Suppl. Material

Aluminum-Specific Upregulation of *GmALS3* in the Shoots of Soybeans: A Potential Biomarker for Managing Soybean Production in Acidic Soil Regions

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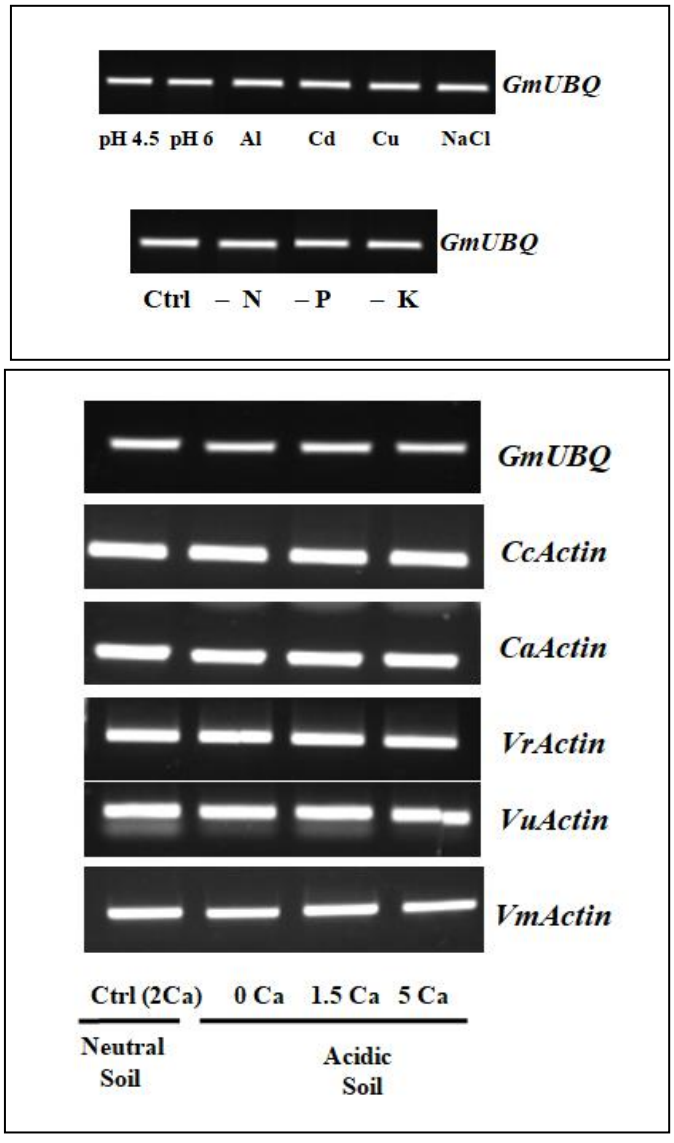
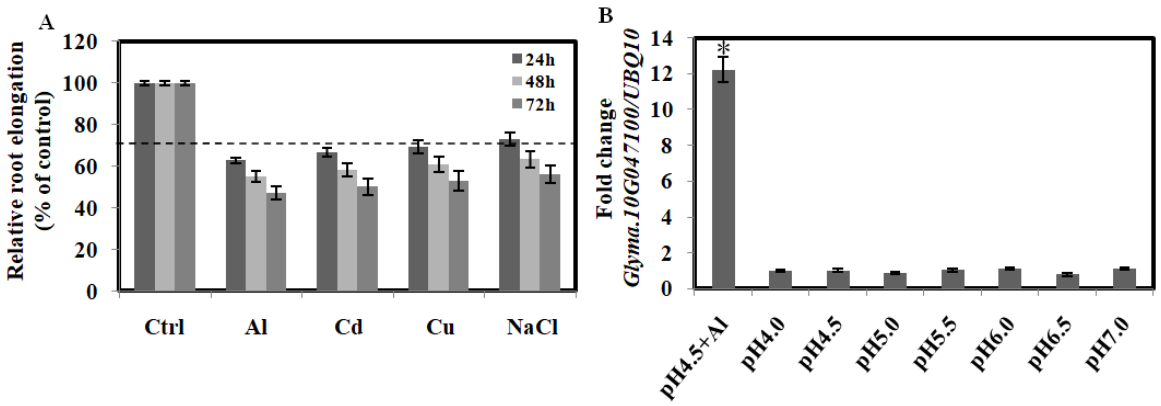
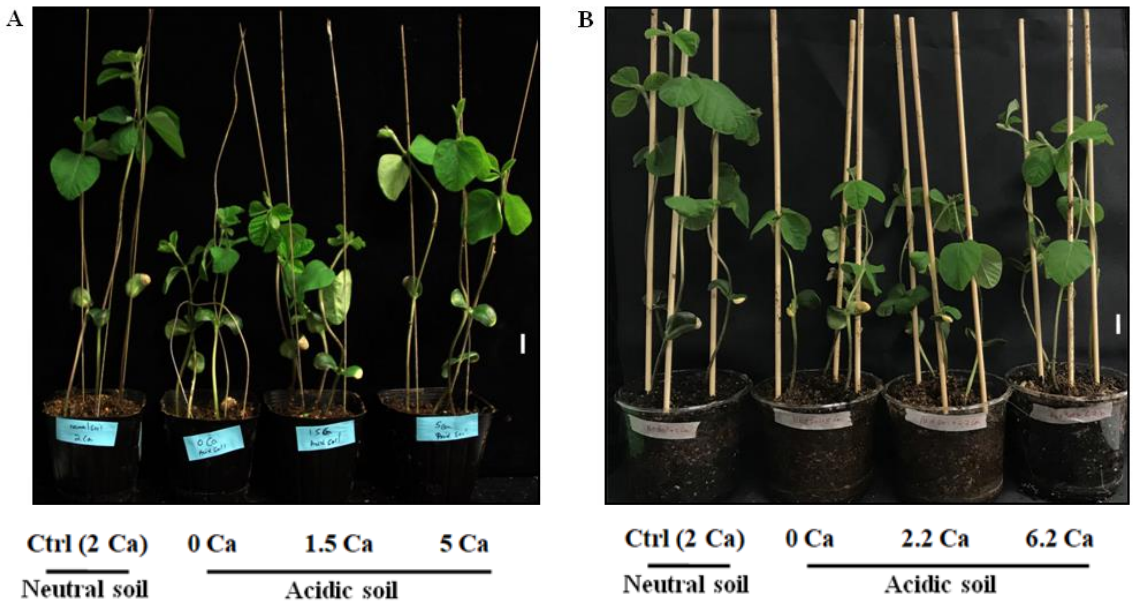


