

DNA-binding domain in MYB transcription factor family in date palm

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Abstract

Date palm (*Phoenix dactylifera* L.) is one of the most important economic plants which belongs to the Arecaceae family in arid and semi-arid areas. The genetic map of the date palm genome is incomplete, which hindered the expansion of molecular studies. Bioinformatics is a way that the researcher compares different plant species to find similarities and differences between them. MYB is a transcription factor family that has well-known functions in many plants, especially *Arabidopsis*. DNA-binding domains are pieces of peptides (a group of amino acids) that are folded independently and are highly attracted to DNA. The study aims to identify the DNA-binding domains for the MYB family in Date Palm to diagnose drought-related genes in the date palm genome through bioinformatics. The main approach followed in the research is to use a set of approved sites to align members of MYB proteins in *Arabidopsis* with the date palm genome to determine the most closely related MYB proteins in date palms. The results indicated a group of date palm proteins, which considered candidate proteins or genes to have an effective role in resisting or reducing the negative effects of water stress on date palms. Also, numbers of palm proteins have the same alignment effect and DNA-binding domains, which means that they may be close to each other in function and structure.

Keywords: *Arabidopsis*, Bioinformatics, *Phoenix dactylifera*.

INTRODUCTION

Date palm (*Phoenix dactylifera* L.) is a monocotyledonous, monosexual, and dioecious plant that belongs to the Arecaceae family (Al-Asadi, Abdulwahid, & Al-Mayahi, 2019; Ibrahim, Al-Taha, & AL-ALI, 2017).

Transcription Factor (TF) is a protein that performs several functions, the most important being: directing the enzyme RNA polymerase II to the promoter. It also separates the two DNA strands to facilitate the transcription process (TFII) (Song, Li, & Chen, 2022).

MYB transcription factors are a kind of DNA-binding protein that can specifically interact with the promoter region. Members of MYB TFs are widely involved in plant growth and development, secondary metabolism, stress response, and hormone signal transduction (Wang, Niu, & Zheng, 2021). However, there is no report of comprehensive bioinformatics analysis on the MYB family and its relation in Date Palm. The first encoding gene of the TFs was identified in the plant, thirteen years ago. A locus coding for the MYB domain protein, required for anthocyanin synthesis, was found in maize kernels (Paz-Ares, Ghosal, Wienand, Peterson, & Saedler, 1987). Ten years later, the sequences of the *Arabidopsis* genome were fully known, which provided the first comprehensive and taxonomic description of MYB genes in plants (Davuluri et al., 2003; C. Dubos et al., 2010; Palaniswamy et al., 2006). The functional properties of MYB proteins were studied in many plants such as rice, maize, poplar, *Arabidopsis* (Christian Dubos et al., 2010), petunia, grapevine (Bai et al., 2019), barley, and apple through the use of both molecular and genetic analyzes applications (Leng & Zhao, 2020). R2R3 is a protein subfamily of the MYB domain found in plants.

MYB transcription factors family has diverse functions within the plant genome. The key function of MYB is a transcription factor for DNA binding due to the presence and plurality of its repeat domain. The plant kingdom is distinguished from the animal kingdom by the presence of a selectively expanded family (R2R3-MYB). These proteins (R2-R3) play a key role in the

regulatory process which controls stress responses, especially abiotic stress. Understanding the potential structure of MYB proteins will make it easy to predict the functioning of this family in plant biology (C. Dubos et al., 2010).

