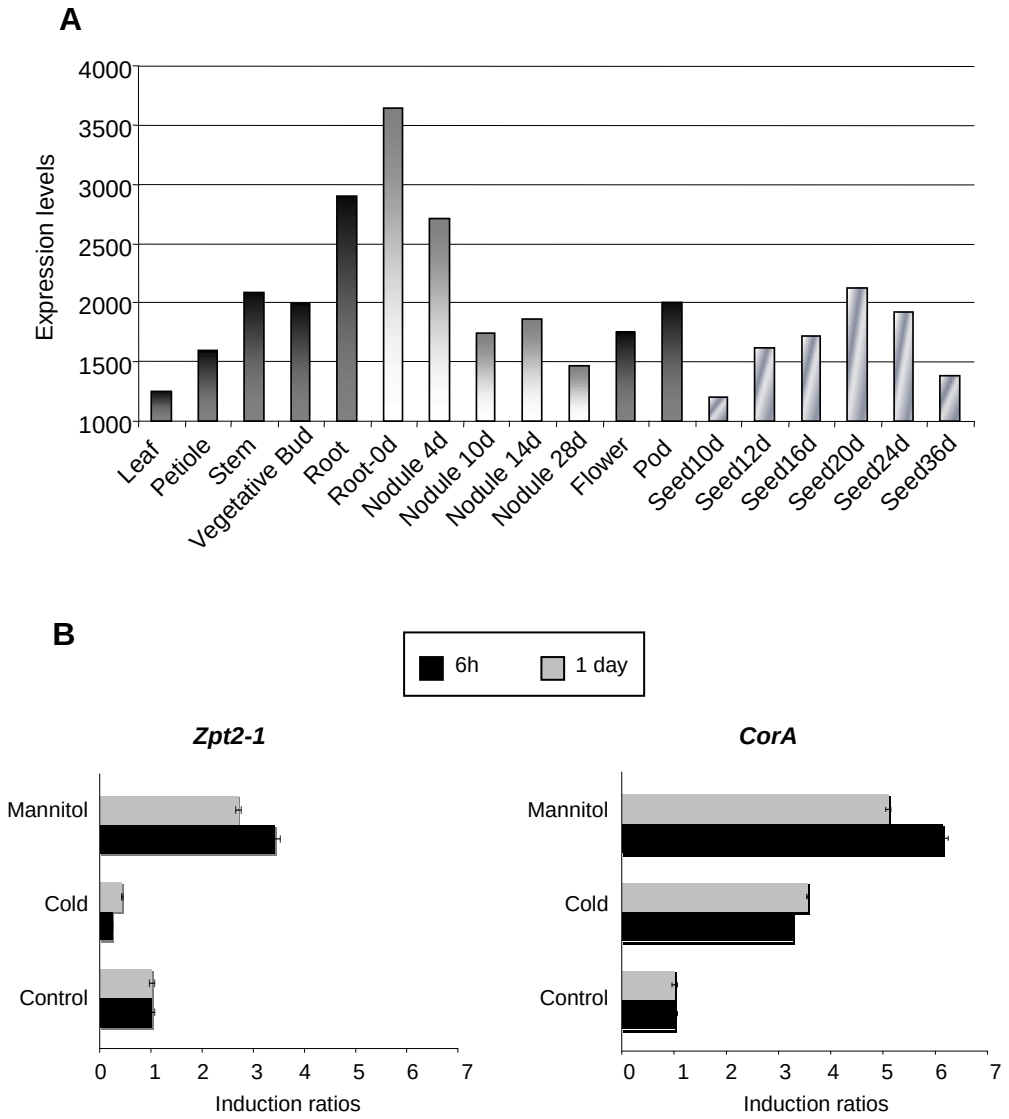
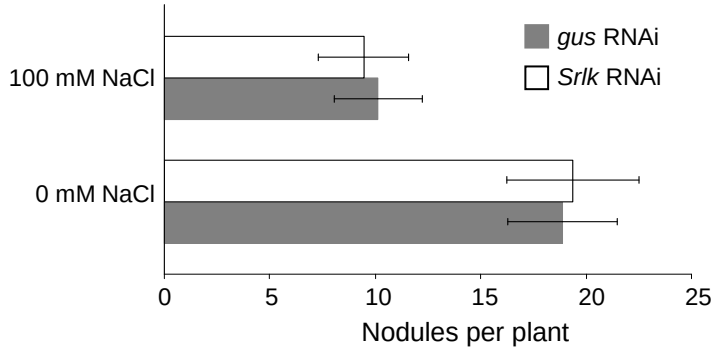
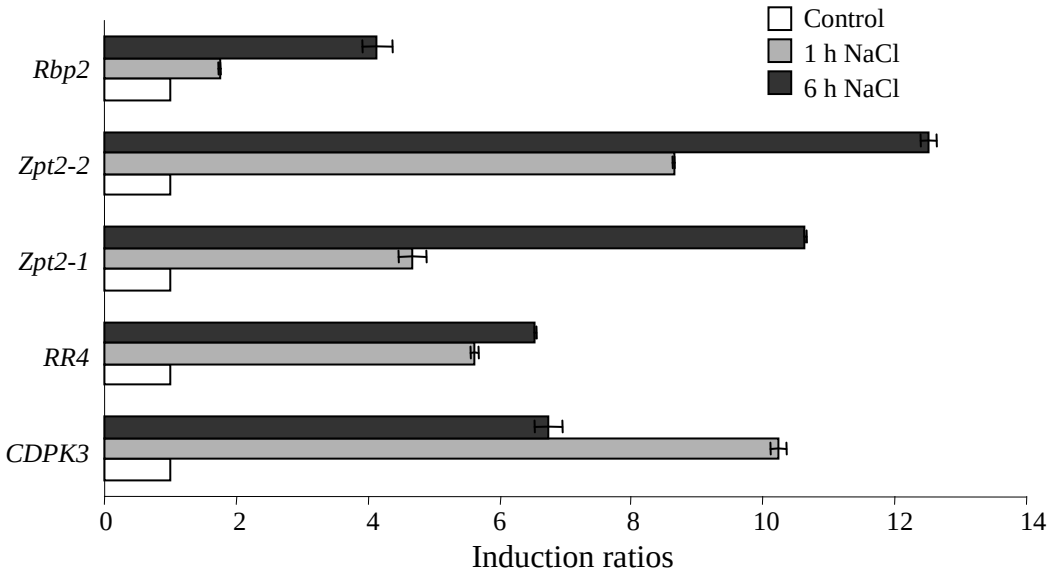
Supplemental Data. de Lorenzo et al. (2009). A novel plant LRR receptor kinase regulates the response of *Medicago truncatula* roots to salt stress



