THE ROLE OF R2R3MYB TRANSCRIPTION FACTORS IN PLANT STRESS TOLERANCE

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ABSTRACT

The plant-specific R2R3MYB proteins constitute a largest subfamily in MYB transcription factors and are widely involved in growth and developmental processes. In spite of their highly conserved DNA-binding domains, the biologic functions are strikingly varied across plant species. Recently, their roles in making plant more survivable under adverse environmental conditions including cold, drought, pathogen, salinity have received considerable attention. Nowadays, the R2R3MYBs in response to stresses, here termed as stress-responsive R2R3MYBs (SR-R2R3MYBs), were continuously identified from different plant species. This review mainly focused on the recent progresses in SR-R2R3MYBs gene structure, biological functions and their expression pattern under varied stresses. Additionally, we have also discussed the genetic relationship of SR-R2R3MYBs proteins, and their role in integrating a diversity of pathways including metabolic, SOS, signal transduction pathways etc, with an aim to use them for crop improvement via transgenic technologies.

Key words: R2R3MYB, transcription factor, stress signaling, biologic function, target gene.

INTRODUCTION

MYB proteins constitute a super-family of transcription factors (TFs) in plant, and they are characterized by the presence of a functional DNA binding domain (MYB domain) composed of one to three imperfect repeats (R1, R2, and R3), each repeat contains about 52 amino acid residues (Du et al., 2009). According to the number of adjacent repeats in MYB domain, these MYBs can be classified into three subfamilies: R1R2R3MYB, R2R3MYB, and R1MYB (Du et al., 2009). Among them, R2R3MYB subfamily is the largest and specific to higher plants (Du et al., 2012a,b). Through genome-wide analysis, we identified 112 MYB genes in Citrus, of which 97 belong to R2R3MYB genes and their biological bio-functions are in progress to be analyzed. Taking a cue from the above-mentioned findings, we feel that numerous new plant R2R3MYB members remain to be discovered.

In recent years, functional analyses revealed that numerous R2R3MYBs could enhance plant tolerance to stresses (He et al., 2012; Yang et al., 2012). Due to their important role in stress adaptation, the isolation and characterization of SR-R2R3MYBs from plants, especially from fruit tree crops such as citrus, pear, Chinese bayberry etc. become a very active area of research.

SR-R2R3MYBs as TFs can up- or/and down-regulate the expression of many stress-related genes (Xie et al., 2010), and subsequently alter multiple signal transduction pathways and metabolic processes, eventually leading to enhanced plant tolerance; in turn, they are also directly regulated by hormones, stresses and TFs (Mao et al., 2011). The above-mentioned findings indicated that the plant SR-R2R3MYBs perform as nodes to weave a complicated stress-responsive network that enable plant to avoid stress injuries. While, dramatic variations in biologic functions were observed among different SR-R2R3MYBs. In recent years, several reviews on MYBs have been published, briefly introducing their molecular structure, evolution, function and interaction with their target DNA binding sites (Jin et al., 1999; Rtracke et al., 2001; Dubos et al., 2010; Prouse et al., 2012; Ambawat et al., 2013). While, to date, a comprehensive review on plant SR-R2R3MYBs how to regulate plant adaptation to adverse conditions has not been dealt with. Therefore, this review focused on the roles of SR-R2R3MYBs in stress response, mainly including sensing and transducing stress signals as well as activating targeted genes. Our aims are to provide useful information for future research and to develop effective strategies for improving stress adaptability of plants.

