

SUPPLEMENTAL RESULTS

C-terminal Domain of StMYB1R-1 Functions as Transcriptional Activation

To test whether StMYB1R-1 has transcriptional activity in yeast, we cloned the full-length and its deletion forms of *StMYB1R-1* in the pGBKT7 vector and introduced them into the AH109 yeast strain. Yeast strains harboring constructs containing 234 to 267 amino acids of StMYB1R-1 showed the transcriptional activity, but yeast strains harboring construct missing this domain did not (Supplemental Fig. S3). Therefore, this result indicates that the transcriptional activation domain of StMYB1R-1 exists between 234 and 267 amino acids.

Effect of *StMYB1R-1* Expression in Potato

To examine whether the expression of *StMYB1R-1* influences on potato agricultural traits, we expressed it under the control of the CaMV 35S promoter. Twenty-four independent transgenic potato lines were generated and confirmed by hygromycin selection and Northern blot analysis. Among these transgenic lines, we selected three independent transgenic lines in which the *StMYB1R-1* transgene was more expressed than in the wild type (Supplemental Fig. S5A). And 3-week-old T0 progenies grown on MS medium were transferred to plant hydroponic culture growth chambers for 1 week to increase their adaptation ability to the environmental changes in soil, and then were planted in soil and raised in a greenhouse. After cultivating for 8 weeks under normal condition in the greenhouse, the plants were analyzed for growth characteristics and tuber formation. *StMYB1R-1* overexpression in transgenic potato lines 1 and 2 showed reduced plant heights of about 5~8% compared to wild-type potato plants, but line 8 grew to a similar height as wild-type plants (Supplemental Fig. S5B and C). Analysis of morphology indicated that the leaf size, shape, and leaf number of the foliar tissue were similar between wild-type and transgenic potatoes. After cultivating for 12 weeks in the greenhouse, potato tubers from wild-type and transgenic plants were harvested

and the crop yields were compared. The tuber sizes were very diverse for single plants, but tuber numbers per potato plant were about 7 to 12, with an average of 9.2 ± 1.07 . The total tuber weight of the wild-type potatoes was about 123g, but the potatoes from transgenic lines 1, 2, and 8 weighed 109.4 g, 99.7g, and 111.8 g (Supplemental Fig. S3B and D), respectively. Although transgenic plants showed a little reduced total tuber yield (about 10 - 20%), these results suggest that the expression of *StMYBIR-1* did not significantly change growth characteristics compared to wild-type potato plants.

SUPPLEMENTAL MATERIALS AND METHODS

Data analysis of Quadruple 9-mer protein binding microarray

The distribution of all log intensities were plotted as histograms which resembled a Gaussian distribution for the left side of the mode and a heavy tailed skewed distribution for its right side of the mode. The log intensity values less than the median were fit to a Gaussian distribution and the maximum likelihood estimates of the mean, standard deviation and probabilities were obtained. In order to correct for multiple hypothesis testing, the p-values for the *i*-th largest individual was adjusted by multiplying $(k-i+1)$ following the Holm modification of the Bonferroni adjustment (Holm, 1979) where *k* is the total number of 131,072. This modification of p-value was equivalent to changing the level of significance into $\alpha/(k-i+1)$ for the *i*-th largest log intensity values. Because 9mer core sequences are present 9 times due to the base shift, the probe sequences with p value of median less than the significance level were selected as significant probe sequences. Among the probe sequences, the sequences containing homopolymer stretches including G stretches of more than 4 G's due to the presence of single strand region by the difficulty of double strand synthesis. The selected significant probe sequences were clustered with clustalW program and the sequence logo for the clustered sequence was generated (Crooks et al., 2004).

SUPPLEMENTAL LITERATURE CITED

Holm S (1979) A simple sequentially rejective multiple test procedure. *Scandinavian Journal of Statistics* 6: 65-70

Crooks GE, Hon G, Chandonia JM, Brenner SE (2004) WebLogo: a sequence logo generator. *Genome Res* **14**: 1188-1190

Supplemental Table S1. Nine probes presenting same DNA sequences were grouped as one probe set.

