Identification of 30 MYB transcription factor genes and analysis of their expression during abiotic stress in peanut (Arachis hypogaea L.)

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The MYB superfamily constitutes one of the most abundant groups of transcription factors and plays central roles in developmental processes and defense responses in plants. In the work described in this article, 30 unique peanut MYB genes that contained full-length cDNA sequences were isolated. The 30 genes were grouped into three categories: one R1R2R3-MYB, nine R2R3-MYBs and 20 MYB-related members. The sequence composition of the R2 and R3 repeats was conserved among the nine peanut R2R3-MYB proteins. Phylogenetic comparison of the members of this superfamily between peanut and Arabidopsis revealed that the putative functions of some peanut MYB proteins were clustered into the Arabidopsis functional groups. Expression analysis during abiotic stress identified a group of MYB genes that responded to at least one stress treatment. This is the first comprehensive study of the MYB gene family in peanut.

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1. Introduction

Plant growth and yield are strongly influenced by abiotic stresses such as drought, salt and cold. Plants respond and adapt to these conditions with an array of biochemical and physiological changes (Hsieh et al., 2004; Zhu et al., 2007). Many adaptation processes are regulated by stress-responsive gene expression. Transcription factors (TFs) regulate gene expression, which provides plants with a complicated control mechanism for responding to abiotic and biotic stresses and modulating developmental processes (Mitsuda and Ohme-Takagi, 2009). A previous study has uncovered a group of TF genes — such as DREB, MYB and bZIP — which play important roles in plant molecular stress regulation (Ahuja et al., 2010).

MYB TFs are widely distributed in eukaryotic organisms, and constitute one of the largest TF families in the plant kingdom (Du et al., 2012). The distinguishing property of MYB proteins is a highly conserved MYB domain consisting of 1–4 imperfect tandem repeats (MYB repeat) at the N-terminus. The MYB repeat is 50–53 amino acids in length and contains three regularly distributed tryptophan (or phenylalanine) residues. Each MYB repeat encodes three α-helices, with the second and third helices forming a helix-turn-helix structure, which recognizes and binds to the DNA major groove at the specific recognition site C/TAACG/TG (Lipsick, 1996; Stracke et al., 2001). The MYB family is divided into different types according to the number of MYB repeat(s): 4R-MYB has four repeats, 3R-MYB (R1R2R3-MYB) has three consecutive repeats, R2R3-MYB has two repeats and the MYB-related type usually, but not always, has a single MYB repeat (Dubos et al., 2010; Jin and Martin, 1999; Rosinski and Atchley, 1998).

The first plant MYB gene C1 was isolated in maize (Paz-Ares et al., 1987). Since then different aspects of the MYB gene family, including gene number, sequence characterization, evolution and functions, have been widely studied in plants (Chen et al., 2006; Du et al., 2012, 2013; Dubos et al., 2010; Matus et al., 2008; Wilkins et al., 2009; Zhang et al., 2011). So far, the MYB proteins have been shown to be involved in many significant physiological and biochemical processes, including regulation of primary and secondary metabolism, control of cell development and the cell cycle, participation in defense and response to various biotic and abiotic stresses, flavonoid biosynthesis, hormone synthesis and signal transduction (Czemmel et al., 2012; Du et al., 2009; Dubos et al., 2010; Feller et al., 2011; Ma et al., 2009; Stracke et al., 2001).

Extensive studies in various plant species have provided a better understanding of the MYB gene family; however, little is known about this family in peanut (Arachis hypogaea L.). Until now, only one MYB family gene, whose expression was induced by cold stress, has been reported in peanut (Tang et al., 2011). The cultivated peanut is an important oil crop and its production is severely affected by adverse environmental stresses. Unfortunately, little is known about the network of gene expression regulation related to abiotic stress in peanut, except for several genes shown to be stress regulated (Chen et al., 2012; Dave and Mitra, 1998, 2000; Jain et al., 2006; Rudrabhatla and Rajasekharan, 2002).
Identifying stress-responsive genes will elucidate the molecular mechanisms of peanut stress response and tolerance, and offer a number of candidate genes as potential markers of tolerance to environmental stresses.