The binding domain of MYB proteins is highly conserved and characterized in these proteins. In general, this binding domain consists of approximately 4-52 incomplete sequence repeats of amino acid, each repeat forming three alpha-helices. Helix–Turn–Helix (HTH) structure with three residues of hydrophobic or tryptophan regularly spaced, constructed from the second and third helix for each repetition. Tryptophan forms a hydrophobic core in the three-dimensional conformation of HTH (Yuan, Shi, & Tang, 2020). Many R2R3MYB members are recognized because of their genetic strategies; these members can be a part of the responses to environmental conditions changes or biotic stresses. The subgroup3 of R2R3MYB as AtMYB96, AtMYB60, and AtMYB30 are involved in this approach. AtMYB96 and AtMYB60 are involved in the drought stress response and stomatal activity (opening and closing) regulation through the ABA signaling (Seo & Park, 2010; Wilkins, Nahal, Foong, Provar, & Campbell, 2009). AtMYB30 act in plant cell elongation control through its activity in the Brassinosteroid pathway. Also, it involves pathogen resistance by encoding an operator in the cell death process, which is involved in fatty acid synthesis by regulating its long chains (Raffaele et al., 2008). Subgroup22 such as (AtMYB44, AtMYBR2, AtMYB73, and AtMYB70) is involved in biotic stress response through the control of stomata closure regulation. AtMYB13 acted in ABA-moderates responses to stress signals (Jung et al., 2008; Reyes & Chua, 2007). More than 125 members of the AtR2R3-MYB transcription factors were dispersed over various chromosomes, while several expressed in specific patterns. AtMYB44 can be expressed in the generality of plant tissues, where it is found to have an expression (positive or negative) under a range of biotic and abiotic stresses (Baldoni, Genga, & Cominelli, 2015). Also, AtMYB60 has a specific expression pattern in the guard cells under drought stress, which indicates an effective role in regulating stomatal activity (Cominelli et al., 2005; Du et al., 2009).

R2R3 has a function of many MYB transcription factors members in Arabidopsis, such as (MYB6, MYB13, MYB14, MYB44, MYB60, MYB77, and MYB96), which are expressed under water stress (Kirik, Kölle, Wohlfarth, Miséra, & Bäumlein, 1998).

The research aims to identify the DNA-binding domains in the MYB transcription factors family in date palms. Also, it aims to diagnose drought-related genes through bioinformatics and obtain a database of drought-related date palm genes.

MATERIALS AND METHODS

Identification of MYB Date Palm

Protein sequences of the Arabidopsis thaliana MYB were used as queries to identify related sequences in the Date Palm (*Phoenix dactylifera*) obtained from The Arabidopsis Information Resource (TAIR) online database (Rhee et al., 2003). This step is done by the NCBI databases and Phytozome v10.3 (Goodstein et al., 2012). Due to the unavailability of *Phoenix dactylifera* protein sequences online, the entire mRNA of MYB was obtained from the NCBI database and translated using EMBOSS TRANSEQ tool (http://www.ebi.ac.uk/Tools/st/emboss_backtranseq). MYB-related sequences were searched within this dataset using Blastp (Altschul et al., 1997; Zhang, Miller, & Lipman, 1997).

Prediction of putative functional domains in PdMYB

The amino acid sequence of each AtMYB protein aligned with corresponding sequences of PdMYB using the Multalin tool (<http://multalin.toulouse.inra.fr/multalin/>) (version 5.4.1) (Corpet, 1988) to identify conserved DNA-binding motifs in the PdMYB protein sequences. For the online tool, all default parameters are kept.

RESULTS AND DISCUSSION

Identification of MYB transcription factor family in Date Palm

Eight sequences of AtMYB members were used to query for related sequences in *Phoenix dactylifera* genomes (via BLASTP). A total of 58 members of the R2R3-MYB-related sequences were identified in Date Palm. Data shown in table (1) was collected from NCBI (blastp suite) database after running the blast for individual AtMYB proteins with Date Palm databases. Table (1)

shows the complete identification of each member of the Arabidopsis proteins and their corresponding proteins in the Date Palm genome, noting that both proteins are within the MYB transcription factors family. The table shows the following information: the identity of the gene, the gene symbol, the name of the gene, the copy of the gene (transcripts), and the protein identity.