Probe ID	Signal intensity	Probe sequence (5'-3')
UPB_056122	42902	AATCTGGATAATCTGGATAATCTGGATAATCTGGATA
UPB_056124	45097	ATCTGGATAATCTGGATAATCTGGATAATCTGGATAA
UPB_056126	36111	TCTGGATAATCTGGATAATCTGGATAATCTGGATAAT
UPB_056128	40614	CTGGATAATCTGGATAATCTGGATAATCTGGATAATC
UPB_056130	31599	TGGATAATCTGGATAATCTGGATAATCTGGATAATCT
UPB_056132	28939	GGATAATCTGGATAATCTGGATAATCTGGATAATCTG
UPB_056134	22942	GATAATCTGGATAATCTGGATAATCTGGATAATCTGG
UPB_056136	24869	ATAATCTGGATAATCTGGATAATCTGGATAATCTGGA
UPB_056138	43765	TAATCTGGATAATCTGGATAATCTGGATAATCTGGAT

(continuous)

PID	ASI	Probe sequence	PID	ASI	Probe sequence
068	9378	TAAGCTAGATAAGCTAGATAAGCTAGATAAGCTAGA	Group 7		
069	8782	TAACATAGATAACATAGATAACATAGATAACATAGA	096	13024	TCAAATGGATCAAATGGATCAAATGGATCAAATGGA
070	12674	TAACTTAGATAACTTAGATAACTTAGATAACTTAGA	097	7529	TCAAACGGATCAAACGGATCAAACGGATCAAACGGA
071	7559	TAACGAAGATAACGAAGATAACGAAGATAACGAAGA	098	16745	TCAAGTGGATCAAGTGGATCAAGTGGATCAAGTGG
072	16297	TAACGTAGATAACGTAGATAACGTAGATAACGTAGA	099	8023	TCAACTGGATCAACTGGATCAACTGGATCAACTGGA
073	11301	TAACGCAGATAACGCAGATAACGCAGATAACGCAGA	100	8238	TCATTCGGATCATTTCGGATCATTTCGGATCATTTCGGA
Group 3			Group 8		
074	8512	TAAAGTTGATAAAGTTGATAAAGTTGATAAAGTTGA	101	21000	TATTATGGATATTATGGATATTATGGATATTATGGA
075	21274	ATATAATGATAATAATGATAATAATGATAATAATGG	Group 9		
076	13622	TAATGTTGATAATGTTGATAATGTTGATAATGTTGA	102	20740	TAGATTGGATAGATTGGATAGATTGGATAGATTGGA
077	9306	TAATCCTGATAATCCTGATAATCCTGATAATCCTGA	103	13228	TAGTTTGGATAGTTTGGATAGTTTGGATAGTTTGGA
078	30772	ATAGAATGATAAGAATGATAAGAATGATAAGAATGG	Group 10		
079	9618	TAAGATTGATAAGATTGATAAGATTGATAAGATTGA	104	12984	TTTAATGGATTTAATGGATTTAATGGATTTAATGGA
080	9618	TAAGGTTGATAAGGTTGATAAGGTTGATAAGGTTGA	105	15650	TTTAGTGGATTTAGTGGATTTAGTGGATTTAGTGGA
081	10764	TAACGTTGATAACGTTGATAACGTTGATAACGTTGA	106	8355	TTTTGTGGATTTTGTGGATTTTGTGGATTTTGTGGA
Group 4			Group 11		
082	9628	TAAATCCGATAAATCCGATAAATCCGATAAATCCGA	107	10273	TTGAACGGATTGAACGGATTGAACGGATTGAACGGA
083	16688	ATATAACGATAATAACGATAATAACGATAATAACGG	108	16214	TTGAGTGGATTGAGTGGATTGAGTGGATTGAGTGGA
084	13052	TAATATCGATAATATCGATAATATCGATAATATCGA	109	6766	TTGACTGGATTGACTGGATTGACTGGATTGACTGGA
085	11711	TAATGTCGATAATGTCGATAATGTCGATAATGTCGA	110	5922	TTGTTTGGATTGTTTGGATTGTTTGGATTGTTTGGA
086	7926	TAATGCCGATAATGCCGATAATGCCGATAATGCCGA	111	11363	TTGGATGGATTGGATGGATTGGATGGATTGGATGGA
087	6173	TAATCTCGATAATCTCGATAATCTCGATAATCTCGA	112	8114	TTGGGTGGATTGGGTGGATTGGGTGGATTGGGTGGA
088	10169	TAATCCCGATAATCCCGATAATCCCGATAATCCCGA	Group 12		
089	19377	ATAGAACGATAAGAACGATAAGAACGATAAGAACGG	113	9235	TGTAATGGATGTAATGGATGTAATGGATGTAATGGA
Group 5			114	6667	TGTTATGGATGTTATGGATGTTATGGATGTTATGGA
090	20393	TTAAATGGATTAAATGGATTAAATGGATTAAATGGA	115	8876	TGTTGTGGATGTTGTGGATGTTGTGGATGTTGTGGA
091	10592	TTAAACGGATTAAACGGATTAAACGGATTAAACGGA	Group 13		
092	10902	TTAATCGGATTAATCGGATTAATCGGATTAATCGGA	116	15880	TTAAGTAGATTAAGTAGATTAAGTAGATTAAGTAGA
Group 6			117	7125	TTAACTAGATTAAGTAGATTAAGTAGATTAAGTAGA
093	11928	TGAAATGGATGAAATGGATGAAATGGATGAAATGGA			
094	15015	TGAAGTGGATGAAGTGGATGAAGTGGATGAAGTGGA			
095	13132	TGATTTGGATGATTTGGATGATTTGGATGATTTGGA			