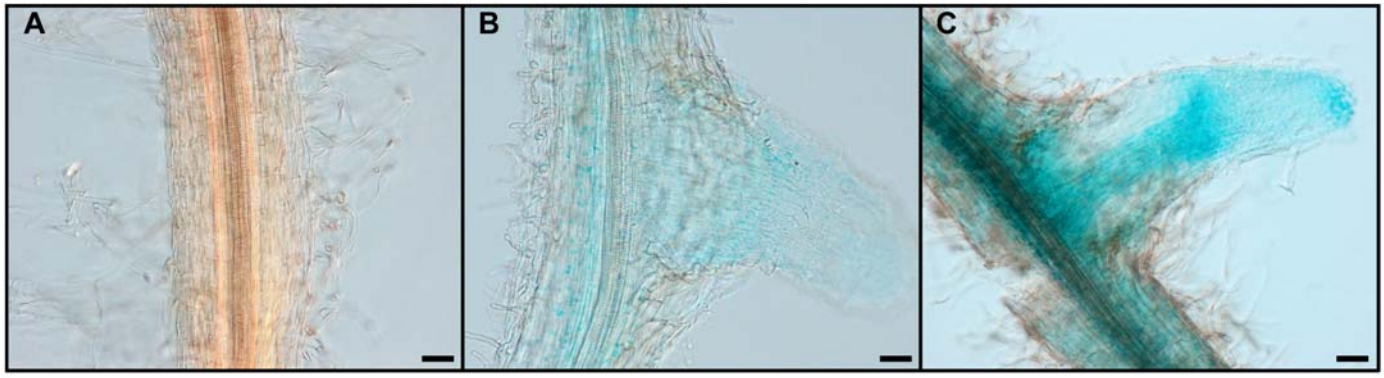
Supplemental Figure 1. (A) Expression levels of *Srlk* gene in *Medicago* organs (roots, nodules, stems, petioles, leaves, vegetative buds, flowers, seeds and seed pods) with detailed developmental time-series for nodules and seeds. Expression data was obtained from the *Medicago truncatula* Transcriptome Investigator (MtTI) Web Server (http://bioinfo.noble.org/gene-atlas/) using the gene expression atlas for *Medicago truncatula* based on Affymetrix Medicago Gene Chip® Genome Array as described by Benedito et al. (2008). **(B)** Expression levels of *Zpt2-1* (left chart) and *CorA* (right chart) genes in response to mannitol and cold stress (300 mM and 4 ºC, respectively). *M. truncatula* Jemalong A17 roots (4-days grown) were transferred to each stress-induced medium for different times. Real time RT-PCR analysis of *Zpt2-1* and *CorA* expression in roots at 0, 6 h, and 1 day of treatment is shown. Histogram represents quantification of specific PCR amplification products for these genes normalised against the constitutive control *actin*11. Numbers on the X-axis indicate fold-induction of gene expression. A representative example out of two biological experiments is shown, and error bars represent standard deviation between three technical replicates.



