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

| Gene | treatment | 0h | 2h | 5h | 10h | 24h |
|---|---|---|---|---|---|---|
| *Actin* | salt | 17.81±0.21 | 16.45±0.45 | 17.84±0.49 | 17.38±0.12 | 17.67±0.24 |
| MtMYB14 | salt | 24.43±0.46 | 24.76±0.16 | 24.28±0.06 | 23.71±0.18 | 23.17±0.15 |
| MtMYB22 | salt | 28.14±0.71 | 25.43±0.23 | 26.64±0.12 | 25.55±0.37 | 24.83±0.04 |
| MtMYB23 | salt | 29.51±0.29 | 27.82±0.20 | 26.04±0.19 | 26.81±0.28 | 29.26±0.27 |
| MtMYB29 | salt | 29.21±0.15 | 25.58±0.12 | 25.83±0.26 | 25.95±0.06 | 29.11±0.11 |
| MtMYB48 | salt | 23.24±0.34 | 20.01±0.10 | 20.75±0.10 | 23.51±0.72 | 24.45±0.04 |
| *Actin* | drought | 17.55±0.42 | 16.72±0.17 | 17.91±0.17 | 17.01±0.33 | 16.86±0.30 |
| MtMYB14 | drought | 24.19±0.31 | 24.05±0.20 | 26.26±0.13 | 23.23±0.56 | 22.17±0.16 |
| MtMYB22 | drought | 29.02±0.64 | 29.97±0.38 | 31.54±0.34 | 29.34±0.32 | 26.59±0.17 |
| MtMYB23 | drought | 29.98±0.23 | 30.01±0.36 | 29.62±0.34 | 29.08±0.18 | 29.54±0.12 |
| MtMYB29 | drought | 25.95±0.17 | 23.92±0.06 | 25.05±0.15 | 22.57±0.08 | 21.79±0.07 |
| MtMYB48 | drought | 23.20±0.26 | 21.68±0.08 | 23.81±0.09 | 23.56±0.12 | 24.41±0.05 |
| Gene | treatment | 0h | 2h | 6h | 12h | 24h |
| *Actin* | cold | 17.81±0.18 | 17.98±0.12 | 18.08±0.09 | 17.32±0.35 | 18.28±0.11 |
| MtMYB14 | cold | 24.03±0.40 | 25.45±0.34 | 25.91±0.09 | 27.45±0.13 | 27.46±0.31 |
| MtMYB22 | cold | 27.95±0.13 | 28.45±0.78 | 31.13±0.44 | 28.83±0.15 | 28.59±0.31 |
| MtMYB23 | cold | 29.51±0.29 | 27.82±0.20 | 28.04±0.19 | 26.81±0.28 | 29.26±0.27 |
| MtMYB29 | cold | 27.87±0.01 | 26.97±0.12 | 26.86±0.22 | 26.26±0.13 | 28.69±0.49 |
| MtMYB48 | cold | 23.21±0.27 | 21.55±0.54 | 21.59±0.09 | 21.67±0.25 | 26.30±0.40 |
| *Actin* | ABA | 16.82±0.13 | 17.05±0.34 | 17.84±0.29 | 16.93±0.06 | 17.27±0.04 |
| MtMYB14 | ABA | 26.56±0.25 | 24.98±0.21 | 23.89±0.04 | 23.38±0.49 | 22.01±0.37 |
| MtMYB22 | ABA | 29.96±0.48 | 29.34±0.55 | 30.31±0.41 | 27.93±0.02 | 26.42±0.12 |
| MtMYB23 | ABA | 28.94±0.42 | 25.53±0.38 | 27.44±0.23 | 25.55±0.37 | 27.67±0.69 |
| MtMYB29 | ABA | 23.63±0.33 | 23.56±0.22 | 22.51±0.24 | 23.85±0.38 | 22.68±0.36 |
| MtMYB48 | ABA | 24.19±0.12 | 23.69±0.57 | 22.99±0.95 | 22.91±0.59 | 25.25±0.24 |

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

| Gene | Forward primer | Reverse primer |
|---|---|---|
| *MtMYB14* | GTTAGGGCTCCTTGTTGTGAAA | CTATGGCTGACCACCTGTTC |
| *MtMYB22* | GCAAAGGAAAATGAGACAGGG | CGTAAATCGGGACGCAAGTA |
| *MtMYB23* | GGGAAGAACCGACAATGAAATAA | AACCCCTGACGAGTACGATGA |
| *MtMYB29* | GGGAAACCGATGGTCTAAAAT | CAAATGAATCCGATGATGCTG |
| *MtMYB48* | AGCAGGGTTGTTAAGGTGTGG | TTCGTCCAGGTAGATGAGATGC |
| *MtActin* | CCACATGCCATCCTTCGTTT | TGTCACGGACAATTTCCCG |