Table 1: ID genes and proteins of AtMYB and PdMYB

Gene ID	Symbol	Gene name	Transcripts	Proteins
837127	AtMYB13	myb domain protein 13	NM_100499.2	NP_172108.1
103719944	LOC103719944	transcription factor MYB30-like	XM_008809436.4	XP_008807658.1
103718266	LOC103718266	transcription factor MYB30	XM_008806997.4	XP_008805219.1
103709857	LOC103709857	transcription factor MYB13-like	XM_008795381.2	XP_008793603.2
103696754	LOC103696754	myb-related protein Zm1-like	XM_008778468.3	XP_008776690.1
120110921	LOC120110921	myb-related protein Zm1-like	XM_039126966.1	XP_038982894.1
103721840	LOC103721840	MYB-like transcription factor ODO1	XM_008812200.4	XP_008810422.1
103712648	LOC103712648	transcription factor MYB1-like	XM_008799230.4	XP_008797460.2
817674	AtMYB14	myb domain protein 14	NM_128674.4	NP_180676.1
103708442	LOC103708442	transcription factor MYB30	XM_008793371.4	XP_008791593.2
120110921	LOC120110921	myb-related protein Zm1-like	XM_039126966.1	XP_038982894.1
103705419	LOC103705419	transcription factor MYB13-like	XM_008789127.4	XP_008787349.2
108510996	LOC108510996	myb-related protein P-like	XM_039124514.1	XP_038980442.1
103703278	LOC103703278	transcription factor MYB1-like	XM_008786075.4	XP_026658863.1
103710554	LOC103710554	transcription factor MYB17-like	XM_008796324.3	XP_008794546.2
103721840	LOC103721840	MYB-like transcription factor ODO1	XM_008812200.4	XP_008810422.1
103720134	LOC103720134	transcription factor MYB61-like	XM_008809710.4	XP_008807932.1
836865	AtMYBR1(44)	myb domain protein r1	NM_126130.2	NP_201531.1
103714181	LOC103714181	transcription factor MYB44-like	XM_008801354.4	XP_008799576.1
103711951	LOC103711951	transcription factor MYB77-like	XM_008798286.3	XP_008796508.2
103709098	LOC103709098	transcription factor CSA-like	XM_008794294.4	XP_008792516.2
103709300	LOC103709300	myb-related protein Hv33	XM_008794582.3	XP_008792804.1
120110921	LOC120110921	myb-related protein Zm1-like	XM_039126966.1	XP_038982894.1
103714577	LOC103714577	myb-related protein Zm1-like	XM_008801869.4	XP_008800091.1
838438	AtMYB51	myb domain protein 51	NM_101715.4	NP_173292.1
103716377	LOC103716377	transcription factor MYB106-like	XM_008804346.4	XP_008802568.1
103716128	LOC103716128	transcription factor MYB93	XM_039127781.1	XP_038983709.1
103711165	LOC103711165	transcription factor MYB102	XM_008797215.4	XP_008795437.2
103710568	LOC103710568	transcription factor LAF1-like	XM_008796344.2	XP_008794566.1
120110921	LOC120110921	myb-related protein Zm1-like	XM_039126966.1	XP_038982894.1
103695454	LOC103695454	myb-related protein 308-like	XM_008776791.4	XP_008775013.1
103697922	LOC103697922	transcription factor MYB36-like	XM_008779863.4	XP_008778085.2
837403	AtMYB60	myb domain protein 60	NM_001331790.1	NP_001318958.1

103696222	LOC103696222	transcription factor MYB60-like	XM_008777765.3	XP_008775987.1
103715730	LOC103715730	myb-related protein 306-like	XM_008803465.4	XP_008801687.1
103718266	LOC103718266	transcription factor MYB30	XM_008806997.4	XP_008805219.1
103714577	LOC103714577	myb-related protein Zm1-like	XM_008801869.4	XP_008800091.1
103704749	LOC103704749	transcription factor MYB92-like	XM_026803643.2	XP_026659444.2
103711807	LOC103711807	transcription factor MYB41-like	XM_026806457.2	XP_026662258.2
103702783	LOC103702783	transcription factor MYB106-like	XM_008785347.4	XP_008783569.2
103710296	LOC103710296	transcription factor MYB17-like	XM_008795960.3	XP_008794182.1
824168	AtMYB77	myb domain protein 77	NM_114866.4	NP_190575.1
103714181	LOC103714181	transcription factor MYB44-like	XM_008801354.4	XP_008799576.1
103711951	LOC103711951	transcription factor MYB77-like	XM_008798286.3	XP_008796508.2
103706746	LOC103706746	transcription factor MYB77-like	XM_008790957.4	XP_008789179.4
103704090	LOC103704090	transcription factor MYB1-like	XM_008787241.4	XP_008785463.1
103715801	LOC103715801	MYB-like transcription factor EOBII	XM_008803559.3	XP_008801781.1
103718814	LOC103718814	transcription factor CSA	XM_008807802.4	XP_008806024.2
120110921	LOC120110921	myb-related protein Zm1-like	XM_039126966.1	XP_038982894.1
103714577	LOC103714577	myb-related protein Zm1-like	XM_008801869.4	XP_008800091.1
836367	AtMYB96	myb domain protein 96	NM_125641.4	NP_201053.2
103715730	LOC103715730	myb-related protein 306-like	XM_008803465.4	XP_008801687.1
120113276	LOC120113276	myb-related protein 306-like	XM_039134308.1	XP_038990236.1
103696222	LOC103696222	transcription factor MYB60-like	XM_008777765.3	XP_008775987.1
103709082	LOC103709082	transcription factor MYB60	XM_008794273.4	XP_008792495.2
103714577	LOC103714577	myb-related protein Zm1-like	XM_008801869.4	XP_008800091.1
103721840	LOC103721840	MYB-like transcription factor ODO1	XM_008812200.4	XP_008810422.1
103713671	LOC103713671	transcription factor MYB4-like	XM_008800677.4	XP_008798899.2

