

Aluminum tolerance mechanisms in Kenyan maize germplasm are independent from the citrate transporter ZmMATE1

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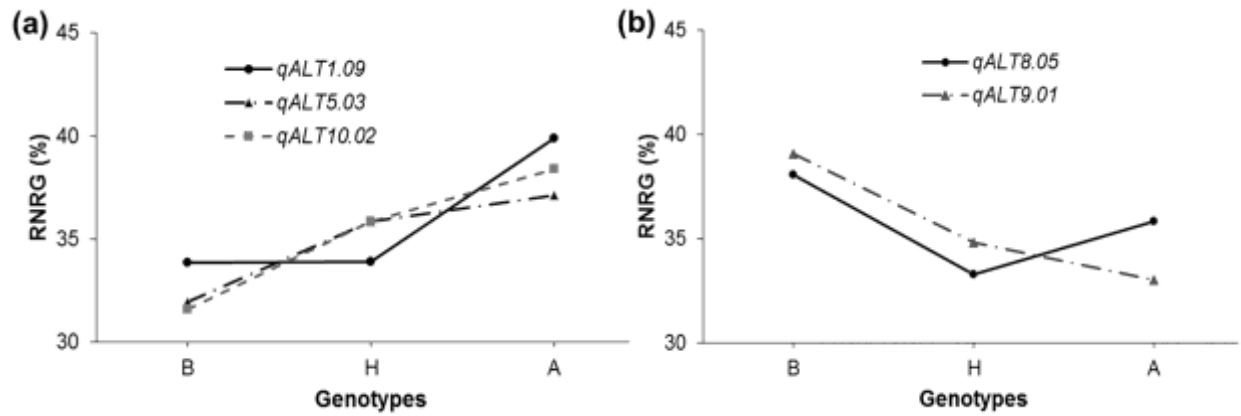
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Supplementary material



Supplementary Figure S1. Relative net root growth (RNRG %) mean for F_{2:3} progeny showing genotypic classes of the AI tolerance QTLs alleles. Progeny homozygous for the allele donated by the AI-sensitive parent, SCH3, are shown in B, whereas progeny homozygous B for the AI-tolerant parent, 203B-14, are shown in A. Means for heterozygous are shown in H. **(a)** QTLs with favorable allele derived from 203B-14. **(b)** QTLs with favorable allele derived from SCH3.

Supplementary Table S1. Variance analysis for relative net root growth in the F_{2:3} families derived from the cross 203B-14 x SCH3.

Source of Variation	D.F.^a	M.S.^b	F^c
Experiments	5	1650.29	157.32**
Replicates/Experiments	12	26.86	2.56*
Checks x Experiments	5	103.66	9.88**
Families adjusted	183	715.48	68.11**
Checks	1	50912.11	4853.39**
Families	176	357.79	34.11**
Checks x Families	6	2841.73	270.90**
Residual	376	10.49	
Families Mean		35.21	
Coefficient of genetic variation (CVg%)		20.24	
Coefficient of experimental variation (CVe%)		8.82	
Broad sense heritability (H^2)		0.97	

^a D.F. degrees of freedom

^b M.S. mean square

^c *,** Significant at $p \leq 0.05$ and 0.01 , respectively.

Supplementary Table S3. Maize predicted proteins similar to the Al tolerance genes previously characterized in plants (*ZmMATE1*, *OsNrat1*, *SbWRKY1* and *OsART1*). Percent of identity is based protein sequence alignment and genomic positions are in base pairs (bp).

Al Tolerance Gene	Gene ID ^a	Identity (%)	Chromosome	Position (bp)
ZmMATE1 (GRMZM5G870170)	GRMZM2G163154 (ZmASL)	56.4	1	27,099,590
	GRMZM5G890665	43.3	2	153,549,513
	GRMZM2G080450	40.3	3	57,084,587
	GRMZM2G065154 (ZmMATE3)	21.9	5	71,724,077
	GRMZM2G170128 (ZmMATE2)	21.8	5	20,622,821
OsNrat1 (Os02g03900)	GRMZM2G168747 (ZmNrat1)	81.8	5	74,616,918
	GRMZM2G147560	60.3	7	26,963,762
	GRMZM2G366919	52.6	2	170,851,138
	GRMZM2G069198	49.5	5	43,146,138
SbWRKY1 (Sb09g023500)	GRMZM2G034421 (ZmWRKY)	80.1	8	118,496
	GRMZM2G003551	30.3	9	18,108,182
	GRMZM2G004060	25.4	10	12,108,516
OsART1 (Os12g0170400)	GRMZM2G068710 (ZmART1)	59.4	10	10,146
	GRMZM2G075956	48.6	8	165,627
	GRMZM2G129428	46.8	3	174,504

^a Gene names in parenthesis were given by Krill *et al.* (2010) for *ZmASL*, Maron *et al.* (2010) for *ZmMATE2* and the others genes are described in this current work.

References

Krill, A. M., Kirst, M., Kochian, L. V., Buckler, E. S. & Hoekenga, O. A. Association and linkage analysis of aluminum tolerance genes in maize. *Plos One*, e9958 (2010).

Maron, L. G. et al. Two functionally distinct members of the MATE (multi-drug and toxic compound extrusion) family of transporters potentially underlie two major aluminum tolerance QTLs in maize. *Plant J.* **61**, 728-40 (2010).

Supplementary Table S4. Sequence of the primers used for the expression analysis using quantitative RT-PCR of each candidate gene mapped within the AI tolerance QTLs.

Gene ID	Name	Primer Sequence 5'-3'
GRMZM2G065154	<i>ZmMATE3</i>	F - ATGACGTCCAAGGTGGCATT R- TGATGGGCTGCAAGCGTTAT
GRMZM2G168747	<i>ZmNrat</i>	F - CGCGCTTCTGATCCAAACA R - GCGAGATGCTTGCCTGTCTT
GRMZM2G034421	<i>ZmWRKY</i>	F - AGCTCATCCCGGAGCCTAA R - GGAGAGCGACGACTCCATAG
GRMZM2G068710	<i>ZmART1</i>	F - CCCGTCACTGAAGAACTCGT R - TCGCATTAGGCACTAGGCTC
<i>18S rRNA</i>		F - CGTCCTAGTCTCAACCATAAACG R - CCCCAGGAACCCAAAGACT