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

| Name | Clade No. | Gene locus | Chromosome location | Intron no. | Protein length (aa) | Molecular weight (D) | Theoretical *Pi* | Domain position | Probeset |
|---|---|---|---|---|---|---|---|---|---|
| *MtMYB0* | C1 | Medtr0001s0360.1 | scaffold0001:148343 - 149371 | 2 | 253 | 29218.22 | 8.08 | 9-56, 62-107 | |
| *MtMYB1* | C25 | Medtr0008s0280.1 | scaffold0008:117749 - 119885 | 2 | 231 | 27173.29 | 6.68 | 14-61, 67-112 | |
| *MtMYB2* | C25 | Medtr0008s0390.1 | scaffold0008:236494 - 237588 | 1 | 272 | 31187.69 | 5.64 | 15-62, 68-111 | |
| *MtMYB3* | C25 | Medtr0008s0470.1 | scaffold0008:261364 - 262450 | 1 | 291 | 33299.87 | 5.21 | 15-62, 68-111 | |
| *MtMYB4* | C25 | Medtr0063s0090.1 | scaffold0063:54059 - 56064 | 2 | 224 | 25835.84 | 6.1 | 14-61, 68-112 | |
| *MtMYB5* | C16 | Medtr0140s0030.1 | scaffold0140:9682 - 11671 | 2 | 334 | 37966.26 | 7.1 | 15-62, 68-113 | |
| *MtMYB6* | C1 | Medtr0193s0090.1 | scaffold0193:23279 - 25596 | 2 | 207 | 23782.04 | 9.59 | 9-56, 62-107 | |
| *MtMYB7* | C1 | Medtr0197s0010.1 | scaffold0197:3158 - 6293 | 2 | 229 | 26232.60 | 6.04 | 5-50, 56-98 | |
| *MtMYB8* | C25 | Medtr0251s0050.1 | scaffold0251:9249 - 10577 | 1 | 272 | 31594.95 | 4.87 | 15-62, 68-111 | |
| *MtMYB9* | C16 | Medtr0489s0020.1 | scaffold0489:9594 - 10964 | 2 | 334 | 37966.26 | 7.1 | 15-62, 68-113 | |
| *MtMYB10* | C18 | Medtr1g017000.1 | chr1:4609270 - 4612231 | 2 | 252 | 28048.60 | 6.67 | 14-62, 69-111 | |
| *MtMYB11* | C19 | Medtr1g017140.1 | chr1:4685126 - 4687287 | 2 | 341 | 38782.00 | 7.64 | 14-62, 69-111 | Mtr.5541.1.s1_at |
| *MtMYB12* | C31 | Medtr1g021230.1 | chr1:6319810 - 6322818 | 2 | 444 | 50317.66 | 5.13 | 59-106, 112-156 | Mtr.20290.1.s1_at |
| *MtMYB13* | C24 | Medtr1g043050.1 | chr1:16126632 - 16128228 | 2 | 254 | 29323.76 | 5.66 | 14-61, 67-112 | Mtr.15339.1.s1_at |
| *MtMYB14* | C24 | Medtr1g043080.1 | chr1:16137369 - 16139502 | 2 | 255 | 29233.59 | 5.68 | 14-61, 67-112 | Mtr.15332.1.s1_at |
| *MtMYB15* | C32 | Medtr1g045610.1 | chr1:17117606 - 17119422 | 2 | 258 | 30264.09 | 9.64 | 23-70, 76-120 | Mtr.33162.1.s1_at |
| *MtMYB16* | C32 | Medtr1g062940.1 | chr1:27632125 - 27633748 | 3 | 218 | 25855.74 | 5.76 | 8-55, 62-106 | |
| *MtMYB17* | C32 | Medtr1g073170.1 | chr1:32447073 - 32448262 | 2 | 257 | 30223.95 | 7.94 | 22-69, 75-119 | Mtr.50305.1.s1_at |
| *MtMYB18* | C24 | Medtr1g076150.1 | chr1:33730166 - 33733155 | 2 | 258 | 29415.05 | 5.12 | 14-61, 67-112 | Mtr.10636.1.s1_at |
| *MtMYB19* | C35 | Medtr1g083630.1 | chr1:37214098 - 37220601 | 10 | 369 | 41435.17 | 7.5 | 14-61, 67-109 | Mtr.49995.1.s1_at |
| *MtMYB20* | C28 | Medtr1g085040.1 | chr1:37962367 - 37964302 | 2 | 369 | 41435.17 | 7.5 | 14-61, 67-111 | Mtr.20337.1.s1_at |
| *MtMYB21* | C28 | Medtr1g085640.1 | chr1:38268101 - 38269978 | 2 | 388 | 44196.28 | 6.51 | 14-61, 67-111 | Mtr.13636.1.s1_at |
| *MtMYB22* | C34 | Medtr1g086510.1 | chr1:38706430 - 38707982 | 2 | 265 | 30327.89 | 7.25 | 23-70, 76-119 | Mtr.15010.1.s1_s_at |
| *MtMYB23* | C34 | Medtr1g086530.1 | chr1:38719605 - 38720569 | 1 | 278 | 31891.50 | 5.66 | 35-82, 88-131 | Mtr.15018.1.s1_at |
| *MtMYB24* | C20 | Medtr1g100653.1 | chr1:45593395 - 45595446 | 2 | 295 | 33352.56 | 7.55 | 14-61, 67-112 | |
| *MtMYB25* | C26 | Medtr1g100667.1 | chr1:45604161 - 45606367 | 2 | 272 | 31237.22 | 9.9 | 15-62, 68-113 | |

