Research Progress on the Physiological and Molecular Response Mechanisms of the Medicinal Plant *Citrus medica* to Adverse Stresses

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Abstract *Citrus medica* is an important medicinal plant belonging to the genus *Citrus* in the Rutaceae family. Its fruit is rich in bioactive compounds such as flavonoids and coumarins, and has significant medicinal and economic value. However, during cultivation, *C. medica* often suffers from various adverse stresses, including pests, diseases, and abiotic factors, which significantly affect its yield and quality. This paper systematically reviews the research progress on the physiological and molecular response mechanisms of *C. medica* to these adverse stresses. The paper also explores the signal interactions between biotic and abiotic stresses, the trade-off of defense resource allocation, and comprehensive prevention and control strategies, including exogenous regulation, grafting, and intelligent monitoring. In light of current research challenges such as insufficient understanding of mechanisms, limited studies on multi-stress interactions, and delayed validation of key gene functions, it is proposed that future efforts should focus on strengthening multi-omics integrative analysis, gene-editing breeding, phenomics monitoring, and the development of green control technologies. These measures will provide theoretical foundations for stress-resistant breeding and the efficient cultivation of *C. medica*.

Key words *Citrus medica*, Adverse stress, Huanglongbing (HLB), Low-temperature stress, Coumarin compounds, Transcriptional regulation, Defense mechanism

0 Introduction

Citrus medica L. var. *sarcodactylis* Swingle, a species in the genus *Citrus* of the Rutaceae family, is one of the precious traditional Chinese medicinal materials in China due to its unique fruit shape, rich aroma, and medicinal, edible, and ornamental uses[1]. The fruit of *C. medica* is rich in various bioactive compounds such as flavonoids, coumarins and volatile oils, and has pharmacological effects such as anti-inflammation, anti-oxidation and anti-depression. Besides, it is widely used in the food, cosmetics and pharmaceutical industries[1-2]. In recent years, with advances in plant stress biology and molecular biology techniques, scholars at home and abroad have conducted extensive studies on the stress response mechanisms of *C. medica*, covering multiple levels such as the determination of physiological and biochemical indicators, secondary metabolite analysis, transcriptome sequencing, key gene cloning and expression regulation[3-4]. *C. medica* can form a defense system by regulating secondary metabolic pathways to accumulate defensive compounds, activating the antioxidant enzyme system, regulating osmotic protective substances, and activating specific transcription factor networks[5-6].

C. medica is highly susceptible to both biotic and abiotic stresses during its growth and development, which severely restrict its yield and quality[7-10]. However, compared to model plants or major crops, systematic reviews of stress response mechanisms in *C. medica* remain relatively scarce. In particular, summaries about multi-stress interactions, integration of signaling pathways, and functional validation of key genes are still incomplete. Therefore, this paper systematically reviews the physiological and molecular response mechanisms of *C. medica* from four perspectives: pest stress, disease stress, abiotic stress, and multi-stress interactions, and summarizes exogenous regulation methods and cultivation defense strategies. Finally, the paper identifies gaps in current research and proposes directions for future development, aiming to provide a scientific foundation for the innovation of stress-resistant germplasm, the development of green control technologies, and the sustainable cultivation of *C. medica*.

1 Response mechanisms of *C. medica* to pest stress

1.1 Major pest species and their damage characteristics The main pests of *C. medica* exihibit host specificity and regional distribution patterns. Research on deep learning-based image recognition indicates that the common field pests of *C. medica* 'Fingered' mainly include *Papilio polytes*, aphids, red spiders, leaf miners, *Icerya purchasi*, among others[11]. The larva of *P. polytes* is an specialist pest of *C. medica*. When present in large numbers, these larvae can eat up all the leaves, seriously affecting the photosynthesis and growth and development of *C. medica*[12]. In cultivation bases such as Wanzhou, Chongqing, *Dichocrocis punc-*

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tiferalis has been reported to cause damage to *C. medica* fruits, resulting in fruit damage rates up to 48.8% and significantly reducing the economic value of the fruits[3]. In addition, although *Bactrocera cucurbitae* and *Bactrocera tau* mainly pose greater threats to cucurbitaceous plants, they also show selectivity in oviposition on the fruits of *C. medica*, resulting in "maggot fruit"[13]. The above-mentioned pests directly damage *C. medica* through their piercing-sucking mouthparts (such as aphids and red spiders) or chewing mouthparts (such as larvae of *P. polytes*), while facilitating the entry of pathogenic bacteria.