Evolutionary conservation of DNA binding domains in PdMYB

The full-length sequence of each AtMYB subgroup aligned with its corresponding PdMYB sequences based on phylogeny. Multalin tool was used to investigate whether previously identified functional domains in Arabidopsis proteins also are conserved in Date Palm proteins or not (Hooper, Castleden, Tanz, Aryamanesh, & Millar, 2017; Hooper et al., 2014).

The initial alignment of the R2R3-MYB TFs sequence in Arabidopsis reveals a short peptide stretch that is conserved in all eight AtMYB members. This peptide region appears of two short peptides (R2 and R3) shown in red (Figure 1).

The date palm orthologues of MYB are not published, but in comparison with AtMYB, the multiple sequence alignments show different responses in these domain regions. These regions can be highly conserved (>90%) indicated in red, while the other amino acids are partially conserved (>50%) shown in blue. The protein region immediately upstream of the potential MYB-binding domain is less conserved. However, it is noticeable that the N-terminus and central region between amino acids 1-120 are highly conserved (Hooper et al., 2017; Hooper et al., 2014). Even so, the DNA-binding regions of PdMYB have not been studied extensively.

AtMYB6 DNA-domain shown in two peptides stretch: the first one (R2) between 14-61 amino acids, a total of 26 out of 45 amino acids (aa) in this domain are highly conserved(>90%), but a total of 11 out of 45 (aa) are partially conserved (50%). The second peptides stretch (R3) is between amino acids 67-112, a total of 27 out of 45 (aa) in this domain are highly conserved(>90%), and a total of 13 out of 45 amino acids partially conserved (50%) as shown in figure (1A). The Date Palm orthologues of PdMYB (XP_008807672.1 and XP_008799190.2) have highly conserved amino acids predicted DNA-binding domain in contrast with other PdMYB proteins.

AtMYB13 DNA-domain shown in two peptides stretch: the first one (R2) between amino acids 14-61, a total of 23 out of 45 (aa) in this domain are highly conserved (>90%), and a total of 12 out of 45 aa are partially conserved (50%). The second peptides stretch (R3) is between amino acids 67-112, a total of 30 out of 45 (aa) in this domain are highly conserved (>90%), and a total of 9 out of 45 aa partially conserved (50%) as shown in figure (1B). The Date Palm orthologues of PdMYB (XP_008810422.1 and XP_008807658.1) have highly conserved amino acids in the predicted DNA-binding domain compared to other PdMYB proteins.

AtMYB14 DNA-domain shown in two peptides stretch: the first one (R2) between amino acids 14-61, a total of 21 out of 45 aa in this domain are highly conserved(>90%), but a total of 15 out of 45 amino acids are partially conserved (50%). The second peptides stretch (R3) is between amino acids 67-112, a total of 24 out of 45 (aa) in this domain are highly conserved (>90%), but a total of 9 out of 45 aa partially conserved (50%) as shown in figure 1C. The Date Palm orthologues of PdMYB (XP_008791593.2 and XP_008810422.1) have highly conserved amino acids, in the predicted DNA-binding domain, in contrast with other PdMYB proteins.

AtMYB44 DNA-domain shown in two peptides stretch: the first one (R2) between amino acids 6-52, a total of 20 out of 48 aa in this domain are highly conserved(>90%), but a total of 12 out of 48 aa are partially conserved (50%). The second peptides stretch (R3) is between amino acids 58-103, a total of 19 out of 45 aa in this domain are highly conserved (>90%), but a total of 12 out of 45 amino acids are partially conserved (50%) as shown in figure 1D. The Date Palm orthologues of PdMYB (XP_008799576.1 and XP_008796508.2) have highly conserved amino acids, in the predicted DNA-binding domain, in contrast with other PdMYB proteins.