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|---|---|---|---|---|---|---|---|---|---|
| *MtMYB26* | C34 | Medtr1g110460.1 | chr1:49847994 - 49849320 | 2 | 311 | 35803.00 | 7.55 | 28-75, 81-124 | Mtr.34421.1.s1_at |
| *MtMYB27* | C28 | Medtr1g112760.1 | chr1:51044088 - 51045093 | 2 | 230 | 26476.21 | 8.62 | 15-62, 68-111 | |
| *MtMYB28* | C12 | Medtr2g011660.1 | chr2:2891380 - 2892991 | 2 | 345 | 39590.84 | 6.23 | 15-61, 68-112 | |
| *MtMYB29* | C34 | Medtr2g033170.1 | chr2:12558787 - 12561118 | 2 | 329 | 37139.06 | 5.64 | 26-73, 79-122 | Mtr.38757.1.s1_at |
| *MtMYB30* | C8 | Medtr2g034790.1 | chr2:13342703 - 13345610 | 2 | 332 | 37985.75 | 5.51 | 14-61, 67-112 | |
| *MtMYB31* | | Medtr2g064160.1 | chr2:27171680 - 27173646 | 2 | 313 | 35920.33 | 6.58 | 13-60, 66-109 | |
| *MtMYB32* | C23 | Medtr2g067420.1 | chr2:28203900 - 28207822 | 2 | 329 | 37350.33 | 4.47 | 13-63, 69-114 | |
| *MtMYB33* | C31 | Medtr2g088170.1 | chr2:37135155 - 37138193 | 2 | 459 | 51733.05 | 4.81 | 31-78, 84-127 | |
| *MtMYB34* | C25 | Medtr2g089420.1 | chr2:37788683 - 37793627 | 2 | 451 | 52176.74 | 4.56 | 13-60, 66-110 | |
| *MtMYB35* | C25 | Medtr2g089450.1 | chr2:37807548 - 37809745 | 2 | 250 | 29000.68 | 7.89 | 14-61, 67-110 | Mtr.42274.1.s1_at |
| *MtMYB36* | C12 | Medtr2g089620.1 | chr2:37880385 - 37882262 | 2 | 326 | 36661.06 | 6.92 | 14-61, 67-111 | |
| *MtMYB37* | C19 | Medtr2g095520.1 | chr2:40818577 - 40820355 | 2 | 320 | 36454.86 | 7.39 | 14-62, 69-111 | Mtr.10159.1.s1_at |
| *MtMYB38* | C32 | Medtr2g096380.1 | chr2:41173925 - 41175393 | 2 | 236 | 27425.67 | 6.63 | 8-55, 61-105 | Mtr.18579.1.s1_at |
| *MtMYB39* | C27 | Medtr2g097910.1 | chr2:41830130 - 41832026 | 1 | 319 | 36585.90 | 5.7 | 22-69, 75-118 | Mtr.23404.1.s1_at |
| *MtMYB40* | C30 | Medtr2g099740.1 | chr2:42787607 - 42789410 | 1 | 401 | 45878.16 | 6.77 | 14-61, 67-110 | Mtr.17139.1.s1_at |
| *MtMYB41* | C31 | Medtr3g011610.1 | chr3:2911993 - 2917319 | 2 | 520 | 57543.48 | 6.74 | 39-86, 92-135 | |
| *MtMYB42* | C27 | Medtr3g028740.1 | chr3:9231780 - 9234575 | 1 | 343 | 39282.00 | 5.94 | 24-71, 77-121 | |
| *MtMYB43* | C16 | Medtr3g039990.1 | chr3:13992780 - 13994587 | 2 | 311 | 35476.99 | 5.28 | 15-62, 68-113 | Mtr.11711.1.s1_at |
| *MtMYB44* | C31 | Medtr3g052430.1 | chr3:20730678 - 20733390 | 2 | 538 | 60381.33 | 6.39 | 43-90, 96-139 | Mtr.19398.1.s1_s_at |
| *MtMYB45* | C31 | Medtr3g052450.1 | chr3:20738432 - 20741241 | 2 | 530 | 59724.63 | 6.38 | 42-89, 95-138 | Mtr.19398.1.s1_s_at |
| *MtMYB46* | C17 | Medtr3g461490.1 | chr3:24436965 - 24438138 | 2 | 325 | 36584.16 | 6.78 | 14-61, 68-110 | |
| *MtMYB47* | C45 | Medtr3g463400.1 | chr3:25378599 - 25381933 | 2 | 451 | 50929.99 | 9.15 | 132-181, 184-232 | |
| *MtMYB48* | C11 | Medtr3g065440.1 | chr3:29570157 - 29572286 | 2 | 311 | 35663.78 | 4.56 | 14-61, 71-111 | Mtr.38413.1.s1_at |
| *MtMYB49* | C14 | Medtr3g074520.1 | chr3:33695099 - 33696602 | 2 | 346 | 38466.95 | 6.18 | 14-61, 67-112 | |
| *MtMYB50* | C28 | Medtr3g077110.1 | chr3:34625727 - 34626994 | 1 | 349 | 39939.94 | 6 | 14-61, 67-110 | Mtr.25748.1.s1_at |
| *MtMYB51* | C45 | Medtr3g077650.1 | chr3:34915543 - 34917386 | 1 | 447 | 51906.12 | 7.13 | 181-227, 236-275 | Mtr.48147.1.s1_at |
| *MtMYB52* | C5 | Medtr3g083540.1 | chr3:37705148 - 37706195 | 2 | 303 | 33756.22 | 7.34 | 43-90, 101-141 | Mtr.20924.1.s1_at |
| *MtMYB53* | C41 | Medtr3g089045.1 | chr3:40751456 - 40752741 | 0 | 292 | 33310.20 | 9.2 | 15-64, 67-115 | Mtr.11386.1.s1_at |
| *MtMYB54* | C10 | Medtr3g097450.1 | chr3:44673757 - 44675359 | 2 | 297 | 33365.60 | 8.48 | 14-61, 67-111 | Mtr.38547.1.s1_at |
| *MtMYB55* | C39 | Medtr3g101290.1 | chr3:46598864 - 46599544 | 2 | 226 | 25223.84 | 6.88 | 37-82, 91-133 | Mtr.38547.1.s1_at |