1.2 Physiological and biochemical responses of *C. medica* to pest stress When plants are attacked by pests, they can rapidly activate physiological and biochemical defense mechanisms. The defense response of *C. medica* against pests is closely related to changes in its secondary metabolites. Bergapten and xanthotoxin, which are two important furanocoumarin compounds in *C. medica*, show significant antifeedant activity. Liu Junqi *et al.*[14-15] found that bergapten and xanthotoxin derivatives, both isolated and synthesized from *Zanthoxylum*, exhibit good antifeedant activity against the third instar larvae of *Spodoptera exigua*. These active compounds may interact with the amino acid residues in the active site of carboxylesterase (CarE) in *S. exigua* through van der Waals forces, hydrogen bonding, and alkylation. This interaction significantly inhibited CarE activity (with an inhibition rate of up to 94.97%), thereby interfering with the detoxification metabolism and nervous system of the pest, ultimately leading to food refusal or mortality. The above findings suggest that plants of the Rutaceae family, such as *C. medica*, accumulate furanocoumarins, which constitute an important part of their chemical defense system.

1.3 Defense signaling pathways and gene expression of *C. medica* induced by pests *Phyllocoptruta oleivora* is a major pest of *C. medica*. The transcriptional regulatory mechanisms of *C. medica* in response to pest infestation have gradually been revealed. Lei Mengying *et al.*[7] used Illumina sequencing technology to conduct transcriptome analysis on the fruit peel of *C. medica* 'Fingered' subjected to damage by *P. oleivora*. This analysis identified a total of 1 005 differentially expressed genes, of which 271 were upregulated and 734 were downregulated. Gene Ontology (GO) functional enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis revealed that these differentially expressed genes were significantly enriched in metabolic pathways, biosynthesis of secondary metabolites, and plant-pathogen interaction pathways. In the plant-pathogen interaction pathway, the expression levels of 42 genes exhibited significant changes, most of which were auxin early response protein genes and auxin response factors, indicating that the auxin signaling pathway may be involved in the response of *C. medica* to mite infestation. Furthermore, 81 transcription factors (TFs) were differentially expressed after mite infestation, involving families such as MYB, WRKY, and bHLH, with a predominance of down-regulated genes. These transcription factors may act as upstream regulatory switches to coordinate the expression of downstream defense-related genes. These findings provide important transcriptome data for understanding the molecular defense network of *C. medica* against pests.

Transcriptome analysis of *C. reticulata* Orah in response to damage caused by *P. oleivora* also revealed that differentially expressed genes were significantly enriched in pathways such as plant hormone signal transduction and flavonoid biosynthesis, which corroborated previous research on *C. medica* 'Fingered'[16]. These studies show that *C. medica* and its closely related species have established a precise defense system against pests through the regulation of auxin signaling, activation of specific transcription factors, and synthesis of coumarin-like antifeedant compounds. Recent research progresses concerning the chewing pest *P. polytes* include the following: (i) research on deep learning-based image recognition has shown efficient identification of its larvae across different instar stages[1]; (ii) adult *P. polytes* assess host suitability by identifying common volatile compounds in host plants and larval feces, with a ternary mixture of linalool, citronellal, and geraniol exhibiting a strong repellent effect[17]. These findings lay a foundation for subsequent studies on the molecular interactions between *P. polytes* and *C. medica*.

2 Response mechanism of *C. medica* to disease stress

2.1 Main disease species and their infection characteristics Huanglongbing (HLB) is the most devastating disease affecting the production of *C. medica* and is caused by *Candidatus Liberibacter asiaticus* (CLas)[18-19]. Infected *C. medica* plants typically exhibit symptoms such as mottled yellowing, uniform yellowing, and leathery leaves, along with smaller, deformed, and abnormally colored fruits, which can result in a yield reduction exceeding 72%[18,20-21]. The distribution of pathogenic bacteria in the plant shows spatiotemporal dynamics. Bacterial concentrations are relatively higher in the older leaves of branches and in leaves adjacent to the fruit, but the fruit itself does not display significant bacterial accumulation. This pattern differs from the distribution observed in diseased Gongguan citrus plants, indicating that *C. medica* may possess a certain level of disease resistance[20-21].

Anthracnose and brown spot disease are common fungal diseases affecting the leaves and fruits of *C. medica*. Anthracnose in *C. medica* leaves is mainly caused by the fungus *Colletotrichum gloeosporioides*. This pathogen shows broad temperature adaptability, with optimal mycelial growth occurring between 25 and 28 ℃. It tends to germinate and infect host tissues under high humidity and warm conditions, and secretes cell wall-degrading enzymes to destroy the host tissue[9,22-24]. In addition, bacterial anthracnose caused by *Bacillus anthracis* has also been observed on the fruit of *C. medica*[25]. The leaf brown spot disease of *C. medica* is caused by *Alternaria gossypina*, which shows high sensitivity to fungicides such as prochloraz and difenoconazole[9].