Supplemental Table S3. Upregulated genes in transgenic plant overexpressing *StMYB1R-1*.

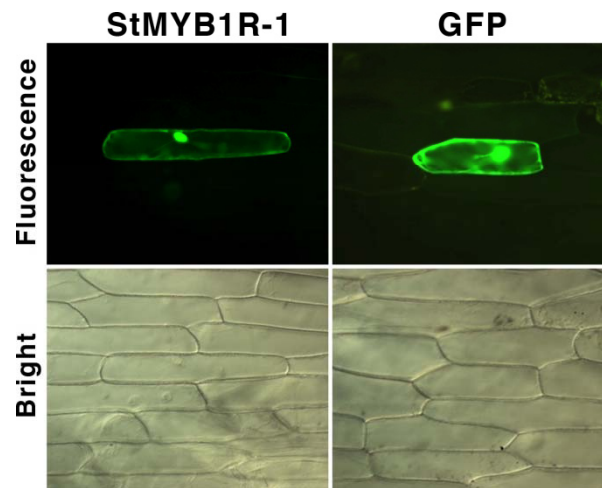
Accession number	Gene information	Induction Fold (Ox/Wt)	Accession number	Gene information	Induction Fold (Ox/Wt)
BQ111672	DNA repair protein-like	3.32	BQ506877	Tospovirus resistance protein B	2.42
BQ112220	Proton pump interactor	3.36	BQ509005	Putative glucosyltransferase	2.34
BQ112876	G protein alpha subunit 3	2.34	BQ509008	GRF1-interacting factor 1	2.19
BQ113469	Double WRKY type transfactor	2.86	BQ510295	Cullin 3a	2.06
BQ113600	CUL1	2.14	BQ511700	Blight resistance protein SH10	2.08
BQ113754	Oxygen-evolving enhancer protein 1	2.92	BQ511710	Homeobox-leucine zipper protein ATHB-7	2.01
BQ113873	Putative nicotianamine aminotransferase A	3.84	BQ512152	Copper chaperone	3.10
BQ114030	Putative Ubiquitin ligase SINAT5	2.80	BQ512667	Dihydroneopterin aldolase	2.74
BQ114179	Ethylene-responsive nuclear protein	2.27	BQ513064	Early light inducible protein	2.36
BQ114885	Ammonium transporter 1, member 2	3.94	BQ513130	Late blight resistance protein Rpi-blb2	2.67
BQ115059	Putative acid phosphatase	4.68	BQ513184	Putative Pto-like serine/threonine kinase	2.01
BQ115512	Inorganic pyrophosphatase	2.17	BQ513478	Putative serine/threonine protein kinase	4.09
BQ115542	Putative Yippee-like protein	2.78	BQ513881	HECT ubiquitin-protein ligase 3	3.50
BQ115565	CCCH type domain-containing protein ZFN1	2.35	BQ513951	Na ⁺ dependent ileal bile acid transporter	2.20
BQ115584	Phytoene synthase	3.31	BQ513961	Salicylic acid-induced protein 19	2.23
BQ115668	Glyceraldehyde-3-phosphate dehydrogenase	2.32	BQ514012	Temperature-induced lipocalin'	2.53
BQ115681	Seed maturation-like protein	3.51	BQ514087	Dehydration-induced protein ERD15	2.33
BQ115951	Nuclear transcription factor Y subunit C-1	3.39	BQ514325	Trehalose-6-phosphate synthase	2.82
BQ116142	Phosphoglycerate kinase-like	3.11	BQ514498	4-coumarate--CoA ligase 1	5.22
BQ116304	Putative CCAAT-binding transcription factor	2.47	BQ515101	Major intrinsic protein 2	3.19
BQ116364	Zinc finger, RING-type; Thioredoxin-related	3.03	BQ515352	Ultraviolet-B-repressible protein	2.20
BQ116595	Mitogen-activated protein kinase	2.50	BQ515888	Osmosensor histidine-aspartate kinase	2.00
BQ117354	Protein kinase-like	2.30	BQ516203	Homoserine kinase	2.36
BQ117445	U1 snRNP-interacting 70 kDa protein	2.62	BQ516292	Betaine aldehyde dehydrogenase-like	4.39
BQ118215	Fructokinase-like	2.00	BQ517304	DNA-binding protein-like	2.05
BQ119584	Plasma membrane intrinsic protein PIP2-like protein	2.91	BQ517628	Histone H2B	2.62
BQ119706	Oxidation protection protein-like	2.50	BQ517857	Transmembrane protein kinase	2.17
BQ121774	Phospho-2-dehydro-3-deoxyheptonate aldolase	3.21	BQ518519	DnaJ-like protein	2.14
BQ121945	Class IV chitinase	4.76	BQ518826	Calcium sensor calcineurin B-like protein	2.45
BQ506128	Sulfate transporter 2	2.51	BQ519147	Bacterial-induced peroxidase precursor	2.43

