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Study on resistance of MdMYB gene family in apple

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Abstract. The growth and development of apples are susceptible to environmental stress. The MdMYB gene family is one of the largest gene families in apples. It is widely involved in various resistance signaling pathways in apples and can interact with a variety of genes. Through anthocyanin synthesis, lignin synthesis, epidermal wax synthesis, Na + / H + pump regulation, hormone interaction, etc., the resistance of plants to abiotic and biotic stresses is improved. The MdMYB gene is regulated by multiple factors. It is regulated by epigenetic factors such as methylation and miRNA before transcription. At the transcriptional level, it is regulated by transcription factors such as MdHY5, MdWRKY11 and MdWRKY41. After translation, it is regulated by ubiquitin ligase, which is involved in MdSIZ, MdMEIL and other proteins. This paper reviews the physiological responses and signaling pathways of MdMYB-mediated apple stress resistance and the gene function regulation mechanism of MdMYB, in order to provide new ideas for the study of apple stress resistance.

Keywords: apple; MdMYB; stress; signal pathway.

1. Introduction

The planting area of apples in China ranks second, only lower than citrus, with an area of more than 30 million mu. The main producing areas are: Bohai Bay, Loess Plateau, Old Yellow River, Southwest Cold Highland, Xinjiang and Northeast China[1]. As one of the most commonly eaten fruits, it is rich in malic acid, vitamins, carotene and trace mineral elements[2], has important economic value and nutritional value. And its rich flavonoids can also reduce the risk of cancer, which has strong health care function. In addition, apples also play an important ecological function, with developed roots and rapid growth, which can stabilize the soil structure. To sum up, apples are rich in nutrients, with high economic value, and help to prevent soil erosion. Therefore, how to improve the quality of apples and increase the yield has become an urgent problem to be solved[3,4].

Apple is easily affected by many abiotic stresses in the production process because of its perennial nature and difficult transfer[5]. Apple production in China is concentrated in the Loess Plateau of northwest China, which has excellent planting properties such as abundant sunshine, large temperature difference between day and night and loose soil. However, the production in this area is threatened by drought and salinity, which will lead to the accumulation of reactive oxygen species (ROS) in cells and membrane lipid peroxidation[6]. In addition, there is still freezing injury in apple planting, which is caused by the damage of cell membrane system caused by the accumulation of active oxygen[7].It often occurs in the characteristic producing areas of small apples in Northeast China[8], seriously restricting the development of intensive apple orchards. Except for biological stress, infections caused by viruses, bacteria and fungi also affect the production of apples and reduce profits[9].

Plants have developed various effective strategies to cope with and adapt to environmental stress, such as the elimination of ROS, the promotion of ABA synthesis, and the accumulation of lignin and anthocyanins. The existing research shows that some transcription factors, such as bZIP, WRKY, AP2/ERF and MYB, belong to different families, but they all play a vital role in stress signal transmission and participate in regulating stress response[10]. MYB transcription factors are widely distributed in higher plants. A complete MYB consists of three parts: DNA structural binding region, transcription activation region and negative regulatory region[11].MYB is involved in plant

secondary metabolism, the response of various hormones and environmental factors, and plays an important regulatory role in plant response to environmental stress. For example, Arabidopsis thaliana AtMYB2 is involved in the expression of ABA-mediated drought stress response[12].GmMYB76 and GmMYB1 genes in soybean are involved in low temperature response[11]. At present, it is known that MdMYB gene family plays an important role in the synthesis and accumulation of anthocyanins in apple, but MdMYB gene family has many members and participates in very complicated signal pathways. Therefore, this paper summarizes the process and regulation mechanism of MdMYB-mediated apple stress resistance.

2. Study on MYB resistance in apple

2.1 overview of MdMYB gene resistance

The research on MdMYB gene family focuses on its promotion of anthocyanin synthesis. When plants are affected by one or more abiotic stresses, they will generate reactive oxygen species (ROS), which will cause oxidative damage to plants, and anthocyanin production can promote the removal of ROS. In apple, ROS production is influenced by transcription regulator MdMYB. The MdMYB gene families that promote anthocyanin synthesis include MdMYB1,MdMYB10,MdMYB308L, etc. At present, the research on such MdMYB genes has been relatively perfect.