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|---|---|---|---|---|---|---|---|---|---|
| *MtMYB56* | C19 | Medtr3g103570.1 | chr3:47845866 - 47847116 | 2 | 347 | 39315.49 | 6.7 | 14-62, 69-111 | |
| *MtMYB57* | C40 | Medtr4g019370.1 | chr4:6040214 - 6045853 | 1 | 350 | 37642.01 | 6.51 | 37-86, 89-137 | Mtr.42655.1.s1_at |
| *MtMYB58* | C36 | Medtr4g029540.1 | chr4:10211682 - 10213682 | 2 | 296 | 34320.72 | 7.76 | 15-65, 68-108 | |
| *MtMYB59* | C19 | Medtr4g057635.1 | chr4:21173502 - 21175255 | 2 | 324 | 36839.08 | 7.17 | 14-62, 69-111 | |
| *MtMYB60* | C32 | Medtr4g063100.1 | chr4:23344337 - 23345778 | 2 | 253 | 29491.60 | 5.41 | 9-56, 62-106 | Mtr.12401.1.s1_s_at |
| *MtMYB61* | C27 | Medtr4g065017.1 | chr4:24357763 - 24360022 | 1 | 332 | 37524.41 | 5.22 | 19-66, 72-115 | |
| *MtMYB62* | C10 | Medtr4g073420.1 | chr4:27808040 - 27809137 | 1 | 285 | 32250.40 | 8.42 | 14-61, 67-111 | Mtr.15080.1.s1_at |
| *MtMYB63* | C15 | Medtr4g478180.1 | chr4:30007102 - 30008882 | 2 | 353 | 39560.45 | 6.84 | 15-62, 68-113 | Mtr.5071.1.s1_at |
| *MtMYB64* | C41 | Medtr4g082040.1 | chr4:31789875 - 31791702 | 2 | 427 | 47975.94 | 5.47 | 105-150, 157-199 | Mtr.18999.1.s1_at |
| *MtMYB65* | C22 | Medtr4g082230.1 | chr4:31939369 - 31942857 | 2 | 407 | 46280.83 | 6.45 | 14-61, 67-112 | |
| *MtMYB66* | C22 | Medtr4g082290.1 | chr4:31999665 - 32001816 | 2 | 407 | 46075.53 | 6.1 | 14-61, 67-112 | |
| *MtMYB67* | C9 | Medtr4g485530.1 | chr4:33337309 - 33339901 | 2 | 193 | 22063.31 | 10.32 | 14-61, 67-111 | |
| *MtMYB68* | C42 | Medtr4g088015.1 | chr4:34653348 - 34657396 | 0 | 442 | 50039.92 | 9.51 | 221-267, 273-315 | |
| *MtMYB69* | C12 | Medtr4g091490.1 | chr4:36245993 - 36247326 | 2 | 358 | 41018.50 | 6.3 | 15-62, 68-112 | Mtr.21051.1.s1_at |
| *MtMYB70* | C39 | Medtr4g094982.1 | chr4:39427139 - 39427954 | 2 | 271 | 29507.28 | 5.82 | 5-50, 59-101 | Mtr.43277.1.s1_at |
| *MtMYB71* | C19 | Medtr4g097570.1 | chr4:40253459 - 40254681 | 3 | 294 | 33680.66 | 7.98 | 14-63, 70-112 | Mtr.11740.1.s1_at |
| *MtMYB72* | C19 | Medtr4g097570.2 | chr4:40253459 - 40254681 | 1 | 293 | 33581.52 | 7.98 | 14-62, 69-111 | Mtr.11740.1.s1_at |
| *MtMYB73* | C3 | Medtr4g100720.1 | chr4:41544004 - 41544940 | 2 | **175** | 20435.03 | 8.22 | 15-62, 68-113 | Mtr.48481.1.s1_at |
| *MtMYB74* | C11 | Medtr4g102380.1 | chr4:42422167 - 42423418 | 2 | 272 | 30751.50 | 5.09 | 14-61, 69-111 | |
| *MtMYB75* | C28 | Medtr4g105130.1 | chr4:43559456 - 43560815 | 2 | 361 | 41023.65 | 5.59 | 14-61, 67-111 | Mtr.6076.1.s1_at |
| *MtMYB76* | C45 | Medtr4g105660.1 | chr4:43865250 - 43867027 | 2 | 430 | 49843.57 | 6.87 | 180-226, 232-274 | |
| *MtMYB77* | C8 | Medtr4g121460.1 | chr4:50199327 - 50201231 | 2 | 339 | 38531.49 | 5.02 | 14-61, 67-112 | |
| *MtMYB78* | C34 | Medtr4g123040.1 | chr4:50776917 - 50778655 | 2 | 315 | 35996.10 | 7.44 | 26-73, 79-122 | Mtr.42808.1.s1_at |
| *MtMYB79* | C4 | Medtr4g125520.1 | chr4:52070924 - 52072039 | 2 | 307 | 34938.29 | 8.21 | 14-61, 67-112 | |
| *MtMYB80* | C19 | Medtr4g128670.1 | chr4:53557002 - 53558487 | 2 | 350 | 39502.90 | 5.26 | 14-62, 69-111 | Mtr.41980.1.s1_at |
| *MtMYB81* | C15 | Medtr5g007370.1 | chr5:1317895 - 1319526 | 1 | 299 | 33780.02 | 6.71 | 14-61, 67-112 | Mtr.16038.1.s1_at |
| *MtMYB82* | C29 | Medtr5g010020.1 | chr5:2590584 - 2592324 | 2 | 339 | 38788.19 | 6.22 | 14-61, 67-111 | Mtr.40201.1.s1_at |
| *MtMYB83* | C19 | Medtr5g014990.1 | chr5:5113168 - 5114523 | 2 | 309 | 34817.24 | 7.63 | 14-62, 69-111 | Mtr.13258.1.s1_at |
| *MtMYB84* | C39 | Medtr5g016510.1 | chr5:5923364 - 5924314 | 0 | 316 | 34214.85 | 8.34 | 5-50, 59-101 | Mtr.40383.1.s1_at |
| *MtMYB85* | C13 | Medtr5g029840.1 | chr5:12551834 - 12553088 | 1 | 334 | 37824.44 | 6.72 | 14-61, 67-110 | Mtr.49230.1.s1_at |

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|---|---|---|---|---|---|---|---|---|---|
| *MtMYB86* | C42 | Medtr5g038910.1 | chr5:17124080 - 17128774 | 11 | 466 | 53059.51 | 6.51 | 25-74, 77-125 | Mtr.41037.1.s1_at |
| *MtMYB87* | C42 | Medtr5g038910.2 | chr5:17125457 - 17128774 | 9 | 390 | 44854.18 | 6.63 | 25-74, 77-125 | Mtr.41037.1.s1_at |
| *MtMYB88* | C41 | Medtr5g041570.1 | chr5:18244022 - 18246575 | 2 | 438 | 50303.31 | 6.17 | 134-179, 186-228 | |
| *MtMYB89* | | Medtr5g042030.1 | chr5:18477810 - 18479574 | 2 | 289 | 32748.04 | 8.61 | 9-55, 62-104 | |
| *MtMYB90* | C45 | Medtr5g049190.1 | chr5:21549233 - 21551259 | 2 | 242 | 28245.96 | 6.77 | 18-67, 70-118 | |
| *MtMYB91* | C35 | Medtr5g062790.1 | chr5:26032596 - 26033729 | 2 | 304 | 35294.15 | 4.43 | 11-58, 64-107 | |
| *MtMYB92* | C45 | Medtr5g070020.1 | chr5:29670807 - 29672960 | 2 | 355 | 41325.38 | 8.87 | 121-170, 173-221 | |
| *MtMYB93* | C11 | Medtr5g078140.1 | chr5:33378245 - 33380528 | 2 | 320 | 36893.49 | 4.9 | 14-61, 71-111 | |
| *MtMYB94* | C1 | Medtr5g078800.1 | chr5:33679342 - 33680505 | 2 | 265 | 30724.70 | 8.67 | 9-56, 62-106 | |
| *MtMYB95* | C1 | Medtr5g078860.1 | chr5:33697936 - 33699039 | 2 | 306 | 35356.91 | 8.78 | 9-56, 62-106 | Mtr.32975.1.s1_at |
| *MtMYB96* | C1 | Medtr5g078910.1 | chr5:33733725 - 33736557 | 2 | 300 | 34340.90 | 9.23 | 9-56, 62-107 | |
| *MtMYB97* | C1 | Medtr5g078930.1 | chr5:33748446 - 33750303 | 2 | 219 | 25592.47 | 10.3 | 12-58, 67-109 | |
| *MtMYB98* | C1 | Medtr5g078950.1 | chr5:33762292 - 33763827 | 2 | 265 | 30460.67 | 9.37 | 9-56, 62-107 | |
| *MtMYB99* | C1 | Medtr5g079120.1 | chr5:33821199 - 33824299 | 3 | 306 | 35363.39 | 9.26 | 63-110, 118-161 | |
| *MtMYB100* | C1 | Medtr5g079220.1 | chr5:33865422 - 33867467 | 2 | 276 | 31744.12 | 8.05 | 23-70, 76-121 | |
| *MtMYB101* | C1 | Medtr5g079290.1 | chr5:33903955 - 33904999 | 2 | 264 | 30481.92 | 9.42 | 19-66, 73-117 | |
| *MtMYB102* | C9 | Medtr5g079670.1 | chr5:34066783 - 34068436 | 2 | 213 | 24065.64 | 9.18 | 13-60, 69-110 | Mtr.16432.1.s1_at |
| *MtMYB103* | C40 | Medtr5g082910.1 | chr5:35747836 - 35748702 | 0 | 288 | 32142.18 | 8.45 | 19-64, 73-115 | Mtr.1108.1.s1_at |
| *MtMYB104* | C35 | Medtr5g088080.1 | chr5:38180518 - 38181517 | 2 | 266 | 30550.20 | 4.83 | 14-61, 67-110 | Mtr.52032.1.s1_s_at |
| *MtMYB105* | C35 | Medtr5g088150.1 | chr5:38189614 - 38190613 | 2 | 266 | 30550.20 | 4.83 | 14-61, 67-110 | Mtr.52032.1.s1_s_at |
| *MtMYB106* | C35 | Medtr5g488170.1 | chr5:38207742 - 38208675 | 2 | 244 | 28147.96 | 8.44 | 14-61, 67-110 | Mtr.52032.1.s1_s_at |
| *MtMYB107* | C35 | Medtr5g488210.1 | chr5:38216839 - 38217838 | 2 | 266 | 30550.20 | 4.83 | 14-61, 67-110 | |
| *MtMYB108* | C35 | Medtr5g088610.1 | chr5:38446993-38448346 | 2 | 355 | 40752.76 | 4.82 | 10-60,63-112 | |
| *MtMYB109* | C35 | Medtr5g088640.1 | chr5:38455878 - 38456905 | 2 | 275 | 31627.28 | 4.84 | 14-61, 67-110 | Mtr.52032.1.s1_s_at |
| *MtMYB110* | C33 | Medtr6g006030.1 | chr6:968999 - 973160 | 2 | 209 | 24316.21 | 7.32 | 16-63, 69-112 | Mtr.44263.1.s1_at |
| *MtMYB111* | C17 | Medtr6g009430.1 | chr6:2790201 - 2792486 | 2 | 336 | 37673.70 | 5.79 | 14-61, 69-110 | Mtr.50091.1.s1_at |
| *MtMYB112* | C16 | Medtr6g012180.1 | chr6:3615813 - 3617651 | 2 | 318 | 35763.11 | 5.81 | 14-61, 67-112 | Mtr.29990.1.s1_at |
| *MtMYB113* | C22 | Medtr6g012690.1 | chr6:3910276 - 3912642 | 2 | 357 | 39753.70 | 5.71 | 14-61, 67-112 | Mtr.21878.1.s1_at |
| *MtMYB114* | | Medtr6g015455.1 | chr6:5126272 - 5138191 | 9 | **1513** | 171571.78 | 7.02 | 4-53, 56-104 | |
| *MtMYB115* | C26 | Medtr6g055910.1 | chr6:20067485 - 20070426 | 2 | 283 | 32750.77 | 7.06 | 16-63, 71-114 | |