Considering the multiple functions of MYB family proteins, especially their important roles in response to abiotic stresses in plants, research was conducted concerning the evolution and expression properties of the MYB gene family in peanut. In this study, 30 full-length cDNA sequences encoding peanut MYB proteins were isolated. A phylogenetic tree combining peanut and Arabidopsis MYB proteins was constructed to examine their evolutionary relationships and the putative functions of peanut MYB proteins based on Arabidopsis MYB proteins with known functions. The expression of the MYB family genes' response to abiotic stress and abscisic acid (ABA) was analyzed to identify potential genes that participated in the stress signal transduction pathway in peanut. This is the first comprehensive study of the MYB gene family in peanut and provides valuable information for further exploration of the functions of this significant gene family in this plant.

2. Materials and methods

2.1. Plant materials

Peanut seeds (Arachis hypogaea L. cultivar Huayu19) were germinated in a mixture of nutrient soil and vermiculite (2:1) and grown under conditions of 16 h light/8 h dark (28 °C/22 °C). Seedlings at the trefoil leaf stage were used in subsequent experiments.

The stress treatment processes were performed according to the study reported by Chi et al. (2012). For the cold treatment, seedlings in soil were maintained at 4 °C in a light incubator. For the NaCl, PEG6000 and ABA treatments, the roots of seedlings grown in soil were pulled out carefully to avoid injury, flushed carefully to remove all soil and then dipped into 200 mM NaCl, 20% PEG6000 or 100 μM ABA solution. The leaves and roots for all kinds of treatments were collected at 0 h, 1 h, 3 h, 6 h, 12 h, 24 h, 48 h and 72 h. All the samples were immediately frozen in liquid nitrogen and then stored at −80 °C until required.

2.2. Identification of peanut MYB genes in the peanut cDNA library using BioEdit software

The cDNA sequences used in this study came from three cDNA libraries from three laboratories (data not shown). All ESTs (Expressed Sequence Tags) of the 36,741 cDNA sequences were saved in FASTA format. The amino acid sequences of the MYB domain of Arabidopsis were used to search for homologous genes from the peanut cDNA library. Before searching for the MYB family genes, a local nucleotide database file was created using BioEdit software. A local BLAST procedure was then run to find the homologous genes of the MYB family. We found 74 ESTs that may encode MYB proteins via this method. To remove redundant sequences, the assemblies were assembled using the ‘Alignment’ function of the DNAman software and adjusted manually. Sequences that shared >95% matches were considered redundant. The ORFs (open reading frames) and putative amino acids of all nonredundant sequences were analyzed using DNAman software and 30 members were shown to contain a complete ORF. Finally, to confirm that the obtained sequences were MYB members, all of the amino acid sequences of the primary that identified 30 MYB members were submitted to the website http://pfam.sanger.ac.uk to predict the MYB domains. Only sequences that contained the conserved MYB domain were confirmed to be MYB members (Coghill et al., 2008).

2.3. Amino acids conservative analysis of the MYB repeats of R2R3-MYB proteins

To analyze the features of the MYB domain of peanut R2R3-MYB proteins, the sequences of R2 and R3 MYB repeats corresponding to nine R2R3-MYB proteins were aligned respectively with the ClustalW method using BioEdit software, and adjusted manually. The sequence logos for R2 and R3 MYB repeats were obtained by submitting the multiple alignment sequences to the website http://weblogo.berkeley.edu/logo.cgi (Crooks et al., 2004).