With the deepening of research, some MdMYB genes that can improve plant resistance through other ways have been discovered one after another, such as MdMYB46, which can regulate lignin synthesis and improve apple salt resistance, and MdMYB94, which can promote wax synthesis and improve apple drought resistance. The discovery of these genes has broadened people's understanding of MdMYB genes.

Table 1. Overview of MdMYB gene family resistance

function	Gene name	summary
Cold stress	MdMYB308L	Interacting with MdbHLH33, positively regulating anthocyanin synthesis An et al,2020.
	MdMYB23	It interacts with MdCBF1\MdCBF2 to positively regulate anthocyanin synthesis. An et al,2018
	MdMYB88/124	Activate the expression of MdCCA1\MdCSP3 to improve the stress resistance. Niu et al,2022
	MdMYB10	Positive regulation of anthocyanin accumulation Zhang et al,2020
	MdMYB108L	*Combine with MdCBF3 to improve cold resistance. Wang et al,2019*
	MdMYB9/11/12	Mdbx 22-mir 858-mdmyb 9/11/12 module regulates the accumulation of PA in apples. Zhang et al,2022
	MdMYB2	Participated in the sumo process of MdMYB1, and maintained the stability of MDM YB1. jiang et al.2022
	MdMYB16 MdMYB15L MdMYB6	*Negative regulation of anthocyanin synthesis Xu et al,2017; Xu et al,2018; Gao et al,2011*
Salt stress	MdMYB4/44	*Interact with MdNHX1 to reduce the salt tolerance of plants. Wang et al,2017; Wu et al,2018*
	MdMYB63	*Interact with MdSOS1 to improve salt tolerance. Yu et al,2018*
	MdMYB46	Activate the expression of genes such as MdABRE1A, MdDREB2A, MdRD22 and MdRD29A, and promote lignin accumulation. Chen et al,2019
Drought stress	MdMYB94	*Inhibit the expression of MdGH3.6 and promote wax synthesis. Jiang et al,2022*
	MdFLP	Activation of MdNAC019 expression, introduction of activation, indirect activation of MdERF6 and MdZAT10 expression. Wang et al,2022
Iron deficiency stress	MdMYB58	*Inhibition of MdMATE43 expression is regulated by MdSAT1. Wang et al,2018*
fight the disease	MdMYB73	*Interact with MdWRKY31 to improve disease resistance. Gu et al,2021*

2.2 MdMYB gene regulates anthocyanin synthesis

Anthocyanins play an important role in plant drought resistance, salt resistance, disease resistance, cold resistance and corresponding light stress. Under strong light, anthocyanin acts as an optical filter, which can transfer redundant high-energy quantum from the saturated photosynthetic electron transport chain, so anthocyanin cell vacuole can not only protect chloroplasts from photoinhibition and photooxidation under strong light, but also prevent the catabolism of photodegradable defense compounds[13]. Anthocyanins also reduce the oxidative load in plant cells by filtering yellow and green light, and eliminate free radicals and reactive oxygen species to reduce the oxidative damage of leaves, thus coping with the accumulation of reactive oxygen species caused by cold, drought and salt stress. In addition, anthocyanin can be used as osmotic pressure regulator to deal with water stress caused by drought and salt stress[14]. Anthocyanin is a flavonoid, which is mainly synthesized by phenylpropanoid pathway[15].It consists of R2R3-MYB, basic helix-loop helix (bHLH) and WD40 protein (called MBW complex).The Transcription activation complex has been proved to control the expression of anthocyanin structural genes[16]. MdCBF gene is an important downstream gene of MdMYB gene, which can induce the expression of COR gene and promote the accumulation of anthocyanins[17]. MdMYB gene can be expressed by combining with bHLH[18].Transcription factors such as MdCBF, MdCCA1 and so on interact to activate the expression of MdCBF, or directly activate the expression of mdcbf gene, thus indirectly regulating anthocyanins. In addition, MdMYB gene can promote anthocyanin synthesis by interacting with MdUGT83L3, MdNAC42, MdCSP3 and other genes. Recent studies show that MYB repressor can negatively regulate anthocyanin synthesis by binding to MBW complex and directly binding to target gene promoter. The molecular mechanism of apple MYB repressor inhibiting anthocyanin synthesis needs further study.