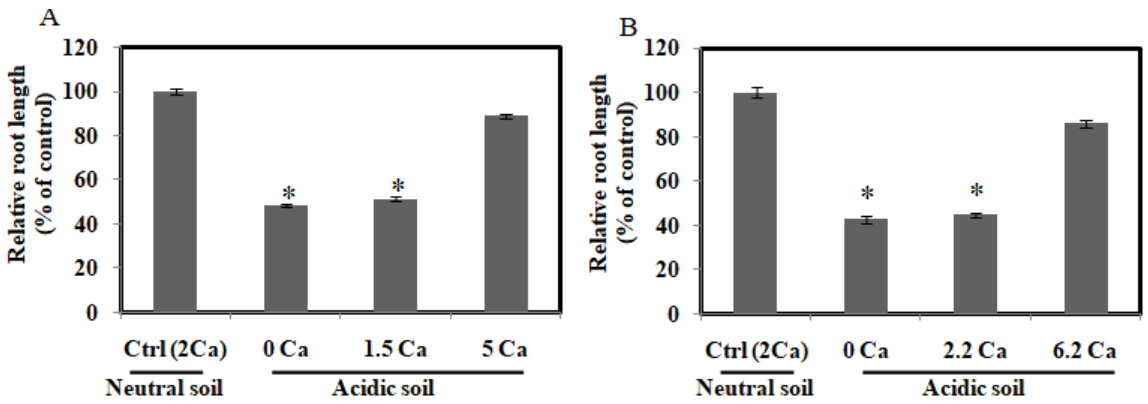
Suppl Fig.S1: Semi-quantitative PCR expression of internal standards used in this study to normalize the gene of interest in qRT-PCR. PCR products were separated by 3% agarose gel electrophoresis and visualized with GelRed staining.



