SUPPLEMENTARY DATA

TABLE S1. Transformation of wheat with *TaALMT1*

Exp. no.	Number of embryos	Embryo age (days after anthesis)	Recovered plants	Positive for transgene	Efficiency
1	255	14	2	1	0.39%
2	117	14	1	1	0.85%
3	56	14	0	-	0%
4	125	14/15	0	-	0%
5	95	15	8	6	6.31%
6	145	15/16	7	5	3.44%
7	83	16	0	-	0%
Total	876		18	13	
Mean					1.5%

Shown for each of the experiments are the number of embryos bombarded, the number of recovered plants, the number of recovered plants shown to contain the transgene by PCR and the calculated efficiency of transformation.

TABLE S2. Scoring T_2 families for Al^{3+} resistance

Families of T2_1 line	**1_1**	**1_2**	**1_6**	**1_7**	**1_8**	**1_9**	**1_11**	**1_13**	**1_14**	**1_19 (null)**
No. of resistant seedlings	10	10	19	8	15	11	13	11	13	0
No. of sensitive seedlings	6	6	0	6	0	6	4	6	4	20
Total seedlings	16	16	19	14	15	17	17	17	17	20
Probably homozygous	No	No	Yes	No	Yes	No	No	No	No	Negative control
Families of T2_2B line	**2B_1**	**2B_2**	**2B_3**	**2B_4**	**2B_7**	**2B_8**	**2B_9**	**2B_10**	**2B_13**	**2B_19 (null)**
No. of resistant seedlings	20	12	13	15	16	11	13	16	16	0
No. of sensitive seedlings	0	7	7	5	4	7	7	4	4	19
Total seedlings	20	19	20	20	20	18	20	20	20	19
Probably homozygous	Yes	No	No	No	No	No	No	No	No	Negative control
Families of T2_3 line	**3_1**	**3_2**	**3_3**	**3_4**	**3_5**	**3_6**	**3_7**	**3_8**	**3_9**	**3_20 (null)**
No. of resistant seedlings	17	19	19	21	18	18	16	16	14	0
No. of sensitive seedlings	4	0	2	0	2	0	4	4	6	20
Total seedlings	21	19	21	21	20	18	20	20	20	20
Probably homozygous	No	Yes	No	Yes	No	Yes	No	No	No	Negative control
Families of T2_4 line	**4_1**	**4_2**	**4_3**	**4_4**	**4_5**	**4_6**	**4_7**	**4_8**	**4_9**	**4_13 (null)**
No. of resistant seedlings	14	19	16	20	15	20	10	18	15	0
No. of sensitive seedlings	7	0	8	0	6	0	11	2	2	20
Total seedlings	21	19	24	20	21	20	21	20	17	20
Probably homozygous	No	Yes	No	Yes	No	Yes	No	No	No	Negative control
Families of T2_5 line	**5_1**	**5_2**	**5_3**	**5_4**	**5_5**	**5_6**	**5_7**	**5_8**	**5_9**	**5_15**
No. of resistant seedlings	10	19	19	19	0	0	19	15	19	20
No. of sensitive seedlings	10	0	0	0	0	0	5	2	1	0
Total seedlings	20	19	19	19	0	0	24	17	20	20
Probably homozygous	No	Yes	Yes	Yes	missing	missing	No	No	No	Yes
Families of T2_8 line	**8_1**	**8_2**	**8_3**	**8_4**	**8_5**	**8_7**	**8_9**	**8_11**	**8_15**	**8_16 (null)**
No. of resistant seedlings	16	15	17	15	21	14	12	11	16	0
No. of sensitive seedlings	3	3	5	1	0	6	4	5	0	20
Total seedlings	19	18	22	16	21	20	16	16	16	20
Probably homozygous	No	No	No	No	Yes	No	No	No	Yes	Negative control
Families of T2_12 line	**12_1**	**12_2**	**12_3**	**12_4**	**12_5**	**12_6**	**12_7**	**12_8**	**12_9**	**12_19(null)**
No. of resistant seedlings	12	19	13	12	16	12	18	17	13	0
No. of sensitive seedlings	4	0	3	3	2	3	0	3	5	18
Total seedlings	16	19	16	15	18	15	18	20	18	18
Probably homozygous	No	Yes	No	No	No	No	Yes	No	No	Negative control
Families of T2_14 line	**14_1**	**14_2**	**14_3**	**14_4**	**14_5**	**14_6**	**14_7**	**14_8**	**14_9**	**14_19(null)**
No. of resistant seedlings	19	18	16	20	19	16	20	16	20	0
No. of sensitive seedlings	4	3	3	0	4	7	0	3	0	20
Total seedlings	23	21	19	20	23	23	20	19	20	20
Probably homozygous	No	No	No	Yes	No	No	Yes	No	Yes	Negative control
Families of T2_18 line	**18_1**	**18_2**	**18_3**	**18_4**	**18_7**	**18_9**	**18_11**	**18_12**	**18_15**	**18_18(null)**

