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RESEARCH PROGRESS IN TRANSCRIPTOMICS

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Abstract: the results of the use of transcriptomics technology for understanding the molecular mechanism of stress resistance in wheat plants, as well as the cultivation of highly resistant varieties by genetic engineering, are described.

Keywords: wheat, transcriptional sequencing, transcription activity, adaptation mechanism, genes, RNA sequencing, cold resistance**.**

Transcriptomics is a fast and direct approach to the genes themselves. Using transcriptomics technology to understand the molecular mechanism of stress resistance in plants, and breeding highly resistant varieties through genetic engineering has become one of the hot topics in the field of botany. Transcriptome sequencing based on next-generation high-throughput sequencing technology can detect the overall transcription activity of any species at the single nucleotide level . During the long-term natural evolution of wheat, a relatively complete mechanism for adapting to low temperature environments has been formed. In the low temperature environment, wheat firstly senses the low temperature region through various complex signal pathways, and transmits the low temperature signal step by step to regulate the expression of downstream genes. Eventually, it causes the cell to produce low temperature response and physiological and biochemical changes, making the wheat adapt to the low temperature environment.

Robinson and Parikin used the SAGE technique to identify 920 cold-induced differentially expressed genes from Arabidopsis, of which 24% of the differentially expressed genes overlap with the identification results of the previous microarray technology . Molina et al. Used Super SAGE technology to identify 7 532 drought stress response genes and 880 drought stress response genes from roots of drought-tolerant chickpea cultivars. These drought response genes are involved in signal sensing and transmission, reactive oxygen species clearance, metabolism, osmotic and ionic stress-related pathways, and water and ion balance regulatory genes .However, salt stress or drought stress response genes identified using stress-sensitive plants such as Arabidopsis thaliana may reflect death or senescence rather than adaptation. therefore. Transcriptome analysis of plants that are more resistant to stress has become an important strategy for studying molecular mechanisms of plant stress and identifying related genes. For example, a large number of salt stress-responsive genes have been identified from halophytes using transcriptomics: Wang et al. Established a cDNA library of Stargrass under 450 mmol / L NaHCO3 stress and performed ESTs analysis to identify differential expression. Gene and found that the metabolic functional group accounted for the largest proportion of all responding gene functional groups.

The use of related species or different salt-tolerant genotypes of the same species can better clarify the salt-tolerance mechanism of plants, especially using genotypes with similar genetic backgrounds and only some genes. Walia et al. Used two rice recombinant inbred lines (RILs) with large differences in salt tolerance as research materials and used a rice genomic chip containing 55515 probes to study rice gene expression under salt stress during vegetative growth. The results showed that more genes were induced in sensitive genotypes than in salt-tolerant genotypes, which involved riboflavin synthesis pathway genes. Differential expression of cell wall synthetic genes was found in both genotypes, indicating that cell wall reconstruction plays an important role in the adaptation of plants to salt stress.

With the development of RNA sequencing technology and its application in the study of plant transcriptomics, the study of plant stress-resistant molecular biology has also ushered in new opportunities for development. Xu Shuo used the Solexa sequencing platform to obtain significantly different salt stress-related miRNAs from the mi RN A differential expression analysis of soybean Williams82. Olga et al. Used the Illumina sequencing platform to deeply sequence two alfalfa varieties with different salt tolerance under 250 mmol / L Na Cl treatment, and found that 1165 genes, including 86 transcription factor genes were significantly regulated. Analysis of common response genes and functional annotation, mining candidate genes for salt tolerance in alfalfa . Pang et al. Used the Illumina sequencing platform for the first time to perform transcriptome sequencing of sargassum under low temperature stress (−30℃), and also the first time to study the transcriptome of non-model species under low temperature stress. Through the study of differentially expressed genes, Shadongqing was found to be involved in low temperature metabolic pathways and important transcription factor genes. Xiu et al. Used the Illumina sequencing platform and bioinformatics tools to study gene expression changes of soybean inbred line HJ-1 after 48 hours of salt stress, saline-alkali stress, and drought stress, providing new insights into soybean stress tolerance.