Supplemental Figure 2. Evaluation of nodulation capacity of transgenic *gus*-RNAi and *Srlk*-RNAi *M. truncatula* roots. Total number of nodules per plant observed 21 d.p.i. with *S. meliloti* 2011 in *M. truncatula* roots expressing *gus*-RNAi and *Srlk*-RNAi constructs. Nodule numbers were determined in composite plants grown in control (0 mM of NaCl) or in salt-stress conditions (100 mM of NaCl). The Student's t-test has been used, $P < 0{,}001$ and $n > 30$.



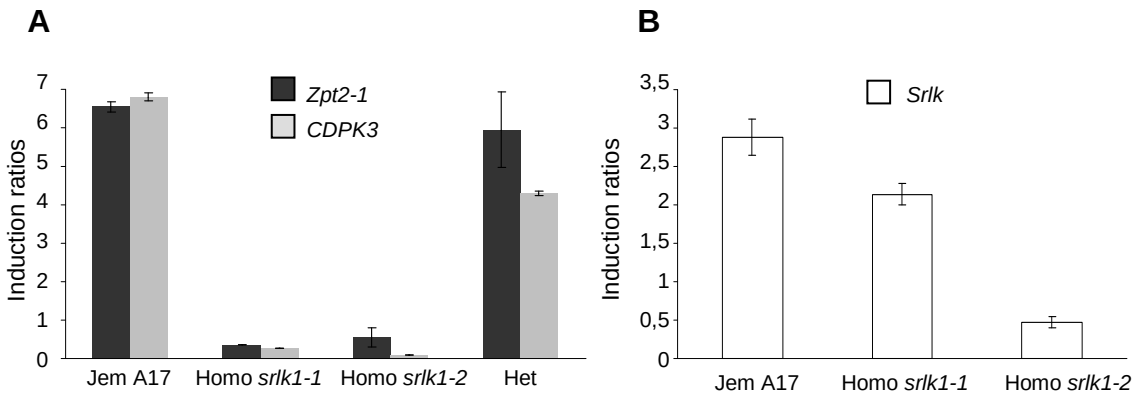
Supplemental Figure 3. Expression levels of different salt-related genes in response to short-term salinity treatments. The *M. truncatula* Jemalong A17 roots were grown in low-nitrogen liquid medium without salt for 4 days, and then transferred to the same medium supplemented with 150 mM of salt during different times. Real time RT-PCR analysis of *CDPK3*, *RR4*, *Zpt2-1*, *Zpt2-2* and *Rbp2* expression in roots at 0, 1 and 6h of treatment is shown. Histogram represents quantification of specific PCR amplification products for these genes normalised against the constitutive control *actin11*. Numbers above the X-axis scale indicate fold-induction of gene expression. The value of untreated roots (t = 0) was set to 1 as reference. A representative example out of two biological experiments is shown, and error bars represent standard deviation of three technical replicates.



