Research Progress of miR164 and Target Gene *NAC* in Regulating Sugarcane Stress Tolerance

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Abstract Studies have shown that miR164 is a highly conserved miRNA family between monocot and dicotyledonous plants, and it plays an important role in the growth and development of sugarcane organs and in response to stress. As the main target gene of miR164, *NAC* transcription factors are mainly regulated at the post-transcriptional level. MiR164: NAC module may play an important role in determining the adaptive response of sugarcane to stress. MiR164 has a regulatory effect on the expression of target gene *NAC*, and may be closely related to the resistance process of sugarcane to abiotic stress, which provides a reference for using miRNA to carry out sugarcane resistance molecular breeding.

Key words miRNA164; NAC; Stress tolerance

1 Introduction

miRNA (MicroRNA) is a small endogenous non-coding RNA with a length of 20 to 24 nucleotides. In 2002, Reinhart *et al.*[1] discovered plant miRNA for the first time in the model plant *Arabidopsis thaliana*. Plant miRNAs mainly regulate downstream gene expression through two ways of post-transcriptional gene silencing, that is, cutting target mRNA that is reverse complementary to its sequence or inhibiting the translation of target mRNA[2]. On the one hand, when the sequences of plant miRNA and target mRNA are completely or almost completely complementary (only 0 – 5 mismatch bases), miRNA cuts downstream target mRNA under the action of RISC, HASTY, *etc.* to degrade it. On the other hand, when the sequences of the plant miRNA and the target miRNA are not completely complementary, the miRNA regulates the expression of downstream genes by inhibiting the translation of target miRNA[3]. In addition to the above two main mechanisms of action, the third mechanism of action of plant miRNAs on their target genes is based on the methylation of DNA and histones, which regulates them at the transcriptional level, thereby causing post-transcriptional silencing of target genes[4]. miRNAs and their target genes are the main regulators in response to various stresses. miRNAs almost regulate all biological and metabolic processes of plants, and are of great significance for regulating the expression of endogenous resistance genes. With the advancement of research technology, high-throughput and next-generation sequencing methods have become the first choice for miRNA analysis under low temperature stress.

2 miR164 participates in the research of plant resistance to stress

miR164 was first discovered in *A. thaliana*, and then through high-throughput sequencing, homology alignment, clone sequencing and other methods, many miRNAs with similar or identical sequences have been discovered in different plants, and they together constitute the miR164 family. The miR164 family is a type of conserved microRNA that exists widely in plants. In recent years, there have been more and more reports about miR164 family members participating in plant resistance to stress. The expression of miR164 is tissue and developmental stage specific, and plays an important regulatory role in plant cell growth, tissue development, and organ formation[5]. Under adversity stress conditions, plant miR164 responds to adversity stress, and may play an important role in the defense system as a regulatory molecule. Under abiotic stress conditions, mechanical damage would inhibit the expression of *Populus pilosa* miR164, and salt and drought stress treatments would inhibit the expression of miR164 in sweet poplar seedlings[6].

3 Research on the anti-adversity effect of NAC transcription factor

The target genes regulated by plant miR164 belong to plant NAC (NAM, ATAF1/2 and CUC2) transcription factors. NAC transcription factors are a family of plant-specific transcription factors that can be regulated at transcription, post-transcriptional and protein levels. As the main target gene of miR164, NAC transcription factors are mainly regulated at the post-transcriptional level. In recent years, NAC protein has been deeply studied because of its multiple roles in the process of plant growth and adaptation to the environment[7]. NAC transcription factors can be regulated by certain cis-acting elements and trans-acting factors at the transcription level, miRNAs at the post-transcriptional level, and post-translational regulation levels including phosphorylation, protein degradation, and dimerization[8]. In wheat, the *TaNAC29* gene of the

