Supplementary Materials for

Polyploids exhibit higher potassium uptake and salinity tolerance in Arabidopsis

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This PDF file includes:

Materials and Methods
Figs. S1, S2
Tables S1, S2
Captions for databases S1 to S4

Other Supplementary Materials for this manuscript includes the following:

Databases S1 – S4 (separate files)
Additional Data table S1. ICP-MS data for plant samples described in fig. 1 G and fig. S2.
Additional Data table S2. Survival data for plants described in fig. 2 A.
Additional Data table S3. ICP-MS data for plant samples described in fig. 2 B & C.
Additional Data table S4. Seed count data for plants described in fig. 2 E & F.

Materials and Methods

Plant growth conditions and treatments

Plants for elemental analysis were grown in a controlled environment as previously described (*2*) unless specially indicated. For experiments assessing survival after NaCl treatment plants were grown in potting mix and irrigated as needed with 0.25-strength Hoagland solution as previously described (*2*). After 10 days, plants were irrigated weekly with 0.25-strength Hoagland solution supplemented with 200 mM NaCl and the time in days for the above ground portion of each plant to become 100% necrotic after first NaCl treatment recorded as the days-to-death. For experiments assessing fitness after NaCl treatment through seed production two diploid and two tetraploid plants were grown in potting mix in the same pot and irrigated with 0.25-strength Hoagland solution as needed with 5 – 6 pots of plants grown per genotype. After one week plants were irrigated weekly with 0.25-strength Hoagland solution supplemented with 100 mM NaCl. Treatment with NaCl was continued until all plants had finished setting seed. As individual plants matured seeds were collected and pooled by cytotype for each pot, separated from the siliques and counted.

Elemental analysis

The leaf tissue concentration of Li, B, Na, Mg, P, S, K, Ca, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Rb, Sr, Mo and Cd, were measured by inductively couple plasma mass spectrometry (ICP-MS) as described previously (*2*). All samples were normalized to calculated weights, as determined with a heuristic algorithm using the best-measured elements, the weights of seven weighed samples, and the solution concentrations, and the method and validation data sets are presented at www.ionomicshub.org.

QTL analysis

QTL analysis was performed using WinQTL Cartographer 2.5 with the composite interval mapping method (*8*). The genotypic map and the ploidy level for each RIL was obtained from published sources (*3,4*). Ploidy level was treated as a genetic marker, and a pseudo-linkage group for ploidy was created by adding 4 pseudo-linkage markers for ploidy. The median value of the concentration of each of the quantified elements was used as a trait. 1000 permutations per trait were run to determine the significance level (P=0.01) of the logarithm of the odds ratio (LOD).

Statistical analysis

Variation in seed production was analysed using a lognormal Poisson generalised linear mixed model (GLMM) with treatment, cytotype, genotype and all interactions between these variables as fixed effects and the pot in which the plants were grown as a random effect. Pot was included as a random effect to account for between pot variability not accounted for by the explanatory variables. A lognormal Poisson GLMM was used in preference to a Poisson GLMM in order to better account for the overdispersed nature of these data. The significance of the fixed effect parameter estimates was assessed using likelihood ratio tests between nested models and model adequacy was determined using standard residual plots. All analysis was conducted using R (Version 2.14.2) (*9*). Both linear and generalised linear mixed models were fitted using the lme4 package in R (Version 0.999375-42) (*10*).