2.2 Changes in metabolism and enzyme activity of *C. medica* under disease stress Pathogenic bacterial infection significantly interferes with the normal physiological metabolism of *C. medica*. Yuan Meng *et al.*[18] found a significant reduction in the levels of chlorophyll a, chlorophyll b, and carotenoids in the

leaves of infected plants, which then hinders photosynthesis. In terms of carbohydrate metabolism, the content of starch and sucrose in diseased leaves and stems was significantly increased, while the content of starch and fructose in fruits was significantly decreased, indicating that the pathogen may form a "metabolic reservoir" by manipulating the host's sugar transport and distribution to meet its own proliferation needs. Meanwhile, the malondialdehyde (MDA) content in diseased plants was increased, indicating intensified lipid peroxidation of the cell membrane. The activities of defense enzymes such as superoxide dismutase (SOD) and peroxidase (POD) were significantly increased in the leaves, suggesting that *C. medica* activated its antioxidant system to cope with oxidative stress induced by pathogenic infection[18]. The infection of HLB also remarkably increased the content of flavonoid components such as naringin and hesperidin in the fruits of *C. medica*[1]. This response might be a plant defense response involving the activation of secondary metabolic pathways under stress or may reflect a pathological metabolic disorder. Mahmoud *et al.*[26] found that the pathogen titer of HLB in the leaves of disease-resistant hybrids was relatively low, while the contents of chlorophyll, total phenols, total flavonoids and antioxidant activity were significantly increased. Transcriptome analysis revealed that differentially expressed genes were enriched in pathways related to cell wall structure, redox homeostasis, and biotic stress response. Genes such as PAL1, jasmonic acid-associated genes, and WRKY transcription factors were upregulated in disease-resistant hybrid species, thereby confirming a positive correlation between the accumulation of secondary metabolites and HLB tolerance from the perspective of related species.

2.3 Disease-induced immune-related genes and regulatory networks of *C. medica* Transcriptome sequencing offers a novel perspective for revealing the molecular mechanisms underlying disease resistance in *C. medica*. Transcriptome analysis of different organs (leaves, stems, and fruits) of *C. medica* after HLB infection showed that differentially expressed genes were significantly enriched in pathways related to defense response, carbohydrate metabolism, and signal transduction[27]. Four *CmNI* genes were cloned, and it was observed that HLB infection significantly upregulated the expression of *CmNI2* and *CmNI3* in leaves and stems, which may be the molecular basis for the disorder of glucose metabolism in susceptible plants[27]. Meanwhile, genes related to defense responses, including phenylalanine ammonia-lyase (PAL), chitinase, and β-1,3-glucanase, were also observed to be upregulated after pathogen infection[28]. These enzymes facilitate the synthesis of phytoalexins and the degradation of fungal cell walls, thereby enhancing the broad-spectrum resistance of plants. Previous studies have determined the inhibitory effects of 18 coumarin compounds on citrus *Colletotrichum*. Furanocoumarins such as bergapten and xanthotoxin demonstrated varying degrees of antifungal activity[29]. *C. medica* is abundant in furanocoumarin compounds, such as bergapten and xanthotoxin, suggesting its potential use of similar coumarin compounds for chemical defense[29]. Coumarin compounds such as isobergapten and pimpinellin, found in plants like Radix Angelicae Pubescentis, have been shown to strongly inhibit the growth of *Colletotrichum* species in citrus. Microscopic observation showed that these compounds led to an increase in mycelial branches, deformity of the pathogen, and the disappearance of certain septa[28].

3 Response mechanism of *C. medica* to abiotic stress

3.1 Response mechanism of *C. medica* to drought stress *C. medica* shows a high sensitivity to water deficiency. Under water stress, the relative water content (RWC) and leaf water potential (ψw) of *C. medica* leaves decrease, resulting in stomatal closure to reduce transpiration. This physiological response causes a significant reduction in net photosynthetic rate (Pn)[2,30]. Meanwhile, cells produce a large amount of reactive oxygen species (ROS), which leads to membrane lipid peroxidation, an increase in MDA content, an increase in relative electrical conductivity (REC), and damage to the cellular membrane system[30]. Drought stress induces the expansion of chloroplasts, disintegration of grana lamella, and distortion of mitochondrial cristae, resulting in severe impairment of organelle function[30]. To alleviate oxidative damage, the activity of SOD in *C. medica* shows a trend of first increasing and then decreasing[30]. *C. medica* mainly relies on stomatal regulation and a limited antioxidant system to cope with drought, but its drought tolerance is relatively weak. Therefore, ensuring an adequate water supply is essential in production.