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NAC family is involved in the response to salt, drought and ABA treatment. TaNAC2L can enhance the heat tolerance of wheat. The *NAC* gene can be used to fine-tune senescence to improve yield and quality[9]. Many *NAC* proteins play a role in abiotic stress response. *Arabidopsis* ANAC019, 055 and 072 respond to abiotic stresses such as drought, salt and ABA, and *Arabidopsis* NAC transcription factor LOV1 responds to cold stress. Overexpression of rice SNAC1 significantly improves the drought resistance of transgenic rice[10]. In *Arabidopsis*, overexpression of TaNAC67 significantly improves tolerance to freezing stress[11]. As an important upstream regulator of plant abiotic stress response, TsNAC1 plays a role in adversity stress[2]. TaNAC30 negatively regulates resistance to stripe rust in wheat[12]. TaNAC69-1 is the transcriptional repressor of TaSHY2 and TaIAA7, which is homologous to the *Arabidopsis* root growth regulator, and may be involved in promoting root elongation in dry soil[13]. TaNAC2-5A can be directly combined with the promoter regions of genes encoding nitrate transporter and glutamine synthetase. TaNAC2-5A overexpression transgenic wheat has higher grain yield and higher nitrogen accumulation in the aerial part[14].

Abiotic stress strongly induces the expression of NAC1. These NAC transcription factors involved in stress response improve the abiotic stress resistance of transgenic or mutant plants. Under the conditions of drought, high salinity and ABA treatment, the expression levels of *Arabidopsis* ANAC019, ANAC055 and ANAC072 were significantly up-regulated; the RNA expression of ATAF1 was also strongly induced by drought and ABA treatment[15]. The overexpression of ANAC019, ANAC055 or ANAC072 in *Arabidopsis* significantly improved the drought tolerance of plants; overexpression of ATAF increased the sensitivity to salt and ABA stress, and the mutants ataf1-1 and ataf1-2 showed better results than wild-type plants. In rice, high salt induced the expression of OMTN1/ONAC027, OMTN2/ONAC004, OMTN3/ONAC060, OMTN4/ONAC011 and OMTN6/ONAC104, while the expression of OMTN1, OMTN3 and OMTN4 were also induced by ABA; however, under drought stress conditions, the expression of OMTN1, OMTN3, OMTN4 and OMTN6 was significantly reduced[16]. ONAC004 and ONAC092/OMTN5 are up-regulated under drought, salt and cold stress conditions. Gibberellin treatment can enhance the expression of OSNAC2/ONC004. In rice, the overexpression of OMTN2, OMTN3, OMTN4 and OMTN6 leads to a decrease in drought tolerance[17].

4 Study on the regulation of miR164 and target gene *NAC* on sugarcane stress tolerance

4.1 miR164 regulates *NAC* gene and participates in sugarcane growth and stress resistance research miRNA does not directly act in regulating the response of plants to low temperature stress. On the contrary, as mentioned above, miRNA acts as a regulatory factor for gene expression through endonucleic acid cleavage or translational inhibition of target genes. Therefore, identifying the target genes involved in the low temperature response is essential for revealing the regulatory functions of miRNAs and delineating the complex network of genes that respond to stress. The *Arabidopsis* miR164 family (ath-miR164a, b, and c) regulates the mRNA degradation of five NAC transcription factor genes (*CUC*1, *CUC*2, *NAC*1, *at*5*g*07680, and *at*5*g*61430) for the formation of plant organ borders, lateral root production, nutrition and flowers required for organ formation and age-related cell death[18]. Wheat miR164 and its target gene *NAC* (miR164-NAC) are negatively correlated in response to drought stress[19]. *Populus euphratica* peu-miR164 negatively regulates PeNAC070 to improve tolerance to drought stress and salt stress[20]. tae-miR164 negatively regulates TaNAC21/22 and plays an important role in regulating wheat stripe rust resistance[21]. In wheat, miR164 and its target are involved in the drought stress tolerance mechanism of wheat[22]. In maize, miR164 directed cleavage of ZmNAC1 to regulate lateral root development. Somatic embryogenesis studies have shown that at the stage of embryogenesis, the expression of miR164 in callus is significantly higher than that in non-embryogenic callus, and miR164 and some new miRNAs play an important role in callus formation[23]. The overexpression of PeNAC070 in *Arabidopsis* promotes lateral root development, delays stem elongation, and improves the sensitivity of transgenic plants to drought and salt stress[24]. In *Arabidopsis*, NAC4 promotes allergic cell death by inhibiting the expression of its target genes, and this immune process is negatively regulated by miR164, which increases the sensitivity of transgenic plants to drought and salt stress[25]. The role of miR164-NAC protein is to attenuate the auxin signal related to lateral root development in *Arabidopsis*. Research on rice senescence-related genes found that the rice miR164 family mediates rice anti-aging by regulating the target gene *NAP* (a member of the NAC family)[26]. In addition to the role of growth and development, the *NAC* gene targeted by the conservative miR164 is also a negative regulator of drought resistance in rice[27]. In the *Arabidopsis* miR164 mutant, the expression of *NAC1* gene was increased, and the number of lateral roots of the plant was significantly increased compared with that of the wild type. Overexpression of miR164 resulted in a decrease in the expression of the *NAC1* gene, and the number of lateral roots of the plant was significantly reduced compared to the wild type. miR164 can also participate in the formation of plant organ borders and meristems by regulating *NAC* genes. CUC1 and CUC2 are transcription factors that can regulate the leaf morphology, flower morphology and apical meristem formation of *A. thaliana*[28]. Overexpression of MIR164a/b can reduce the expression levels of CUC1 and CUC2, and plants show the fusion of cotyledons and floral organs, and the abnormal formation of apical meristems[27]. Mutating miR164 or changing the binding site of miR164 can cause the nicks of plant leaves to deepen. ORE1 transcription factor (a transcription factor belonging to the NAC family) can promote leaf senescence and cell apoptosis. In the process of leaf senescence induced by EIN2, it was found that the expression of miR164 decreased, and the increase of ORE1 expression was negatively correlated with MIR164[29].