Supplemental Table S4. Primer sequences of the drought stress-responsive genes and housekeeping gene (*β-Tubulin*) used for semi-quantitative RT-PCR.

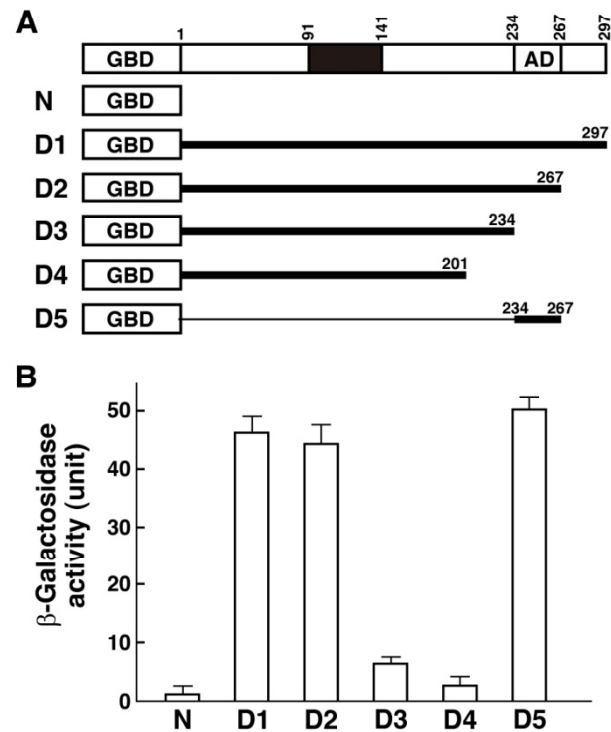
Gene name	Accession number	Primer sequence (5'-3')
<i>StMYB1R-1</i>	AU279205	Forward: ATGTCGAGCGTTTACAGCGAT Reverse: TCATGCCACACGGATGATGC
<i>WRKY-like</i>	BQ113469	Forward: CAAATTGTCCCACCAAGAAGAAG Reverse: GCTCGTCCTTAGTTCTCGAAAACAC
<i>AtHB7-like</i>	BQ511710	Forward: CTTGCTTATTTCAGTTGCAAAACTGA Reverse: CTATTAGGCTGATGGTTTAAGATACCAT
<i>NAC-like</i>	BQ513961	Forward: ATGAGTAACAACAGCAGCTTGAGC Reverse: GACTTTCATATATCATAGCCCACATC
<i>TIL</i>	BQ514012	Forward: AGATGGTGTAACACAAGAGCCAC Reverse: CAAGATGACTATTTCCCAAGATTGAT
<i>EDR15-like</i>	BQ514087	Forward: AGATCAACATTGAATCCAAATGC Reverse: CTGAAAGTAAACCAAGAGACTGGGAAT
<i>DnaJ</i>	BQ518519	Forward: ATGTTTGGGAAGAGCACCGAAGAAG Reverse: TTAGTGTGTGCACATTGGACTCTC
<i>PEX5</i>	BQ112493	Forward: ATTCCTACAATACAATCACTGAATG Reverse: TCTGAGCTGTGCATCTCGAAATG
<i>RD28</i>	BQ515101	Forward: AGTATTCCGCAAAGGATTACACTGATC Reverse: TATGCACTTTGGAATGCCTTCAC
<i>CUL3</i>	BQ510295	Forward: GATTGAACAGGCCACGGAGATC Reverse: TATGCAAGGTAGCGATAACAATCGTC
<i>ALDH22a1</i>	BQ516292	Forward: TGATGCAGGAGGAGGCTTTTG Reverse: TGTGGTCTTAGTCGTTTCTCCTCC
<i>β-Tubulin</i>	Z33382	Forward: ATATCTCTAACAGTGCCAGAGCTTACTCA Reverse: TCTGCAACCGGGTCATTCAT