3.2 Response mechanism of *C. medica* to temperature stress

3.2.1 Physiological threshold and injury characteristics of low-temperature stress. *C. medica* is extremely sensitive to temperature stress, especially low-temperature damage, and is widely recognized as a plant sensitive to low temperatures[31]. The REC method was used to assess this sensitivity, revealing that the REC of *C. medica* leaves rose in a typical S-shaped curve as the temperature decreased[32]. The study identified a critical period of 12 to 24 h for the onset of cold damage in *C. medica*. REC increased sharply after 24 h, while the activity of SOD showed an initial rise followed by a decline, peaking at 12 h and then dropping rapidly. The levels of MDA and free proline (Pro) progressively increased with prolonged treatment time, indicating that when membrane lipid peroxidation intensifies, cells initiate osmotic regulation in response to the damage[32].

Photosynthesis is highly susceptible to low-temperature stress. Guo Weidong *et al.*[2] simulated short-term low-temperature and low-light conditions in winter facilities in the central Zhejiang region and found that exposure to a low temperature of 15 ℃ significantly reduced the Pn and stomatal conductance (Gs) in *C. medica* leaves, while the intercellular CO_2 concentration increased, indicating that the decline in photosynthetic rate is mainly caused by non-stomatal restriction (*i.e.*, the decrease in photosynthetic activity of mesophyll cells). When the temperature dropped below 10 ℃, the maximum photochemical efficiency (Fv/Fm) of photosystem II (PSII) began to decline significantly, and the initial fluorescence (Fo) increased markedly, indicating that the reaction center of PSII suffered irreversible damage[2].

Furthermore, low temperatures also reduced the carboxylation efficiency (CE) and maximum photosynthetic rate (P_{max}) in *C. medica* leaves, and led to a decrease in the light saturation point, indicating that the plant is susceptible to photoinhibition under reduced light intensity[2].

3.2.2 Antioxidant and metabolic responses of *C. medica* under low-temperature stress. In terms of the antioxidant system, *C. medica* shows a relatively weak protective ability under low-temperature stress. Compared to the cold-resistant species *Poncirus trifoliata*, the activities of SOD, POD and CAT in the leaves of *C. medica* all showed a downward trend during exposure to −4 ℃, while the increases in MDA content and REC were much greater in *C. medica* than in *P. trifoliata*[2,31]. These findings suggest that lipid peroxidation in the cell membranes of *C. medica* was more severe, resulting in greater damage to the membrane system. Cytochemical localization analysis revealed that hydrogen peroxide (H_2O_2) induced by low temperature mainly accumulated in mesophyll cells, whereas superoxide anions were widely distributed across the epidermis, palisade tissue, spongy tissue, vascular bundles, and chloroplasts. Chloroplasts were identified as the major organelles for ROS production[5]. The excessive accumulation of ROS led to the disintegration of the chloroplast thylakoid lamellar structure and the blurring of mitochondrial cristae, ultimately causing cell death[5,33].

3.2.3 Molecular regulatory network of *C. medica* under low-temperature stress. *C. medica* responds to low temperatures by regulating a series of cold-responsive genes. Chen Wenrong *et al.*[3] isolated 121 differentially expressed fragments from *C. medica* leaves treated at −4 ℃ using mRNA differential display technology (DDRT-PCR). The functions of these genes are associated with cell wall modification for plant defense and stress response, signal transduction, redox reactions, photosynthesis, *etc.* Genes related to photosynthesis were significantly downregulated under low-temperature conditions, consistent with the observed decrease in photosynthetic rate[3].