AtMYB51 DNA-domain shown in two peptides stretch: the first one (R2) between amino acids 15-62, a total of 23 out of 47 aa in this domain are highly conserved (>90%), but a total of 14 out of 47 amino acids are partially conserved (50%). The second peptides stretch (R3) is between amino acids 68-113, a total of 23 out of 45 aa in this domain are highly conserved (>90%), but a total of 11 out of 45 amino acids are partially conserved (50%) as shown in figure 1E. The Date Palm orthologues of PdMYB (XP_008778085.2 and XP_008802568.1) have highly conserved amino acids, in the predicted DNA-binding domain, in contrast with other PdMYB proteins.

AtMYB60 DNA-domain shown in two peptides stretch: the first one (R2) between amino acids 14-61, a total of 31 out of 45 amino acids (aa) in this domain are highly conserved(>90%), but a total of 7 out of 45 amino acids are partially conserved (50%). The second peptides stretch (R3) is between amino acids 67-112, a total of 26 out of 45 amino acids (aa) in this domain are highly conserved (>90%), but a total of 9 out of 45 amino acids are partially conserved (50%) as shown in figure 50F. The Date Palm orthologues of PdMYB (XP_008775987.1 and XP_008801687.1) have highly conserved amino acids, in the predicted DNA-binding domain, in contrast with other PdMYB proteins.

AtMYB77 DNA-domain shown in two peptides stretch: the first one (R2) between amino acids 6-52, a total of 19 out of 46 amino acids (aa) in this domain are highly conserved(>90%), but a total of 15 out of 46 amino acids are partially conserved (50%). The second peptides stretch (R3) is between amino acids 58-103, a total of 16 out of 45 amino acids (aa) in this domain are highly conserved (>90%), but a total of 18 out of 45 amino acids are partially conserved (50%) as shown in figure 4G. The Date Palm orthologues of PdMYB (XP_008799576.1 and XP_008789179.4) have highly conserved amino acids, in the predicted DNA-binding domain, in contrast with other PdMYB proteins.

AtMYB96 DNA-domain shown in two peptides stretch: the first one (R2) between amino acids 14-60, a total of 12 out of 46 amino acids (aa) in this domain are highly conserved(>90%), but a total of 21 out of 46 amino acids are partially conserved (50%). The second peptides stretch (R3) is between amino acids 66-111, a total of 22 out of 45 amino acids (aa) in this domain are highly conserved (>90%), but a total of 15 out of 45 amino acids are partially conserved (50%) as shown in figure 50H. The Date Palm orthologues of PdMYB (XP_008801687.1 and XP_008775987.1) have highly conserved amino acids in the predicted DNA-binding domain in contrast with other PdMYB proteins.