Genetic Relationships of SR-R2R3MYBs: Based on the amino acid sequence of all Arabidopsis R2R3MYBs and the SR-R2R3MYBs from other plant species, a phylogenetic tree was constructed using MEGA 5.0 (NJ, bootstrap=1000) (Figure 1), which may facilitate the identification of new SR-R2R3MYBs and potential functions in stress response because members of the same subgroups are generally suggested to have similar function. According to Figure 1, it was showed that the
Structural Feature of R2R3MYBs: The N-terminus of R2R3MYBs is a highly conserved region containing a DNA-binding MYB domain. The MYB domain has two consecutive repeats i.e. R2 and R3. Generally, the MYB repeat is about 50 to 53 amino acids in length and encodes three helices, with the second and third helices forming a helix-turn-helix structure (Figure 2). Using heteronuclear multidimensional NMR, Ogata et al. (1994) demonstrated that R2 and R3 each contain three helices, and the third helix in each is a recognition helix. These two repeats are closely packed in the major groove of DNA, leading to contact each other directly to cooperatively bind the specific sequence (Ogata et al., 1994), while which not contributes to the functional activity and specificity according to the quadruple 9-mer-Based protein binding microarray analysis of AtMYB44 (Jung et al., 2012). By GFP fusion protein analysis, two basic amino acid regions i.e. KRGK and RKKAQEKKR in R3 repeat were found to be concurrently required for localizing to the nucleus, likely serving as bipartite nuclear localization signals, and these two regions were highly conserved in almost all the Arabidopsis R2R3MYB proteins (Li et al., 2006). Besides KRGK and RKKAQEKKR, we believed that the functions of other amino acid regions were also worthy to be characterized.

Outside of the DNA-binding domain (N-terminus), the proteins are highly divergent with the exception of some short conserved amino acid sequence motifs. Based on these motifs, Kranz et al.(1998) have divided Arabidopsis R2R3MYBs into 22 subgroups; for instance, the members in subgroup 22, one of subgroups involved in stress response, including AtMYB73, AtMYB44, AtMYB77, AtMYB70 share a common conserved motif i.e. GEFMxVQEMIxxEVRSYM. Because the C-terminus is the transcriptional activation domain responsible for functional activity or/and specificity (Chen et al., 2013), these conserved motifs could facilitate the functional identification of R2R3MYBs, and explain the molecular basis of functional difference among R2R3MYB members. Through aligning the amino acid sequences of SR-R2R3MYBs from different plant species, we found that no conserved motifs existed in C-terminal domain. These SR-R2R3MYBs used to be aligned here generally responded to salts or drought or both, including TaMYB30-B, AtMYB2, TaMYB73, TaMYB33, TaMYB32, CmMYB2, JAMYB, AtMYB2, and AtMYB20. From them, two members with the same functions reported recently i.e. TaMYB33 and CmMYB2 were used to further analyze in order to discover whether a conserved motif exists in their C-terminal domain, but we failed. Therefore, the conserved motifs do not necessarily associated with the biological functions in response to stresses. As an example, AtMYB44 and AtMYB73 act as positive and negative TFs, respectively, showing an obvious difference in biologic functions, but both of them actually contain a common conserved motif (Seo et al., 2012; Kim et al., 2013). In addition, the promoter of target-genes may also affect SR-R2R3MYBs functional activity and specificity of R2R3MYB proteins, which need to be explored in future.

Expression of SR-R2R3MYB Genes: The differences in expression profiles were observed among different SR-R2R3MYB genes treated by the same stresses or hormones as well as within the same SR-R2R3MYB genes under different stresses or hormones. By gene expression profiles, their biologic functions can be predicted. Based on the data obtained in previous studies, a large number of stresses, such as draught, cold, salt, pathogen and so on, can positively or/and negatively regulated the expression of SR-R2R3MYBs (Oh et al., 2011; Shin et al., 2011). For example, AtMYB14 is down-regulated under cold stress (Chen et al., 2013); whereas MYB15 is up-regulated by cold treatment (Agarwal et al., 2006). Feng et al. (2004) revealed that the AtMYB68 could especially respond to heat stress; the same case was detected to AtMYB62, which was especially response to Pi deprivation (Devaiah et al., 2009). In addition, some of SR-R2R3MYBs are expressed in specific organ under stress condition, such as AtM YaB60, whose transcripts just is induced in guard cells by drought (Oh et al., 2011).

Besides stresses, hormones can also affect the expression profiles of SR-R2R3MYBs. For instance, AtMYB96 expression was up-regulated in different organs by IAA and ABA, respectively (Seo et al., 2009). According to the data reported previously, the R2R3MYBs in response to ABA, were generally related with abiotic tolerance, especially drought (Oh et al.,
2011). However, some, for example OsMYB4, not in response to ABA still exhibit tolerance to abiotic stresses (Cheng et al., 2007). Likewise, the R2R3MYBs related to biotic stresses generally can be regulated by SA. Thus, according to hormone-responsive expression profiles, we could roughly predict the biologic role of one SR-R2R3MYB.