CTTCTTTCTTCCTTTCAATTTCAATTTCAATAGGTGTGTACGGTTTCCGTTTGACGGAGT	60
GTATGTTTAGTCATCACCACCTTTGTATCAATGATTTTTATTGATTCGTGGAAACTTCC	120
AGAACGATGTCGAGCGTTTACAGCGATAAGTCGTGTCGACGCCGGCAGTTACCGGCGGC	180
M S S V Y S D K S S S T P A V T G G	18
GGTTTGGAGGAGAAATCATGTTGTTGGTGTGAGAGTAAAAGTGGATCCTATGAGGAAG	240
G F G G E I M L F G V R V K V D P M R K	38
AGCGTGAGTCTGAACGATCTTTACAGTACGAGCATCCGAATGCTAACAACAACAACAAC	300
S V S L N D L S Q Y E H P N A N N N N N	58
GGCGGTGATAACAATGAATCCTCTAAAGTGGCTCAGGATGAAGGTTATGCCTCTGCAGAT	360
G G D N N E S S K V A Q D E G Y A S A D	78
GACGCTGTTCAACATCAGTCCAACAGTGGTCGCGAGCGTAAGAGAGGAGTTCCGTGGACA	420
D A V Q H Q S N S <u>G R E R K R G V P W T</u>	98
GAGGAAGAGCATAAGTTATTCCTTTTAGGATTGCAGAAAGTGGGAAAAGGAGACTGGAGA	480
<u>E E E H K L F L L G L Q K V G K G D W R</u>	118
GGAATCTCTCGTAACTTCGTAAAGACTCGCACACCGACTCAGGTTGCAAGTCATGCTCAG	540
<u>G I S R N F V K T R T P T Q V A S H A Q</u>	138
AAATACTTCCTCCGGCGAAGCAACCTCAACCGTCGCCCGCTCGATCTAGCCTCTTTTGAT	600
<u>K Y F</u> L R R S N L N R R R R R S S L F D	158
ATCACCACTGACTCGGTATCAGTAATGCCAATAGAAGAGGTGGAAAATAAGCAAGAAATC	660
I T T D S V S V M P I E E V E N K Q E I	178
CCAGTTGTAGCACCAGCAACATTACCAACTACCAAAACCAATGCATTTCCGGTGGCACCA	720
P V V A P A T L P T T K T N A F P V A P	198
ACTGTTGGTCCTATCATATTTCCAGTACAGATTGACAAGTCAAGAGAGTATCCAACCTG	780
T V G P I I F P V Q I D K S R E Y P T L	218
TTGCGACATGATCATGGGAATTCATCGATGCTAGTTGGTCTGTTCCCTATGTTTTCAATG	840
L R H D H G N S S M L V G P V P M F S M	238
CCTAATCCATCCACAGCAATTGACCTTAACGCCAACCACAACCTCAACAATTGAGCCATCG	900
P N P S T A I D L N A N H N S T I E P S	258
TCTTTGTCACTGAGATTATCATTGTCACTTGATCAGGGACAAGCATCATCTACTAGACAC	960
S L S L R L S L S L D Q G Q A S S T R H	278
TCGGCATATAACGTGATGTCAGTTTCAGTAATGGAGAAAAGCATCATCCGTGTGGCATGA	1020
S A Y N V M S S F S N G E S I I R V A *	297
GATCAAAGGATCTCTTGAGAAAAAAGAAGAAGAAAAAACTAGGACAAGAGTGAGTAAGC	1080
TGGGTTTATAATTATTAATCTTATATATTGAGGAAAAGTTTATGATATCCATGTGGTG	1140
ATTAATTGACTAACTTTTGGTCCTG	1166