MdMYB308L interacts with MdbHLH33 physically, which enhances the combination of MdbHLH33 with MdCBF2 and MdDFR promoters, thus positively regulating cold tolerance and anthocyanin biosynthesis[19]. MdMYB23 directly binds to MdCBF1 and MdCBF2 promoters and activates their expression. MdMYB23 interacts with MdANR promoter, a key regulator of proanthocyanidins biosynthesis, to activate its expression and promote proanthocyanidins accumulation and ROS scavenging[20]. Genes with the same function are MdMYB88[4], MdMYB10[21], MdMYB124[17,22–24], MdMYB108L [25]. MYB repressor can inhibit anthocyanin accumulation. For example, MdMYB16 can form homodimer, which binds to the promoter of MdbHLH33 and negatively regulates anthocyanin accumulation[26], which have similar effects: MdMYB6 [24] and MdMYB15L[27].These genes will down-regulate the cold resistance of apples.

2.3 MdMYB promotes the synthesis of other secondary metabolites

In addition to regulating anthocyanin synthesis, MdMYB gene family can also regulate the synthesis of secondary metabolites such as epidermal wax, cellulose, lignin and secondary wall. The component of cuticular cutin of cuticular wax plants covers the non-woody parts of land plants, which has the functions of preventing water evaporation, providing mechanical support and preventing biological attacks[28].The biosynthesis of epidermal wax can be divided into three reactions: (1) de novo synthesis of C16 and C18 fatty acids; (2) extension of VLCFAs; (3) synthesis of VLCFA derivatives. Studies have shown that MYB gene family may promote the synthesis of epidermal wax by regulating the expression of key enzyme structural genes in the process of epidermal wax synthesis[29].Whether this molecular mechanism exists in apple needs further exploration. The deposition of lignin is closely related to secondary wall synthesis and stress resistance of plant cells. Providing structural barrier for cell wall, basic biological functions such as mechanical support, impermeability and resistance to biodegradation, and establishing biological defense mechanism are the main biological functions of lignin; The synthesis process of lignin is mainly regulated by a tertiary regulatory network, and MYB transcription factor belongs to a secondary regulatory factor, which can regulate the expression of primary regulatory factors and structural genes [30] . At present, the regulation of MYB transcription

factor on lignin synthesis and accumulation in Arabidopsis thaliana has been systematically studied, but the regulation network of apple lignin metabolism is lacking.

The existing research shows that MdMYB94 can promote wax synthesis of apple leaves, increase peroxidase activity and reduce hydrogen peroxide content, thus improving the drought resistance of apple [28] . MdMYB30 binds to MdKCS1 promoter, activates its expression and regulates wax biosynthesis[18]. MdMYB46 promotes the biosynthesis of secondary cell walls and the deposition of lignin by directly binding to the promoter SMRE and M46RE sites of genes related to lignin biosynthesis[30]. MdMYB88 and MdMYB124 were directly combined with MdMYB46 promoter to increase the expression of MdMYB46, thus promoting lignin, cellulose deposition and secondary wall synthesis, and improving the drought resistance of apples[31].

2.4 MdMYB regulates ion transporter activity

Calcium-dependent protein kinase pathway, that is, SOS pathway of salt stress signal and Na+ tolerance, plays an important role in plants' response to salt stress. This pathway helps plants pump toxic sodium ions out of cells, maintain ion homeostasis in cells, and avoid ion poisoning and generation of reactive oxygen species[32]. In this pathway, SOS3 senses the cytoplasmic calcium signal induced by salt stress. It interacts with serine/threonine protein kinase SOS2 and activates SOS2, which is phosphorylated and activated on plasma membrane. After being activated SOS1, SOS1 can discharge sodium ions from roots into soil solution or transport them to leaves through vascular tissues for a long distance[33,32]. SOS1 and NHX1 belong to NHX-type Na(K)/H exchange family genes (NHXS), and NHXS family is very important for the balance of Na and K in plants. The main function of NHX1 is to compartmentalize sodium ions in vacuoles and reduce the toxicity of sodium ions in cells[34].