2.4. Phylogenetic analysis

The amino acid sequences of Arabidopsis (five R1R2R3-MYB, 126 R2R3-MYB and 80 MYB-related members) MYB proteins were downloaded from the Plant TFDB website (http://planttfdb.chi.edu.cn/) (He et al., 2010). The complete amino acid sequences of MYB proteins were used to construct phylogenetic trees. Sequence alignments were performed with ClustalW using MEGA4.0 software. Neighbor-joining (NJ) trees for 3R-MYB and R2R3-MYB subfamilies and the MYB-related subfamily combining peanut and Arabidopsis MYB members were constructed individually using the MEGA4.0 program (Tamura et al., 2007), and internal branch support was estimated with 1000 bootstrap replicates.

2.5. RNA isolation and cDNA synthesis

Total RNA was isolated and purified from samples using the RNeasy Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer’s instructions. Before cDNA synthesis, RNA was treated with RQ1 RNase-free DNase (Qiagen, Hilden, Germany) to avoid DNA contamination as recommended by the vendor. Only RNA preparations having an A260/A280 ratio of 1.8–2.0 and an A260/A230 ratio >2.0 were used for subsequent analysis. RNA integrity was verified by 2% agarose gel electrophoresis.

The cDNA was synthesized by the use of M-MLV Reverse Transcriptase (Promega, Madison, WI) in a 25-μL reaction system containing 2 μg total RNA. Reverse transcription reactions were carried out at 42 °C for 60 min followed by chilling on ice for 5 min.

2.6. Primer design and quantitative real-time RT-PCR

ACT11 was used as a reference gene to normalize all data (Chi et al., 2012). Primers (Online Resource 1) for ACT11 and AhMYB1–30 were designed according to the nucleotide sequences of ACT11 and AhMYB1–30 using Beacon Designer V 7.0 (Premier Biosoft International, Palo Alto, CA, USA) with melting temperatures of 58–60 °C, primer lengths of 20–25 bp and amplicon lengths of 60–200 bp.

For real-time PCR, the cDNA samples were diluted to 8 ng μL⁻¹. The qPCR analysis was performed using a LightCycler 2.0 instrument system (Roche, Germany), based on SYBR Premix Ex Taq polymerase (TaKaRa, Toyoto, Japan). Each 20 μL reaction comprised 2 μL cDNA template, 10 μL 2× SYBR Premix and 0.4 μL (200 nM) of each primer. The reactions were subjected to an initial denaturation step of 95 °C for 10 s, followed by 40 cycles of 95 °C for 5 s, 60 °C for 30 s and 72 °C for 10 s. A melting curve analysis was performed at the end of the PCR run over the range 60–95 °C, increasing the temperature stepwise by 0.5 °C every 10 s. Baseline and quantification cycles (Cq) were automatically determined using the LightCycler Software. Zero template controls were included for each primer pair, and each PCR reaction was carried out in triplicate. The raw Cq values obtained from LightCycler 2.0 were converted into relative quantities via the delta-Cq method.

3. Results

3.1. Isolation of 30 genes encoding MYB family TFs in peanut

74 ESTs that probably encode MYB family proteins were identified from the peanut cDNA library using BioEdit software, of which 44 were removed, as they were either did not contain complete ORFs or
were redundant with other sequences. The remaining 30 full-length sequences represented unique peanut MYB genes (Online Resource 2).

These 30 full-length MYB sequences belonged to three subfamilies: one R1R2R3-MYB, 9 R2R3-MYB and 20 MYB-related members; no 4R-MYB was found (Online Resource 2). The 20 MYB-related proteins contained one or two MYB repeat(s). Sequences of the three members with two MYB repeats did not have the normative character of the R2R3-MYB family, and so were included in the MYB-related family. The other 17 MYB-related members contain a single MYB repeat. All the 30 MYB sequences were designated as AhMYB1–30 and were submitted to GenBank with accession numbers KF208655–KF208684.

