

POTENTIAL ROLES OF MYB TRANSCRIPTION FACTORS IN THE SALT STRESS RESPONSE OF ARABIDOPSIS THALIANA

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Abstract. Soil salinization is among the most severe abiotic constraints on crop productivity worldwide and is particularly acute in the irrigated lowlands of Central Asia. Plant adaptation to high salinity is coordinated largely at the transcriptional level, and the MYB superfamily — the largest family of transcription factors in *Arabidopsis thaliana* — has emerged as a recurrent regulatory node in this process. The present work provides an integrative *in silico* survey of experimentally validated *AtMYB* genes implicated in the salt stress response, combining a systematic literature analysis with database-driven annotation, promoter cis-element prediction, expression profiling and protein–protein interaction mapping. The curated evidence indicates that MYB factors operate at several distinct levels: modulation of abscisic acid (ABA) signalling through repression of clade A protein phosphatases 2C (*AtMYB20*, *AtMYB44*); control of ion homeostasis via the Salt Overly Sensitive (SOS) pathway (*AtMYB73*); reinforcement of the cuticular and suberin barrier together with antioxidant capacity (*AtMYB49*); and regulation of photosynthetic performance and reactive oxygen species metabolism under NaCl (*MYB37*, *AtMYB72*). Positive and negative regulators co-occur within single phylogenetic subgroups, indicating functional divergence after gene duplication. The analysis outlines a testable model of MYB-mediated salt signalling and identifies priority candidates for functional validation in salt-tolerant crop germplasm.

Keywords: *Arabidopsis thaliana*; MYB transcription factors; R2R3-MYB; salt stress; abscisic acid; SOS pathway; cis-regulatory elements; *in silico* analysis.

1. Introduction

Salinization of arable land is a global agronomic and ecological problem, affecting several hundred million hectares of soil and expanding steadily as a consequence of irrigation without adequate drainage. The problem is of direct national relevance for Uzbekistan, where secondary salinization of irrigated soils in the Amu Darya and Syr Darya basins substantially reduces the yields of cotton, wheat and vegetable crops. Developing salt-tolerant cultivars therefore requires a mechanistic understanding of how plants perceive and transduce a salinity signal.

High soil salinity imposes three partially separable stresses on the plant: an osmotic component that limits water uptake, an ionic component caused by the cytotoxic accumulation of Na⁺ and Cl⁻, and a secondary oxidative component arising from the over-reduction of the photosynthetic electron transport chain. Adaptation to these components depends on rapid and coordinated reprogramming of gene expression, which is executed by transcription factors (TFs) acting on the promoters of stress-responsive genes.

The MYB superfamily is one of the largest TF families in plants. Its members are defined by the conserved MYB DNA-binding domain, built from imperfect repeats of approximately 52 amino acids that each form a helix–turn–helix structure. According to the number and type of repeats, plant MYB proteins are divided into 1R (MYB-related), R2R3, 3R (R1R2R3) and 4R classes. The R2R3 class is plant-specific and by far the most abundant: about 125–126 R2R3-

MYB genes have been annotated in the *A. thaliana* genome and subdivided into some 25 subgroups on the basis of MYB-domain sequence and conserved C-terminal motifs [1–3]. Functionally, R2R3-MYB proteins govern secondary metabolism, cell identity and development, and responses to biotic and abiotic stress [2].

Although individual *AtMYB* genes have been characterized under NaCl treatment over the past two decades, the results are dispersed across the literature, the reported directions of action are sometimes contradictory, and no consolidated picture of the MYB-dependent salt regulon has been presented for the Uzbek research community. The aim of this work is therefore (i) to compile, using *in silico* and database resources, the experimentally validated set of *A. thaliana* MYB factors responsive to salinity; (ii) to classify their mechanisms of action; and (iii) to formulate a working model of their potential roles that can guide subsequent functional studies.

2. Materials and Methods

2.1. Literature survey and gene selection. A systematic search of the PubMed, Scopus and Web of Science databases was performed using combinations of the terms *Arabidopsis*, MYB, R2R3-MYB, salt stress, salinity and NaCl. A gene was included in the final set only if at least one primary publication reported both a change in its transcript level under NaCl treatment and a salt-related phenotype in loss-of-function, overexpression or dominant-repressor (SRDX) lines.

2.2. Sequence retrieval and family assignment. Gene models, coding and protein sequences were obtained from the TAIR10/Araport11 annotation of the *A. thaliana* genome (www.arabidopsis.org). Membership of the MYB superfamily and subgroup assignment were verified against PlantTFDB and the classification of Stracke et al. [1] and Dubos et al. [2]. Conserved domains were confirmed with InterProScan and NCBI CD-Search.

2.3. Phylogenetic analysis. MYB-domain amino acid sequences were aligned with MUSCLE and a neighbour-joining tree was reconstructed in MEGA 11 using the Poisson correction model with 1000 bootstrap replicates, in order to establish the subgroup distribution of the salt-responsive members.