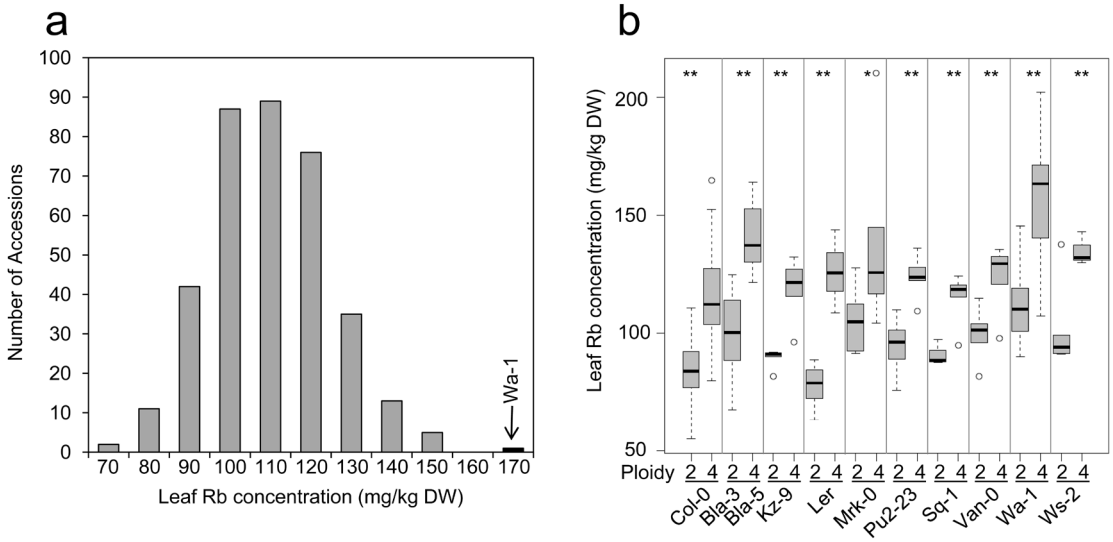

Fig. S1. Ploidy contributes to Rb accumulation in *A. thaliana* leaves. (**A**) Leaf Rb concentration among 349 accessions. Arrow and back bar indicate the tetraplod, Wa-1. (**B**) Box-plots (the minimum, 1st quartile, median, 3rd quartile and maximum with data > 1.5 interquartile ranges denoted with circles) for leaf Rb concentration in natural accessions and artificial diploid or tetraploid plants. Asterisks (**B**) indicate the significance of pair-wise comparisons (Student's *t*-test * $p < 0.05$; ** $p < 0.01$). Data collected from 6 - 12 biological replicates for each accession and cytotype. All leaf Rb concentration data is accessible using the digital object identifier (DOI) 10.4231/T9H41PBV and 10.4231/T93X84K7.

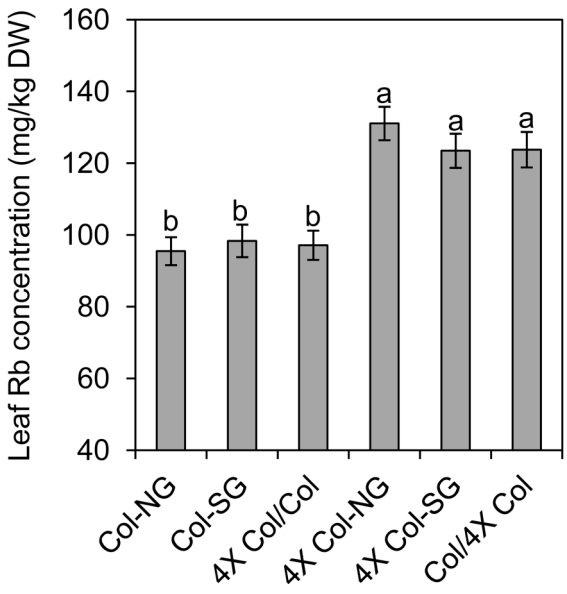

Fig. S2. Reciprocal grafting establishes that elevated leaf Rb in tetraploids is driven by the ploidy of the root. Leaf Rb concentration of grafted diploid and tetraploid plants. NG, non-grafted; SG, self-grafted; Col/4xCol, grafted with Col-0 as scion and 4xCol-0 as root stock; 4xCol/Col, grafted with 4xCol-0 as scion and Col-0 as root stock. Letters above bars indicate statistically different groups (one-way ANOVA with groupings by Tukey's HSD with a 95% confidence interval). 2x, diploid; 4x, tetraploid. Data collected from 14 - 18 biological replicates and represented as mean ± standard error.

Table S1.

Arabidopsis thaliana accessions and their ploidy.