3.2. Sequence conservation within the MYB domain of R2R3-MYB proteins

The R2R3-MYB proteins share significant sequence conservation within the MYB domain regions. To investigate the homologous domain sequence features, and the frequency of the most prevalent amino acids at each position within each repeat of the peanut R2R3-MYB domain, sequence logos were produced using the nine homologous domain amino acid sequences of R2 and R3 repeats (Fig. 1). The 19 and 18 highly conserved amino acid residues were identical among all the members detected in the R2 and R3 repeat regions, respectively.

A typical MYB protein contains three evenly distributed tryptophan (Trp) residues, which play significant roles in interaction between the MYB protein and specific DNA sequences (Ogata et al., 1995; Stracke et al., 2001). For the nine peanut R2R3-MYB proteins, all R2 repeat sequences contained three tryptophan residues (Fig. 1a). However, in the R3 repeats, the first tryptophan residue was replaced by phenylalanine or isoleucine (Fig. 1b). The second and third tryptophan residues were conserved in the R3 repeats of all nine members (Fig. 1b). In addition to the highly conserved Trp amino acid residue, there were many other highly conserved residues in the nine peanut R2R3-MYB domains. These included Gly-4, Glu-9, Glu-10, Asp-11, Gly-22, Arg-37, Gly-39, Lys-40, Ser-41, Cys-42, Arg-43, Leu-44, Arg-45, Asn-48, Leu-50 and Pro-52 in the R2 repeat (Fig. 1a); and Glu-10, Ile-14, His-18, Gly-22, Asn-23, Ile-28, Ala-29, Leu-32, Gly-34, Arg-35, Thr-36, Asp-37 and Asn-38 in the R3 repeat (Fig. 1b).

3.3. Phylogenetic analysis of the MYB family proteins from peanut and Arabidopsis

To evaluate the evolutionary relationships within the MYB gene family, we performed a combined phylogenetic analysis of Arabidopsis and peanut MYB proteins to obtain a NJ tree (Fig. 2). Previous studies indicated that the MYB-related proteins had undergone differentiation and evolution to a larger extent than R2R3-MYB and 3R-MYB in plants.
3.4. Expression analysis of the 30 peanut MYB genes in salt-treated roots

Increasing evidences suggest that MYB TFs play important roles in the response to abiotic stresses (Dubos et al., 2010). To search for MYB genes that responded to abiotic stress in peanut, we first tested the expression pattern of the 30 peanut MYB genes in peanut roots under salt stress (Figs. 3 and 4). The expression of the four R2R3-MYB genes including AhMYB1, AhMYB2, AhMYB6 and AhMYB7 was clearly enhanced under salt stress (Fig. 3). The expression of 3R-MYB and the other five R2R3-MYB genes had no obvious change (Fig. 3). The expression of the 20 MYB-related proteins showed different patterns in roots under salt stress. The mRNA abundance of AhMYB12, AhMYB18, AhMYB28 and AhMYB30 increased under salt stress, while expression of AhMYB11, AhMYB15 and AhMYB17 decreased (Fig. 4). The other 13 peanut MYB-related proteins showed no obvious change in expression under salt stress (Fig. 4). Our results indicated at least eight peanut MYB genes were induced by salt stress: AhMYB1, AhMYB2, AhMYB6, AhMYB7, AhMYB12, AhMYB18, AhMYB28 and AhMYB30.

3.5. The expression kinetic analysis of the eight salt-induced peanut MYB genes under salt, drought, cold and ABA stresses in peanut roots and leaves

To determine the expression patterns of the eight salt-induced peanut MYB genes in detail, we monitored their expression kinetics in peanut roots and leaves respectively during 0–72 h of different abiotic stress conditions (Figs. 5–8).

All eight MYB genes were induced quickly after 1 h of salt treatment in peanut roots (Figs. 5a–h). Five genes had the highest expression level at 72 h: AhMYB1, AhMYB7, AhMYB12, AhMYB18 and AhMYB30 (Figs. 5a, d–f and h). However, most of the eight genes were not induced in peanut leaves during salt stress, except for AhMYB1, AhMYB2 and AhMYB30 (Figs. 5a, b' and h'). AhMYB6 was obviously repressed in salt-stressed leaves—a different expression pattern to that in roots (Figs. 5c and c').