Cold resistance refers to the adaptability of plants to low-temperature environments formed by domestication and genetic variation for a long period of time. Cold resistance is regulated by multiple genes, and the cold resistance gene is a type of inducible gene. Only under the induction of low temperature and short sunlight, can it be expressed in large quantities and then show cold resistance. The cold resistance genes of wheat were first proposed by Kaperperska when studying the cold resistance of wheat seedlings. Since then, various genes related to cold resistance in wheat have been continuously discovered. At present, there are more than 450 cold resistance genes in wheat. It was isolated and identified. The expression of cold-resistant genes in plant cells means the synthesis of new cold-resistant proteins. These cold-resistant proteins play an important role in improving the cold resistance of plants. Many studies have confirmed that, due to the huge genetic background of wheat, cold resistance in wheat is controlled and exerted by micro-effects of multiple genes. Researchers have located alleles related to cold resistance in wheat on more than 10 pairs of chromosomes such as 1B, 1D, 2B, 2D, 4D, 5A, 5D, and 7A. Among them, 5A and 5D chromosomes are related to wheat. Cold resistance is most closely related. In recent years, with the rapid development of modern biotechnology, researchers have used various methods such as isozyme labeling, protein labeling, DNA molecular labeling, and Several types of genes with multiple cold resistance genes were isolated and identified in wheat . Fridovich research suggested that the cold resistance genes of wheat are located on 4D, 5D and 7A of the chromosome, and their effects have additive effects. Cahalan's freeze tolerance test in hexaploid and tetraploid wheat showed that the alleles on the 5A and 5D chromosomes of the Chinese spring variety had weak cold-resistance genes, while the variety Chanizhong Both the 5A and 5D alleles in Hope are closely related to freezing tolerance .

The mRNA of the cold-treated wheat variety "Shixin 828" and the control material was detected by differential display technology. A gene regulating the cold-regulated protein wpebp of wheat was found. After quantitative quantitative PCR analysis, the expression level of this gene changed with the low-temperature treatment time. The increase is increasing, indicating that this gene is related to the cold resistance of wheat. High cold-tolerant wheat varieties Dongnong Dongmai 1 and normal winter wheat Jimai 22 were treated at low temperature of 4 ℃, -10 ℃ and -20 ℃. According to the expression of Ta EXPA5, Ta EXPA6, Ta EXPA7 in wheat root tissue, These genes are associated with resistance to low temperature stress . Studies by Feng Yulei and others show that the root system of Dongnong Dongmai 1 is more developed and has a certain resistance to low temperature and other stresses, becoming the key to the successful wintering of winter wheat in high and cold regions . The low temperature sensitive wheat anti-white line and the control variety Aibian No. 1 were used as materials. Four chloroplast genes pet N, trn C, pet D, and rrn 16S that were different under low temperature conditions and promoters of these genes (TaMET1, TaDRM) were found , TaCMT). The expression levels of these four genes were detected by fluorite quantitative PCR using sulfite sequencing method. The results showed that the expression of pet N was sensitive to low temperature and the gene expression was down-regulated. TaMET1 and TaCMT genes in Dwarf No. 1 Expression is down-regulated.

Chen Lu concluded that TSOD-3, HAPX-2 and HAPX-3 genes are important genes involved in low-temperature response. Among them, TSOD-3 gene plays a major role in the early stage of cold stress, and HAPX-2 and HAPX-3 genes play a major role under continuous freezing. The yellow crane study showed that the relative expression of TPS2, TPS3, and TPS6 genes reached the highest during freezing, and then decreased. It is speculated that the accumulation of trehalose is regulated by temperature, but it is not that the lower the temperature, the higher the expression of TPS, and the stronger the cold resistance, but there is a certain threshold value. If the temperature is too low, it will interfere with the normal metabolism of trehalose in wheat. Lu Yan concluded through research that ABA is positively regulating the expression of the TabZIP1 gene and is helpful to improve the cold resistance of winter wheat . The TaHD2C gene improved the cold resistance of Arabidopsis thaliana and could be stably inherited in the offspring. The expression level of wcor14a was significantly higher than that of the control under low temperature stress, which is consistent with previous research results, further verifying the sensitivity of the gene to low temperature Sex. Glutathione S-transferase (GST) is a multifunctional protease. The three GST genes are significantly up-regulated in roots and leaves in response to low temperature stress . After low temperature stress at 4 ℃, the expression of Ta CIPK8 gene in wheat leaves decreased and reached the lowest value at 8 h; while in the root, the expression of Ta CIPK8 gene was induced up to 12 h . Wheat Ta Di19ATa Di19A may improve cold tolerance of transgenic plants by regulating downstream cold stress response-related genes expression . A total of 19 cold-resistant genes were screened using Chinese spring as materials. Among them, significant correlations of AA changes in CBF-A3, CBF-A15, VRN-A1, VRN-B3, PPD-B1, and PPD-D1 were found to assist cold-resistant wheat Breeding . Hsp90, BBI and REP14 may play important roles in conferring cold tolerance in bread wheat .

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