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Identification and characteristics of MYB4 transcription factor related to regulation of abiotic stress tolerance in peanut

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Abstract. Peanut (*Arachis hypogaea* L.), an economically valuable crop, provides protein and oil for human and animal consumption. The transcription factor MYB4 has been identified as a potential drought tolerance gene in peanut. This study aimed to isolate and characterize the *MYB4* gene in the L14 peanut cultivar. The isolated *AhL14_MYB4* gene was found to be 1.1 kb long, with a 663 bp coding sequence containing 3 exons and 2 introns. *In silico* analysis showed that AhL14_MYB4 possesses a nuclear localization signal and two DNA-binding domains characteristic of transcription factors. The findings revealed key molecular features of AhL14_MYB4 and provided insights into improving drought resistance in peanut varieties. Further research on AhL14_MYB4 may aid efforts to enhance drought tolerance in local peanut cultivars through molecular breeding or genetic engineering. Overall, this finding about preliminary characterization of the peanut *MYB4* gene lays the groundwork for potential genetic improvements to this economically important crop.

Keywords: *MYB4,* peanut, abiotic stress, PCR cloning

1 Introduction

Peanut (*Arachis hypogaea* L.) is an important crop in agriculture, widely used in food and animal feed production [1]. However, peanut plants are often subjected to drought stress, which is one of the major constraints affecting crop productivity and quality. Developing peanut varieties with improved drought tolerance is therefore of great importance for sustainable agriculture. In recent years, significant progress has been made in understanding the molecular mechanisms based on their sequences underlying drought tolerance in peanuts, which has led to the identification of potential genes and pathways involved in this process [2, 3].

MYB transcription factors play a critical role in regulating plant responses to abiotic stresses. Under stress conditions such as drought, salinity, and cold, MYB transcription factors are involved in the modulation of gene expression that contributes to stress tolerance in plants [4]. The *MYB4* gene encodes a transcription factor belonging to the R2R3-MYB family, which plays a crucial role in plant responses to various abiotic stresses, such as cold, freezing, salt, and drought stress, as well as in cadmium tolerance and flavonoid biosynthesis [5-11]. This family of transcription factors has been widely studied in recent years due to their potential for enhancing stress tolerance in plants, which is of great significance for crop productivity and food security in the face of global climate change.

Specifically, transgenic expression of the rice *Osmyb4* gene in *Arabidopsis thaliana* can enhance tolerance to low temperature and

freezing conditions in this plant [5]. The MYB4 transcription factor has also been introduced into potato plants, resulting in enhanced abiotic stress tolerance and altered gene expression [6]. The apple MdMYB4 transcription factor plays a vital role in cold and salt stress tolerance in apple calli [7], and its overexpression has been shown to improve salt tolerance in apple callus by increasing *MdNHX1* expression levels [8].

A recent study has shown that the transgenic expression of *MbMYB4*, a MYB transcription factor from *Malus baccata*, increases *A. thaliana*'s tolerance to cold and drought stressors [9]. The MYB4 transcription factor has been found to regulate cadmium tolerance in Arabidopsis [10], and it also plays dual roles in flavonoid biosynthesis [11]. Various studies have explored the role of MYB transcription factors in enhancing drought tolerance in plants [12, 13], with some specifically focusing on the *Panax ginseng MYB4* gene [14].

Additionally, a study has examined the *R2R3-MYB* gene family in *Santalum album* and provided insights into the involvement of these transcription factors in cold stress response pathways in this species [15]. Other work has shown that the expression of BrMYB4 transcription factor has been found to be down-regulated by UV-B but not by pigment-inducing sunlight in turnip plants [16]. Additional research has demonstrated that the N-terminal MYB domains play a role in stabilizing the proper folding of the MYB4 transcription factor protein from *A. thaliana* under heat stress conditions [17]. Furthermore, FvMYB24, a strawberry R2R3-MYB transcription factor, has been demonstrated to improve salt stress tolerance in transgenic Arabidopsis plants [18], further emphasizing the importance of transcription factors, including MYB family members, in enhancing drought stress tolerance in plants [19].