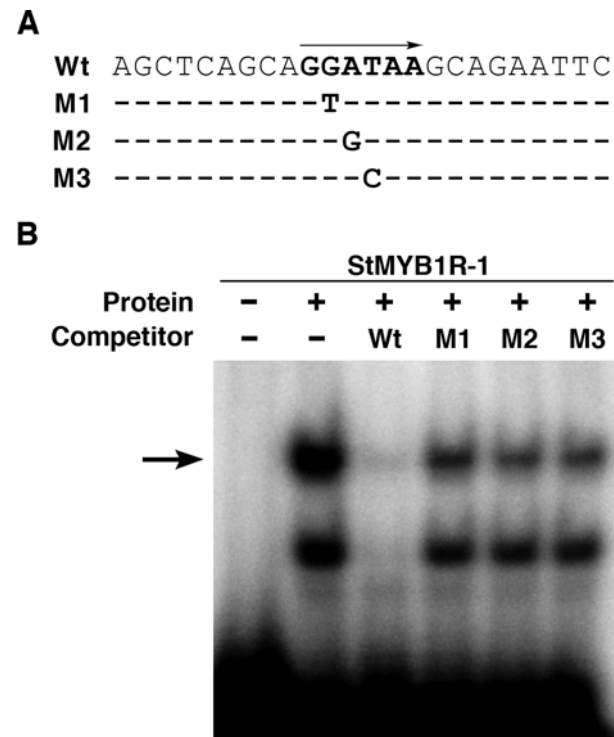
Supplemental Fig. S1. Nucleotide and predicted amino acid sequence of *StMYB1R-1* cDNA. Under line represents single MYB-like domain.



Supplemental Fig. S2. StMYB1R-1 localizes to the nucleus. cDNA of the *StMYB1R-1* segment cloned into the pBIN121-GFP plasmid. The constructs were transfected into the onion epidermal cells by transient bombardment assay and then were observed by fluorescence microscopy at 24 hours after transfection. GFP was used as a control.