No. of resistant seedlings	20	19	13	16	15	11	20	19	14	0
No. of sensitive seedlings	0	1	7	4	4	4	0	0	4	20
Total seedlings	20	20	20	20	19	15	20	19	18	20
Probably homozygous	Yes	No	No	No	No	No	Yes	Yes	No	Negative control
Families of T2_20A	**20A_1**	**20A_2**	**20A_4**	**20A_6**	**20A_8**	**20A_9**	**20A_11**	**20A_13**	**20A_15**	**20A_18(null)**
No. of resistant seedlings	20	16	19	18	20	0	17	20	0	0
No. of sensitive seedlings	0	0	0	1	0	20	0	0	19	16
Total seedlings	20	16	19	19	20	20	17	20	19	16
Probably homozygous	Yes	Yes	Yes	No	Yes	No	Yes	Yes	No	Negative control

Ten seeds from each of nine T_1 transgenic lines were grown to yield. PCR tests had previously established that nine of these T_1 seed contained the transgene and one was a null segregant. The seed generated from each of the T_1 plants comprised a T_2 family that was either segregating for the transgene or homozygous for the transgene. Between 16 and 24 seed from each of the nine T_2 families for each transgenic line were grown in 70 mL of nutrient solution (pH 4.3) with 30 μM $AlCl_3$ on a platform shaker (100 rpm). Solutions were replaced daily. Aluminum resistance of each seedling in each family was scored after 5 d by assessing root length and by examining the root apices for tissue damage under a dissecting microscope. Likely homozygous families were identified as those in which all seedlings had good root growth and undamaged root apices.

FIG. S1 Relationship between *TaALMT1* expression and malate efflux in the T_1 and T_2 lines. *TaALMT1* expression was measured by qRT–PCR using the endogenous *GAPDH* gene as a reference in plants of the (A) T_1 and (B) T_2 generations. Since the data were collected over several experiments, the expression data are presented here relative to the parental line BW26 included to account for the variation between experiments. Expression data for the T_1 lines represent the mean and s.e. of biological replicates (n = 3) and for T_2 it represents the mean of technical replicates (n = 3). The Al^{3+}-activated malate efflux was measured from excised root apices in the presence of 50 μM $AlCl_3$. Malate efflux was only detected in the presence of Al^{3+} treatment and data show means and standard errors (n = 4). Open circles represent each of the (A) T_1 and (B) T_2 lines and the open triangle in (B) represents a null-segregant T2_1-19. Filled circles represent ET8 and closed triangles represent BW26.

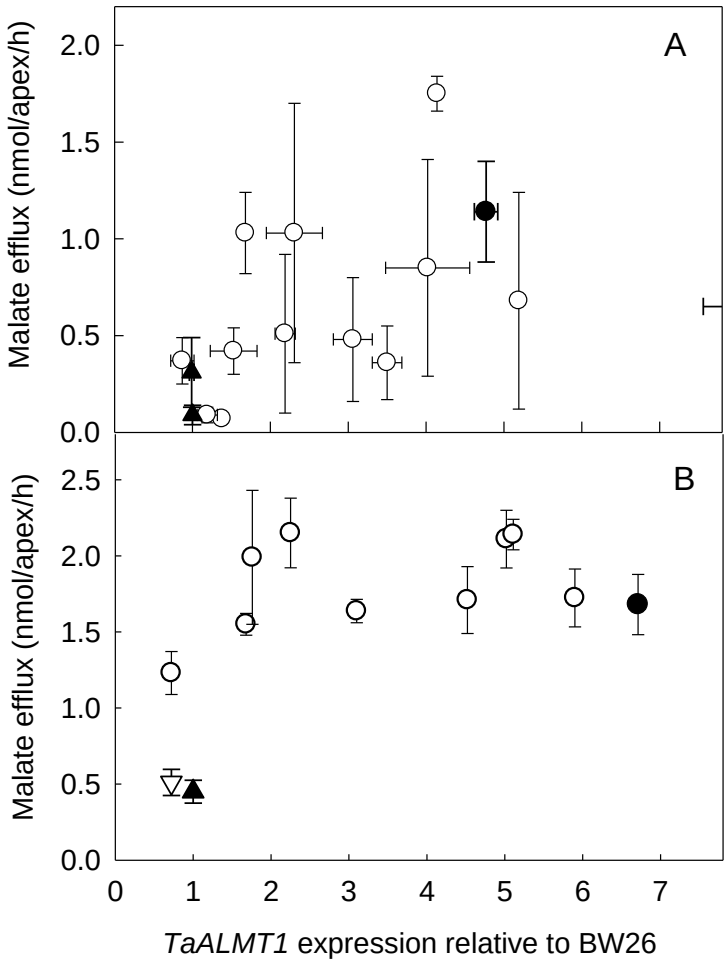

FIG. S2. Southern analysis of the T_2 transgenic plants. Genomic DNA (10 µg) isolated from the T_2 lines identified as being probably homozygous for the transgene (based on an Al^{3+} resistance assay) was digested with *Bam*HI, run on an agarose gel and transferred to a membrane filter using the alkaline blotting method. The membrane was probed with a labelled PCR product targeting the maize ADH intron region of the plasmid. The column numbers show the following lines: (BW26) Bob White 26 (parent line); (1) T2_1.6; (2) T2_2B.1; (3) T2_3.4; (4) T2_4.4; (5) T2_5.4; (6) T2_8.5; (7) T2_12.7; (8) T2_18.1; (9) T2_20A.8. Size markers are included on the right-hand side and the two arrows on the left-hand side show bands cross-reacting with the control parental line BW26.