**Regulation of SR-R2R3MYBS:** The transcript abundance of a gene can be firstly up- or down-regulated by binding of specific TFs to its cis-element in the promoter. Although numerous putative stress-responsive cis-elements are present in SR-R2R3MYBs promoter, far less is known how they are regulated by other transcription factors. These cis-elements mainly include GCC-box, MYC, AG-motif-like, Box-P, ABRE, CE1, RY-motif and so on (Denekamp et al., 2003; Maeda et al., 2006; Jung et al., 2008; He et al., 2012). The GCC-box and MYC binding sequence in DcMYB1 promoter might be necessary for UV-B irradiation, and the AG-motif-like and/or Box-P for elicitor treatment (Maeda et al., 2006). Further analysis found that some cis-elements neighboring the TATA-box such as MYB binding sequence and W-box, might take part to influence promoter activity (Maeda et al., 2006). Miyahara et al. (2010) reported that binding DcEIL (ethylene insensitive3-like protein) to an inconclusive regulatory region in promoter repressed DcMYB1 expression, suggesting ethylene was involved in stress response. The stress-responsive expression of NtMYB2 was induced by binding AGP1 (GATA-type zinc finger protein) to AG-motif i.e. AGATCCAA (Sugimoto et al., 2003), while that of AtMYB44 and TaMYB2 might be partly due to the presence of RY motif and MBS (Jung et al., 2008; Mao et al., 2011). Over-expressing NST3 (a secondary cell wall-associated NAC thickening factor) up-regulated AtMYB20 and AtMYB85, indicating these two MYB genes were directly or indirectly regulated by NST3 (Zhong et al., 2008). The ABRE sequence is extensively present in the promoter of SR-R2R3MYBs, which is necessary for ABA-responsiveness (Qin et al., 2012); however, there are several exceptions. For example, some ABREs in the BcMYB1 promoter, but exogenous ABA just slightly induced the expression of BcMYB1 (Chen et al., 2005). Based on abovementioned findings, the regulatory role of these cis-elements in SR-R2R3MYBs transcription remains to be further investigated in detail. At the same time, lots of novel TFs that regulate stress-responsive SR-R2R3MYBs also need to be discovered.

Recently, several studies have shown that the MYB genes are post-transcriptionally regulated by microRNA (miRNA) through cleaving target gene. It is well known that miR159 target to anther or pollen development-related R2R3MYB genes such as AtMYB33, AtMYB35, AtMYB65 and AtMYB101 (Allen et al., 2007; Addo-Quaye et al., 2008). However, none microRNA, to date, has been reported to control SR-R2R3MYBs. Most recently, the primary transcript of a SR-R2R3MYB gene, AtMYB60, was found to be alternatively spliced, forming two splice variants, one of which lacks the first two exons encoding the first MYB DNA binding domain; these two splice variants locate to the nucleus and enable plant to adapt to drought (Oh et al., 2011). Thus, besides microRNA, the alternative splicing how to affect SR-R2R3MYB genes could also be worthy to study.

To the best our knowledge, no other information about regulation of SR-R2R3-MYB is available, specifically, post-translational regulation. Therefore, intensive works, in future, need to elucidate the molecular mechanism underlining the regulation of SR-R2R3MYBs.

**SR-R2R3MYB Genes for plant stress tolerance:** Stresses, such as drought, salt, cold and pathogen, adversely affect crop productivity and quality, which have received considerable attention of plant scientists. Recently, the major aim of agronomic researches is to enhance or stabilize agriculture yields under various environmental conditions. A significant approach is to use stress-associated genes to improve crop tolerance to different stresses. Increasing plant SR-R2R3MYB genes have been found to have an important role in stress tolerance (Table 1), showing a promise for improving plant adaptation to stresses.

