Article title: OsRMC, a negative regulator of salt stress response in rice, is regulated by two AP2/ERF transcription factors

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Authors: Tânia S. Serra, Duarte D. Figueiredo, André M. Cordeiro, Diego Almeida, Tiago Lourenço, Isabel A. Abreu, Alvaro Sebastián, Lisete Fernandes, Bruno Contreras-Moreira, M. Margarida Oliveira, Nelson J.M. Saibo

Corresponding Author:

Nelson J. M. Saibo
Tel.: +351-214469644
Fax: +351-214411277
e-mail: saibo@itqb.unl.pt

Table S1. Oligonucleotide sequences used in the cloning procedures, gene expression studies and recombinant protein expression. The underlined region of the primer corresponds to adapter sequences and the remaining region is specific to the target DNA.

Primer name	Primer sequence 5´- 3´
OsRMC-Fw	GTTCGACATCACGCTGGA
OsRMC-Rv	ATAATCCGGTTACAGCTTAGATAGAT
HybriZAP-Fw	CCCCACCAAACCCAAAAAAAG
HybriZAP-Rv	GTTGAAGTGAACTTGCG
OsEREBP1-Fw	TGCAGCTTCTTCAGCACTGT
OsEREBP1-Rv	ACTTCGAGGAGTTCGAGGTG
OsEREBP2-Fw	GTACCTGCGCTACCAGATGC
OsEREBP2-Rv	CATCTCCGTCTCTCCGTCTC
OsACT1-Fw	GTCGCACTTCATGATGGAGTTG
OsACT1-Rv	CATGCTATCCCTCGTCTCGAC
OsUBC-Fw	CAAAATTTTCCACCCGAATG
OsUBC-Rv	ATCACATGAATCAGCCATGC
qOsEREBP1-Fw	ACGTCGTCGAGATCAAGCC
qOsEREBP1-Rv	TTTGGCAGACTTTGCAGCAG
qOsEREBP2-Fw	TCGGAGTCGAGCTATCACCA
qOsEREBP2-Rv	AATCTGCGACGTCCATCTCC
q25S-Fw	AAGGCCGAAGAGGAGAAAGGT
q25S-Rv	CGTCCCTTAGGATCGGCTTAC
pRMCF3-Fw	ATCTGCAGCTTGACGAGCAGGCATAGGT
pRMCF3-Rv	ATGTCGACTGCCTGCGTTCTATGGTCTG
GW-OsEREBP1-Fw	GGGGACAAGTTTGTACAAAAAAGCAGGCTACAAGCAATCCACCACTGCA
GW-OsEREBP1-Rv	GGGGACCACTTTGTACAAGAAAGCTGGGTTGCCTCCCAATCTCCAATAG
GW-OsEREBP2-Fw	GGGGACAAGTTTGTACAAAAAAGCAGGCTATATGACGGTGGCGGGGGCGTCG GAGCT
GW-OsEREBP2-Rv	GGGGACCACTTTGTACAAGAAAGCTGGGTGGACAGAATCCGGCGGCTACTGC GTGTGC
GX-OsEREBP1-Fw	ATATGAATTCATGTGCGGCGGCGCCATCATCC
GX-OsEREBP1-Rv	ATATCTCGAGGAATTCCTCAATAGAAATCGCTAACGGGCAT
GX-OsEREBP2-Fw	ATATGAATTCATGACGGTGGCGGGGGCGTCGGAGCT

GX-OsEREBP2-Rv	ATATCTCGAGGGACAGAATCCGGCGGCTACTGCGTGTGC
PET-OsBWMK1-Fw	CACCATGGGGGGAGGGGGCACGCT
PET-OsBWMK1-Rv	ATCATCGTCATCGTTGTGCATTAGGAGTGC
S1-Fw	GCTTGACGAGCAGGCATAGGTATATTTG
S1-Rv	TCGACAAATATACCTATGCCTGCTCGTCAAGCTGCA
S1M-Fw	TTCTTGACTATCATTCATAGGTATATTT
S1M-Rv	TTAAATATACCTATGAATGATAGTCAAG
S2-Fw	GCGCATCCAATGGCAGCACTGGTTCCTAG
S2-Rv	TCGACTAGGAACCAGTGCTGCCATTGGATGCGCTGCA
S2M-Fw	TTCGCATCCAATTTCATCACTGGTTCCTA
S2M-Rv	TTTAGGAACCAGTGATGAAATTGGATGCG
GCC-Fw	GCATAAGAGCCGCCACTAAAATAAGACCGATCAAATAAGAGCCGCCATG
GCC-Rv	TCGACATGGCGGCTCTTATTTGATCGGTCTTATTTTAGTGGCGGCTCTTATGCTGCA