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|---|---|---|---|---|---|---|---|---|---|
| *MtMYB116* | C41 | Medtr6g074860.1 | chr6:27792058 - 27795296 | 2 | 241 | 28725.99 | 8.98 | 4-49, 56-99 | |
| *MtMYB117* | C19 | Medtr6g090405.1 | chr6:34357171 - 34358762 | 2 | 255 | 28665.95 | 5.62 | 14-62, 70-111 | |
| *MtMYB118* | C16 | Medtr7g010210.1 | chr7:2460471 - 2462227 | 2 | 325 | 36399.76 | 7.36 | 14-61, 67-112 | Mtr.14368.1.s1_at |
| *MtMYB119* | C16 | Medtr7g010210.2 | chr7:2460471 - 2462227 | 3 | 271 | 30579.64 | 8.91 | 14-61, 67-112 | Mtr.14368.1.s1_at |
| *MtMYB120* | C22 | Medtr7g011170.1 | chr7:2948411 - 2950759 | 2 | 309 | 34859.76 | 7.05 | 14-61, 67-112 | Mtr.50403.1.s1_at |
| *MtMYB121* | C1 | Medtr7g017260.1 | chr7:5468456-5471716 | 2 | 275 | 31780.25 | 7.82 | 27-76,82-127 | |
| *MtMYB122* | C33 | Medtr7g035075.1 | chr7:13292900 - 13298140 | 2 | 208 | 23806.60 | 6.51 | 16-63, 69-112 | Mtr.11143.1.s1_at |
| *MtMYB123* | C42 | Medtr7g035350.1 | chr7:13467452 - 13470094 | 2 | 450 | 51394.95 | 5.83 | 224-273,276-324 | |
| *MtMYB124* | C42 | Medtr7g037130.1 | chr7:13641861 - 13644153 | 2 | 358 | 41004.49 | 8.67 | 145-191, 197-239 | |
| *MtMYB125* | C42 | Medtr7g037260.1 | chr7:13704241 - 13706602 | 2 | 358 | 41173.63 | 9.37 | 145-191, 197-241 | |
| *MtMYB126* | C42 | Medtr7g450950.1 | chr7:17067325 - 17069310 | 0 | 338 | 38857.42 | 5.62 | 157-203, 209-253 | |
| *MtMYB127* | C42 | Medtr7g451170.1 | chr7:17173579 - 17176966 | 2 | 412 | 47441.39 | 9.68 | 191-237, 243-285 | |
| *MtMYB128* | C38 | Medtr7g061550.1 | chr7:22184367 - 22187445 | 2 | 359 | 40855.49 | 9.92 | 6-58,61-109 | Mtr.40836.1.s1_at |
| *MtMYB129* | C20 | Medtr7g076740.1 | chr7:28944308 - 28947368 | 2 | 304 | 34084.15 | 8.14 | 14-62, 68-113 | Mtr.51999.1.s1_at |
| *MtMYB130* | C41 | Medtr7g086960.1 | chr7:33824855 - 33826525 | 2 | 356 | 39821.29 | 8.97 | 89-134, 141-183 | Mtr.5259.1.s1_at |
| *MtMYB131* | | Medtr7g087130.1 | chr7:33927808 - 33929283 | 2 | 338 | 38049.36 | 7.04 | 14-61, 67-112 | |
| *MtMYB132* | C24 | Medtr7g096930.1 | chr7:38915326 - 38917919 | 2 | 278 | 31792.90 | 5.36 | 14-61, 67-112 | |
| *MtMYB133* | C19 | Medtr7g102110.1 | chr7:41207378 - 41208914 | 2 | 272 | 30203.73 | 6.51 | 14-62, 69-111 | Mtr.9331.1.s1_at |
| *MtMYB134* | C8 | Medtr7g109320.1 | chr7:44676112 - 44678587 | 2 | 355 | 40428.25 | 4.65 | 14-61, 67-112 | Mtr.47437.1.s1_at |
| *MtMYB135* | C28 | Medtr7g110830.1 | chr7:45437737 - 45439145 | 2 | 367 | 41559.30 | 5.08 | 14-61, 67-111 | Mtr.22767.1.s1_at |
| *MtMYB136* | C28 | Medtr7g111290.1 | chr7:45674983 - 45676384 | 1 | 381 | 42774.38 | 6.72 | 14-61, 67-110 | Mtr.28710.1.s1_at |
| *MtMYB137* | C43 | Medtr7g115550.1 | chr7:47751336 - 47758260 | 2 | 836 | 113524.13 | 4.78 | 6-55, 58-105 | Mtr.6897.1.s1_at |
| *MtMYB138* | C43 | Medtr7g115550.2 | chr7:47751993 - 47755106 | 1 | 729 | 82945.11 | 6.39 | 6-55, 58-106 | Mtr.6897.1.s1_at |
| *MtMYB139* | | Medtr7g115650.1 | chr7:47812694 - 47813779 | 2 | 207 | 23496.37 | 8.46 | 14-61, 67-112 | Mtr.9637.1.s1_at |
| *MtMYB140* | C6 | Medtr7g117730.1 | chr7:48892047 - 48893136 | 2 | 256 | 29915.21 | 9.57 | 14-61, 67-112 | |
| *MtMYB141* | C36 | Medtr8g006470.1 | chr8:571945 - 573793 | 2 | 287 | 33414.62 | 6.63 | 12-57, 68-107 | |
| *MtMYB142* | C30 | Medtr8g017340.1 | chr8:5841430 - 5843448 | 2 | 492 | 56439.56 | 4.74 | 14-62, 68-111 | |
| *MtMYB143* | C30 | Medtr8g017350.1 | chr8:5847584 - 5850190 | 3 | 643 | 73605.24 | 4.58 | 14-62, 86-111 | |
| *MtMYB144* | C30 | Medtr8g017390.1 | chr8:5885779 - 5887633 | 2 | 475 | 54691.14 | 5.46 | 14-62, 68-111 | Mtr.42362.1.s1_at |
| *MtMYB145* | C30 | Medtr8g017440.1 | chr8:5919956 - 5921521 | 2 | 341 | 38898.05 | 6.95 | 14-61, 67-110 | |