Name or Stock No.	Native Name	Origin	Ploidy
11ME1-32	11ME1.32	USA	2x
11PNA4-101	11PNA4.101	USA	2x
328PNA054	328PNA054	USA	2x
627ME-4Y1	627ME-4Y1	USA	2x
CS76409	Agu-1	Spain	2x
Alc-0	Alc-0	Spain	2x
ALL1-2	ALL1-2	France	2x
ALL1-3	ALL1-3	France	2x
CS28013	Alst-1	UK	2x
CS28014	Amel-1	Netherlands	2x
An-1	An-1	Belgium	2x
CS28017	An-2	Belgium	2x
CS28018	Ang-0	Belgium	2x
CS28049	Ann-1	France	2x
App1-16	App1-16	Sweden	2x
CS28053	Ba-1	UK	2x
Bå1-2	Bå1-2	Sweden	2x
CS28054	Baa-1	Netherlands	2x
CS22756	Bak-2	Kaukasus	2x
CS22760	Bak-7	Kaukasus	2x
CS28063	Be-1	Germany	2x
Belmonte-4-94	Belmonte-4-94	Sweden	2x
CS28064	Benk-1	Netherlands	2x
Bg-2	Bg-2	USA	2x
CS28081	Bla-3	Spain	2x
CS28083	Bla-5	Spain	4x
CS28090	Blh-2	Czechoslovakia	2x
CS28091	Boot-1	UK	2x
Bor-4	Bor-4	Czech	2x
Brö1-6	Brö1-6	Sweden	2x
CS28097	Bs-2	Switzerland	2x
Bu-0	Bu-0	Germany	2x
CS28108	Bu-8	Germany	2x
BUI	BUI	France	2x
CS28128	Ca-0	Germany	2x
CAM-16	CAM-16	France	2x
CAM-61	CAM-61	France	2x
CS76410	Cdm-0	Spain	2x

CS28133	Cha-0	Switzerland	2x
CS28135	Chat-1	France	2x
CS28140	CIBC-2	UK	2x
CS28141	CIBC-4	UK	2x
CS76360	Ciste-2	Italy	4x
CLE-6	CLE-6	France	2x
CS28163	Co-2	Portugal	2x
CS28165	Co-4	Portugal	2x
CS28193	Com-1	France	2x
CS28181	CSHL-5	USA	2x
CUR-3	CUR-3	France	2x
CS28201	Da(1)-12	Czechoslovakia	2x
CS28200	Da-0	Germany	2x
CS28890	Del-10	East Europe	2x
CS28208	Di-1	France	2x
CS22698	Dog-4	Kaukasus	2x
CS76411	Don-0	Spain	2x
CS28214	Dra-2	Czechoslovakia	2x
Dra3-1	Dra3-1	Sweden	2x
DraII-1	DraII-1	Czech	2x
DraIV 1-14	DraIV 1-14	Sweden	2x
DraIV 1-5	DraIV 1-5	Sweden	2x
DraIV 1-7	DraIV 1-7	Sweden	2x
DraIV 6-16	DraIV 6-16	Sweden	2x
DraIV 6-35	DraIV 6-35	Sweden	2x
Duk	Duk	Czech	2x
CS28217	Ede-1	Netherlands	2x
Eden-2	Eden-2	Sweden	2x
CS28241	Es-0	Finland	2x
Est-1	Est-1	Estonia	2x
CS76399	Ey15-2	Tuebingen	2x
Fei-0	Fei-0	Portugal	2x
CS28252	Fi-1	Germany	2x
Fjä1-1	Fjä1-1	Sweden	2x
Fjä1-2	Fjä1-2	Sweden	2x
Fjä1-5	Fjä1-5	Sweden	2x
CS28268	Fr-4	Germany	2x
CS28274	Ga-2	Germany	2x
Ge-0	Ge-0	Switzerland	2x
CS28279	Gel-1	Netherlands	2x
CS28280	Gie-0	Germany	2x