A comparative study of two principal transcription factor families has revealed the molecular basis underlying the cold sensitivity of *C. medica*. Cao Yibin *et al.*[31] successfully cloned the ethylene response factor gene *CmsERF6* (GenBank: HQ698835) from *C. medica*, which encodes a protein containing a conserved AP2/EREBP domain. Under exposure to low-temperature treatment at 4 ℃, the expression level of *CmsERF6* in *C. medica* leaves only increased by approximately 4.3-fold after 12 h, followed by a rapid decline. In contrast, the expression levels of homologous genes in the cold-resistant species *P. trifoliata* increased by approximately 4 000-fold after 36 h of treatment[34−35]. This significant difference in gene expression suggests that ERF6 is the key factor determining the cold sensitivity of *C. medica*. Shi Rui *et al.*[34] cloned the *CmsGRAS* transcription factor from *C. medica* (GenBank: JF440647). Under exposure to 4 °C, the expression level of *CmsGRAS* in *C. medica* increased by only about 9.6-fold at 36 h, while that in *P. trifoliata* increased remarkably by 1 506-fold[9]. Further analysis of the promoter sequences revealed that although the promoter regions of *CmsERF6* and *CmsGRAS* in *C. medica* contain ICE1 binding sites, CBF binding sites and LTR low-temperature response elements, differences were observed in the number, position, or nucleotide sequences of these cis-regulatory elements compared to those in *P. trifoliata*, which may reduce their binding affinity with upstream transcription factors and hinder the effective activation of downstream cold-responsive genes[35−36]. These results provide a fundamental explanation for the low cold tolerance observed in *C. medica*.

3.2.4 Response of *C. medica* to high-temperature stress. Compared to low temperatures, *C. medica* has a certain tolerance to high temperatures. Zheng Jianshu[5] found that exposure to 35 ℃ had no significant effect on the growth of *C. medica*. At 40 ℃, although the photosynthetic rate decreased, it was capable of recovery. However, exposure to an extreme high temperature of 45 ℃ for 6 h led to a sudden decrease in net photosynthetic rate, severe inhibition of PSII activity, and destruction of chloroplast ultrastructure (loosened thylakoid lamellae and disappearance of stroma lamellae). Meanwhile, MDA content and REC increased sharply. Notably, treatment at 45 ℃ did not cause plant mortality, indicating that *C. medica* possesses a certain degree of short-term adaptability to high-temperature stress.

3.3 Signal transduction and key regulatory factors of *C. medica* in response to abiotic stress The signaling network of *C. medica* in response to abiotic stress mainly includes ROS signals, calcium signals, and hormone signals.

ROS signals: stress factors such as low temperature and drought induce the outbreak of ROS. ROS acts not only as toxic molecules but also as second messengers that activate the expression of downstream antioxidant enzyme genes (such as *SOD*, *CAT*, *APX*) and defense genes, thereby participating in the amplification and transduction of stress signals[5,37].

Hormone signals: abscisic acid (ABA) is a central hormone involved in responses to drought and low-temperature stress. Although direct evidence remains limited, exogenous application of ABA can enhance stress resistance in various plants, suggesting that ABA also plays a key role in *C. medica*. Moreover, the induced expression of ERF6, a key transcription factor in the ethylene signaling pathway, indicates that ethylene is also involved in the low-temperature response of *C. medica*[31].

Key regulatory factors: ERF6 and GRAS are two key transcription factors. The MAPK cascade reaction is an upstream signaling node that transmits environmental stimuli to downstream transcription factors. Calcium-dependent protein kinases (CDPKs) serve as key components of the calcium signaling pathway. At present, the upstream activation mechanism of calcium signals and the identification of their downstream target proteins require further research[18,27].

4 Response mechanism of *C. medica* to multiple-stress interactions

4.1 Cross-response and signal dialogue of *C. medica* to biotic and abiotic stresses The response of *C. medica* to HLB over-

laps with its response to low temperature stress. Both stresses can cause the outbreak of ROS and membrane lipid peroxidation, leading to similar changes in the activities of antioxidant enzymes (SOD, POD)[26,31]. Transcriptome data show that both HLB infection and low-temperature exposure induce the expression of PR protein genes such as PAL and chitinase in *C. medica*[3,18]. The overlapping responses described above indicate that the salicylic acid (SA) and jasmonic acid (JA)/ethylene (ET) signaling pathways may interact through cross-communication. In classic plant defense mechanisms, the SA pathway is usually associated with biotic stress (especially biotrophic pathogens), while the JA/ET pathway is more closely related to pests and necrotrophic pathogens. Low temperature, as an abiotic stress, can also activate these pathways, indicating that these defense signals are universal.