As shown in Figs. 6 and 7, the eight salt-induced genes showed different expression patterns in response to drought and cold stresses.
The expressions of *AhMYB1* and *AhMYB2* were distinctly enhanced in both roots and leaves during drought stress (Figs. 6a,a',b and b'). *AhMYB6* and *AhMYB28* were induced in PEG-treated roots but repressed in leaves; and *AhMYB7* and *AhMYB18* were induced in PEG-treated leaves but changed little in roots (Figs. 6c,c',d,d',e,e',f and f'). Four genes were obviously induced in both cold-treated roots and leaves: *AhMYB2*, *AhMYB6*, *AhMYB7* and *AhMYB18* (Figs. 7b–d,b',d' and f'). The expressions of *AhMYB1* and *AhMYB28* were also quickly induced within 6 h by PEG treatment. *AhMYB2* and *AhMYB6* also quickly responded in cold-treated peanut roots and leaves. However, some genes responded slowly to drought or cold stress, such as *AhMYB1* in PEG-treated leaves and cold-treated roots, and *AhMYB18* and *AhMYB28* in cold-treated roots.

Stress-responsive genes have been proposed to be regulated by both ABA-dependent and -independent signaling pathways (Shinozaki and Yamaguchi-Shinozaki, 2000; Zhu, 2002). To analyze the relationship between the abiotic stress-induced MYB genes and ABA, we determined the expression of the eight genes in peanut roots and leaves treated with exogenous ABA. All eight genes, except for *AhMYB12* and *AhMYB18*, were induced by ABA in roots, and the transcripts of the most accumulated within 6 h (Figs. 8a–h). The expression of *AhMYB1* and *AhMYB2* also increased in ABA-treated leaves (Figs. 8a' and b'). Three genes displayed
repressed expression patterns in ABA-treated peanut leaves: AhMYB6, AhMYB12 and AhMYB18 (Figs. 8c,e and f).

As a whole, the expression of AhMYB1, AhMYB2, AhMYB6 and AhMYB28 were all enhanced in roots treated with the three abiotic stresses. AhMYB2 was also induced by the three kinds of abiotic stresses in peanut leaves. The expression of AhMYB6 showed contrasting patterns in peanut roots (increased) and leaves (decreased) during salt and drought stress. The other genes showed more complex patterns in the two peanut tissues under all three abiotic stresses, suggesting complicated functions of the MYB family proteins in peanut abiotic stress regulation. During exogenous ABA treatment, all the eight genes except AhMYB12 and AhMYB18 were induced in either peanut roots or leaves, implying these MYB proteins may participate in the stress regulation of peanut through ABA-mediated signaling pathway.

4. Discussion

4.1. Characterization of the peanut MYB gene family

The MYB superfamily has been described as the richest TF family in plants, with at least 204 and 218 members in Arabidopsis and rice, respectively (Chen et al., 2006). However, no related information has been reported in the cultivated peanut. In this study, 30 peanut MYB genes containing full-length gene sequences were identified. These 30 full-length MYB sequences belonged to three subfamilies: one R2R3-MYB, nine R2R3-MYB and 20 MYB-related members; no 4R-MYB was found (Online Resource 2). The R2R3 family contained fewer members of MYB genes than the MYB-related family in the present study — which is inconsistent with the annotation results for the Arabidopsis and rice genomes (Chen et al., 2006). However, our data are not exact because we only searched for the MYB genes with full-length cDNAs. The exact number of MYB subfamily genes in peanut will be determined when the whole-genome sequencing of peanut is complete and all peanut MYB genes are isolated from its genome.

