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Functional Analysis of the Promoter pBnMYB69-1 in Brassica Napus

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Abstract:

MYB transcription factors play a crucial role in regulating plant development and adaptation. Brassica napus, a major oil crop, often faces challenges such as lodging and diseases. In this study, using the genome of Brassica napus as a template, we cloned a 2000 bp promoter sequence upstream of the start codon of the pBnMYB69-1 gene. Prediction and analysis of cis-regulatory elements revealed that the promoter contains essential core regulatory elements of eukaryotic promoters, such as the TATA box and CAAT box. Additionally, it includes various functional elements, such as light-responsive elements (ATCT-motif, Box 4, TCT-motif), anaerobic induction elements (ARE), methyl jasmonate (MeJA)-responsive elements (CGTCA-motif, TGACG-motif), and gibberellin-responsive elements (P-box). These findings provide a solid foundation for the application of this promoter in transgenic improvement of crop quality and the artificial creation of germplasm resources in Brassica napus.

Keywords: Brassica napus, promoter, function, pBnMYB69

1. Introduction

The MYB family represents a large group of transcription factors (TFs) (Feller et al., 2011). Due to the diversity in gene numbers and functions, it has been a central focus of research on plant transcription factor functionality. Based on the number of repeats, MYB TFs are categorized into four groups: 1R-MYB, R2R3-MYB, 3R-MYB, and 4R-MYB (Dubos et al., 2010). Among these, R2R3-MYB transcription factors have been demonstrated to play critical roles in plants, including cell fate and identity determination, developmental processes, and responses to both biotic and abiotic stresses (Hajiebrahimi et al., 2017).

Although members of the MYB superfamily have been annotated in Arabidopsis and many other plants, the majority remain functionally uncharacterized. BnMYB69, an R2R3 transcription factor, is known to regulate lignin synthesis, mediate secondary growth and plant height limitations by restricting cell division and cell cycle processes, and constrain indole-3-acetic acid (IAA) synthesis through the shikimate pathway for IAA precursor production. Additionally, it plays a role in regulating glucose metabolism in plants.

Promoters are essential genetic elements involved in the regulation of gene expression. Cis-acting elements within promoters interact with corresponding transcription factors to either activate or repress gene

expression, thereby enabling precise control of gene activity. Analyzing promoter elements is thus a critical approach for predicting upstream regulatory genes (Yao Q et al., 2011).

Rapeseed, a member of the genus Brassica in the family Brassicaceae, is one of the four major oil crops and ranks among the top five global economic crops. It serves as an essential source of edible vegetable oil. Since the advent of the first genetically modified rapeseed in 1985, rapeseed genetic engineering has advanced rapidly, with molecular breeding techniques offering a more precise and efficient way to improve crop quality compared to traditional breeding methods (Odell JT et al., 1985). However, the lack of stable and highly efficient endogenous constitutive promoters in rapeseed has limited the potential of rapeseed genetic engineering (Ooms G et al., 1985). Therefore, studying endogenous constitutive promoters in dicotyledonous crops like rapeseed holds significant application value for genetic improvement and molecular breeding in dicotyledons.

In this study, we cloned approximately 2 kb of the upstream promoter sequence of the BnMYB69-1 gene. Using online tools, we predicted the cis-acting elements within this promoter and analyzed the distribution characteristics of its regulatory elements. This work provides a theoretical foundation for understanding the functions of the MYB transcription factor family in the physiological and biochemical processes of Brassica napus.

2. Materials and Methods

1) Cloning of Target Fragment

Using whole-genome sequencing data as a reference, primers were designed using the online primer design tool available at NCBI. The molecular cloning of the pBnMYB69-1 promoter was successfully completed based on the designed primers.

2) Sequencing of target fragments

The pBnMYB69-1 promoter was sequenced and the sequencing results were obtained (Figure 1)