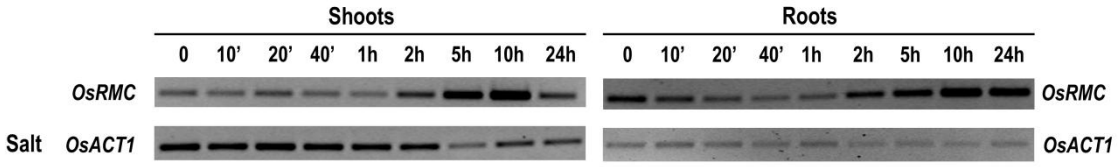


Figure S1. Analysis of *OsRMC* gene expression in response to high salinity conditions. RT-PCR reactions were performed with cDNA prepared from 1 µg of total RNA extracted from shoots and roots of 14-day-old rice seedlings (cv. Nipponbare) subjected to salt (200 mM). *OsRMC* was amplified with 30 cycles. *OsACT1* was used as internal control and amplified using 25 and 20 cycles for the shoot and root samples, respectively.

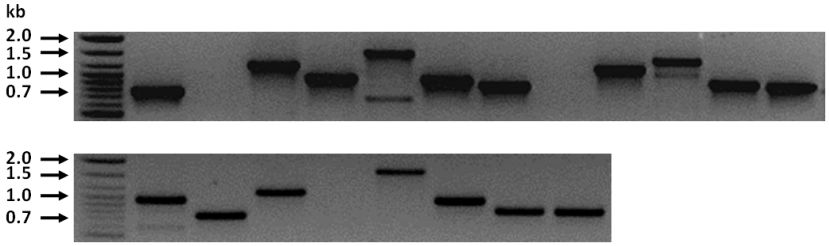


Figure S2. Analysis of the inserts present in the salt-induced rice cDNA expression library. Twenty plaques were randomly picked from the cDNA expression library and analyzed by PCR, using the HybriZAP primers described in Supplementary Table 1.

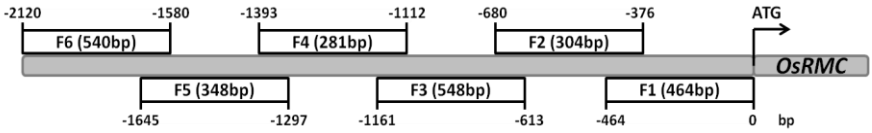


Figure S3. Schematic representation of the *OsRMC* promoter fragments (F1-F6) used to prepare the yeast bait strains. The *OsRMC* promoter region was defined as the 2120 bp sequence upstream the translation start codon (ATG).

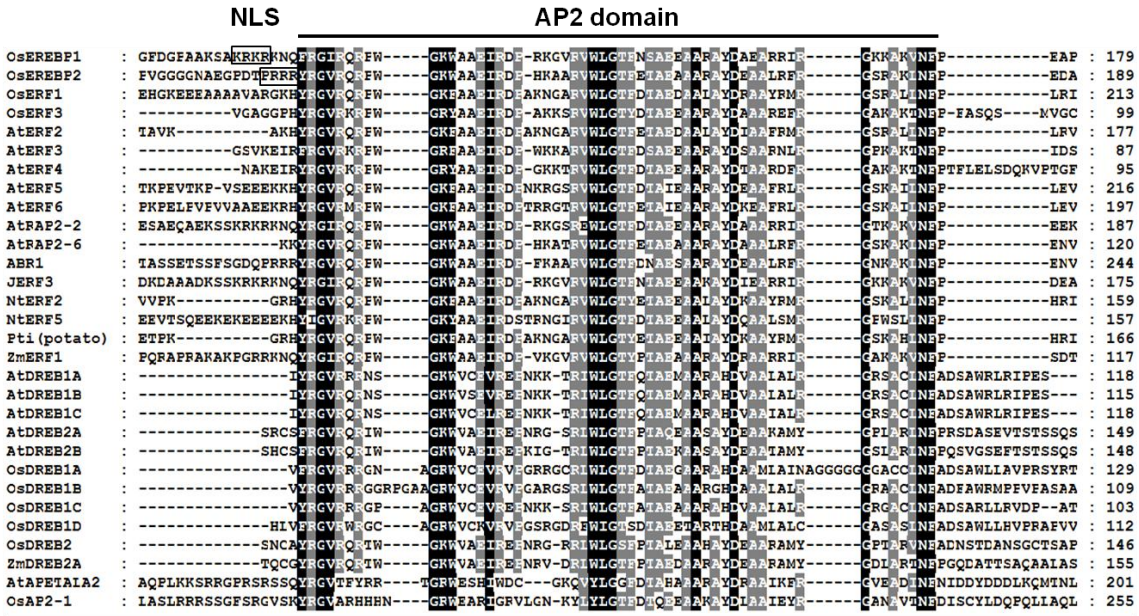
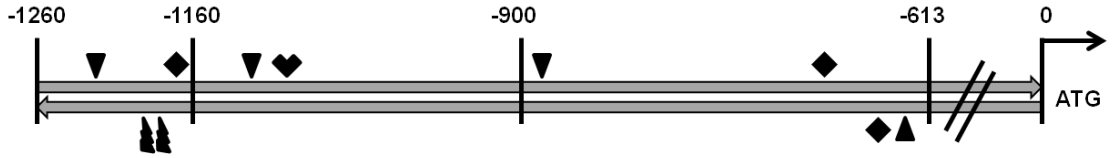