The *MYB4* gene and its orthologs in several plant species have been identified as significant participants in the control of plants' responses to a variety of abiotic stressors, according to the above review of these studies. Overexpression of *MYB4* and related genes have been shown to enhance tolerance to cold, freezing, salt, drought, and cadmium stress, as well as modulate flavonoid biosynthesis and abscission zone separation. The studies highlight the potential of MYB4 transcription factors as targets for genetic manipulation and breeding strategies aimed at improving abiotic stress tolerance in crop plants, which could have significant implications for agriculture in the context of global climate change.

In the present work, we identified and characterized the MYB4 transcription factor in the drought-tolerant peanut L14 cultivar. Our results provide an initial investigation into the properties of the *MYB4* gene in peanut plants. This research aimed to provide information that can guide further studies to improve the drought tolerance and yield of local peanut cultivars in Vietnam.

2 Material and methods

2.1 Material

Peanut L14 cultivar seeds were bought from the Center for Development of High-Tech Seeds, Vietnam Academy of Agriculture, Vietnam. The peanut cultivar was then naturally cultured in a greenhouse at the Institute of Bioactive Compounds, University of Sciences, Hue University.

2.2 Methods

Primer

The primer for the isolation of *MYB4* was designed based on the corresponding gene in NCBI (Accession number: XM_025747717.1) and

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the Peanutbase database. After designing the primer with Primer3, it was confirmed to be specific using the Primer-BLAST tool with *A. hypogaea* databases. The primer sequences (5′-3′) were as follows: MYB4.L14_F: ATGGCAAAAACTCCTTGTTGTGAG and MYB4.L14_R: TGCCCTTTAAGATAGCTGTCCT, with an expected size of around ~1.1 kb.

Molecular cloning

The total DNA was extracted from root tissues of the peanut L14 cultivar using the GeneJET Genomic DNA Purification Kit (Thermo Scientific, USA) following the manufacturer's instructions. The *MYB4* transcription factor gene was isolated by polymerase chain reaction (PCR) using DreamTaq DNA polymerase (Thermo Scientific, USA). The PCR product was purified using the GeneJET Gel Extraction kit (Thermo Scientific, USA) and ligated to the pGEM-T Easy vector. The ligation solution was incubated overnight at 4°C before transformation into *E. coli* TOP10 competent cells. The transformants were screened on LB agar selection medium containing 50 mg/L ampicillin. The plasmid DNA was extracted and PCR was performed to confirm the successful transformants. The confirmed transformants were then sequenced using the T7 and SP6 universal primers (First BASE, Malaysia).

Bioinformatic methods

The DNA sequences of the forward and reverse strands were assembled using the CAP3 tool (https://doua.prabi.fr/software/cap3). The introns and exons of the DNA sequence were predicted using the Augustus tool (https://bioinf.uni-greifswald.de/augustus/) based on the *A. thaliana* database. The AhL14_MYB4 and their protein orthologs from various plants were used to generate a phylogenetic tree using the neighbour-joining method (1000 replications). In addition, the biological function and localization of MYB4 were analyzed using InterPro (https://www.ebi.ac.uk/interpro/result/) and Deeploc 2.0 (https://services.healthtech.dtu.dk/services/DeepLoc-2.0/), respectively. The secondary structure of MYB4 predicted by PHYER2 (http://www.sbg.bio.ic.ac.uk/phyre2) and the 3D structure of its DNA binding domains were built using SWISS-MODEL (https://swissmodel.expasy.org/) and visualized by PyMOL.

3 Results and discussion

3.1 DNA isolation

The total DNA was isolated and analyzed on 1% agarose gel along with a DNA ladder marker (DNA ladder 1 kb, Thermo Scientific, USA) (Fig. 1A). The results indicated that the total DNA isolation had good quality with a clear and bright DNA band with minimal breakage and less RNA contamination. The extracted DNA was then stored at -70°C for further experiments.