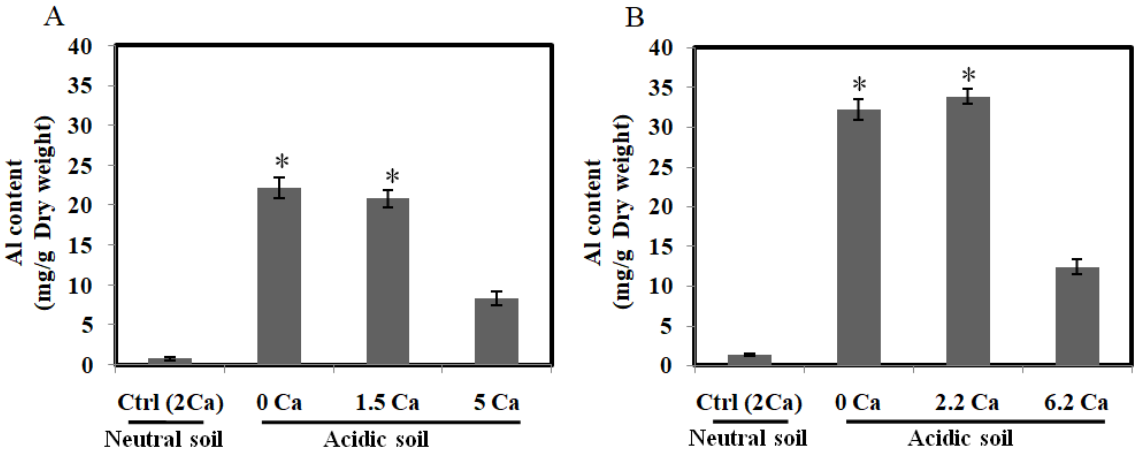
Suppl Fig.S2: Rhizotoxic ions effects on root growth and *GmALS3* expression in soybean. (A) Relative root elongation; soybean plants were exposed to rhizotoxic solutions containing $AlCl_3$ (50 µM), $CdCl_2$ (25 µM), $CuSO_4$ (10 µM), or NaCl (75 mM) at pH 4.5 for 24, 48, and 72 h. Values are the means ± SE (n=3). The dashed line indicates 30% inhibition of relative root elongation; (B) *GmALS3* expression in the shoots of soybean plants after exposure of the roots to nutrient solution with different pH for 24 h (+Al used as a positive control). The expression levels were quantified using real time PCR. *UBQ10* was used as an internal standard. Data are presented as the means ± SE (n=3). Asterisks indicate a significant difference compared with the treatment solution (-Al different pH) (Student's *t*-test, $P<0.05$).



Suppl Fig.S3: Soybean shoots growth inhibition in neutral and acidic soil. (A) Shoot growth observed after two weeks of artificial soils (generally used for altering the color of hydrangea flowers in Japan). Plants were grown on neutral soil (addition of 2 g $CaCO_3$ kg^{-1} soil) as a control and acidic soil with addition of different levels of liming (0, 1.5 and 5 g $CaCO_3$ kg^{-1} soil) as treatment; (B) Shoot growth observed after 2 weeks of soybean grown in Indian field soils. Plants were grown on neutral soil (addition of 2 g $CaCO_3$ kg^{-1} soil) as a control and acidic soil with addition of different levels of liming (0, 2.2, and 6.2 g $CaCO_3$ kg^{-1} soil) as treatment. Scale bar =1cm.



Suppl. Fig. S4: Soybean roots growth inhibition in neutral and acidic soil. (A) Relative root elongation of soybean in artificial soils. Plants were grown on neutral soil (addition of 2 g $CaCO_3$ kg^{-1} soil) as a control and acidic soil with addition of different levels of liming (0, 1.5 and 5 g $CaCO_3$ kg^{-1} soil) as treatment; (B) Relative root elongation of soybean grown in Indian field soils. Plants were grown on neutral soil (addition of 2 g $CaCO_3$ kg^{-1} soil) as a control and acidic soil with addition of different levels of liming (0, 2.2, and 6.2 g $CaCO_3$ kg^{-1} soil) as treatment. Mean and ± SE (n=3), asterisks indicate significant difference (Student's *t*-test, $P<0.05$) compared to neutral soil (Ctrl).



