Appendix A. The Ct-value and standard deviation of *R2R3-MYB* genes

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Appendix B. The primer sequences used for qPCR of stress-responsive *R2R3-MYB* genes

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# Appendix C. Characteristics of R2R3-MYB gene family members in *Medicago truncatula*

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<td>MtMYB3R-4</td>
<td>C46</td>
<td>chr7:22157813 - 22161933</td>
<td>7</td>
<td>530</td>
<td>59080.52</td>
<td>9.39</td>
<td>25-71, 77-123, 129-172</td>
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<td>MtMYB3R-5</td>
<td>C46</td>
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<td>MtMYB4R-1</td>
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<td>chr6:30262070 - 30267432</td>
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<td>94205.47</td>
<td>9.78</td>
<td>205-255, 258-307, 309-357, 360-408</td>
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</tbody>
</table>
Appendix D. Sequence alignment of 155 typical R2R3-MYB domains in *M. truncatula* using MUSCLE. If the similarity score assigned to a column is greater than 70%, residues are considered highly similar and are colored in red and framed in blue. The strictly identical residues are shown in white on a red background. At the bottom of the alignment, the regions corresponding to the three α-helices that form each MYB repeat are annotated as Helix 1 to Helix 3.

Appendix E. The function-known R2R3-MYB genes in other plant species

<table>
<thead>
<tr>
<th>Gene</th>
<th>Species name</th>
<th>Accession ID</th>
<th>Function reported</th>
</tr>
</thead>
<tbody>
<tr>
<td>MYB2</td>
<td><em>Oryza sativa</em></td>
<td>BAA23338.1</td>
<td>Salt, cold, and dehydration stress</td>
</tr>
<tr>
<td>MYB4</td>
<td><em>Oryza sativa</em></td>
<td>BAA23340.1</td>
<td>Confers tolerance to chilling and freezing stress</td>
</tr>
<tr>
<td>MYB3R-2</td>
<td><em>Oryza sativa</em></td>
<td>BAD81765.1</td>
<td>Participates in the cold signalling pathway by targeting the cell cycle and a putative DREB/CFB; Increases Tolerance to Freezing, Drought, and Salt Stress</td>
</tr>
<tr>
<td>MYB2P-1</td>
<td><em>Oryza sativa</em></td>
<td>BAS92185.1</td>
<td>In response to deficiencies in Pi and Fe as well as cold stress, regulate Root Development</td>
</tr>
<tr>
<td>MYB5</td>
<td><em>Oryza sativa</em></td>
<td>BAS95240.1</td>
<td>High temperature</td>
</tr>
<tr>
<td>AMYB</td>
<td><em>Oryza sativa</em></td>
<td>CAA67000.1</td>
<td>Flower development</td>
</tr>
<tr>
<td>A</td>
<td><em>Oryza sativa</em></td>
<td>BAS71534.1</td>
<td>Anther development and pollen maturation</td>
</tr>
<tr>
<td>MYBAS1</td>
<td><em>Oryza sativa</em></td>
<td>XP_015617959.1</td>
<td></td>
</tr>
<tr>
<td>MYBAS2</td>
<td><em>Oryza sativa</em></td>
<td>XP_015620227.1</td>
<td></td>
</tr>
<tr>
<td>MYBF1</td>
<td><em>Vitis vinifera</em></td>
<td>ACV81697.1</td>
<td>Controlling flavonol synthesis in fruit</td>
</tr>
<tr>
<td>MYB5a</td>
<td><em>Vitis vinifera</em></td>
<td>AAS68190</td>
<td>Regulates the Phenylpropanoid pathway</td>
</tr>
<tr>
<td>MYB5b</td>
<td><em>Vitis vinifera</em></td>
<td>NP_001267854.1</td>
<td>Anthocyanin and Proanthocyanidin biosynthesis</td>
</tr>
<tr>
<td>MYBA1</td>
<td><em>Vitis vinifera</em></td>
<td>BAD18977.1</td>
<td>Control the last biosynthetic step of anthocyanin synthesis</td>
</tr>
<tr>
<td>MYBA2</td>
<td><em>Vitis vinifera</em></td>
<td>BAD18978.1</td>
<td>Control the last biosynthetic step of anthocyanin synthesis</td>
</tr>
<tr>
<td>MYBPA2</td>
<td><em>Vitis vinifera</em></td>
<td>ACK56131.1</td>
<td>Promotes proanthocyanidin biosynthesis</td>
</tr>
<tr>
<td>MYB14</td>
<td><em>Vitis vinifera</em></td>
<td>ABW34392.1</td>
<td>Regulation of stilbene biosynthesis in response to biotic and abiotic stresses</td>
</tr>
<tr>
<td>MYB15</td>
<td><em>Vitis vinifera</em></td>
<td>AHA83524.1</td>
<td>Regulation of stilbene biosynthesis in response to biotic and abiotic stresses</td>
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<tr>
<td>MYB4-like</td>
<td><em>Vitis vinifera</em></td>
<td>XP_002273328.1</td>
<td>Suppresses the anthocyanin accumulation</td>
</tr>
<tr>
<td>MYBPA1</td>
<td><em>Vitis vinifera</em></td>
<td>CAJ90831.1</td>
<td>Regulates proanthocyanidin synthesis during fruit development; regulates the early flavonoid biosynthetic genes</td>
</tr>
<tr>
<td>PL</td>
<td><em>Zea Mays</em></td>
<td>AAN12277.1</td>
<td>Anthocyanins synthesis</td>
</tr>
<tr>
<td>MYB111</td>
<td><em>Zea Mays</em></td>
<td>DAA62458.1</td>
<td>Regulate the PAL gene in phenylpropanoid metabolism</td>
</tr>
</tbody>
</table>
**Zea Mays**