3.2 Gene cloning

MYB4 gene was isolated by PCR method based on the specific primer pairs. The PCR product analyzed on a 1% agarose gel showed a specific band of approximately 1.1 kb, which is

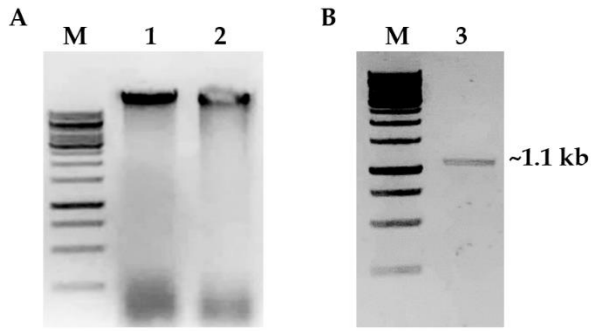

Fig. 1. Total DNA (A), and eluted PCR product on agarose gel. M: DNA marker 1kb plus ladder (Thermo Scientific, USA), Lanes: 1-2: extracted DNA, Lane 3: eluted *MYB4*

consistent with the theoretical size of the *MYB4* gene. The PCR product was purified by GenJET Gel Extraction Kit (Thermo Scientific, USA) according to the instructions of the manufacturer. The purified product on 1% agarose gel showed a quite pure and high concentration with a DNA band approximately 1.1 kb in length (Fig. 1B).

The three transformants were randomly selected for colony PCR. The results showed that two out of three transformants carried the target gene with a size of approximately 1.1 kb, suggesting that the recombination process was successfully carried out. These transformants were then stored in a glycerol solution at -70°C.

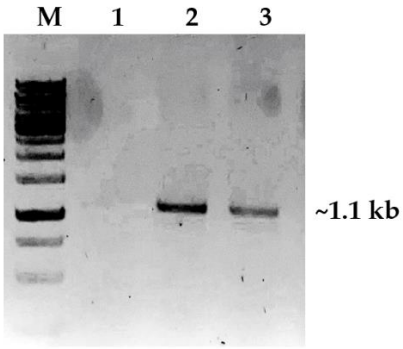

Fig. 2. Verification of the transformants with colony PCR. The DNA marker 1kb plus ladder (Thermo Scientific, USA). Lanes 1, 2, and 3 represent the PCR products from three transformants

3.3 *In silico* characteristic analysis

The DNA plasmid carrying the putative *MYB4* gene was sequenced and analyzed (Fig. 3). The putative *MYB4* gene is 1111 bp long and contains two introns and three exons. Its CDS has 663 bp, which encodes 220 amino acids with a molecular weight of 24.5 kDa. *In silico* functional analysis showed that MYB4 contains two MYB domains (9 – 61 aa and 62 – 116 aa) (Fig. 4A and 4B) with the specific DNA binding motif described in Fig .5. Specifically, the DNA binding site located at residues 70 (F), 99 (R), 100 (T), 102 (N), 103 (E), 105 (K), 106 (N), 107 (F), 109 (H), 110 (T), 111 (H). These are important domains of MYB transcription factors for the regulation of stresses induced genes in plants.

In addition, the AhL14_MYB4 was predicted as location in nuclear with a probability of 91.3% (Table 1). Besides, biological function analysis with Interpro indicated that MYB4 has two catalogues at biology process and molecular function. The putative MYB4 was performed blast to transcription factor database showed the best fit to MYB4 of *A. thaliana*. From the above analysis, this gene was named *AhL14_MYB4*.

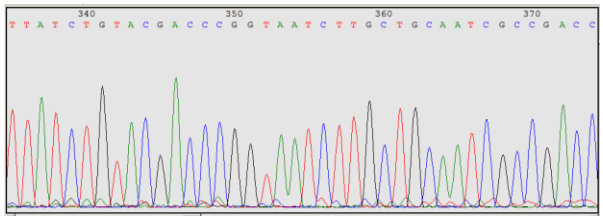

Fig. 3. Illustration of Sanger sequencing chromatogram of *AhL14_MYB4*.

Table 1. Prediction of AhL14_MYB4 protein localization

Loc	Cyto	Nuc	Extr	CM	Mito	Plast
Pro	0.193	0.914	0.010	0.063	0.056	0.069

Localization (Loc), Probability (Prob), Cytoplasm (Cyto), Nucleus (Nuc), Extracellular (Extracel), Cell membrane (CM), Mitochondrion (Mito), and Plastid (Plast).