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|---|---|---|---|---|---|---|---|---|---|
| *MtMYB146* | C30 | Medtr8g017500.1 | chr8:5958601 - 5960679 | 2 | 348 | 40006.68 | 6.43 | 14-61, 67-112 | |
| *MtMYB147* | C30 | Medtr8g017540.1 | chr8:5983119 - 5985752 | 3 | 394 | 45177.96 | 6.54 | 60-107, 113-156 | |
| *MtMYB148* | C4 | Medtr8g020490.1 | chr8:7194946 - 7196190 | 2 | 254 | 28853.45 | 8.62 | 10-57, 63-108 | Mtr.50541.1.s1_at |
| *MtMYB149* | C6 | Medtr8g027345.1 | chr8:9621348 - 9622704 | 1 | 300 | 34713.59 | 7.41 | 14-61, 67-112 | Mtr.42416.1.s1_at |
| *MtMYB150* | C20 | Medtr8g031360.1 | chr8:11745382 - 11748030 | 2 | 297 | 33422.17 | 6.26 | 15-63, 69-114 | |
| *MtMYB151* | C31 | Medtr8g042410.1 | chr8:16333311 - 16335916 | 2 | 546 | 61291.69 | 5.14 | 39-86, 92-135 | Mtr.5616.1.s1_at |
| *MtMYB152* | C1 | Medtr8g060940.1 | chr8:21305461 - 21306435 | 2 | 244 | 28084.60 | 6.06 | 9-56, 62-106 | |
| *MtMYB153* | C10 | Medtr8g095390.1 | chr8:39916388 - 39917565 | 2 | 315 | 34259.58 | 9.14 | 14-61, 67-111 | Mtr.5681.1.s1_at |
| *MtMYB154* | C29 | Medtr8g098860.1 | chr8:41349758 - 41351654 | 2 | 320 | 36509.41 | 6.26 | 14-61, 67-111 | Mtr.34412.1.s1_at |
| *MtMYB3R-1* | C46 | Medtr1g026870.1 | chr1:8820780 - 8826887 | 10 | 983 | 108667.55 | 5.47 | 33-79, 85-131, 137-180 | Mtr.9335.1.s1_at |
| *MtMYB3R-2* | C46 | Medtr3g110028.1 | chr3:50889355 - 50895583 | 11 | 903 | 100754.87 | 4.86 | 33-79, 85-131, 137-180 | Mtr.36181.1.s1_at |
| *MtMYB3R-3* | C46 | Medtr7g061330.1 | chr7:22157494 - 22161933 | 8 | 566 | 62466.01 | 8.62 | 61-107, 113-159, 165-208 | Mtr.5069.1.s1_at |
| *MtMYB3R-4* | C46 | Medtr7g061330.2 | chr7:22157813 - 22161933 | 7 | 530 | 59080.52 | 9.39 | 25-71, 77-123, 129-172 | Mtr.5069.1.s1_at |
| *MtMYB3R-5* | C46 | Medtr7g461410.1 | chr7:22216202 - 22220641 | 6 | 566 | 62466.01 | 8.62 | 61-107, 113-159, 165-208 | Mtr.5069.1.s1_at |
| *MtMYB4R-1* | C37 | Medtr6g080360.1 | chr6:30261554 - 30269213 | 8 | 1046 | 117719.52 | 7.03 | 425-475, 478-527, 529-577, 580-628 | Mtr.13840.1.s1_at |
| *MtMYB4R-2* | C37 | Medtr6g080360.2 | chr6:30262070 - 30267432 | 9 | 825 | 94205.47 | 9.78 | 205-255, 258-307, 309-357, 360-408 | Mtr.13840.1.s1_at |