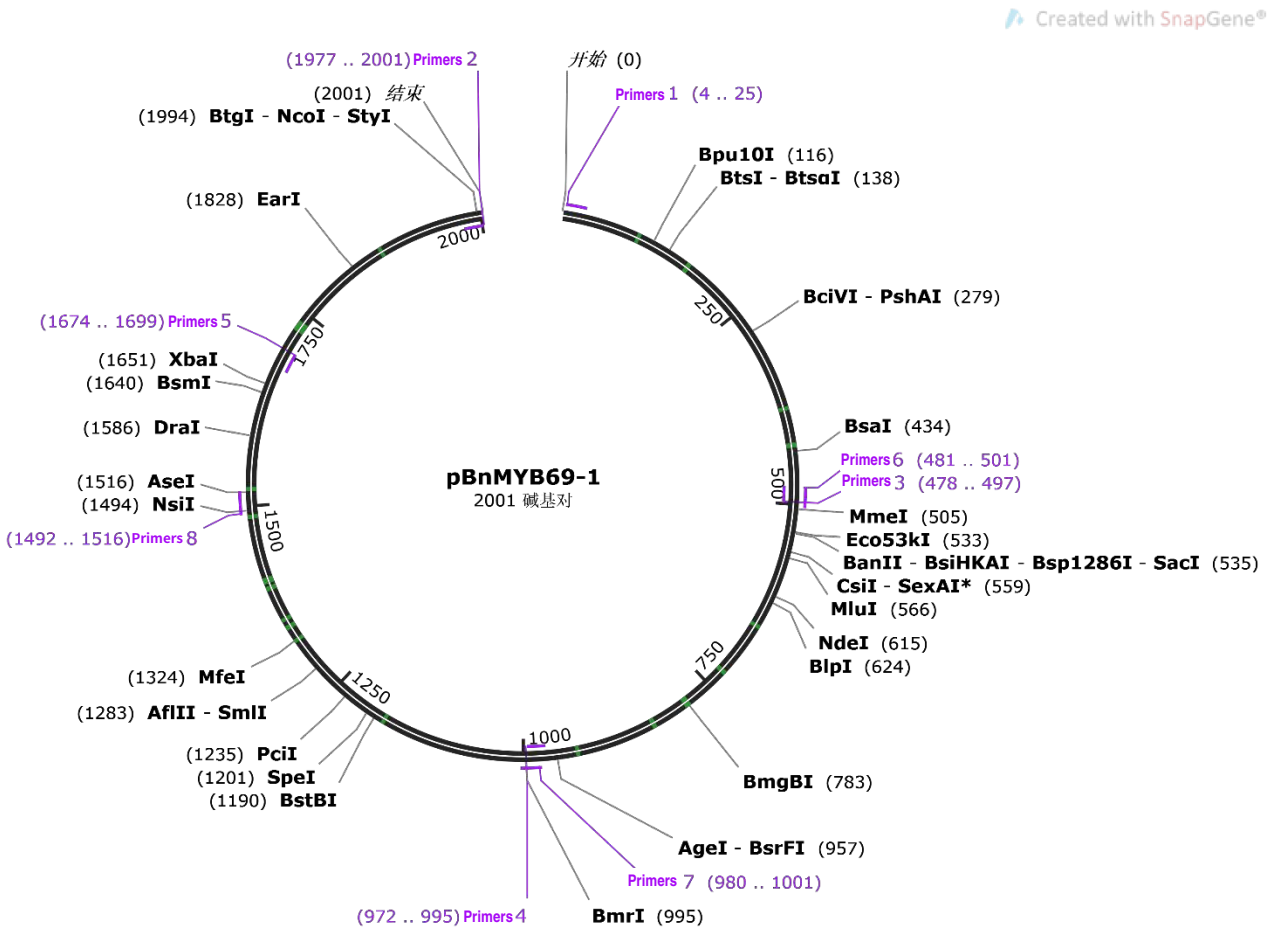

Figure 1 Demonstration of pBnMYB69-1 promoter sequencing results

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3. Results and Analysis

PlantCare online bioinformatics analysis of the sequencing results of the pBnMYB69-1 promoter (Figure 2, Table 1) showed that the sequence contained a large number of functional elements of various types, including 47 core promoter elements TATA-box, 33 core elements CAAT-box common to promoters and enhancers, 8 light signal response related elements AE-Box, GA-motif, G-Box, GT1-motif, GATA-motif and TCT-motif, 2 anaerobic regulatory elements ARE, 1 abscisic acid regulatory response element ABRE, 4 jasmonic acid response regulatory elements CGTCA-motif and TGACG-motif, 2 gibberellin response regulatory elements GARE-motif and P-Box, 1 low temperature response regulatory element LTR, 2 drought-induced combined MBS response elements, and 65 other functional elements of unknown function.