4.2. The evolution of the MYB proteins in peanut and Arabidopsis

Phylogenetic analysis of the MYB proteins had been conducted extensively in Arabidopsis, rice, wheat and grape, and the evolutionary relationships of this gene family within and among the different species has been systematically studied (Chen et al., 2006; Matus et al., 2008; Zhang et al., 2011). To obtain an overall picture of the 30 peanut MYB proteins and their relationships with those of Arabidopsis, phylogenetic trees combining the peanut and Arabidopsis MYB proteins were constructed, which divided the 30 MYB members into 16 groups (Fig. 2). Most plant MYB genes encode proteins of the R2R3-MYB class, which are thought to have evolved from an R1R2R3-MYB gene ancestor by the loss of the sequences encoding the R1 repeat and subsequent expansion of the gene family (Rosinski and Atchley, 1998). An appreciable number of R2R3 peanut MYB proteins (four of nine) were clustered into group 5, which was evolutionarily close to the 3R-MYB group, indicating that these proteins may have evolved from 3R-MYB in peanut. The other five R2R3 MYB members were dispersed into four groups of greater evolutionary distance from 3R-MYB. Whether these proteins evolved from 3R-MYB cannot be determined in peanut. The 3R-MYB subfamily was considered to share the conservative function of controlling cell development between plants and animals (Jin and Martin, 1999). So far, five Arabidopsis and four rice 3R-MYB proteins have been identified and, among them, AtMYB3R-1 and AtMYB3R-4 positively regulate cytokinesis (Haga et al., 2007). In the present study, only one peanut MYB protein, AhMYB10, was grouped with all the five Arabidopsis 3R-MYB members (Fig. 2a). It is noteworthy that the R1 repeat in AhMYB10 proteins is incompletable, therefore, whether the AhMYB10 protein functions in cell-cycle regulation requires further exploration.

Several peanut MYB proteins were clustered into Arabidopsis functional groups, providing valuable information for studying the functions of peanut MYB genes. Previous studies indicated that the MYB proteins were involved in many significant physiological and biochemical processes, including response to various biotic and abiotic stresses (Czemiell et al., 2012; Du et al., 2009; Dubos et al., 2010; Feller et al., 2011; Ma et al., 2009; Stracke et al., 2001). For example, R2R3-MYB proteins such as AtMYB44/AtMYB1 (group 5) regulate ABA-mediated stomatal closure in response to abiotic stresses and three other members of this subgroup (AtMYB70, AtMYB73 and AtMYB77) are likely associated with stress responses (Jung et al., 2008). AtMYB3, AtMYB4 and AtMYB8 (group 12) are regulatory factors related to different biotic or abiotic stress responses (Bang et al., 2008; Hemm et al., 2001; Jin et al., 2000; Zhao et al., 2007; Zhu et al., 2005). AtMYB13 and AtMYB15 (group 17) are involved in ABA-mediated responses to environmental signals (Reyes and Chua, 2007). AtMYB15 is also involved in cold stress tolerance (Agarwal et al., 2006). Interestingly, the majority of the peanut R2R3 MYB proteins (seven of nine) were clustered into these stress response groups (Fig. 2a), which thus stimulated us to explore the functions of MYB proteins in peanut abiotic stress regulation.

4.3. Peanut MYB proteins respond to multiple abiotic stresses

Plants experience various environmental stresses including drought, salinity and extremes of temperature. During the response and adaptation to abiotic stress, there are many changes in biochemical and physiological processes. Many genes are activated, leading to the accumulation of numerous proteins involved in resistance to abiotic stress, such as late embryogenesis abundant proteins (Ma et al., 2010). The expression of stress-induced genes is largely regulated by specific TFs (Hu et al., 2008). Among these TFs, members of the APETELA2 (AP2), bZIP, NAC and MYB families have been well characterized for their regulatory roles in the response of plants to abiotic stress (Hu et al., 2008; Takasaki et al., 2010).