4.2 Priority defense strategies and resource allocation trade-offs of *C. medica* under complex stress conditions When multiple stresses coexist, plants must allocate resources by making "trade-offs" based on the severity and type of each stress. When *C. medica* is simultaneously exposed to low-temperature stress and HLB infection, it remains to be investigated whether the limited energy and metabolic products are prioritized to activate cold-resistant pathways (such as synthesis of osmotic regulatory substances) or disease-resistant pathways (such as synthesis of phytoalexins). Existing research has found that the content of flavonoids in *C. medica* fruits infected with HLB increases significantly[25]. The synthesis of flavonoids uses phenylalanine as a precursor and is generated through the phenylpropanoid metabolic pathway. This process may consume a large amount of carbon skeleton resources, thereby weakening the plant's resistance to other stresses (such as low temperature). The response mechanism of *C. medica* under simultaneous multiple stresses, especially the interactions and resource allocation strategies among different stress signaling pathways, is a research direction that urgently requires further investigation in the future.

5 Defense response and regulatory strategies of *C. medica* against adverse stress

5.1 Physical and chemical defense reactions Physical defense: the wax layer and cuticle of the leaf epidermis in *C. medica* serve as the first barrier against pathogen invasion and help reduce water evaporation. Under conditions of low temperature or drought stress, the thickening of cell walls and enhanced lignification effectively prevent the expansion of pathogenic bacteria[14].

Chemical defense: *C. medica* is rich in various secondary metabolites, among which coumarins and flavonoids serve as the primary chemical defense substances[1]. These compounds not only have direct antimicrobial and insecticidal activities[11-12,38], but also can act as signaling molecules that activate the expression of defense genes. In addition, terpene compounds such as D-limonene and γ-terpinene in volatile oils also have potential allelopathic effects and contribute to defense mechanisms[39].

5.2 Enzymatic and non-enzymatic antioxidant systems and osmotic regulation defense reactions When *C. medica* is subjected to adverse stress, the balance between the production and clearance of ROS within its cells is disrupted. The plant mitigates this damage through enzymatic and non-enzymatic antioxidant systems, as well as the accumulation of osmotic regulatory substances.

5.2.1 Dynamic changes of enzymatic antioxidant system. Under different adverse conditions, the response patterns of antioxidant enzyme activities in *C. medica* vary notably. (i) Under low-temperature stress, the overall activities of key antioxidant enzymes such as SOD, POD and CAT in *C. medica* leaves show a declining trend, which contrasts markedly with that observed in the cold-resistant species *P. trifoliata* (with increased enzyme activities)[2,31]. The failure of the antioxidant enzyme system in *C. medica* is an important factor contributing to its susceptibility to severe cold damage. (ii) Antioxidant enzyme response under drought/water stress: Yang Ling *et al.*[40] demonstrated that with the intensification of water stress, the SOD activity in *C. medica* leaves showed a trend of slightly increasing first and then decreasing. During mild drought conditions, SOD activity increased by approximately 15%, while during severe drought, SOD activity decreased by more than 30% compared to the control, and the MDA content increased by 2.5 times[30]. These findings indicate that *C. medica* is capable of tolerating only short-term and mild water deficiency. (iii) Antioxidant enzyme response under HLB infection: the activities of SOD and POD in *C. medica* leaves infected with HLB were significantly increased, showing increases of approximately 40% and 65%, respectively, compared to those in healthy plants[18]. This might be a defensive mechanism whereby plants actively upregulate their antioxidant systems under pathogen stress to inhibit the proliferation of pathogens, but prolonged high levels of ROS can also cause damage to the plants' own cells. (iv) Antioxidant enzyme response under fluorine pollution: Xu Lishan *et al.*[41] reported that fluorine pollution led to a reduction of POD activity in *C. medica* leaves. However, after the application of protective agents, POD activity was restored to 80% -95% of the control level, and the chlorophyll content and relative conductivity were also significantly enhanced.

5.2.2 Non-enzymatic antioxidant system. *C. medica* is abundant in a variety of non-enzymatic antioxidant compounds, mainly including ascorbic acid (AsA), reduced glutathione (GSH), flavonoids and phenolic acids. Studies have confirmed that flavonoids from *C. medica* have notable scavenging activity against both DPPH and ABTS free radicals, with a significant dose-effect. Besides, polysaccharides from *C. medica* also effectively neutralize superoxide anions and hydroxyl radicals, indicating that the non-enzymatic antioxidant system of *C. medica* operates through a synergistic interaction among multiple components[39]. Sangdan[21] investigated various parts of the *C. medica* fruit and found that the total phenol, flavonoid and vitamin C contents in the exocarp were significantly higher than those in the mesocarp. Moreover, as the fruit matured, the contents of these compounds and the total an-