Recent studies show that MdMYB gene can promote the expression of SOS1 and NHX1 proteins. MdMYB4 can combine with MdNHX1 promoter to improve its expression[35]. MdMYB108L can also be combined with MdNHX1 promoter to improve the stress resistance of apple[36]. MdERF106 interacts with MdMYB63. The MdMYB63-MdERF106 complex was formed, which combined with MBS cis-elements in MdSOS1, MdSOS2 and MdSOS3 promoters, and significantly promoted the expression of downstream MdSOS1[37].So as to improve the salt tolerance of apples[5].

2.5 MdMYB improves the stress resistance of apple by interacting with plant hormones.

Plant hormones may be the key regulators of customized responses to different stress combinations[38]. Abscisic acid (ABA) can close stomata and prevent plants from losing too much water under drought and salt stress[39]. The accumulation of salicylic acid (SA) can induce the development of systemic acquired resistance,(SAR) in nearby plant cells. SAR is an unprofessional, systematic and lasting defense mechanism[40]. The role of SAR is closely related to the expression of a group of nine genes called SAR gene family. Among them, five genes encode pathogenesis-related (PR) proteins, including chitinase, β-5,9- glucanase and somatin-like protein, which have antifungal activity in vitro when used alone or in combination[41]. In addition, jasmonic acid, ethylene and other plant hormones also have important functions in plant stress resistance. Recent studies show that some transcription factors of MdMYB family can promote hormone synthesis to improve plant stress resistance, and the expression of some MdMYB transcription factors is regulated by hormone level.

The MdMYB73W-box sequence interacts with the MYB binding sequence of the forward regulator MdWRKY31 promoter of B. dothidea. MdWRKY31 and MdMYB73 jointly enhanced the resistance of apple to B. dothidea, which may be affected by regulating SA pathway[42]. The expression of dSIMYB2 was positively induced by ethylene precursor ACC and several hormones including IAA,ABA,MeJA and SA. The results showed that the salt tolerance, drought resistance and cold tolerance of transgenic apple lines overexpressing MdSIMYB1 were higher than those of wild type[43].

3. Regulation of MdMYB gene function

3.1 MdMYB pre-transcriptional level regulation

Pre-transcriptional level regulation refers to the regulation at the gene level. It is found that MdMYB gene family is regulated by methylation, non-coding RNA and so on. Genes such as MdMYB1/10 are regulated by methylation level, and their expression levels increase with the decrease of methylation level. The scalar of MdMYB9/11/12/12 is negatively regulated by mdm-miR858.

Under natural conditions, the allele MdMYB1-2/3 of MdMYB1 gene is not expressed, and the methylation level of these genes is higher than 90% in the upstream of the translation initiation codon from -846 to -555 bp. However, it was found that apple bagging treatment can promote the accumulation of anthocyanins, significantly reduce the methylation level of MdMYB-704 to -555 bp and activate the expression of MdMYB1-2/3[44]. The low methylation of mCHG context in MYB10 leads to transcriptional activation [12] . The results show that the methylation level of MdMYB gene in the green stripe region of apple is higher than that in the red stripe region [27] .

Mdm-mir858, a mirna with multiple functions in plant development. Mdm-miR858 negatively regulates the accumulation of proanthocyanidins (PA) by targeting MdMYB9/11/12 in pericarp. Experiments show that mdm-miR858 can cleave and inhibit the expression of MdMYB9/11/12. In addition, MdBBX22 binds to mdm-miR858 promoter and induces its expression. Under light stress, MdBBX22 induced the expression of mdm-miR858, which inhibited the accumulation of PA, thus indirectly promoting the synthesis of anthocyanins in pericarp[45].

3.2 Regulation of MdMYB transcription level

The expression level of MdMYB gene family is regulated by many transcription factors. MdNAC52 binds to the promoters of MdMYB9 and MdMYB11 to promote the biosynthesis of anthocyanins and PA[45]. In addition, MdHY5 plays a role in the upstream of MdMYB108L, which interacts with the G-box sequence in MdMYB108L promoter to promote the expression of MdMYB108l, and MdMYB108L specifically binds to type-1 motif in MdHY5 promoter to inhibit the expression of MdHY5. The resulting increase in abundance of MdMYB108L down-regulated the transcription of MdHY5[25]. MdWRKY11 combined with the W-box motif in MdMYB9/10/11/12 promoter, positively regulated the expression level, promoted the synthesis of anthocyanins and improved the cold resistance of apples[46]. Overexpression of MdWRKY41 in red-fleshed apple callus inhibited the accumulation of anthocyanins and PA by down-regulating the expression of MYB TF gene (MdMYB12) and specific structural genes (MdLAR, MdUFGT and MdANR). In addition, MdWRKY41 has been proved to interact with MdMYB16 to form a complex, which can further inhibit the expression of MdANR and MdUFGT [47].