Many studies have indicated that the MYB TFs play essential roles in the regulation of gene expression to cope with environmental changes (Ahuja et al., 2010; Dubos et al., 2010; Hirayama and Shinozaki, 2010). In the model plants Arabidopsis and rice a number of MYB genes were characterized to function as key factors in the signaling pathways for plant resistance to abiotic stresses (Dubos et al., 2010; Ma et al., 2009; Vannini et al., 2004; Yang et al., 2012). In recent years, the MYB proteins of many other plants had been demonstrated as involved in abiotic stress regulation (Cai et al., 2011; Garg et al., 2012; Prabu and Prasad, 2012; Qin et al., 2012; Wang et al., 2013). However, functional studies of this gene family in peanut are lacking. Of all 30 peanut MYB genes in salt-treated roots in the present study, the expression patterns indicated that at least eight genes responded to salt stress. Among these eight genes, four encode R2R3 MYB proteins and four encode the MYB-related proteins. The four R2R3 MYB proteins – AhMYB1, AhMYB2, AhMYB6 and AhMYB7 – all clustered into the groups with stress response function categories, suggesting that they may have similar functions to the homologous proteins of Arabidopsis in abiotic stress regulation. Interestingly, four MYB-related genes also responded to some kinds of abiotic stress. In contrast to the R2R3-MYB genes, the MYB-related genes have attracted little attention, and few have been
studied functionally, such as CCA1-like genes involved in the maintenance of circadian rhythms (Alabadi et al., 2001; Green and Tobin, 1999; Kuno et al., 2003; Mizoguchi et al., 2002; Schaffer et al., 1998; Wang and Tobin, 1998) and CPC-like genes involved in control of cellular morphogenesis (Kirik and Bäumlein, 1996; Kirik et al., 2004; Lee and Schiefelbein, 2002; Sawah, 2002; Schellmann et al., 2002; Wada et al., 1997). Shin et al. (2010) proved that a novel potato single MYB-like domain protein StMYB1R-1 played positive roles in potato drought resistance. The present study provides evidence that the MYB-related proteins may be involved in peanut abiotic stress regulation.

Further analysis suggested that most of the eight MYB genes were also induced by drought and cold stress in peanut roots or leaves (Figs. 5–8), indicating that these genes may function in multiple abiotic stress regulation of peanut. Two genes (AhMYB12 and AhMYB18) were specifically induced by salt stress, suggesting distinct roles in peanut salt stress regulation. Additionally, some genes such as AhMYB6 and AhMYB30 exhibited opposite expression patterns under different stress conditions or in different peanut tissues. Some genes, such as AhMYB7, responded to certain stresses only in one of the two tissues and maintained constitutive expression levels in the other tissue. Although considerable studies had been done to investigate the expression response of MYB family genes during abiotic stress in many plant species, little was known to what extent the stress response programs would differ between different tissues such as roots and leaves. Kreps et al. (2002) reported that roots and leaves have very different transcriptome response to cold, osmotic and salt stresses in Arabidopsis. Their study indicated that 86% of the cold-induced changes were not shared between roots and leaves and similar root-leaf differences were also observed with NaCl and mannitol stress (Kreps et al., 2002). We speculated that the expression differences between roots and leaves may partly reflect the fact that only the roots were in direct contact with the solution during NaCl or PEG treatments, while only the leaves were in direct contact with the low temperature during cold treatment. Our results imply that the signaling pathways mediated by MYB proteins in the peanut abiotic stress response are very complicated systems.