tioxidant capacity (determined by FRAP method) basically showed an increasing trend. A highly significant positive correlation was observed between the contents of vitamin C, total phenols, flavonoids, and antioxidant capacity. This finding suggests that the peel of *C. medica* fruit, particularly the exocarp, serves as the primary non-enzymatic antioxidant defensive position. Sandan's research demonstrated that the antioxidant capacity of *C. medica* increased with maturity, but it did not provide a detailed analysis of the underlying material basis. Tan *et al.* [42] conducted a quantitative analysis of flavonoids and coumarins in *C. medica* fruits at various growth stages, identifying a total of 22 coumarins and 26 flavonoids. Among these compounds, limettin exhibited the highest coumarin content, while diosmin and hesperidin were the flavonoids with the highest content. Antioxidant activity assessments using DPPH, ABTS, and FRAP assays indicated that *C. medica* showed peak antioxidant capacity in November, corroborating the findings reported by Sandan. Cell experiments further confirmed that *C. medica* extract significantly reduced the levels of NO, MDA and ROS, increased SOD activity, and alleviated oxidative damage. Among them, diosamine, hesperidin and neohesperidin were the main contributors to the observed antioxidant activity[42].

5.2.3 Osmoregulation and defense responses. In terms of osmotic regulation, *C. medica* mainly accumulates Pro and soluble sugar as osmoprotectants under adverse conditions. Under low-temperature stress, the Pro content in *C. medica* leaves progressively increased with the extension of treatment time. Under drought stress, Pro levels also rose significantly[32]. The accumulation of Pro contributes to the maintenance of cellular turgor pressure, the protection of protein structure and the elimination of hydroxyl radicals. Under low-temperature treatment, the soluble sugar content in *C. medica* leaves also showed an increasing trend, but the increase was relatively small. In the plants infected with HLB, starch and sucrose contents in the leaves and stems increased significantly, while starch and fructose in the fruits decreased significantly, indicating that the pathogen may manipulate the host's sugar distribution by affecting the upregulated expression of sugar transporter genes (such as *CmNI2* and *CmNI3*)[18]. Compared to the cold-resistant species *P. trifoliata*, *C. medica* had a slower accumulation rate and lower absolute content of Pro and soluble sugars under low temperature conditions, which further explains its sensitivity to cold stress[30].

Through the synergistic effect of the aforementioned enzymatic and non-enzymatic antioxidant systems as well as osmotic regulatory substances, *C. medica* can partially resist mild adverse stress. However, under conditions of extreme or prolonged stress, these defense systems are often insufficient to maintain cellular homeostasis, thereby leading to cellular damage. Therefore, enhancing the efficacy of these defense systems through exogenous applications (such as protectants, plant growth regulators) or cultivation measures (such as grafting, stress induction) is an effective strategy to improve the stress resistance of *C. medica*[11].

5.3 Defense-related hormone regulatory networks SA, JA, ET, and ABA are the core hormones involved in the defense response of *C. medica*. Sa-dominated systemic acquired resistance (SAR) mainly resists against biotrophic pathogens (such as those causing HLB). In susceptible plants, activation of the SA signaling pathway induces the expression of PR protein genes. JA/ET-driven induced systemic resistance (ISR) mainly responds to chewing pests (such as *P. polytes* larvae) and necrotrophic pathogens (such as *Colletotrichum*). The inhibitory effect of compounds like bergapten on CarE activity in pests may be associated with the JA/ET signaling pathway[14-15]. ABA, as a stress hormone, is synthesized in large quantities under drought and low-temperature stress, inducing stomatal closure and the expression of stress resistance genes.

5.4 Enhancement of the defense ability of *C. medica* to adverse conditions through exogenous regulation and cultivation measures Enhancing the stress resistance of *C. medica* through the application of exogenous substances and agronomic measures is a viable strategy. The foliar application of protective agents such as $Ca(OH)_2$, vitamin C, and rare earth elements can effectively reduce the damage of fluorine pollution to *C. medica*, while also increasing chlorophyll content and POD activity[41]. Grafting and the selection of appropriate rootstocks is an effective way to enhance the cold and drought resistance of *C. medica*. Research indicates that *C. medica* seedlings grafted onto *P. trifoliata* and *Citrus* × *limon* rootstocks exhibit superior cold resistance compared to those grafted onto *C. medica* L. rootstocks[43]. Stem tip micrografting can effectively eliminate the HLB pathogen in *C. medica*. The survival and virus-free rates associated with stem tip grafting with 3 – 4 leaf primordia are relatively high, establishing this method as a key technology for the propagation of disease-free seedlings[44]. Rain-sheltered cultivation can significantly reduce the occurrence of anthracnose in *Dioscorea polystachya* (an analogue species)[45], suggesting that this technique may also be applicable to disease control in *C. medica* fruits.