Suppl. Fig.S5: Al content of the roots of soybean plants grown in neutral and acidic soil. (A) Al contents of roots of soybean grown in artificial soils. Plants were grown for two weeks on neutral soil (addition of 2 g $CaCO_3$ kg^{-1} soil) as a control and acidic soil with addition of different levels of liming (0, 1.5 and 5 g $CaCO_3$ kg^{-1} soil) as treatment; (B) Al contents in the roots of soybean plants grown in Indian field soils. Plants were grown for 2 weeks in neutral soil (addition of 2 g $CaCO_3$ kg^{-1} soil) as a control and acidic soil with addition of different amounts of lime (0, 2.2 and 6.2 g $CaCO_3$ kg^{-1} soil) as treatments. Al concentration in the shoot was quantified using ICP-MS. Mean and ± SE (n=3), asterisks indicate the significance of difference (Student's *t*-test $P<0.05$) compared to neutral soil (Ctrl).

Suppl. Table S1: Sequence information of primers used for qRT-PCR

Gene ID		Primer Sequence (5′→3′)	
Glyma.10G047100	*(GmALS3)*	Fw	TGCGTGAAAATCACCAGAACA
Glyma.10G047100	*(GmALS3)*	Rv	GCAACAAGCCAAGAAATGAGAGA
Glyma.03G175800		Fw	TGTTGGCCAGCATTCTTCAC
Glyma.03G175800		Rv	GAACTCGAAATCACCGATTCA
Glyma.05G124000		Fw	GCTCATTTGCGCTTAATTACCA
Glyma.05G124000		Rv	CGTGGCACGTTAAATTCTCAG
Glyma.13G044600		Fw	TCCGGTACTTCCGAATTGAG
Glyma.13G044600		Rv	CCTGCCAAGTGAATCCTAGC
Glyma.03G222000		Fw	AAGGTTGGTGGGTCTGTTCA
Glyma.03G222000		Rv	TGGACATGGAATCTGGTTCA
Glyma.07G199900	*(GmUBQ10)*	Fw	GCAATTGGAGGATGGAAGGA
Glyma.07G199900	*(GmUBQ10)*	Rv	ACCACGAAGACGCAACACAA
Vigun01g155100	*(VuALS3)*	Fw	GCCAGCATTCTTCACCAAGG
Vigun01g155100	*(VuALS3)*	Rv	GGATGAATTTGAAATGAGGGAGTTG
Vigun01g124700	*(VuActin)*	Fw	CGCACACACATTCCTTCTTTC
Vigun01g124700	*(VuActin)*	Rv	CCTTGACCATTCCAGTTCCA
Ca_11996	*(CaALS3)*	Fw	GCAACACCGAGACAAGCAAC
Ca_11996	(*CaALS3*)	Rv	GGTGAAGCCCCTCCCATAA
Ca_22693	*(CaActin)*	Fw	GCCTGATGGACAGGTGATCAC
Ca_22693	*(CaActin)*	Rv	GGAACAGGACCTCTGGACATCT
MF377548	*(CcALS3)*	Fw	TTCCTGAAGGGCATGGTGAA
MF377548	*(CcALS3)*	Rv	GCCCTGAGAATGGCAACAAC
109802430	*(CcActin)*	Fw	GGCATACATTGCCCTTGACT
109802430	*(CcActin)*	Rv	GAACCTCGGGACATCTGAAA
106757328	*(VrALS3)*	Fw	TGGTCGACAACACGAAAACA
106757328	*(VrALS3)*	Rv	AATGGCTTCCAATGGTGAGG
106757568	*(VrActin)*	Fw	AGCATGAAGGCTGGCAAGA
106757568	*(VrActin)*	Rv	CAACCAAACCAAATCGGTAACA
KT693208	*(VmALS3)*	Fw	ACTGGTCTGATAATGGGAGGTG
KT693208	*(VmALS3)*	Rv	GACTTTGGTTTGGAGCTGATAGG
JZ078743	*(VmActin)*	Fw	GTTCTGTTCCAGCCATCCAT
JZ078743	*(VmActin)*	Rv	GTGGTGCGACAACCTTGATT
Glyma.01G190700	*(GmNRAMP1a)*	Fw	TCAAGAAGGGTTGACCCCAG
Glyma.01G190700	*(GmNRAMP1a)*	Rv	CTCCTGAAGGTACTGCCCTG

