, limitations, and future optimization strategies based on existing research cases.

Banana, one of the most important tropical fruits, ranks as the fourth largest food crop globally after rice, wheat, and corn. However, bananas are highly sensitive to low temperatures, and periodic cold damage has caused significant economic losses to China's banana industry. Breeding cold-resistant varieties represents a crucial solution to mitigate cold-induced damage. Most cultivated banana varieties are triploid, making conventional breeding methods for cold resistance challenging. In this context, transgenic technology offers a promising alternative pathway. This case confirms that overexpression of exogenous CBF genes can activate downstream COR genes, thereby repairing membrane damage and accumulating osmolytes (e.g., proline). However, the genomic complexity of tropical fruits (e.g., mango, pineapple) may limit direct application of this pathway, necessitating further studies on species-specific regulatory mechanisms.

In the research conducted by Liu, Agrobacterium-mediated transformation was employed to introduce the AtCBF1 gene, driven by the cauliflower mosaic virus 35S promoter, into Embryogenic Suspension Cells (ESCs) of Dongguan Dajiao (Musa spp. ABB group) and Furenzhijiao (Musa spp. AA group). Transgenic plants were successfully regenerated via somatic embryogenesis, and their cold resistance was evaluated. A SSH-cDNA library was constructed to screen for endogenous genes associated with cold tolerance [14]. Transgenic and control plants (20 each) were subjected to 4°C for 5 and 3 days, respectively, and cold damage symptoms were observed. Transgenic Dajiao plants showed no visible cold damage, while controls exhibited wilting and dehydration, though no lethal damage occurred. Transgenic Furenzhijiao plants displayed slight leaf curling but no significant cold damage, whereas control plants suffered severe dehydration, with 90% mortality after recovery at 25°C. In contrast, all transgenic plants survived. These results clearly demonstrate that overexpression of AtCBF1 enhances cold tolerance in both banana varieties.

Arabidopsis CBF1 (AtCBF1) is a critical transcriptional activator of COR genes and plays a pivotal role in activating cold tolerance mechanisms in plants [15]. Building on this, numerous studies have successfully introduced AtCBF1 into various plant species, achieving significant improvements in cold resistance.

In April 2022, Japan's Sanatech Seed Company launched and commercially released the world's first CRISPR/Cas9-edited tomato variety [16], "Sicilian Rouge". This variety contains 4-5 times higher γ-Aminobutyric Acid (GABA) levels than conventional tomatoes, marking the first commercialization of a gene-edited crop. In April 2023, China took a significant step by approving the safety certificate for a high-oleic acid soybean developed through gene editing. This milestone not only highlights the vast potential for industrialization of gene-edited crops but also establishes a foundation for independent innovation and application in this field in China. Looking ahead, it is reasonable to anticipate the emergence of high-quality, independently developed gene-edited tomato varieties in China, injecting new vitality into agricultural technology and market development.

Beyond genetic engineering, significant efforts have been made to identify key molecular markers associated with cold tolerance in existing resistant varieties, providing valuable genetic resources for tropical fruit breeding.

Transcriptomic analysis was performed on cold-resistant and cold-sensitive jackfruit (Artocarpus heterophyllus) varieties to identify key genes involved in cold tolerance, offering insights for breeding cold-resistant jackfruit. Field phenotypic observations under natural low-temperature stress were combined with transcriptome sequencing to compare a cold-resistant Guangxi local variety with a cold-sensitive Thai variety. Bioinformatics analysis revealed 30,585 Differentially Expressed Genes (DEGs), with 17,083 upregulated and 13,502 downregulated. GO enrichment analysis showed significant enrichment in photosynthesis-related processes (light harvesting, photosystem I/II, chlorophyll binding), secondary metabolite metabolism (flavonoids, carotenoids, terpenoids), and stress responses. KEGG pathway analysis highlighted photosynthesis, starch/sucrose metabolism, plant hormone signaling, and MAPK pathways as critical for cold adaptation. Transcription factors such as WRKY, ERF, bHLH, NAC, and MYB were identified as key regulators in jackfruit's response to cold stress.

Similarly, comparative transcriptomic and molecular biology studies between cold-sensitive Thai Nong mango and cold-resistant Jinhuang mango varieties identified DEGs and enriched KEGG pathways under low-temperature stress. These findings provide novel insights into the molecular mechanisms of cold adaptation in mango and advance breeding efforts for stress-resistant tropical fruit varieties. This research not only elucidates the molecular basis of cold tolerance but also drives the development of improved tropical fruit cultivars with enhanced resilience to environmental stresses.

3.2. Limitations

While the sequence of many tropical fruits has not yet been documented completely, and as a result, your chances of finding a good gene editing target is limited. Also, the intricacies of cold resistance in mango plants have not been elucidated to a significant extent. Genome-wide expression profiling has proved that COR genes are numerous even for model plants like Arabidopsis [17].