5.5 Integrated regulation and cultivation application prospects of defense responses The defense response of *C. medica* is a complex network composed of signal perception, transduction, gene expression, metabolic adjustment and morphological changes. Future cultivation and management strategies should be based on the principle of "prevention first, followed by comprehensive control"[46]. A green and sustainable stress control system can be built by integrating multiple technical means: the development of strong seedlings through optimized water and fertilizer management, the use of resistant rootstocks, and the combination of biological control with targeted chemical interventions[11,26].

6 Prospects

6.1 Deficiencies existing in the current research Although significant progress has been made in the study of *C. medica* stress in recent years, there are still many deficiencies. (i) Limited mechanistic depth: most studies have focused primarily on physiological and biochemical aspects, with insufficient analysis of key signaling pathways, transcriptional regulatory networks, and pro-

tein-protein interaction mechanisms. For example, the precise molecular switch responsible for *C. medica*'s sensitivity to cold remains unidentified. It is unclear whether the key enzyme genes involved in the bergapten synthesis pathway have been cloned and functionally verified. (ii) Lack of research on multi-stress interactions: few studies have examined the interaction between biotic and abiotic stresses. In nature, *C. medica* often frequently encounters HLB disease concurrently with low winter temperatures, or drought and pest infestations. The mechanisms underlying the response to such combined stresses remain largely unexplored. (iii) Insufficient exploration of germplasm resources and stress-resistant genes: although some studies have analyzed the genetic diversity of *C. medica* germplasm using ISSR markers[21,39], a systematic evaluation and screening of stress-resistant germplasm resources has not yet been carried out. A large number of differentially expressed genes obtained based on transcriptome sequencing[7,18] have functions that have yet to be validated *in vivo*. (iv) The bottleneck of transformation from mechanism to application: most studies stop at discovering phenomena. However, the translation of stress resistance mechanisms into practical breeding materials and cultivation techniques remains a significant challenge. For example, it is yet to be determined whether the *ERF6* and *GRAS* genes can be effectively used in the genetic engineering of cold-resistant *C. medica* cultivars.

6.2 Future research directions Future research on the biology of *C. medica* stress should develop towards systematization, precision and engineering. (i) Multi-omics joint analysis: by integrating data from genomics, transcriptomics, proteomics and metabolomics, a comprehensive regulatory network for the stress response of *C. medica* can be constructed[3,7,11,26]. For example, through weighted gene co-expression network analysis (WGCNA), key transcription factors and metabolic nodes regulating bergapten synthesis have been identified. (ii) Functional identification and molecular breeding of key genes: virus-induced gene silencing (VIGS) technology can be initially applied to the study of the CRC gene in *C. medica*. In the future, the *CRISPR/Cas9* gene editing technology should be vigorously promoted to conduct targeted knockout of genes that negatively regulate stress resistance or to introduce superior alleles, thereby creating new germplasm with enhanced cold and disease resistance. (iii) Phenomics and intelligent monitoring: by integrating deep learning with high-throughput plant phenotyping platforms, it is possible to achieve non-destructive, real-time, and precise identification and early warning of pests, diseases, and adverse conditions affecting *C. medica*, providing decision support for smart agriculture[11]. (iv) Deepening the research on signal transduction mechanisms: future research should concentrate on pattern recognition receptors (PRRs) and their recognition of pathogen-associated molecular patterns (PAMPs), in addition to elucidating the specific defect mechanisms within the ICE1-CBF-COR cascade pathway in *C. medica*, to better understand its molecular basis for cold sensitivity[15]. (v) Research and development of sustainable green prevention and control technology: the endophytic bacteria resources of *C. medica* should be deeply explored to facilitate the development of microbial preparations that can antagonize pathogenic bacteria and induce systemic resistance[47]; the application of plant-derived extracts (such as the volatile oil of *C. medica*) should be optimized for disease prevention and control to minimize reliance on chemical pesticides[39].

C. medica, as an important medicinal plant, has been the subject of extensive research on its stress response mechanisms, progressing from morphological descriptions to investigations at physiological, biochemical, and molecular levels. Considerable advancements have been made in understanding responses to low-temperature stress and HLB disease. However, in the face of complex field environments, research on multi-stress interactions, the functions of key genes, and stress-resistant breeding remain insufficient and require further development. Future research should employ modern molecular biology and multi-omics techniques to systematically analyze the regulatory networks underlying stress resistance in *C. medica*, providing a solid theoretical foundation for breeding high-quality stress-resistant new varieties and for optimizing efficient cultivation techniques.

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