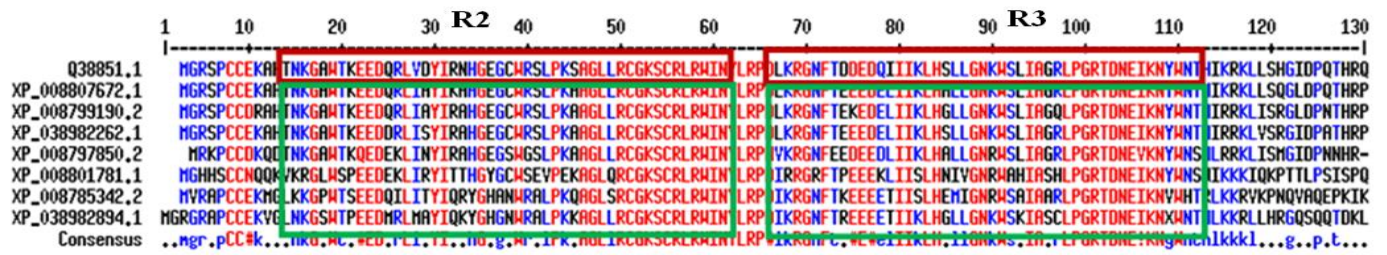


Figure 1A: AtMYB6 DNA-domain aligns with PdMYB

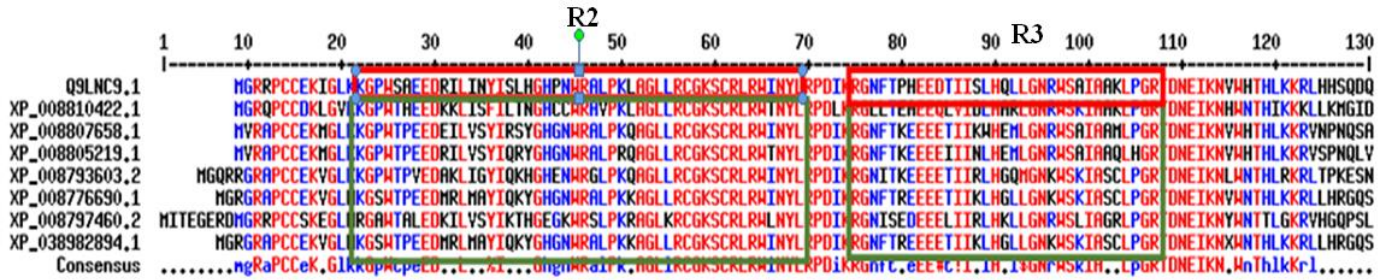


Figure 1B: AtMYB13 DNA-domain aligns with PdMYB

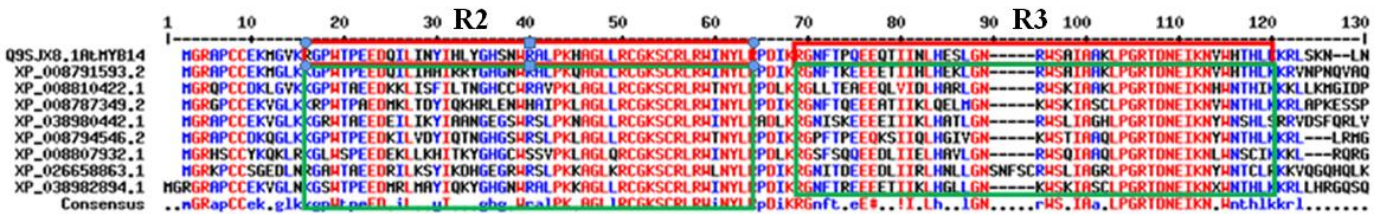


Figure 1C: AtMYB14 DNA-domain aligns with PdMYB

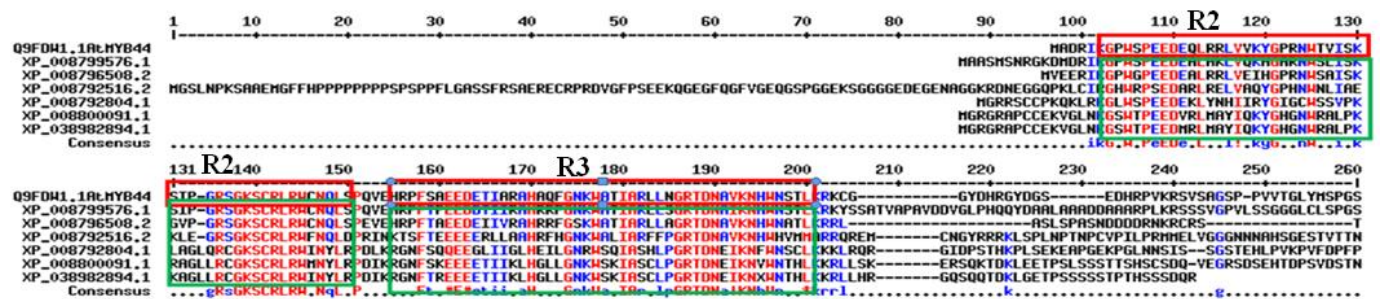


Figure 1D: AtMYB44 DNA-domain aligns with PdMYB

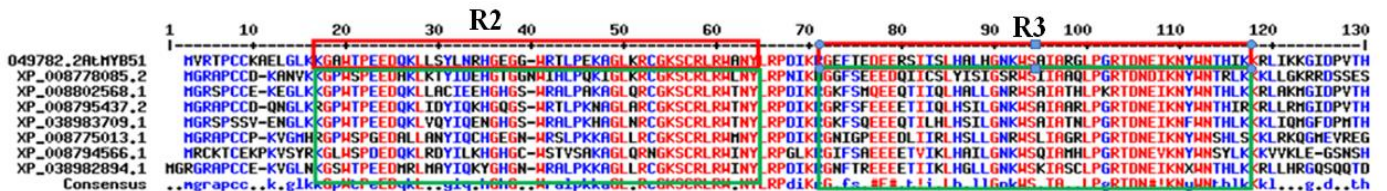


Figure 1E: AtMYB51 DNA-domain aligns with PdMYB

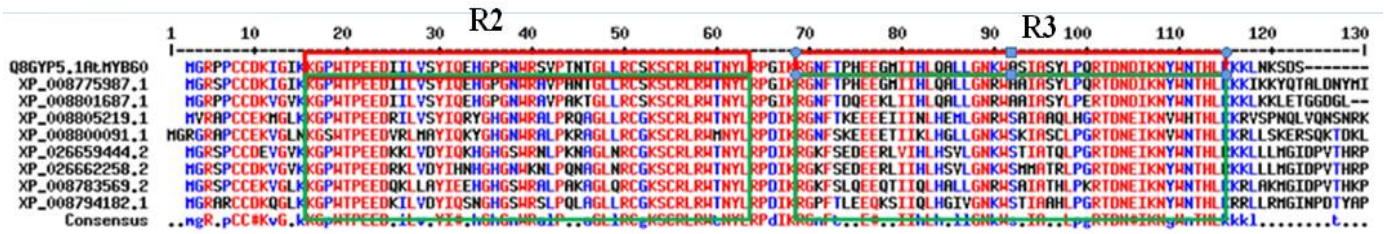


Figure 1F: AtMYB60 DNA-domain align with PdMYB

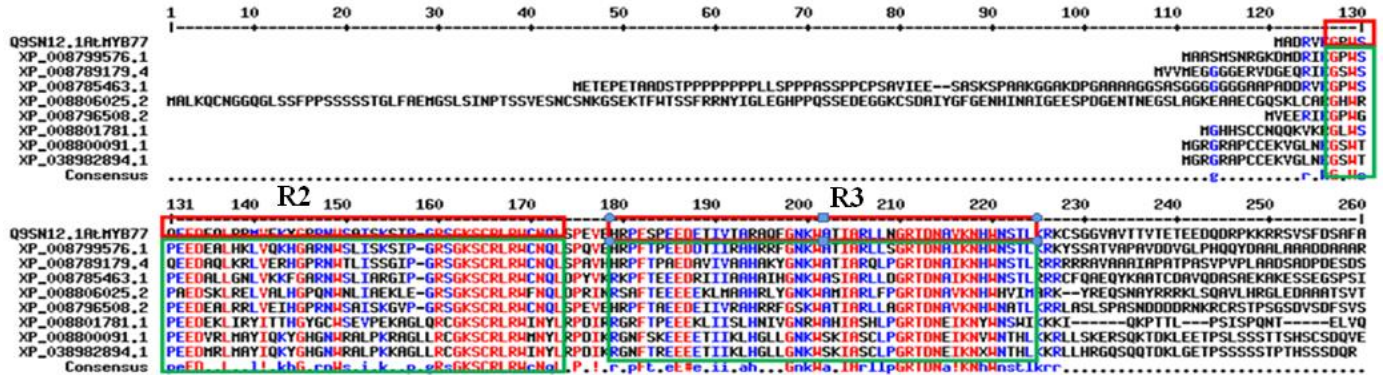


Figure 1G: AtMYB77 DNA-domain aligns with PdMYB

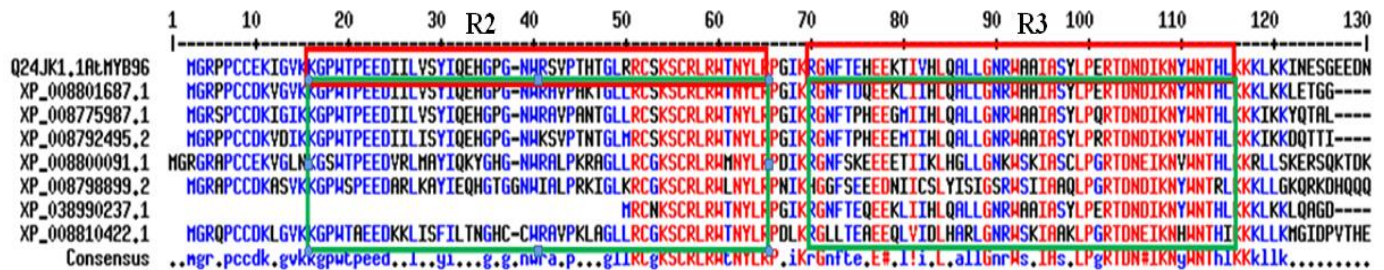


Figure 1H: AtMYB96 DNA-domain aligns with PdMYB

Figure 1: AtMYB the DNA-domains align with PdMYB

The results of the preliminary (Bioinformatics') study showed a close relationship between Arabidopsis proteins (AtMYB) and date palm proteins (PdMYB).

These relationships produced a group of date palm proteins that repeatedly aligned with Arabidopsis proteins, as shown in table2. These results led to select special members of PdMYB for future studies to determine their expression under water stress. On this basis, the XP_038982894 (myb-related protein Zm1-like) gene can consider the most candidate for future studies because its alignment repeats with six Arabidopsis members. Also, XP_008800091.1 (myb-related protein Zm1-like) is a candidate for future studies because of its frequent alignment with four Arabidopsis proteins. In addition, the protein XP_008810422.1 (MYB-like ODO1) is a candidate gene for future studies due to its repeated alignment with three Arabidopsis proteins.