The distribution of cis-acting elements is relatively complex in the promoter 324~1557 bp. The main cis-acting elements in this region are shown in Table 1. The core element CAAT-box in the promoter and enhancer regions appears multiple times and is evenly distributed throughout the sequence; the transcription initiation core promoter element TATA-box is distributed at 842~1861 bp. The hormone regulatory element abscisic acid response element ABRE is distributed at 782 bp; the methyl jasmonate response elements CGTCA-motif and TGACG-motif are distributed at 785 and 1485 bp; the gibberellin response regulatory elements GARE-motif and P-Box are distributed at 35 and 1126 bp; the drought-induced combined MBS response elements are distributed at 165 and 724 bp; the light response-related element GATA-box is distributed at 1558 bp; Box S is distributed at 882 bp; and G-box is distributed at 781 bp. In summary, the functional elements of the pBnMYB69-1 promoter mainly mediate light, temperature and related plant hormones such as gibberellins, abscisic acid and methyl jasmonate to cope with biotic and abiotic stresses of plants.

From the perspective of reference species, similar functional elements mainly come from annual herbaceous plants such as Arabidopsis thaliana, barley (Hordeum vulgare), oats (Avena sativa), parsley (Petroselinum crispum), sunflower (Helianthus annuus), peas (Pisum sativum), mustard (Brassica juncea), rice (Oryza sativa), corn (Zea mays), and sticky tobacco (Nicotiana glutinosa).

Table 2 List of promoter functional elements in pBnMYB69-1 sequence

Functional elements	Sequence	Number	Direction	Reference species	Function Introduction
AE-box	AGAAACAA	1	+	*Arabidopsis thaliana*	Optical signal response related elements
LTR	CCGAAA	1	+	*Hordeum vulgare*	Low temperature stress response element
GT1-motif	GGTTAAT	2	-	*Avena sativa/ Arabidopsis thaliana*	Optical signal response related elements
AAGAA-motif	GAAAGAA	1	-	*Avena sativa*	
MYC	CATGTG	2	+/-	*Arabidopsis thaliana*	
GA-motif	ATAGATAA	1	+	*Arabidopsis thaliana*	Optical signal response related elements
TCT-motif	TCTTAC	1	+	*Arabidopsis thaliana*	Optical signal response related elements
A-box	CCGTCC	1	-	*Petroselinum crispum*	cis-regulatory element
TATA-box	TATA	47	+/-	*Helianthus annuus/ Arabidopsis thaliana/ Pisum sativum/ Brassica juncea/ Oryza sativa/ Zea mays*	Promoter core elements

CAAT-box	CAAT	33	+/-	*Nicotiana glutinosa/ Pisum sativum/ Arabidopsis thaliana*	Core elements common to promoters and enhancers
MBS	CAACTG	2	-	*Arabidopsis thaliana*	MBS drought stress binding element
GARE-motif	TCTGTTG	1	-	*Brassica oleracea*	Gibberellic acid response regulatory element
Myb	TAACTG	4	-	*Arabidopsis thaliana*	
CCGTCC motif	CCGTCC	1	-	*Nicotiana tabacum*	
CGTCA-motif	CGTCA	2	+/-	*Hordeum vulgare*	Jasmonic acid response regulatory element
ABRE	ACGTG	1	+	*Arabidopsis thaliana*	Abscisic acid responsive regulatory element
Myc	TCTCTTA	1	-	*Arabidopsis thaliana*	
ARE	AAACCA	2	-	*Zea mays*	Anaerobic response regulatory element
G-box	CACGTC	1	-	*Zea mays*	Optical signal response related elements
box S	AGCCACC	1	+	*Arabidopsis thaliana*	
MYB	TAACCA	11	+/-	*Arabidopsis thaliana*	
CCGTCC-box	CCGTCC	1	-	*Petroselinum hortense*	
as-1	TGACG	2	+/-	*Arabidopsis thaliana*	
Box 4	ATTAAT	1	-	*Petroselinum crispum*	Optical signal response related elements
TGACG-motif	TGACG	2	+/-	*Hordeum vulgare*	Jasmonic acid response regulatory element
P-box	CCTTTTG	1	+	*Oryza sativa*	Gibberellic acid response regulatory element
Unnamed__4	CTCC	12	+/-	*Petroselinum hortense*	
GATA-motif	AAGATAAGATT	1	+	*Arabidopsis thaliana*	Optical signal response related elements

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Figure 2 Bioinformatics analysis of pBnMYB69 promoter

4. Discussion

Gene promoter is a DNA sequence located upstream of the gene coding region. It has specific binding sites involved in gene transcription initiation and regulation, and contains a variety of cis-acting elements to respond to external biological and non-biological factors. Therefore, promoter function analysis is the basis for understanding plant gene expression regulation. Identification and analysis of functional cis-acting elements of promoters is very important for analyzing gene transcription regulation mechanisms.