- AAK09327.1 Regulate anthocyanin synthesis
- AAL84612.1 Regulate phenylpropanoid biosynthetic genes in flowers

- Flavonoid synthesis
- Inhibits sinapoylmalate and phenylpropanoid biosynthesis
- Regulate phenylpropanoid biosynthetic genes in flowers
- Enhanced resistance to the fungal pathogen Bipolaris sorokiniana and drought stresses
- Positively regulate anthocyanin biosynthesis in wheat coleoptiles
- Regulates lignin biosynthesis
- Abiotic stresses tolerance
- Lateral organ primordia; to maintain knox gene silencing and determinacy during organogenesis
- Regulation of secondary wall biosynthesis
- Control PA synthesis in response to stress
- Participates in the transcriptional control of epidermal cell shape (cell fate specification and differentiation)
- Regulate phenylpropanoid biosynthetic genes in flowers
- Positively regulate anthocyanin biosynthesis in wheat coleoptiles
- Activates Proanthocyanidin biosynthesis
- Enhances resistance to the fungal pathogen Bipolaris sorokiniana and drought stresses
- In response to the oxygen concentration in root environment and abiotic stresses; mediate plant tolerance to the Pi-starvation stress

**Triticum aestivum**

- ABC86569.1 In response to the oxygen concentration in root environment and abiotic stresses; mediate plant tolerance to the Pi-starvation stress
- Enhanced resistance to the fungal pathogen Bipolaris sorokiniana and drought stresses
- Positively regulate anthocyanin biosynthesis in wheat coleoptiles
- Regulates lignin biosynthesis

**Petunia hybrida**

- CAA78386.1 Cell shapes and petal development
- Enhanced resistance to the fungal pathogen Bipolaris sorokiniana and drought stresses
- Positively regulate anthocyanin biosynthesis in wheat coleoptiles
- Activates Proanthocyanidin biosynthesis

**Populus trichocarpa**

- AGT02395.1 Regulation of secondary wall biosynthesis
- Control PA synthesis in response to stress
- Regulates secondary cell wall formation
- Repress the lignin biosynthetic

**Antirrhinum majus**

- CAA06612.1 Growth and dorsoventrality of lateral organs
- CAA55725.1 Participates in the transcriptional control of epidermal cell shape (cell fate specification and differentiation)
- Activates transcription of phenylpropanoid biosynthetic genes in flowers

**Lotus japonicus**

- BAG12893.1
- BAG12894.2
- BAG12895.1

**Nicotiana tabacum**

- AAB41101.1 Disease resistance
- Wounding and elicitors

**Saccharum officinarum**

- ADZ73058.1 Water deficit and salt stress

**Citrus sinensis**

- NP_001275818.1 Activate anthocyanin biosynthesis

**Boea crassifolia**

- AAP32921.1 Drought tolerance (ABA-independent)
<table>
<thead>
<tr>
<th>GenBank Acc.</th>
<th>Species Name</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AHN50422.1</td>
<td>Salicornia brachiata</td>
<td>Response to salinity, desiccation, high temperature, and abscisic acid and salicylic acid treatments</td>
</tr>
<tr>
<td>ALE33742.1</td>
<td>Erythranthe lewisii</td>
<td>Positively regulates carotenoid biosynthesis during flower development</td>
</tr>
<tr>
<td>ACZ48688.2</td>
<td>Salvia miltiorrhiza</td>
<td>Regulate the phenolic acid biosynthetic pathway</td>
</tr>
<tr>
<td>CAD71140.1</td>
<td>Gossypium hirsutum</td>
<td>Plays a direct role in the initiation and elongation of cotton fiber cells</td>
</tr>
<tr>
<td>AAS92346.1</td>
<td>Gossypium hirsutum</td>
<td>Regulates secondary cell wall biosynthesis</td>
</tr>
<tr>
<td>AFX97765.1</td>
<td>Dendrobium hybrida</td>
<td>Involved in the development of conical cell shape of the epidermal cells of flower labellum</td>
</tr>
<tr>
<td>ABP57085.1</td>
<td>Solenostemon scutellarioides</td>
<td>Prolanthocyanidin biosynthesis</td>
</tr>
<tr>
<td>AGT39060.1</td>
<td>Epimedium agitatum</td>
<td>Regulates the Flavonoid biosynthetic pathway</td>
</tr>
</tbody>
</table>
Appendix F. Phylogenetic analysis of R2R3-MYB proteins from *M. truncutula, A. thaliana* and other plant species. The NJ tree was constructed based on the full-length amino acid sequences of 380 R2R3-MYBs, including 162 from *M. truncatula* (R3- and R4-MYBs included), 132 from *A. thaliana* and 86 well-characterized landmark MYB proteins from 23 additional plant species, using MEGA6.06 with 1000 bootstraps. Bootstrap support less than 50% were collapsed in the phylogenetic tree.

Appendix G. Relative quantification of *R2R3-MYBs* gene expression in response to abiotic stresses. Three-week-old seedlings were treated with 200 mM NaCl (A), 300 mM mannitol (B), cold (4°C) (C) and 100 μM ABA (D). The expression level at 0 h was normalized to “1”. The relative expression level of each gene was calculated using the ΔΔCt method and was normalized to the *MtActin* gene (GenBank ID: JQ028731.1). Error bars indicate SE based on three replicates.