Glyma.11G051500 (GmNRAMP1b)	Fw	TCAAGGCAGGTTGATCCCAG
Glyma.11G051500 (GmNRAMP1b	Rv	TCTTGAAGGTACTGCCCTGC
Glyma.17G165200 (GmNRAMP2b)	Fw	GATAGTGGTGGTCGGAGTCG
Glyma.17G165200 (GmNRAMP2b)	Rv	AGGTCCCCCTCTAAGTTTCCA

Fw= Forward primer, Rv= Reverse primer

Suppl. Table S2: Efficiency of designed primer pairs used for qRT-PCR amplification

Gene	Tm(^{0}C)	PCR efficiency (%)	Coefficient of determination(R2)
GmALS3	79.14	109.67	0.995
Glyma.03G175800	79.03	102.43	0.992
Glyma.05G124000	78.97	96.72	0.997
Glyma.13G044600	78.62	98.38	0.989
Glyma.03G222000	79.42	93.65	0.984
GmUBQ10	78.23	110.49	0.998
CaALS3	81.31	102.85	0.993
CcALS3	80.26	103.59	0.986
VrALS3	79.51	104.52	0.982
VmALS3	80.24	100.69	0.992
VuALS3)	79.61	104.28	0.997
CaActin	79.28	103.47	0.994
CcActin	81.61	112.31	0.992
VrActin	78.87	107.68	0.997
VmActin	81.11	106.24	0.993
VuActin	80.39	108.78	0.995
GmNRAMP1a	81.18	96.75	0.991
GmNRAMP1b	78.13	98.13	0.993
GmNRAMP2b	81.26	98.17	0.996

Table S3. Changes in shoots, fresh, and dry weight of soybeans grown in artificial and natural soils

Soil type	Soil condition	CaCO3 (g kg-1 soil)	Fresh weight (g)	% of inhibition	Dry weight (g)	% of inhibition
Artificial	Neutral Soil	2	3.136 ± 0.37	0	0.623 ± 0.11	0
	Acidic Soil	0	1.723 ± 0.19*	45.06	0.252 ± 0.03*	59.55
	Acidic Soil	1.5	1.761 ± 0.24*	43.85	0.261 ± 0.04*	58.10
	Acidic Soil	5	2.812 ± 0.27^{ns}	10.33	0.538 ± 0.06^{ns}	13.64
Field (Natural)	Neutral Soil	2	4.061± 0.25	0	0.718 ± 0.07	0
	Acidic Soil	0	2.131 ± 0.30*	47.52	0.271 ± 0.02*	62.25
	Acidic Soil	2.2	2.174 ± 0.21*	46.46	0.283 ± 0.04*	60.58
	Acidic Soil	6.2	3.462 ± 0.32^{ns}	14.75	0.603 ± 0.03^{ns}	16.01

Asterisks indicate significant differences (Student's *t*-test, $P<0.05$) compared to neutral soil (as a control), ns=not significant at 0.05 level. Mean ± SE; n=3).

Table S4: Changes in roots, fresh, and dry weight of soybeans grown in artificial and natural soils

Soil type	Soil condition	CaCO3 (g kg-1 soil)	Fresh weight (g)	% of inhibition	Dry weight (g)	% of inhibition
Artificial	Neutral soil	2	0.613± 0.08	0	0.039 ± 0.004	0
	Acidic Soil	0	0.224 ± 0.04*	63.45	0.018 ± 0.001*	53.84
	Acidic Soil	1.5	0.229 ± 0.01*	62.64	0.020 ± 0.002*	48.71
	Acidic Soil	5	0.554 ± 0.05^{ns}	9.62	0.033 ± 0.001^{ns}	15.38
Field (Natural)	Neutral soil	2	0.655 ± 0.04	0	0.057 ± 0.002	0
	Acidic Soil	0	0.235 ± 0.03*	64.12	0.023 ± 0.003*	59.64
	Acidic Soil	2.2	0.238 ± 0.07*	63.66	0.025 ± 0.002*	56.14
	Acidic Soil	6.2	0.578 ± 0.05^{ns}	11.75	0.046± 0.005^{ns}	19.29

Asterisks indicate significant differences (Student's *t*-test, $P<0.05$) compared to neutral soil (as a control), ns=not significant at 0.05 level. Mean ± SE; n=3).