TATA box is one of the elements that constitute eukaryotic promoters. Its consistent sequence is TATA(A/T)A(A/T) (non-template chain sequence). It is located upstream of the transcription start point of most eukaryotic genes, basically composed of A-T base pairs, and is the choice that determines the start of gene transcription. It is one of the binding sites of RNA polymerase. RNA polymerase can only start transcription after firmly binding to the TATA box. CAAT box, with a consistent sequence of GGCTCAATCT, is a common regulatory region of eukaryotic genes. It is located about -80bp upstream of the transcription start point. It is the binding site of transcription factor CTF/NF-1 and controls the frequency of transcription initiation.

Gene promoters of eukaryotic organisms generally contain 1-2 TATA-box core promoter elements, but the pBnMYB69-1 promoter is relatively complex, containing dozens of core promoter elements TATA-box and enhancer elements CAAT-box, of which the core promoter element TATA-box is concentrated in 842-1861 bp, and the enhancer element CAAT-box is scattered throughout the promoter region. In addition to the above elements, there are other cis-acting elements on the pBnMYB69-1 promoter, including abscisic acid response element ABRE, methyl jasmonate response element, gibberellin response element, multiple light response elements and drought stress response elements, which indicates that the expression of BnMYB69-1 may be induced by abscisic acid, gibberellin, jasmonic acid and other hormones as well as environmental factors such as light and drought stress.

In the future, further analysis of the promoter activity and function of pBnMYB69-1 is still needed. Based on the above results, a plant expression vector will be constructed to realize GUS expression analysis and functional analysis of the pBnMYB69 gene.

References

[1] Ooms G, Bains A, Burrell M, et al. Genetic manipulation in cultivars of oilseed rape(Brassica napus) using Agrobacterium[J]. Theoretical and Applied Genetics, 1985, 71(2) : 325 - 329.

[2] Yao Q, Lin Y. Cloning and functional analysis of a promoter with temporal - spatial expressing differentiation in rice root[J]. Journal of Agricultural Biotechnology, 2011, 19(2) : 214 - 220.

[3] Battraw M J, Hall T C. Histochemical analysis of CaMV 35S promoter - β - glucuronidase gene expression in transgenic rice plants [J]. Plant Molecular Biology, 1990, 15(4) : 527 - 538.

[4] Ebert P R, Ha S B, An G. Identification of an essential upstream element in the nopaline synthase promoter by stable and transient assays[J]. Proceedings of the National Academy of Sciences, 1987, 84(16) : 5745 - 5749.

[5] Odell J T, Nagy F, Chua N H. Identification of DNA sequences required for activity of the cauliflower mosaic virus 35S promoter[J]. Nature, 1985, 313(6005) : 810.

[6] Zhu Yuxian, Li Yi. Modern Molecular Biology[M]. Beijing: Higher Education Press, 2005.

[7] Irie T, Honda Y, Hirano T, et al. Stable transformation of Pleurotus ostreatus to hygromycin B resistance using Lentinus edodes GPD expression signals[J]. Applied Microbiology and Biotechnology, 2001, 56(5 /6) : 707 - 709.

[8] Feller, A., Machemer, K., Braun, E. L., and Grotewold, E. (2011). Evolutionary and comparative analysis ofMYB and bHLH plant transcription factors. Plant J. 66 (1), 94–116. doi: 10.1111/j.1365-313X.2010.04459.x

[9] Hajiebrahimi, A., Owji, H., and Hemmati, S. (2017). Genome-wide identification, functional prediction, and evolutionary analysis of the R2R3-MYB superfamily in Brassica napus. Genome 60 (10), 797–814. doi: 10.1139/gen-2017-0059

[10] Baldoni, E., Genga, A., and Cominelli, E. (2015). Plant MYB transcription factors: Their role in drought response mechanisms. Int. J. Mol. Sci. 16 (7), 15811–15851. doi: 10.3390/ijms160715811

[11] Wei, X.,Shan, T.,Hong, Y.,Xu, H.,Liu,X., andZhang,Z. (2017). TaPIMP2, apathogen-induced MYB protein in wheat, contributes to host resistance to common root rot caused by Bipolaris sorokinian. Sci. Rep. 7, 1754. doi: 10.1038/s41598-017-01918-7

[12] Dubos, C., Stracke, R., Grotewold, E., Weisshaar, B., Martin, C., and Lepiniec, L. (2010). MYB transcription factors in Arabidopsis. Trends Plant Sci. 15 (10), 573–581. doi: 10.1016/j.tplants.2010.06.005