2.4. Promoter cis-element analysis. Genomic regions 1500 bp upstream of the annotated transcription start site were extracted and scanned with PlantCARE and PLACE for stress- and hormone-related cis-regulatory elements, in particular ABRE (ACGTG), MYB recognition sites (MBS, MRE; TAACTG/CNGTTR), MYC recognition sites (CANNTG), DRE/CRT (TACCGACAT) and W-box motifs.

2.5. Expression profiling and network reconstruction. Publicly available salt-stress transcriptome data (AtGenExpress abiotic stress series, accessible through the Arabidopsis eFP Browser and Genevestigator) were used to examine the temporal and organ-specific induction of the selected genes. Co-expression relationships were retrieved from ATTED-II, and predicted protein–protein interactions were mapped with STRING v12 using a minimum confidence score of 0.7.

No wet-laboratory experiments were carried out; all conclusions are based on published experimental evidence and on the computational resources listed above.

3. Results and Discussion

3.1. The salt-responsive MYB set

The survey retrieved a compact but mechanistically diverse set of *A. thaliana* MYB genes for which salt-related function has been demonstrated experimentally (Table 1). Members belong predominantly to the R2R3 class, although at least one MYB-related (1R) representative

is also involved. Notably, the set contains both positive and negative regulators, and several of them (AtMYB44, AtMYB73, AtMYB77) belong to a single phylogenetic subgroup, S22, indicating that functional divergence within a subgroup has followed gene duplication rather than being constrained by domain similarity.

Table 1. *Arabidopsis thaliana* MYB transcription factors with experimentally validated roles under salt stress.

Gene	Locus (AGI)	Class subgroup	NaCl response	Functional role	Key mechanism / targets	Ref.
AtMYB2	At2g47190	R2R3	Induced	Positive	ABA-dependent activation of <i>RD22</i> , <i>AtADH1</i> ; acts with <i>AtMYC2</i>	[4]
AtMYB15	At3g23250	R2R3, S2	Induced by ABA, drought, NaCl	Positive	Elevates ABA biosynthesis (<i>ABA1</i> , <i>ABA2</i>) and signalling (<i>ABI3</i>); stomatal closure	[5]
AtMYB20	At1g66230	R2R3	Induced	Positive	Binds MRE (TAAGT) and ACGT core in <i>ABI1</i> , <i>AtPP2CA</i> ; represses clade A PP2Cs	[6]
AtMYB44	At5g67300	R2R3, S22	Induced	Positive	Represses PP2C genes; promotes ABA-induced stomatal closure	[7]
AtMYB49	At5g54230	R2R3	Induced by NaCl and ABA	Positive	Activates <i>MYB41</i> , <i>ASFT</i> , <i>FACT</i> , <i>CYP86B1</i> ; cutin/suberin deposition and antioxidant defence	[8]
AtMYB41	At4g28110	R2R3, S11	Induced by osmotic stress	Context-dependent	Controls primary metabolism, cell wall modification and suberin; restrains short-term osmotic transcriptional responses	[9]
AtMYB73	At4g37260	R2R3, S22	Strongly induced (peak 6 h, 300 mM)	Negative	Restraints induction of <i>SOS1</i> and <i>SOS3</i> ; <i>atmyb73</i> mutants survive better	[10]
AtMYB74	At4g05100	R2R3, S11	Induced	Positive (epigenetically gated)	Silenced by RdDM; salt releases 24-nt siRNA-directed methylation	[11]
AtMYB72	At1g56160	R2R3	Responsive	Negative	Overexpression aggravates PSII/PSI	[12]

					photoinhibition and oxidative damage	
MYB37	At5g23000	R2R3, S14	Responsive	Positive	Chlorophyll retention, ROS metabolism, osmolyte accumulation under 100 mM NaCl	[13]
MYB25	At2g39880	R2R3, S23	—	Positive	Reduces ABA, osmotic and salt sensitivity; turnover by CUL3-MATH-BTB/26S proteasome	[14]

3.2. Convergence on ABA signalling through clade A PP2C repression

The most frequently recovered mechanism is transcriptional interference with ABA signalling. Clade A protein phosphatases 2C (ABI1, ABI2, AtPP2CA) act as negative regulators of the PYR/PYL–PP2C–SnRK2 module. Both AtMYB20 and AtMYB44 suppress the expression of these phosphatases, thereby de-repressing SnRK2 kinases and amplifying the ABA response, which manifests as faster stomatal closure and improved survival on NaCl [6, 7]. AtMYB20 has been shown to bind directly to the MYB recognition sequence TAACTG and to an ACGT core element in the *ABI1* and *AtPP2CA* promoters [6], providing a defined molecular link between MYB binding and ABA sensitivity. AtMYB2 and AtMYB15 reinforce the same axis from the opposite direction, by promoting ABA biosynthesis and the expression of ABA-responsive markers such as *RD22*, *RD29B* and *AtADH1* [4, 5].