Figure S4. Alignment of deduced amino acid sequences of OsEREBP1, OsEREBP2 and other proteins from the AP2/ERF family of transcription factors using the Clustal W program and the Genedoc software. Residues in black are 100% conserved and in grey are 80% conserved. Dashes indicate gaps in the amino acid sequences. The line shows the conserved AP2 DNA binding domain. The squares indicate the nuclear localization signal (NLS) predicted for OsEREBP1 and OsEREBP2 by the PSORT program.



Symbol	Putative cis-element	Sequence [a]	Position [b]
▼	ABRE	TGCAC ACGT	-631 -891, -1099, -1211
❤	DRE1	ACCGAGA	-1081
	LTRE1	TTTCGG	-1167, -1175
◆	MYB-recognition motif	CcGTTa CgGTTa	-642 -690, -1164

[a] Sequences are indicated from 5' to 3'
[b] Positions of the *cis*-elements in bp upstream the translation start codon

Figure S5. Putative abiotic stress-related *cis*-elements present in the *OsRMC* gene promoter. The promoter region -1260 bp to -613 bp upstream the translation start codon (ATG) was scanned using the PlantPAN database.

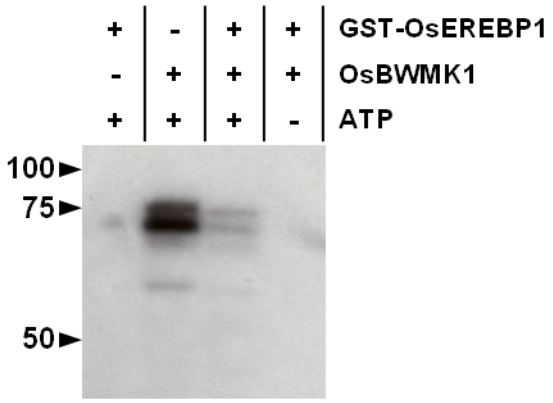


Figure S6. OsBWMK1 protein kinase activity. The activity was measured through incubation of 2.5 µg of purified protein with 5 µCi [γ-^{32}P]ATP and with or without 3 µg of purified GST-OsEREBP1.