On the other hand, the results showed that there is a close relationship between date palm proteins (XP_008799576.1) and (XP_008796508.2) by aligning them with the same Arabidopsis proteins (MYB44 and MYB77) with close matching degrees. There is also a close relationship between date palm proteins (XP_008775987.1) and (XP_008801687.1) through their alignment with the same Arabidopsis proteins (MYB60 and MYB96) with close matching degrees (Table 2).

The results also showed that DNA binding sites are characteristic of TFs, which give them the ability to bind to specific sequences on the DNA called promoters. DNA binding domains have two functions, either the regulation of the transcription process or structural function, which may occur separately or overlap (Lilley and Lilley 1995, Zhu and Huq 2011). Therefore, knowing and distinguishing the active sequences in date palm proteins, which is DNA binding, will open the way to study these proteins in more depth and the extent to which these sequences match with other gene promoters, or these proteins can be considered as candidate genes for future studies.

Table 2: PdMYB proteins alignment repeat with AtMYB

PdMYB Proteins ID	PdMYB Gene name	Alignment Repeats	AtMYB
XP_038982894.1	myb-related protein Zm1-like	6	MYB6 (71%), 13(77.5%), 14(78.2%), 44(47.5%), 51(64.2%), 77(49%)
XP_008800091.1	myb-related protein Zm1-like	4	MYB44 (51%), 60(72.5%), 77(50%), 96(68%)
XP_008810422.1	MYB-like ODO1	3	MYB13 (72.5%), 14(58%), 96(65%)
XP_008805219.1	MYB30	2	MYB13 (75.5%), 60(60%)
XP_008801781.1	MYB-like EOBII	2	MYB6 (75%), 77(49%)
XP_008799576.1	MYB44-like	2	MYB44 (47.5%), 77(89%)
XP_008796508.2	MYB77-like	2	MYB44 (45%), 77(92%)
XP_008775987.1	MYB60-like	2	MYB60 (94%), 96(97%)
XP_008801687.1	myb-related protein 306-like	2	MYB60 (89%), 96(100%)

Conclusion

MYB is an important transcription factor family that plays essential roles in many plants. It is necessary to study the functional and structural roles of PdMYB to identify the possibility of compatibility with other plants, especially AtMYB. DNA-binding domains are highly conserved in the MYB TFs family, which modulate the transcription process and polymerase enzymes. The AtMYB subgroup aligned with its corresponding PdMYB sequences based on phylogeny. The results showed that the DNA-binding domains of AtMYB are identical (in varying degrees) with a group of PdMYB, with two short active domains (peptide). Also, several PdMYB can be considered the most recommended proteins or genes because of their matching with AtMYB members and more compatibility in the active domain, and their frequent alignment with more than one AtMYB protein.

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