4.4. Relationship of stress response MYB genes and ABA signaling in peanut

ABA plays a crucial role in the adaptive response of plants to abiotic stresses. The pathways leading to adaptation to stress can be divided into two major categories: ABA-dependent and -independent pathways (Xiang et al., 2008). Several R2R3-MYB genes have been reported as important mediators of ABA-mediated gene expression under environmental stress conditions. For example, MYB2, MYB41, MYB44, MYB96 and MYB102 were all responsive to ABA and at least one kind of abiotic stress in Arabidopsis (Abe et al., 1997; Cominelli et al., 2008; Denekamp and Smeekens, 2003; Jung et al., 2008; Kranz et al., 1998; Seo et al., 2009). Recently, a MYB-related gene LcMYB1 was also proved to be induced by high salt, drought and ABA stress in Sheepgrass (Cheng et al., 2013). According to our results, the two genes AhMYB1 and AhMYB2 were obviously inducible in both peanut roots and leaves, suggesting they might play roles in ABA-dependent signaling transduction pathways under the three abiotic stress conditions. AhMYB6 was induced in roots and repressed in leaves under exogenous ABA stimulus. This was consistent with the expression pattern of this gene during salt and drought stress, and indicated that AhMYB6 proteins may participate in the two abiotic stresses in the ABA-dependent pathway. Similar conditions also existed in the expression pattern of AhMYB28 and indicated that it may play roles through the ABA-dependent signaling pathway under salt and cold stress. The expressions of other genes – including AhMYB7, AhMYB12, AhMYB18 and AhMYB30 – were not completely consistent for the ABA and three abiotic stress treatments. The relationships of these genes and ABA during abiotic stress need further study.

It is noteworthy that previous investigations also indicated that MYB TFs might function as key mediators of stress responses through complex activities spanning ABA and other multiple stress signaling pathways. For example, AtMYB102 had been shown to respond to ABA, JA, salt stress and wounding (Denekamp and Smeekens, 2003). AhMYB44 mRNA accumulation was induced in most tissues by a variety of hormone treatments (ABA, IAA, ET, JA and GA), environmental conditions (drought, high salinity, and low temperature), and pathogen infections (Jung et al., 2007, 2008; Kranz et al., 1998; Yanhui et al., 2006). These results suggested that MYB family proteins may regulate the stress response through complicated mechanisms in plant and a lot of work need to be done to clarify the functions of these proteins in peanut.

5. Conclusion

30 full-length cDNA sequences of peanut MYB genes were identified, and their expression patterns analyzed under different abiotic stress and exogenous ABA treatments. Phylogenetic comparison of peanut and Arabidopsis MYB members revealed the putative functions of some peanut MYB proteins. The present study provides significant information to use in improving stress tolerance of crops through molecular breeding.

Conflict of interests

All of the authors declare that there are no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.gene.2013.08.092.

References


Fig. 6. Expression kinetic analysis of the eight salt-induced peanut MYB genes in drought treated roots and leaves respectively. (a–h) Time course of AhMYB1, AhMYB2, AhMYB6, AhMYB7, AhMYB12, AhMYB18, AhMYB28 and AhMYB30 expression during treatment with 20% PEG6000, a mimic for drought stress, in peanut roots. (a’–h’) Time course of AhMYB1, AhMYB2, AhMYB6, AhMYB7, AhMYB12, AhMYB18, AhMYB28 and AhMYB30 expression under 20% PEG treatment in peanut leaves. ACT11 was used as reference gene. Data represent means of three replicates. Error bars are SD.
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AhMYB12
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Fig. 7. Expression kinetic analysis of the eight salt-induced peanut MYB genes in cold treated roots and leaves respectively. (a-h) Time course of \( \text{AhMYB1}, \text{AhMYB2}, \text{AhMYB7}, \text{AhMYB8}, \text{AhMYB12}, \text{AhMYB18}, \text{AhMYB28} \) and \( \text{AhMYB30} \) expression under cold (\( -4^\circ\)C) treatment in peanut roots. (a-h') Time course of \( \text{AhMYB1}, \text{AhMYB2}, \text{AhMYB7}, \text{AhMYB8}, \text{AhMYB12}, \text{AhMYB18}, \text{AhMYB28} \) and \( \text{AhMYB30} \) expression under cold treatment in peanut leaves. ACT17 was used as reference gene. Data represent means of three replicates. Error bars are SD.