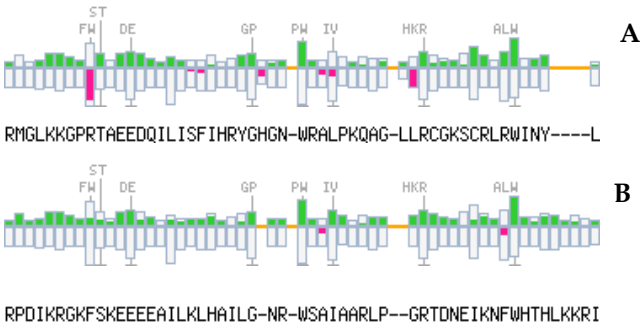

Fig. 4. Two Myb-type HTH DNA-binding domains of AhL14_MYB4 located at 9-61 aa (A) and 62-116 aa (B)

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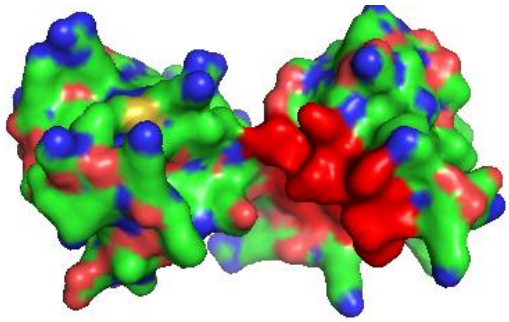

Fig. 5. 3D structure of DNA binding domain of MYB4. Red (DNA binding site), blue (positively charged), green (neutral), pink (negatively charged)

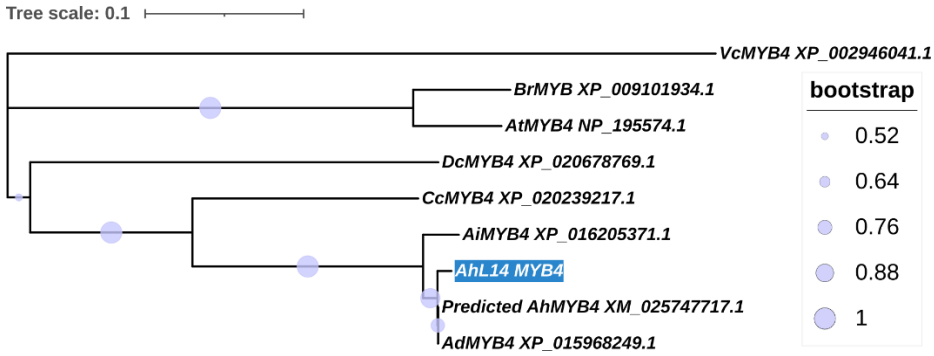

Fig. 6. Phylogenetic tree analysis of MYB4s from various plants. The letters representing the abbreviations for species names are as follows: Vc (*Volvox carteri*), Br (*Brassica rapa*), At (*Arabidopsis thaliana*), Dc (*Dendrobium catenatum*), Cc (*Cajanus cajan*) Ai (*Arachis ipaensis*), Ah (*A. hypogaea*), Ad (*Arachis duranensis*)

3.4 Phylogenetic tree analysis

The phylogenetic tree shows that the AhL14_MYB4 belongs to the same group as its proteins in *A. hypogaea*, *A. duranensis*, and *A. ipaensis* (Fig. 6). The AhL14_MYB4 was found to differ by only 1.4% from the predicted protein sequences of MYB4 found in *A. hypogaea* (XM_025747717.1) in the Genbank. However, there was a 6.5% difference between MYB4 from peanut L14 and the protein sequence of MYB4 found in *Arachis ipaensis* (XP_016205371.1). The result revealed that the AhL14_MYB4 is more closely related to the MYB4 protein in *A. hypogaea* and *A. duranensis* than to the one in *A. ipaensis*, indicating a closer evolutionary relationship between these species. This suggests that MYB4 might have a conserved function in these species. Furthermore, the AhL14_MYB4 has a closer relationship to MYB4 from *Cajanus cajan* and *Dendrobium catenatum* than to those from *Brassica rapa* and *A. thaliana*. These results suggest that MYB4 might have evolved divergently in different plant lineages, possibly acquiring various functions or undergoing subfunctionalization.

4 Conclusion

The DNA sequence of *MYB4* was successfully isolated, and characteristically analyzed from the groundnut L14 cultivar. This finding may provide important information for further research related to the mechanism of its function and support for the selection or generation of local drought-tolerant peanut varieties.

Acknowledgements

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