**Abiotic stress tolerance:** Overexpressing SR-R2R3MYB genes in Arabidopsis generally produces tolerant plant to most abiotic stresses (Cominelli et al., 2005; Jung et al., 2008), while, some serve as negative regulator, showing opposite function (Agarwal et al., 2006). The functions between SR-R2R3MYBs exist obvious discrepancies, of course not exclude redundancy, AtMYB60, AtMYB61 and AtMYB15 enhance plant tolerance to drought by regulating stomatal aperture in dependent- or independent-ABA means, of which AtMYB60 acts as a negative regulator (Cominelli et al., 2005; Liang et al., 2005 Jung et al., 2008). Overexpressing AtMYB15 leads to an increase in salinity tolerance, and a decrease in cold response (Agarwal et al., 2006; Ding et al., 2009), showing functional redundancy. There are a large body of SR-R2R3MYBs from different plant species that can modify plant tolerance to abiotic stresses, such as AtMyb41 (Lippold et al., 2009), OsMYB2 (Yang et al., 2012), TaMYB 30 (Zhang et al., 2012a). Many R2R3-MYBs overexpressed in plants remarkably improved stress tolerance without any distinctively adverse effects; while some could produce detrimental consequences in the transgenics, showing dwarfing, small seeds, later flowering etc. (Table 1). These undesirable phenotypes probably can be overcome by using a proper promoter, for example a tissue- or a stress- specific promoter.
Pathogen resistance: During recent years, RNA knockout/interference and overexpression studies have also revealed the function of SR-R2R3MYBs in various plant-pathogen interactions. For instance, AtMYB30 has been found to regulate plant defense response by activating the hypersensitive cell death program at the infection site (Vailleau et al., 2002). NaMYB8 regulated the accumulation of phenylpropanoid-polyamine conjugates, resulting in local and systemic defense against insect herbivores (Kaur et al., 2010), and could also increase the tolerance to abiotic stresses (Katyar et al., 2012). AtMYB46 can modulate Botrytis cinerea susceptibility likely through integration of cell wall remodeling and activation of pathogen-associated genes (Ramirez et al., 2011). Thus, we could use these SR-R2R3MYBs to obtain genetically modified crops with higher resistance to pests and diseases.

Multifunctional SR-R2R3MYBs: Data from recent studies indicated that some SR-R2R3MYBs were implicated in crosstalk between biotic and abiotic stress. Functional analysis revealed that AtMYB96 as a key regulator is involved in biotic and abiotic stresses by mediating ABA signaling and SA biosynthesis (Seo et al., 2009, 2010). Overexpressing AtMYB44 significantly enhances Pseudomonas syringae resistance as well as tolerance to salt and drought stresses (Zou et al., 2012; Seo et al., 2012). This regulator also affects the resistance to green peach aphid (Liu et al., 2011). Besides the abovementioned multifunctional SR-R2R3MYBs, AtMYB108, JAmyb and TaPIMP1 are also involved in the defense to both biotic and abiotic stress by different regulatory manners (Zhang et al., 2012d; Yokotani et al., 2006). These multifunctional SR-R2R3MYBs are significantly important for making plants survival under extreme stress conditions, since plants often undergo multiple stresses concurrently.

Cis-acting elements and target genes of SR-R2R3MYBs: R2R3MYBs regulate the transcript of their target genes by binding to MYB recognition sequence (MBS). Several years ago, a few studies identified ACC/(A/T)(A/T)CC as MBS in the promoter of PAL or CHS, both of which are involved in phenylalanine metabolism in response to UV-B, nitrogen deficiency, wounding (Miyake et al., 2003, 2004; Maeda et al., 2005; Gális et al., 2006). This MBS highly coincides with the type IIG (H-box-like) which was characterized by YACCCWACC as a recognition sequence of a number of MYB transcription factors (Miyake et al., 2003). Agarwal et al. (2006) also identified (T/C)(T/G)TTA as MBS motif in the promoter of AtCBF genes (Agarwal et al., 2006). Many other MBSs such as MRE4 (TCTCACCTACC), mMREI (CCGGAAAAAAGGAT) and AC element (ACCTAAC or ACCTAC) etc in numerous stress-related gene promoters were gradually identified in recent years (Liao et al., 2008; Michael et al., 2013), indicating SR-R2R3MYBs were widely involved in stress-related processes. Interestingly, the MRE4 sequence contains an AC element i.e. ACCTAC, suggesting that these two MBSs probably were the same and there maybe had several additional nucleotides in MRE4, which need to further assay.