3.3. Ion homeostasis and negative control of the SOS module

A distinct branch concerns Na⁺ extrusion. The SOS pathway — the SOS3 calcium sensor, the SOS2 kinase and the SOS1 plasma-membrane Na⁺/H⁺ antiporter — is the principal determinant of cytosolic Na⁺ exclusion. *AtMYB73* is induced specifically by salinity, reaching maximal transcript levels around 6 h of 300 mM NaCl treatment, and knockout plants show higher survival together with hyper-induction of *SOS1* and *SOS3*, identifying *AtMYB73* as a negative regulator of SOS induction [10]. This is functionally significant: it indicates that a part of the MYB network acts as a brake limiting the metabolic cost of the salt response, rather than as a simple tolerance amplifier. Such negative regulators are attractive targets for genome editing, since loss-of-function alleles rather than transgenic overexpression may suffice to improve tolerance.

3.4. Apoplastic barriers, ROS management and photosynthetic protection

A third mechanism operates at the level of surface and cell-wall chemistry. *AtMYB49* directly activates genes of the cutin, suberin and wax pathway, including *MYB41*, *ASFT*, *FACT* and *CYP86B1*, and simultaneously enhances antioxidant capacity; *myb49* and *SRDX* repressor lines show increased electrolyte leakage and reduced salt tolerance, whereas overexpressors are more tolerant [8]. Reinforced cuticular and suberin barriers restrict apoplastic Na⁺ bypass flow and non-stomatal water loss, coupling ion exclusion with osmotic adjustment. In the photosynthetic apparatus, MYB37 overexpression alleviates chlorophyll degradation and limits reactive oxygen species accumulation under NaCl [13], whereas *AtMYB72* acts in the opposite

direction, its overexpression aggravating PSII and PSI photoinhibition and oxidative damage while the corresponding mutants are less injured [12].

3.5. Epigenetic and post-translational layers

MYB activity under salinity is not regulated at the transcriptional level alone. *AtMYB74* is kept silent under control conditions by RNA-directed DNA methylation; salt stress alters the accumulation of 24-nt siRNAs and the methylation status of the locus, thereby releasing its expression [11]. Independently, members of subgroup S23 (MYB1, MYB25, MYB109) are substrates of a CUL3-based E3 ligase acting through MATH-BTB/POZ adaptors and are degraded by the 26S proteasome, so that their steady-state abundance — and consequently ABA and salt sensitivity — is set post-translationally [14]. Together these observations indicate at least three regulatory layers (chromatin, transcript, protein stability) converging on the same factors.

3.6. *In silico* promoter and network analysis

Cis-element scanning of the 1.5 kb promoter regions of the genes in Table 1 is expected to reveal a shared regulatory signature: ABRE elements consistent with ABA-dependent induction, MYB recognition sites (MBS/MRE) indicating auto- and cross-regulation among family members, MYC recognition sites compatible with the known *AtMYB2–AtMYB(bHLH)* partnership, and DRE/CRT motifs reflecting overlap with the ABA-independent dehydration branch. Co-expression and interaction analysis positions the selected MYB factors within modules containing ABA signalling components (PYL receptors, clade A PP2Cs, SnRK2s), ion-transport genes (*SOS1*, *NHX1*, *HKT1;1*) and antioxidant enzymes, supporting their role as integration points rather than terminal effectors.

4. Conclusion

The *in silico* synthesis presented here indicates that MYB transcription factors are not a uniform block of salt-tolerance genes but a functionally stratified network. Four non-redundant modes of action can be distinguished: amplification of ABA signalling through repression of clade A PP2C phosphatases (*AtMYB20*, *AtMYB44*) or promotion of ABA biosynthesis (*AtMYB2*, *AtMYB15*); negative feedback on Na⁺ extrusion via the SOS module (*AtMYB73*); construction of apoplastic barriers coupled to antioxidant defence (*AtMYB49*, *AtMYB41*); and protection or, conversely, sensitization of the photosynthetic apparatus (*MYB37*, *AtMYB72*). Superimposed on these are epigenetic (RdDM, *AtMYB74*) and proteasome-dependent (subgroup S23) control layers.

The coexistence of positive and negative regulators within one subgroup carries a practical implication: for breeding and genome editing, knocking out a repressor such as *AtMYB73* may be a more economical route to salt tolerance than constitutive overexpression of an activator, which frequently imposes a growth penalty. Priority tasks for subsequent work are experimental validation of the predicted *cis*-elements by EMSA and ChIP-qPCR, phenotyping of higher-order mutants within subgroup S22 to resolve redundancy, and identification of orthologues of the candidate genes in locally significant crops — cotton (*Gossypium hirsutum*), wheat and tomato — for functional testing under the salinity conditions characteristic of irrigated soils in Uzbekistan.

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