3.3 MdMYB post-translation horizontal regulation

Post-translational horizontal regulation refers to the regulation at protein level, which has multiple levels, such as: modifying specific proteins to make them active; Some proteins are specifically inactivated. At present, it is found that MdMYB1 and MdMYB23 proteins are regulated by this level, and MdMYB2 is involved in the regulation process. MdMYB1 protein needs sumo to achieve stability, and can be degraded by ubiquitination, and MdMYB23 can also be degraded.

It was found that MdSIZ1 directly sumo-the MdMYB1 protein in vivo and in vitro, especially at moderate low temperature (17°C), and this sumo-θ transformation was necessary for the stability of MdMYB1 protein. Recent studies have found that MdMYB2 is also involved in the SUMO process of MdMYB1, and low temperature induces MdMYB2, which further activates the expression of MdSIZ1, thus promoting the acylation of MdMYB 1, a key regulator of apple anthocyanin synthesis. MdMYB2 promotes the accumulation of anthocyanins in apple fruit, apple callus and Arabidopsis thaliana in a mdsiz1-dependent manner. In addition, the interaction between MdMYB2 and MdSIZ1 promoter significantly improved the tolerance of plants to cold stress[48,49]. MdMIEL1, as an

ubiquitin E3 ligase, ubiquitinates MdMYB1 protein and then degrades it by 26-s protease, which is an important negative regulation link of MdMYB1 degradation and anthocyanin synthesis[50]. MdCOP1 is also involved in the ubiquitination degradation pathway of MdMYB1[51]. MdBT2 was identified as an interacting protein of MdMYB23 and MdMYB1. MdBT2 inhibits cold tolerance and procyanidins accumulation by promoting the degradation of MdMYB23 and MdMYB1 proteins[52,20].

4. Summary and prospect

Drought, salt, low temperature and diseases will have a great impact on apple production. As transcription factors, MdMYB gene family is widely involved in various signal pathways of apple stress resistance. In production, MdMYB overexpression strains can be constructed to improve the anthocyanin content, fruit quality, apple stress resistance and yield.

The process of MdMYB's stress resistance in apple will be influenced by many factors, and it will also interact with other factors to play a common role. For example, the study of MdMYB1/9/10/11/12 reveals that the transcription level of MdMYB gene family is influenced by epigenetic factors; MdMYB can interact with factors such as MdNAC and MdWRKY to affect apple resistance; MdMYB1 protein needs sumo to achieve stability, and it can affect the stress resistance of apple through ubiquitination degradation. At present, the role of apple MYB gene family in stress-resistant signaling pathway has been well studied, but the stress-resistant process of apple is regulated by multiple genes and is a complex regulatory network. Therefore, it is necessary to further study the MdMYB gene family in order to better apply it to improve the stress resistance of apples.

Apples with MdMYB inhibitors knocked out can accumulate more anthocyanins and improve the cold resistance of apples, but MdMYB inhibitors play an important role in coping with strong light stress and nitrogen deficiency stress. It has been proved in other plant species that MYB inhibitors may interact with MWB complexes, thus inhibiting anthocyanin synthesis, but this signal pathway has not been found in apples, so we should further explore the signal pathway of expression regulation and participation of this kind of MdMYB genes, and make full use of MdMYB inhibitors to make apples have both[53,26].(Finally, we should return to the purpose and significance of the article and the development of the Apple industry.)

At present, China's apples are planted in a wide area, distributed in many regions and greatly influenced by environmental stress. Traditional breeding methods cannot meet the needs of apple production. As a transcription factor, MdMYB can effectively improve the expression of transcription factors related to stress resistance, which has broad application prospects in apple production. However, at present, the research of MdMYB gene family is limited to molecular level, and there is no research on breeding, introduction and cultivation of transgenic apples. In this paper, the MdMYB gene family-mediated apple stress resistance model was summarized, which laid a theoretical foundation for improving apple stress resistance and exploring stress-resistant apple germplasm in production.

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