Helix 1
Helix 2
Helix 3
Helix 1
Helix 2
Helix 3

**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.

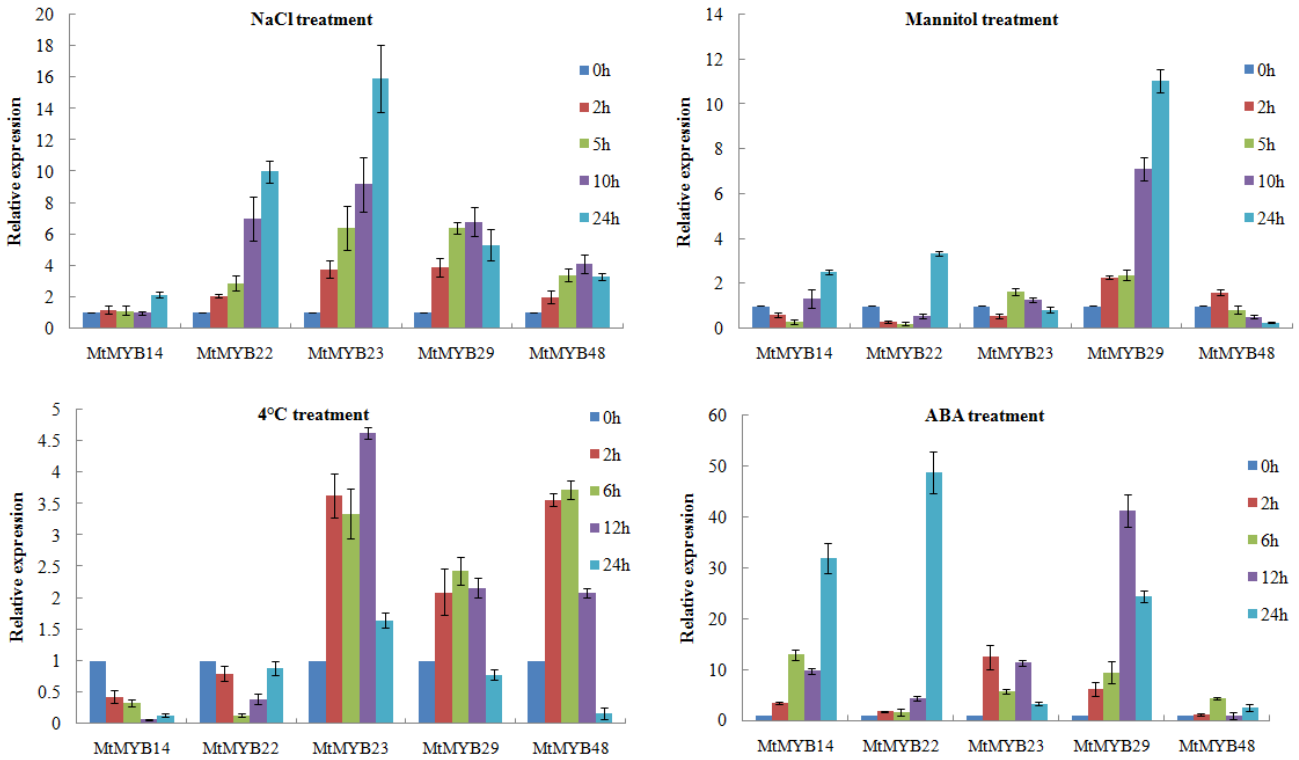
**Appendix E. The function-known *R2R3-MYB* genes in other plant species**

| tein | Specie name | Accession ID | Function reported |
|---|---|---|---|
| D | *Glycine max* | XP_003521289.1 | Nodule formation |
| MYB-G20-1 | *Glycine max* | BAP28594.1 | Control flower color |
| MYBJ1 | *Glycine max* | AGO06072.1 | Enhanced tolerance to drought and cold stresses |
| MYBZ2 | *Glycine max* | ABI73970.1 | Involved in the regulation of flavonoid biosynthesis; Represses catharanthine biosynthesis in hairy roots |
| MYB12B2 | *Glycine max* | AEC13303.1 | Flavonoid biosynthesis |
| MYBJ6 | *Glycine max* | ABI73972.1 | Signal transduction pathways related to ABA and NAA |
| MYBJ7 | *Glycine max* | ABI73973.1 | Signal transduction pathways related to ABA and NAA |
| MYB29B1 | *Glycine max* | NP_001241360.1 | Activate genes for flavonoid biosynthesis like GmCHS in response to UV-B radiation |
| MYB101 | *Glycine max* | XP_006592013.2 | Regulation of flavonoid biosynthesis in response to nitrate starvation |
| MYB90-like | *Glycine max* | XP_003533530.1 | Anthocyanin biosynthesis |
| MYB76 | *Glycine max* | ABH02836.1 | Stress tolerance |
| MYB92 | *Glycine max* | ABH02844.1 | Stress tolerance |
| MYB100 | *Glycine max* | XP_003528525.1 | Negatively regulates plant flavonoid biosynthesis |
| MYB2 | *Oryza sativa* | BAA23338.1 | Salt, cold, and dehydration stress |
| MYB4 | *Oryza sativa* | BAA23340.1 | Confers tolerance to chilling and freezing stress |
| MYB3R-2 | *Oryza sativa* | BAD81765.1 | Participates in the cold signalling pathway by targeting the cell cycle and a putative DREB/CBF；<br>Increases Tolerance to Freezing, Drought, and Salt Stress |
| MYB2P-1 | *Oryza sativa* | BAS92185.1 | Regulation of Phosphate-starvation responses and root architecture;<br>In response to deficiencies in Pi and Fe as well as cold stress, regulate Root Development |
| MYB55 | *Oryza sativa* | BAS95240.1 | High temperature |
| GAMYB | *Oryza sativa* | CAA67000.1 | Flower development |
| A | *Oryza sativa* | BAS71534.1 | Anther development and pollen maturation |
| MYBAS1 | *Oryza sativa* | XP_015617959.1 | |
| MYBAS2 | *Oryza sativa* | XP_015620227.1 | |
| MYBF1 | *Vitis vinifera* | ACV81697.1 | Controlling flavonol synthesis in fruit |
| MYB5a | *Vitis vinifera* | AAS68190 | Regulates the Phenylpropanoid pathway |
| MYB5b | *Vitis vinifera* | NP_001267854.1 | Anthocyanin and Proanthocyanidin biosynthesis |
| MYBA1 | *Vitis vinifera* | BAD18977.1 | Control the last biosynthetic step of anthocyanin synthesis |
| MYBA2 | *Vitis vinifera* | BAD18978.1 | Control the last biosynthetic step of anthocyanin synthesis |
| MYBPA2 | *Vitis vinifera* | ACK56131.1 | Promotes proanthocyanidin biosynthesis |
| MYB14 | *Vitis vinifera* | ABW34392.1 | Regulation of stilbene biosynthesis in response to biotic and abiotic stresses |
| MYB15 | *Vitis vinifera* | AHA83524.1 | Regulation of stilbene biosynthesis in response to biotic and abiotic stresses |
| MYB4-like | *Vitis vinifera* | XP_002273328.1 | Suppresses the anthocyanin accumulation |
| MYBPA1 | *Vitis vinifera* | CAJ90831.1 | Regulates proanthocyanidin synthesis during fruit development; regulates the early flavonoid biosynthetic genes |
| PL | *Zea Mays* | AAN12277.1 | Anthocyanins synthesis |
| MYB111 | *Zea Mays* | DAA62458.1 | Regulate the PAL gene in phenylpropanoid metabolism |