Some reports showed that different type MBSs can be bind with the same one SR-R2R3MYB, and vice versa. For example, TaMYB73 can bind to types I, II, and IIG MYB binding motifs, and then improve corresponding gene expression (He et al., 2012). More interesting, AtMYB15 binds to (T/C)(T/G)TTA in AtCBFs promoter, resulting in a decrease in its expression (Agarwal et al., 2006), while to MBSII element (TAACTAAC), enhancing expression (Maeda et al., 2005), the functional difference of which may be due to the difference of MBSs, or the interaction with other partner factors or both. By quadruple 9-mer based protein binding microarray analysis, Jung et al. (2012) revealed that AtMYB44 and AtMYB77 with quite different biological roles could bind the same MBSs in the promoter of many genes, indicating that the binding of one SR-R2R3MYB to MBS in a gene promoter, not mean that this gene is the target of the corresponding SR-R2R3MYB, and this SR-R2R3MYB may function either by interaction with other transcription factors or through stimulus-induced structural modification.

As mentioned above, some SR-R2R3MYBs do require the coexistence of other partner factors (figure 4), such as GmCaM4, ICE1 and EIL3, however the others don’t, just like as DcMYB1 (Yoo et al., 2005; Agarwal et al., 2006; Ent et al., 2008). According to the findings reported previously, we can speculate the functions of partner factors are as follows: (1) increase the DNA binding activity of SR-R2R3MYBs; (2) confer binding specificity to SR-R2R3MYBs; (3) alter the biologic roles of SR-R2R3MYBs, all functions of which finally result in a change in the level of gene expression.

Identification of the downstream target genes is helpful to elucidate the molecular mechanisms how SR-R2R3MYBs regulate the plant adaptation to various stress conditions. Recently, these target genes are generally revealed by large-scale transcriptome analysis via over-expressing SR-R2R3MYB genes. SR-R2R3MYBs can directly or indirectly regulate a wide range of functional and regulatory genes (table 1) encoding proteins involved in the free radical and superoxide anion scavenging, osmolyte production, material transport, detoxification, signal transduction and regulation of gene expression etc. In general, these stress-related genes contain MBSs in their promoter, while there may exist some genes either without MBS or with a novel MBS, which can also be regulated by stress-responsive R2R3-MYBs, indicating a new mechanism remains to be investigated.
Functional conservation of sr-r2r3mybs in plants: In general, the biological roles of a R2R3-MYB in different plant species are highly conserved. For example, the AtMYB44-overexpressing soybeans exhibited significantly enhanced drought and salt stress tolerance, as observed in Arabidopsis, indicating the tolerance mechanism activated by AtMYB44 is conserved in these two plant species (Seo et al., 2012). Based on this attribute, we can use one plant SR-R2R3MYB gene to modify another plant species tolerance. However, some SR-R2R3MYBs are exceptions. Overexpressing OsMYB4 gene can enhance tolerance to cold and drought in apple, cold in Osteospermum ecklonis, drought but not cold in tomato, salt, cold and drought in rice, and different stresses such as cold, frost, drought, salt, UV and pathogen etc in Arabidopsis (Vannini et al., 2007; Mattana et al., 2005; Pasquali et al., 2008; Laura et al., 2010; Yang et al., 2012). Therefore, it’s better to detailly dissect the function of a SR-R2R3MYB before using it for crop improvement. To date, the reason why the same SR-R2R3MYB gene exhibits different biological roles in different plant species remains to be explored, maybe which can be explained by the genetic discrepancies formed under adverse environmental conditions via a long period of evolution.