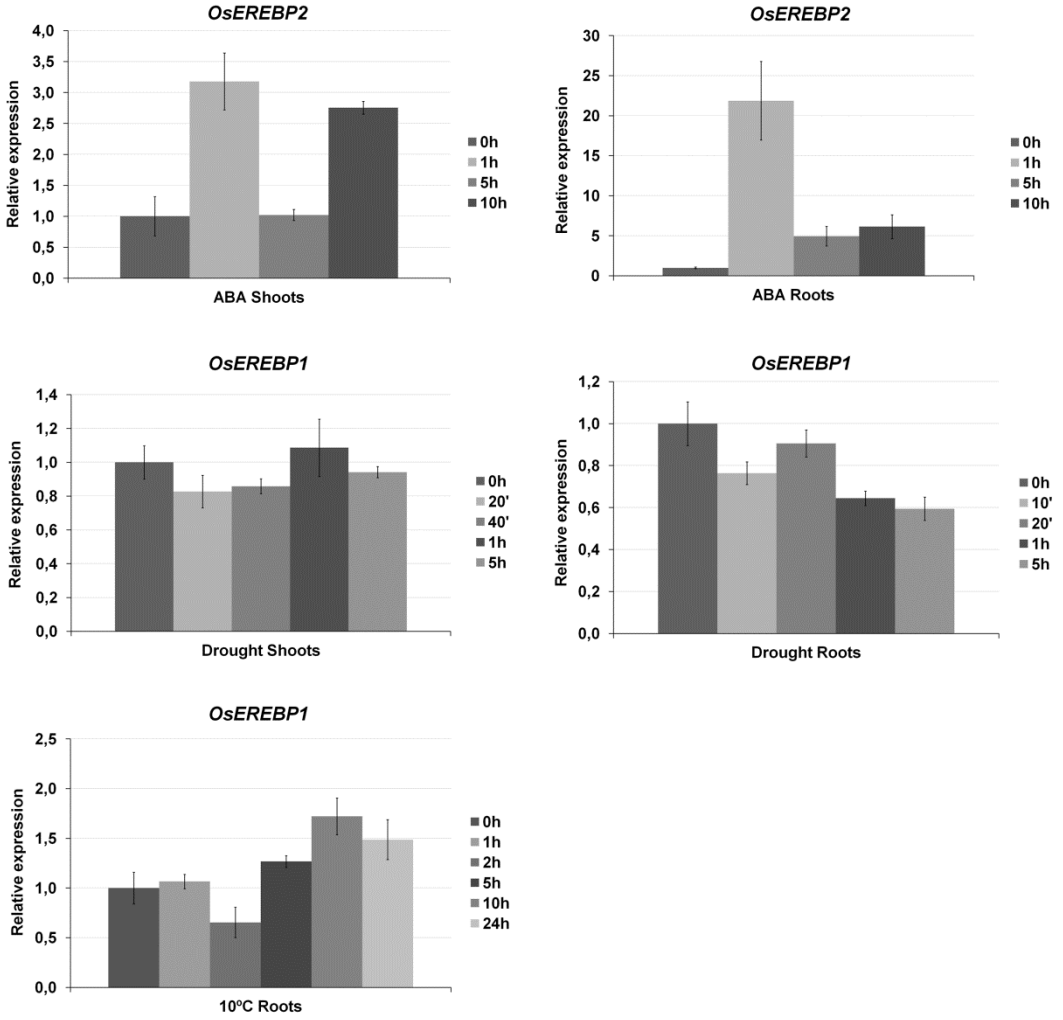


Figure S7. Validation of *OsEREBP1* and *OsEREBP2* gene expression in response to abiotic stress conditions. Quantitative PCR was performed with cDNA prepared from 1 µg of total RNA extracted from shoots and roots of 14-day-old rice seedlings (cv. Nipponbare) subjected to

drought, cold (10ºC) and ABA (100 µM). The data was normalized to the internal control 25S rRNA. Error bars represent standard deviation.

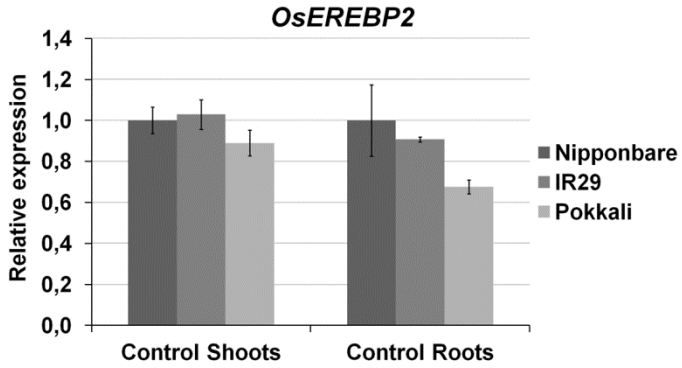


Figure S8. Analysis of *OsEREBP2* gene expression in Nipponbare, IR29 and Pokkali rice varieties under control conditions. Quantitative PCR was performed with cDNA prepared from 2 µg of total RNA extracted from shoots and roots of 11-day-old rice seedlings grown in control conditions. The data was normalized to the internal control 25S rRNA. Error bars represent standard deviation.