Most of the genetic engineering for cold resistance is centred around a single gene, but cold resistance in plants is a trait controlled by multiple genes, and it is difficult to obtain cold-resistant plants by transferring only one gene, and the degree of improvement of cold resistance in transformed single-gene plants is quite limited, so multiple genes must be transferred, but the technology of transforming multiple genes at the same time is not mature at the present time. For example, Du et al. identified

30,585 differentially expressed genes in cold-tolerant jackfruit, involving photosynthesis, hormone signaling, and MAPK pathways—far beyond the scope of CBF alone. However, the technology of transforming multiple genes simultaneously is not yet mature. Moreover, there are not many target genes that can be utilised in the current research. Cold tolerance in plants is controlled by a large regulatory network involving a large number of genes, which makes precision improvement in molecular breeding a great difficulty. The genes isolated so far are mainly protective genes, and most of them are from model plants such as Arabidopsis thaliana. In order to make greater breakthroughs in cold-resistant breeding of plants, more genes must be isolated from different plants, and the molecular mechanism of cold resistance in plants must be studied in depth.

At the technical level, gene transformation efficiency is generally low, and there are problems such as gene silencing and unstable expression, which seriously affects breeding efficiency. Although there are breakthroughs in genome editing technology, the problems of off-target effects and editing accuracy have not yet been completely solved. In terms of biosafety, the ecological safety risk evaluation system of GM crops is still not perfect, especially in terms of uncertainty in the assessment of gene drift and impact on non-target organisms. Meanwhile, the public's low acceptance of GM technology has posed challenges to the promotion and application of breeding products of genetic engineering technology. In addition, genetic engineering technology breeding is costly and requires high R&D investment, which puts greater economic pressure on breeding units.

The regulatory policy on gene-edited crops is not yet perfect, and most countries have not yet established classification standards different from those for traditional GM crops (e.g., the U.S. "SECURE" rule only exempts part of the SDN-1-type edited crops). In terms of safety assessment, the long-term environmental risk of off-target effects is not sufficiently assessed, and there is a lack of uniform protocols for food safety testing, which calls for the development of a scientifically sound regulatory framework.

3.3. Future improvements

For tropical plants, even though a big number of tropical crop plants have had their genomes fully sequenced, the majority of genes still have unknown functions. In this regard, we are still in the early stages, lacking the comprehension of the roles of main plant genes involved in the tropics. Yet, today, with the help of new genome sequencing technologies and the studies of Genome-Wide Associations (GWAS), we can come closer to understanding the many genes granting resistance against diseases for tropical fruit and other crops.

Employing Next-Generation Sequencing (NGS) and GWAS datasets, researchers reported that Cassava Brown Spot Disease (CBSD) is a polygenic and environmentally dependent trait in cassava [18]. Apart from that, they found the protein-coding candidate resistance gene NBS-LRR on chromosome 11. Furthermore, the systematic research also found four cassava MeDELLA genes to be resistance genes for the bacterial spot disease [19].

Moreover, 77 bZIP transcription factors were detected in cassava through RNA Sequencing (RNA-Seq) technology, whereas MebZIP3 and MebZIP5 were recognized as the genes enhancing the resistance to bacterial mottle blotch disease in cassava [20]. The advancement of sequencing technologies, gene expression profiling and small Ribonucleic Acid (sRNA) mapping, proteomics and metabolomics technologies, among other things, will indeed help us to unravel the mechanisms underlying cold tolerance in tropical plants over the next period.

In the future, rather than being limited to omics and disciplines by themselves, the combination of multi-omics and multidisciplinary cross-research will be the path to pursue that will elaborate on the cold tolerance genes and low temperature signaling pathways, which will provide more reference for crop genetic improvement and will also help to solve the problem of plant growing seasons.

The expression system of transgenes is not perfect. At present, cold-resistant genetic engineering mostly adopts the 35S constitutively strong promoter of CaMV to initiate the constitutive expression of exogenous genes in plants, although it can better improve the level of cold resistance of plants under adversity, but under non-adversity, this unnecessary high expression also brings many negative effects to plants. Firstly, over-expression of exogenous genes will consume a large amount of energy, which will lead to a serious growth retardation of transgenic plants even under normal environment; secondly, the exogenous gene expression is not needed when there is no freezing stress, so this kind of promoter can not be used as the optimal promoter for long-term use in the genetic engineering of plant chilling resistance. The key to solving this problem is to find plant endogenous specific promoters, so that the resistance genes can be induced to express in large quantities during chilling stress without affecting the normal growth and development of transgenic plants. It is believed that with the continuous development of molecular biology technology and methods, the use of genetic engineering methods to improve the cold resistance of crops will make promising progress, and will have a wide range of practical applications. At the same time not only genetic engineering, chromosome engineering, molecular marker-assisted selection and haploid breeding and other technologies of integrated application, not only accelerate the process of selection and breeding of excellent varieties, but also for the solution of the key problems in crop production to provide an effective way.