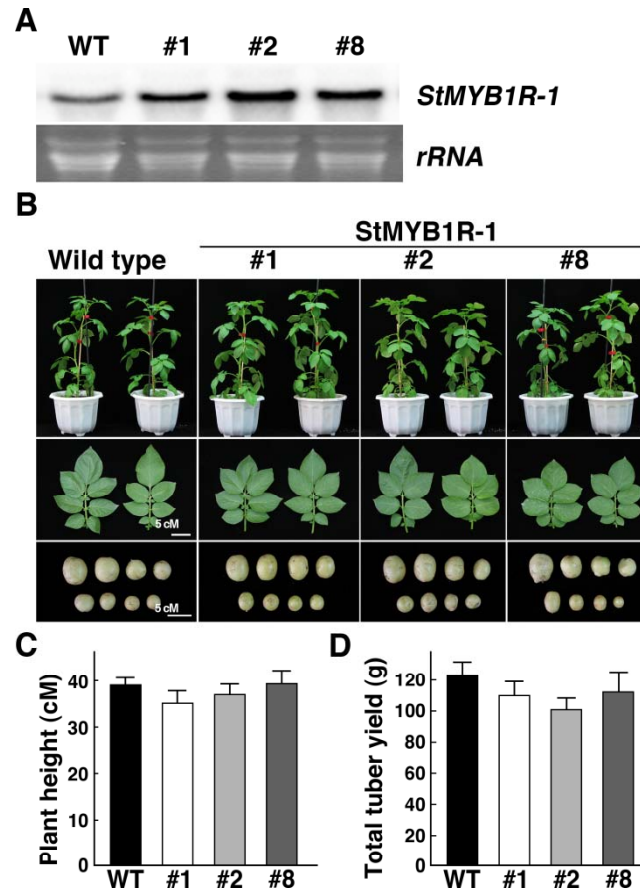
Supplemental Fig. S3. Transcriptional activation test for StMYB1R-1 in yeast. The full-length (D1) and deletion forms of StMYBR1 (D2 to D5) were cloned into the pGBKT7 vector and introduced into the AH109 yeast strain. Yeast cells harboring both genes were cultured at 30 C for overnight, diluted to an O.D.600 of 0.1, and then incubated in SC-Trp⁻ medium for 1 h. Cells were harvested by centrifugation at 3000g for 5 min and resuspended in 1 mL Z-buffer (60 mM Na₂HPO₄, 40mM NaH₂PO₄, 10 mM KCl 1 mM MgSO₄, and 50 mM β-mercaptoethanol, pH 7.0). Cell extracts were prepared, and β-galactosidase activity was measured according to the manufacturer's protocol (Clontech). The vector alone (N) was used as a negative control.



Supplemental Fig. S4. The StMYB1R-1 binds specifically to the GGATAA sequences.

(A) The sequences of oligonucleotides that were used for electrophoresis mobility shift assay. Wt; wild type sequences, M1 through M3; mutated sequences. Mutated sequences are presented in boldface. Sequences identical to the wild-type sequence are indicated with dashes.

(B) For electrophoresis mobility shift assay (EMSA), 250 ng of recombinant StMYB1R-1 protein was incubated with ³²P-labeled probe in the presence or absence of 250-fold excess cold competitor or mutated sequences (M1 to M3). Arrow indicates the positions of the StMYB1R-1 and DNA complexes.

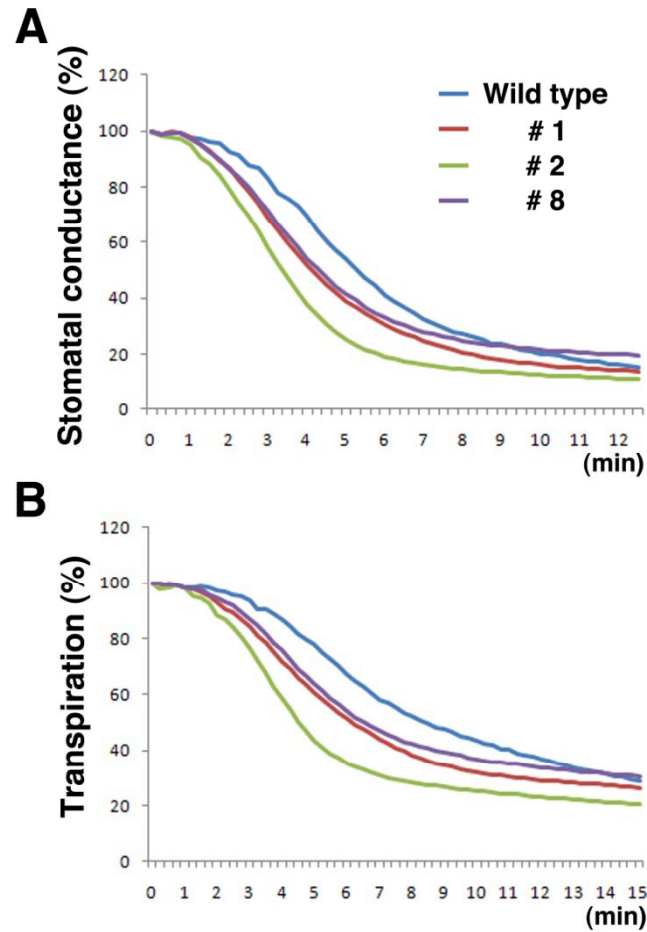


Supplemental Fig. S5. Growth characteristics of transgenic potato plants overexpressing *StMYB1R-1*.