Supplemental Figure 4. Whole mount stainings to show Expression of the *MtSrlk* promoter in lateral root apexes with or without salt (C and B, respectively) as well as a control carrying the vector without promoter (A).Scale Bars: 500 µm.

Jemalong A17 *srlk1-1* *srlk1-2*

Supplemental Figure 5. Effect of a long term NaCl treatment on *M. truncatula* Jemalong A17 and two mutant of *Srlk*. Five days-old plants of the two *Srlk* mutants and Jemalong A17 were grown in the presence of 0 and 100 mM of NaCl in an *in vivo* system. Representative pictures from *M. truncatula* Jemalong A17 and, *srlk1-1* and *srlk1-2* homozygous mutants taken 12 days after treatment with 100 mM of NaCl in growth chamber.



Supplemental Figure 6. Expression of salt-regulated genes in *Srlk* mutants in salt stress condition. Effect of *Srlk* mutation on the expression of early induced salt-responsive genes was determined in leaves treated with 150 mM NaCl during 6 h. RNA samples from *srlk1-1* and *srlk1-2* homozygous lines and their corresponding Jemalong A17 plant or heterozygous lines (control plants), respectively, were analysed by Q-RT-PCR. Histograms represent the expression levels of each gene in TILLING lines in relation to the control plants. The genes studied were *CDPK3* and *Zpt2-1* in salt stress conditions **(A)**, and the expression levels of *Srlk* were also verified in the same condition **(B)**. Values were normalised against the actin gene.

Supplemental Table 1. Sequences of oligonucleotides used for PCR and Quantitative RT-PCR.

Sequence name	Forward primer	Reverse primer
Srlk promoter	GGGGATCCTGGTTTTGAGGTTAAAGGGTTG	GGAATTCTCTAGAGGTACATCCATTTGTGTGTGAG
Srlk cDNA	*MtSrlk*A F: TACCTTCCTTCTCCTCACAC	*MtSrlk*A R: CCATACACATCCCCTTTC
	*MtSrlk*B F: CATTGCTCGTGCGAAAGATA	*MtSrlk*B R: CAAGGGAAATTCGTCATCGT
Srlk LC	TTCGCTTTCTGGGTCACTTT	AACTCCAAACCCAAGACACG
Srlk RNAi	GGGGACAAGTTTGTACAAAAAAGCAGGC TTTCGCTTTCTGGGTCACTTT	GGGGACCACTTTGTACAAGAAAGCTGGGT AACTCCAAACCCAAGACACG
Zpt2-1	AAGTCCGGAAAAGCCGGGAGG	GCACTTAACTCACCCACCACTGC
Zpt2-2	GGCAACGGACTTTCTACCTC	CTCCTCCATCAGCCACCGTG
Rbp2	GGTTGATAGAGTTATGAAGCGTG	CAAAAACTGGAGGCTCTTGC
RR4	GCTGAAGTTCACATGCCTGA	CAGCTCCACAGACCAAACAA
UidA (GUS)	GGCCAGCGTATCGTGCTGCG	GGTCGTGCACCATCAGCACG
CDPK3	AGCATAAAGCAATCCAGT	ACCAACCAAGCCTTCA

* The bases underlined in the primers sequences correspond to substitutions in the genomic sequence.