| | | | |
|---|---|---|---|
| C1 | *Zea Mays* | AAK09327.1 | Regulate anthocyanin synthesis |
| MYB-IF35 | *Zea Mays* | NP_001105092.1 | Flavonoid synthesis |
| P1 | *Zea Mays* | AAU09456.1 | Flavonoid synthesis |
| MYB31 | *Zea Mays* | NP_001105949.2 | Inhibits sinapoylmalate and phenylpropanoid biosynthesis |
| MYB1 | *Zea Mays* | AAL84612.1 | Regulate phenylpropanoid biosynthetic genes in flowers |
| MYB38 | *Zea Mays* | P20025.1 | Anthocyanins synthesis |
| MYBRS2 | *Zea Mays* | AAD25082.1 | Lateral organ primordia;to maintain knox gene silencing and determinacy during organogenesis |
| MYB1 | *Triticum aestivum* | ABC86569.1 | In response to the oxygen concentration in root environment and abiotic stresses; mediate plant tolerance to the Pi-starvation st |
| PIMP1 | *Triticum aestivum* | ABU93236.1 | Enhanced resistance to the fungal pathogen Bipolaris sorokiniana and drought stresses |
| PL1 | *Triticum aestivum* | BAE80456.1 | Positively regulate anthocyanin biosynthesis in wheat coleoptiles |
| MYB4 | *Triticum aestivum* | AEG64799.1 | Regulates lignin biosynthesis |
| MYB19 | *Triticum aestivum* | AEV91143.1 | Abiotic stresses tolerance |
| MYB73 | *Triticum aestivum* | AEW23186.1 | salinity stress tolerance |
| MYB14 | *Trifolium arvense* | AFJ53054.1 | Activates Proanthocyanidin biosynthesis |
| MYB1 | *Petunia hybrida* | CAA78386.1 | Cell shapes and petal development |
| AN2 | *Petunia hybrida* | AAF66727.1 | Anthocyanins synthesis |
| MYB3 | *Petunia hybrida* | CAA78388.1 | Petal epidermis-specific TF |
| MYB3 | *Populus trichocarpa* | AGT02395.1 | Regulation of secondary wall biosynthesis |
| MYB134 | *Populus tremuloides* | ACR83705.1 | Control PA synthesis in response to stress |
| MYB021 | *Populus trichocarpa* | XP_002313334.2 | Regulates secondary cell wall formation |
| MYB1 | *Eucalyptus pilularis* | BAM05596.1 | Repress the lignin biosynthetic |
| GAMYB | *Hordeum vulgare* | AAG22863.1 | Upregulated by GA in anther development |
| MYB1 | *Hordeum vulgare* | CAA50224.1 | Stresses tolerance |
| PL1 | *Hordeum vulgare* | BAJ90148.1 | Anthocyanin biosynthesis |
| kta | *Antirrhinum majus* | CAA55725.1 | Participates in the transcriptional control of epidermal cell shape (cell fate specification and differentiation) |
| AN | *Antirrhinum majus* | CAA06612.1 | Growth and dorsoventrality of lateral organs |
| MYB305 | *Antirrhinum majus* | P81391.1 | Activates transcription of phenylpropanoid biosynthetic genes in flowers |
| MYB340 | *Antirrhinum majus* | P81396.1 | |
| MYB308 | *Antirrhinum majus* | P81393.1 | Phenylpropanoid and lignin biosynthesis |
| MYB330 | *Antirrhinum majus* | P81395.1 | Phenylpropanoid and lignin biosynthesis |
| T2a | *Lotus japonicus* | BAG12893.1 | Up-regulate PA biosynthetic genes |
| T2b | *Lotus japonicus* | BAG12894.2 | |
| T2c | *Lotus japonicus* | BAG12895.1 | |
| MYB1 | *Nicotiana tabacum* | AAB41101.1 | Disease resistance |
| MYB2 | *Nicotiana tabacum* | BAA88221.1 | Wounding and elicitors |
| MYBAS1 | *Nicotiana tabacum* | AAG28526.1 | Positively regulate *gPAL1*expression and phenylpropanoid synthesis in sporophytic (anther-specific) |
| MYBAS2 | *Nicotiana tabacum* | AAG28525.1 | |
| MYB1 | *Daucus carota* | BAE54312.1 | Response to environmental cues: elicitor treatment, UV-B irradiation and the dilution effect |
| MYB1 | *Ipomoea nil* | BAE94391.1 | Synthesis of anthocyanin |
| MYB1 | *Petroselinum crispum* | AAB61699.1 | Light response |
| MYB26 | *Pisum sativum* | CAA71992.1 | Regulate phenylpropanoid biosynthetic genes in flowers |
| MYBAS1 | *Saccharum officinarum* | ADZ73058.1 | Water deficit and salt stress |
| by | *Citrus sinensis* | NP_001275818.1 | Activate anthocyanin biosynthesis |
| MYB1 | *Boea crassifolia* | AAP32921.1 | Drought tolerance ( ABA-independent) |

| | | | |
|---|---|---|---|
| MYB44 | *Salicornia brachiata* | AHN50422.1 | Response to salinity, desiccation, high temperature, and abscisic acid and salicylic acid treatments |
| P1 | *Erythranthe lewisii* | ALE33742.1 | Positively regulates carotenoid biosynthesis during flower development |
| PAP1 | *Salvia miltiorrhiza* | ACZ48688.2 | Regulate the phenolic acid biosynthetic pathway |
| MYB109 | *Gossypium hirsutum* | CAD71140.1 | Plays a direct role in the initiation and elongation of cotton fiber cells |
| MYB7 | *Gossypium hirsutum* | AAS92346.1 | Regulates secondary cell wall biosynthesis |
| MYB1 | *Dendrobium hybrida* | AFX97765.1 | Involved in the development of conical cell shape of the epidermal cells of flower labellum |
| MYB3 | *Solenostemon scutellarioides* | ABP57085.1 | Proanthocyanidin biosynthesis |
| MYBA1 | *Epimedium agittatum* | AGT39060.1 | Regulates the Flavonoid biosynthetic pathway |

**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.