Expression of several *NAC* genes has been found to be induced by different stresses in both ABA-dependent and ABA-independent manner[30]. Functional characterization of different NAC-TFs in response to dehydration, salinity and osmotic stresses supports their role in enhancing tolerance[31]. AtNAC72, AtNAC109, and AtNAC55 were shown to impart drought tolerance by promoting the detoxification of aldehydes in the glyoxalase pathway[32]. Investigation into the mechanisms of other NAC-TFs revealed that they act in a variety of ways by controlling stomatal closure[33], increasing cell membrane stability and enhancing expression of several antioxidant enzymes such as POD (peroxidase), SOD (superoxide dismutase) and P5CS (Pyrroline-5-carboxylate synthase), for increasing ROS (reactive oxygen species) scavenging ability. JUBGBRUNNEN1 (JUB1 or NAC042), a central regulator of plant growth and stress response, confers tolerance to various osmotic and ionic stresses including salt and drought by reducing H_2O_2 levels[34]. In soybean, 31 *NAC* genes were screened and nine of them were found to be induced by drought stress. The expression of GmNAC2/3/4 was significantly induced by osmotic pressure and GmNAC3/4 expression was simultaneously induced by ABA, JA, and salt[35]. Although several advances have been achieved in the study of NAC-TFs, the intricate regulation of different NAC-TFs under salt and drought stress remains largely unknown.

4.2 miR164∶NAC module interaction improves plant resistance to stress miR164∶NAC is the well-studied genetic module in different species like *A. thaliana* (arabidopsis), *Zea mays* (maize), *Nicotiana tabaccum* (tobacco), *Ammopiptanthus mongolicus* (ammopiptanthus) and *Phyllostachys edulis* (moso bamboo)[36]. The role of miR164 in suppressing the transcripts of the development-associated and stress-responsive NAC transcription factors is well-studied[37]. The role miR164∶NAC module in ovule initiation, root formation and various other aspects of plant growth and development has been reported[38]. It affects plant development through regulation of auxin signaling. The tae-miR164 regulating TaNAC21/22 negatively modulates the resistance of *Triticum aestivum* (wheat) to *Puccinia striiformis*[39]. Likewise, miR164 participates in plant defense against *Verticillium dahliae* by post-transcriptionally regulating the expression of a NAC-TF[40]. Comprehensive analysis of miR164∶NAC module also showed its involvement in response to drought and salt stress in several plant species[41]. For instance, in moso bamboo, ped-miR164∶PeSNAC1 controls tolerance to salinity and drought through regulation of lateral root development[42]. This indicates co-evolution of miRNA and its targets to regulate specific functions.

Although there was variability in the number of miRNAs and the NAC targets, the miR164∶NAC module appeared to be conserved across species but showed dynamic expression profile under increasing duration of stress. Considering the conserved nature of miR164∶NAC module, it is a suitable target for future work to understand its intricate regulation at the genome level to decipher the mechanism underlying abiotic stress response. Collectively, our results suggest that genetically determined dynamic modulation of the conserved miR164∶NAC-TF module may play an important role in determining the adaptive response of plants to stress.

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