Integration of various metabolic and signaling pathways by SR-R2R3MYBS: SR-R2R3MYBs usually acting as a key knot integrate a diversity of pathways including metabolic, SOS, signal transduction pathways etc. forming a sophisticated network in respond to various stresses (Fig. 3). TaPIMP1 enable wheat to resist B. sorokiniana and drought stresses by orchestrating ABA- and SA-signaling pathway cross-talk (Zhang et al., 2012d). Seo et al. (2009) reported that AtMYB96 acts as a molecular link that mediates ABA-SA crosstalk, resulting in an increase in plant disease resistance (Seo et al., 2010). At the same time, this gene can also affect lateral roots growth under drought condition through mediating ABA-IAA crosstalk (Seo et al., 2009). Moreover, ABA is able to affect wax biosynthesis via AtMYB96 in response to drought (Seo et al., 2011). These findings show that different SR-R2R3MYBs play different roles in waving tress-related network. Besides TaPIMP1 and AtMYB96, a large amount of other SR-R2R3MYBs, such as AtMYB102, OsMYB4 and JAmyb, also serve as regulatory component that integrate the pathways related to dehydration, osmotic, salinity stress, and wound-signaling etc. to enhance plant tolerance or resistance. However, to date, no one clear and complete schematic of the stress-responsive network weaved by R2R3MYBs was drawing out.

Figure 1 Phylogenetic relationship of Arabidopsis R2R3-MYB proteins and stress-respective R2R3-MYBs from other plant species was clustered using MEGA 5.0 (NJ, bootstrap=1000). The R2R3-MYBs responsive to abiotic and biotic stresses are highlighted by orange and green dot, respectively, and those concurrently responsive to abiotic and biotic stresses are emphasized by blue dot. All of them have been directly tested in vivo.
Figure 2 The three-dimensional (3D) structure of the MYB domain of a citrus R2R3-MYB identified from clementina genome (http://www.phytozome.org), referred as to CitMYB79. This 3D structure was constructed by Phyre2 (http://www.expasy.org/proteomics/protein_structure). The schematic was colored by rainbow from N to C.

Figure 3 A schematic of SR-R2R3MYB TFs as key components in transcriptional regulation of gene expression during abiotic and biotic stresses. Abbreviations: PP, partner protein; MBS, MYB binding site; PC, post-transcriptional processing; PL, post-translational processing.
## Table 1 Summary of stress-associated R2R3-MYB genes and their corresponding biologic functions