Future research should also focus on the role of epigenetic regulation in plant low-temperature response, such as the dynamic changes of DNA methylation, histone modification and non-coding RNA under cold stress. This will help to reveal how plants "remember" the cold environment at the molecular level and adapt to this production challenge.

Strengthening the safety evaluation of genetically engineered crops and formulating appropriate regulatory policies, as well as improving the crop safety evaluation system and regulatory mechanism, are key links in the promotion of biotechnology breeding. It is necessary to establish a scientific and rigorous safety evaluation technical specification covering the whole process, which should not only include all aspects from laboratory research to field trials, but also focus on core issues such as genetic drift risk assessment, ecosystem impact analysis and food safety evaluation. Through the establishment of unified evaluation standards and detailed operating procedures, the scientific and repeatable nature of the evaluation process should be ensured. It is particularly important to establish independent third-party evaluation organisations, which must have professional evaluation capabilities and complete independence, to ensure the safety of biotechnology breeding products through objective and impartial evaluations, and to enhance public trust in the evaluation results. It is also necessary to establish a long-term monitoring mechanism to continuously follow up biotechnology breeding products that have been commercialised, so that potential safety hazards can be detected and resolved in a timely manner, and this dynamic regulatory model can provide a strong guarantee for the healthy development of biotechnology breeding.

4. Conclusion

Genetic engineering technology cultivating tropical fruit varieteis is a revolutionary way to address the challenge of climate change and to expand the cultivation of such important cash crops. This research systematiclly discover the molecular mechanisms of plant response to low temperature stress, which mostly focus on ICE-CBF-COR regulatory pathway and evaluating the capacity of modern genetic teconology on enhencing cold tolerence aspect. The result shows bright prospect and the complex challenge of realisation of practical applications.

In molecular aspects, ICE-CBF-COR is confirmated as a core regulatory system for the cold domestication process in plants achieves practical application. ICE, CBF transcription factor and downstream COR genes and other key components act synergistically activate the protective mechanisms of defending low-temperature stress. The successful overexpression of the AtCBF1 gene in the banana varieties in this research demonstrated that cold resistance can indeed be enhanced without compromising fruit quality. This result highlights the potential of genetic engineering techniques to introduce cold tolerance traits to tropical fruits that are naturally cold-shy, a possibility that is further amplified by the application of CRISPR-Cas9 gene editing technology, whose precise gene modification capability provides a faster and more efficient alternative to traditional breeding methods.

However, developing fruit species which have enhancing cold tolerance and business value still faces many challenges. The first obstacle is there are big amount of gene features of cold tolerance. It involves many genes and controling network interaction. While single-gene modifications have shown results, achieving improvement on whole cold tolerance aspect may require regulating different genes. The recent limitations of the knowledge of tropical fruit genomes, as well as the technical challenges of multi-gene transformation, are important bottlenecks that need to be broken. In addition, side effects such as growth inhibition due to constitutive transgene expression call for the development of stress-inducible or tissue-specific promoters to optimise gene expression.

In addition to technical barriers, the successful promotion of genetically engineered cold-resistant varieties depends on the resolution of biological safety issues and the improvement of public acceptance. Building up a mature regulatory system based on scientific evaluation is the key to ensure that these new crop species are safe for the environment and humans. Communication with policy makers and the public is also essential to build trust and facilitate the translation of innovations. Economic considerations also need to be taken into account to ensure that tropical fruit growers, who are predominantly smallholders, have access to these improved varieties.

Looking for the future, firstly, the combination of multi-omics and machine learning technologies may totally change the identification of cold tolerance genes and the design of targeted modifications. By integrating genomics, transcriptomics, proteomics, and metabolomics data with AI algorithms (e.g., deep learning, random forests), researchers can more efficiently uncover key gene networks underlying complex traits and predict optimal gene-editing strategies. For instance, machine learning models trained on multi-omics datasets from cold-stressed mango could identify novel transcription factors and optimize CRISPR target sites to minimize off-target effects. Second, international research cooperation on combining resources and expertise to accelerate research and design of cold-resistant tropical fruit varieties have irreplaceable value. Furthermore, the study of epigenetic regulation and stress memory mechanisms may provide novel ideas for enhancing low-temperature adaptation in plants.

As a result, althoug there is improtant progress is acquired on understanding and regulation on tropical fruits, there is still a long way to change advances into practical solutions. The potential benefits like expanding areas of suitability, enhancing yields, and safeguarding food security are significant which is deserve continued exploration. By addressing the challenges identified in this research at the scientific, technological and societal levels, we are moving to a future which tropical fruits can thrive on different environment, which will be an important contribution to global agricultural resilience and sustainability. This journey will require sustained investment, interdisciplinary collaboration and careful advancement, but the rewards for science and human society will be immeasurable.

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