(A) Expression of 3 independent transgenic potatoes by Northern blot analysis. Each lane was loaded with 10 μ g of total RNA extracted from 3-week-old **T0 potato plantlets**. The Northern blot was performed with a 32 P-labeled *StMYB1R-1* cDNA probe. Ethidium bromide-stained rRNA was used as a RNA-loading control.

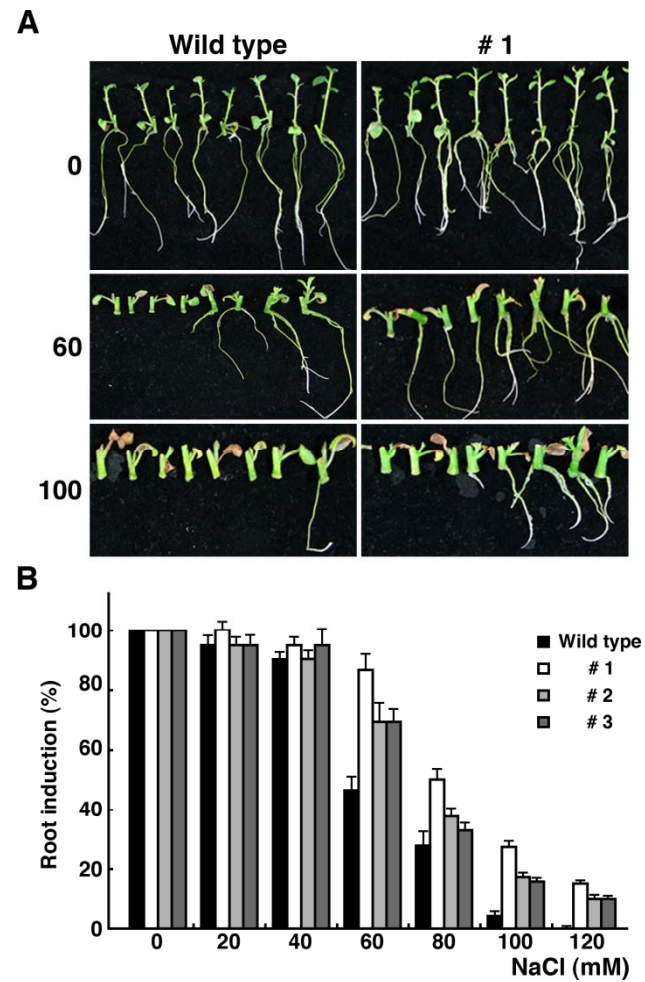
(B) and (C) Whole plant and foliar tissue of three independent lines were evaluated in 12- or 16-week-old plants grown in a greenhouse.

(D) Representative tubers of wild-type and transgenic potato plants are shown for each line that produced tubers.



Supplemental Fig. S6. Physiological characteristics of transgenic potato plants overexpressing *StMYB1R-1*.

Stomatal conductance (A) and transpiration (B) from detached leaves of 8-week-old wild-type and transgenic plant were conducted with Li-Cor LI-6400 console (Li-COR, Nebraska, USA) according to the manufacturer's recommendations (air flow; $300 \mu\text{mol s}^{-1}$, CO_2 mole fraction; $400 \mu\text{mol mol}^{-1}$, temperature; $25 \text{ }^\circ\text{C}$, light; $1000 \mu\text{mol m}^{-2} \text{ s}^{-1}$).



Supplemental Fig. S7. Transgenic potato plants overexpressing *StMYB1R-1* are tolerant to salt stress conditions.

(A) Potato inter-node tissues were transferred to MS medium with or without NaCl (mM) and photographs were taken after 3 weeks.

(B) Root induction was determined by counting rooted or unrooted plants. $n = 3$ independent experiments (20 plants in each experiment).