<table>
<thead>
<tr>
<th>Transgenic plant</th>
<th>Genotype</th>
<th>Result/phenotype</th>
<th>Negative traits of transgenics</th>
<th>Target genes</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. thaliana</td>
<td>CaMV 35S-AtMYB60 overexpression</td>
<td>Promoted root mess and negatively controlled stomatal aperture under drought dress</td>
<td>By overexpression, increased sensitivity to drought</td>
<td>The auxin-responsive DR5, PAP1, PAP2, flavonon-3ß-hydroxylase</td>
<td>Oh et al., 2011</td>
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<td></td>
<td>CaMV 35S-AtMYB15 overexpression</td>
<td>Decreased CBF genes expression, enhanced salt and drought tolerance</td>
<td>Reduces freezing tolerance</td>
<td>CBF1, CBF2, CBF3, ABI3, AtADH1, RD22 etc.</td>
<td>Agarwal et al., 2006; Ding et al., 2009</td>
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<td></td>
<td>CaMV 35S-AtMYB30 overexpression</td>
<td>MYB30 positively regulated pathogen resistance for different bacterial pathogens and a biotrophic fungal pathogen, Cercospora nicotianae</td>
<td>-</td>
<td>hsr203, hsr511 Athsr3, PR-1, PR-5</td>
<td>Vailleau et al., 2002</td>
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<td></td>
<td>CaMV 35S-TaMYB30-B overexpression</td>
<td>Improved drought stress</td>
<td>-</td>
<td>RD29A, ERD1</td>
<td>Zhang et al., 2012a</td>
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<td></td>
<td>CaMV 35S-AtMYB2 overexpression</td>
<td>Improved Stress Tolerance, and Pi uptake</td>
<td>Showed a dwarf phenotype and reduced primary root length</td>
<td>rd22, AtADH1, Cor6.6, rd20, US1, VSP2, BG1, TGG2, miR399f, PSI</td>
<td>Abe et al., 2003; Baek et al., 2013</td>
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<td></td>
<td>AtMYB14 knockdown/overexpression</td>
<td>Negative regulator of low temperature tolerance</td>
<td>-</td>
<td>CBF1, CBF2, and CBF3</td>
<td>Chen et al., 2013</td>
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<td></td>
<td>CaMV 35S-AtMYB41 overexpression</td>
<td>Enhanced sensitivity to desiccation</td>
<td>Showed a mild dwarf phenotype</td>
<td>AtRd29a, AtCor15a, AtKin1, AtDreb2a etc.</td>
<td>Cominelli et al., 2008</td>
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<td></td>
<td>CaMV 35S-AtMYB44 overexpression</td>
<td>Showed enhanced resistance to <em>Pseudomonas syringae</em> pv. tomato DC3000 and to drought, salt and cold stresses</td>
<td>Showed a mild dwarf phenotype; delayed flowering time; small seeds</td>
<td>PR1, ABI1, ABI2, AtPP2CA, HAB1, HAB2 etc.</td>
<td>Jung et al., 2008; Zou et al., 2012</td>
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<td></td>
<td>CaMV 35S-TaMYB73 overexpression</td>
<td>Enhanced salinity tolerance</td>
<td>-</td>
<td>AtCBF3, AtABF3, AtRD29A, AtRD29B</td>
<td>He et al., 2012</td>
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<td></td>
<td>CaMV 35S-TaMYB32 overexpression</td>
<td>Tolerance to salt stress</td>
<td>-</td>
<td>-</td>
<td>Zhang et al., 2012b</td>
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<td></td>
<td>CaMV 35S-MiMYB10 overexpression</td>
<td>Tolerance to osmotic stress</td>
<td>-</td>
<td>chs, chi, f30h, c4h and fls</td>
<td>Gao et al., 2011</td>
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<td></td>
<td>CaMV 35S-CmMYB2 overexpression</td>
<td>Tolerance to drought and salt stress</td>
<td>Delayed in flowering</td>
<td>RD22, RD29A, RAB18, COR47, ABA1, ABA2, CO, FT, SOCI1,LFY and AP1</td>
<td>Shan et al., 2012</td>
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<td></td>
<td>AtMYB46 mutant</td>
<td>Showed enhanced resistance to <em>B. cinerea</em></td>
<td>-</td>
<td>Ep5C</td>
<td>Ramirez et al., 2011</td>
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<td></td>
<td>AtMYB72 knockouts</td>
<td>Showed enhanced resistance to <em>B. cinerea</em></td>
<td>impaired the ability against a broad spectrum of pathogens</td>
<td>-</td>
<td>Katiyar et al., 2012</td>
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<td></td>
<td>CaMV 35S-TaMYB33 overexpression</td>
<td>Enhanced resistance to drought and salt stresses</td>
<td>-</td>
<td>AtP5CS, AtZAT12, AtAAO3, AtABF3, AtABI1</td>
<td>Qin et al., 2012</td>
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<td></td>
<td>CaMV 35S-TaMYB56-B overexpression</td>
<td>Enhanced resistance to freezing and salt stresses</td>
<td>-</td>
<td>DREB1A/CBF3 and COR15a</td>
<td>Zhang et al., 2012c</td>
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<td></td>
<td>CaMV 35S-OsMYB4 overexpression</td>
<td>Enhanced resistance to freezing and chilling stresses</td>
<td>-</td>
<td>PAL2, COR15a, COR78</td>
<td>Vannini et al., 2004</td>
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<td>Authors</td>
<td>Mutation/Overexpression</td>
<td>Associated Phenotypes/Genes</td>
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<td>Xie et al.,</td>
<td>CaMV 35S-AtMYB15</td>
<td>Resulted in activation of the shikimate pathway genes in response to wounding delayed senescence, resulted in a characteristic gibberellic acid (GA)-deficient phenotype, leading to decreased total Pi content in leaf</td>
<td>Chen et al., 2006b</td>
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<td>CaMV 35S-AtMYB62</td>
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<td></td>
<td>CaMV 35S-JAmyb</td>
<td>Enhanced resistance to high-salinity</td>
<td></td>
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<td></td>
<td>AtMYB102 knockout Bos1</td>
<td>Increased susceptibility to P. rapae</td>
<td></td>
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<td></td>
<td>CaMV 35S-AtMYB20</td>
<td>Hypersensitive to multiple abiotic and biotic stress</td>
<td></td>
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<tr>
<td></td>
<td>CaMV 35S-AtMYB96</td>
<td>Enhanced resistance to salinity</td>
<td></td>
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<tr>
<td></td>
<td>CaMV 35S-TaMYB2A</td>
<td>Enhanced resistance to drought; promoted accumulation of epicuticular wax</td>
<td></td>
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<td></td>
<td>CaMV 35S-DsMYB2</td>
<td>Hypersensitive to iron limitation.</td>
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<td></td>
<td>CaMV 35S-AtMYB4 mutant</td>
<td>Accumulated more dark-colored phenolics, when stressed</td>
<td></td>
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<td></td>
<td>N.tabacum</td>
<td>Enhanced resistance to salinity</td>
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<tr>
<td></td>
<td>CaMV 35s-NtMYBJS1</td>
<td>Tolerance to drought, salt, freezing tresses</td>
<td></td>
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<tr>
<td></td>
<td>CaMV 35s-TaPIMP1</td>
<td>Reduced in tolerance to drought</td>
<td></td>
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<td></td>
<td>CaMV 35s-TaPIMP1 silencing</td>
<td>Decreased in tolerance to drought</td>
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<tr>
<td></td>
<td>AtMYB73 mutant</td>
<td>Enhanced adaptation to salt stress</td>
<td></td>
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<tr>
<td></td>
<td>CaMV 35S-OsMYB2</td>
<td>Cold, salt and dehydration tolerance</td>
<td></td>
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<td>Ubiquitin-OsMYB2P-1</td>
<td>Enhanced adaptation to low the low-Pi environment</td>
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<td></td>
<td>T. aestivum</td>
<td>Enhanced resistance to Bipolaris sorokiniana and drought stresses,</td>
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<td></td>
<td>Ubiquitin-TiMYB2R-1</td>
<td>Enhanced resistance take-all disease</td>
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<tr>
<td></td>
<td>G. max</td>
<td>Tolerance to drought, salt stress</td>
<td></td>
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<td></td>
<td>M. pamila</td>
<td>Enhanced resistance to drought and cold stress</td>
<td></td>
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<td></td>
<td>H. vulgare</td>
<td>Enhanced resistance to frost and hypoxic stress</td>
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</tbody>
</table>

DHS, DHQase-SDH, EPSPS ect.  
AtCPS, AtKO, AtGA3ox1, AtKS.  
Vos et al., 2006  
Mengiste et al., 2003  
Yokotani et al., 2013  
Cui et al., 2013  
Chen et al., 2006b  
Seo et al., 2009, 2011  
Mao et al., 2011  
Cai et al., 2006a  
Gális et al., 2006  
Liu et al., 2011  
Huang et al., 2013  
Kim et al., 2013  
Yang et al., 2012  
Dai et al., 2012  
Zhang et al., 2012d  
Liu et al., 2013  
Seo et al., 2012  
Pasquali et al., 2008  
Soltész et al., 2012
Summary: Plants are sessile organisms and are frequently exposed to fluctuating environmental stress, such as drought, low temperature and pathogen that adversely affect plant growth and agricultural production. R2R3MYBs play an important role in plant adaptation to numerous stresses, recently receiving considerable attention. Overexpressing stress-responsive R2R3-MYB genes can modify the expression of stress-related genes, leading to an increase or a decrease in plant resistance. Some of them show the great potential for crop improvement. A growing body of R2R3MYBs has been identified from different plant species and their partial roles in stress tolerance also have been elucidated. However, there still have numerous questions remaining to be answered. Concerning these questions, further researches are needed to detailedly explore the R2R3-MYB structure sequence how to decide its biologic functions, the specificity to activate target gene, and the stress-responsive network waved by R2R3-MYBs. Unraveling the role of R2R3-MYBs in plant adaptation will ultimately contribute to the development and/or selection of crops with high productivity and quality under adverse environment conditions.

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functional characterization of the members of the R2R3-MYB gene family from *Arabidopsis thaliana.* *Plant J.* 16: 263-276.