Got-7	Got-7	Germany	2x
CS28326	Gr-5	Austria	2x
CS28332	Gu-1	Germany	2x
Gul1-2	Gul1-2	Sweden	2x
CS28344	Hey-1	Netherlands	2x
CS28345	Hh-0	Germany	2x
CS76404	HKT2.4	Tuebingen	2x
CS28350	Hn-0	Germany	2x
Hod	Hod	Czech	2x
Hov4-1	Hov4-1	Sweden	2x
Hovdala-2	Hovdala-2	Sweden	2x
HR-5	HR-5	UK	2x
Hs-0	Hs-0	Germany	2x
HSm	HSm	Czech	2x
CS76373	ICE1	East Europe	2x
CS76423	ICE102	Italy	2x
CS76367	ICE104	Italy	2x
CS76365	ICE106	Italy	2x
CS76364	ICE107	Italy	2x
CS76361	ICE111	Italy	2x
CS76363	ICE112	Italy	2x
CS76424	ICE119	Italy	2x
CS76425	ICE120	Italy	2x
CS76385	ICE127	Central Asia	2x
CS76384	ICE130	Central Asia	2x
CS76383	ICE134	Central Asia	2x
CS76426	ICE138	Central Asia	2x
CS76379	ICE150	Central Asia	2x
CS76380	ICE152	Central Asia	2x
CS76381	ICE153	Central Asia	2x
CS76353	ICE163	Tyrol	2x
CS76357	ICE169	Tyrol	2x
CS76358	ICE173	Tyrol	2x
CS76354	ICE181	Tyrol	2x
CS76370	ICE21	East Europe	2x
CS76355	ICE212	Tyrol	2x
CS76351	ICE216	Tyrol	2x
CS76349	ICE226	Tyrol	2x
CS76419	ICE29	East Europe	2x
CS76372	ICE33	East Europe	2x
CS76369	ICE36	East Europe	2x

CS76347	ICE49	Spain	2x
CS76348	ICE50	Spain	2x
CS76377	ICE60	Russia	2x
CS76378	ICE61	Russia	2x
CS76420	ICE63	East Europe	2x
CS76371	ICE7	East Europe	2x
CS76421	ICE70	Russia	2x
CS76375	ICE71	Russia	2x
CS76374	ICE72	Russia	2x
CS76376	ICE73	Russia	2x
CS76422	ICE75	Russia	2x
CS76352	ICE79	Tyrol	2x
CS76362	ICE91	Italy	2x
CS76366	ICE92	Italy	2x
CS76368	ICE93	Italy	2x
CS76359	ICE97	Italy	2x
CS22730	Istisu-1	Kaukasus	2x
CS28364	Je-0	Germany	2x
JEA	JEA	France	2x
CS28369	Jl-3	Czechoslovakia	2x
CS28373	Jm-1	Czechoslovakia	2x
Ka-0	Ka-0	Austria	2x
CS22807	Kastel-1	East Europe	2x
KBS-Mac-8	KBS-Mac-8	USA	2x
CS28382	Kelsterbach-2	Germany	2x
Kelsterbach-4	Kelsterbach-4	Germany	2x
CS28394	Kl-5	Germany	2x
CS28395	Kn-0	Lithuania	2x
CS28407	KNO-11	USA	2x
Kno-18	Kno-18	USA	2x
CS22823	Koch-1	East Europe	2x
Köln	Köln	Germany	2x
CS28423	Krot-2	Germany	2x
Kulturen-1	Kulturen-1	Sweden	2x
LAC-3	LAC-3	France	2x
LAC-5	LAC-5	France	2x
CS22747	Lag2.2	Kaukasus	2x
LDV-25	LDV-25	France	2x
LDV-34	LDV-34	France	2x
LDV-58	LDV-58	France	2x
CS76413	Leo-1	Spain	2x

CS22712	Lerik1-3	Kaukasus	2x
CS28457	Li-5:2	Germany	2x
CS28459	Li-6	Germany	2x
CS28461	Li-7	Germany	2x
Liarum	Liarum	Sweden	2x
Lillö-1	Lillö-1	Sweden	2x
LI-OF-095	LI-OF-095	USA	2x
Lis-1	Lis-1	Sweden	2x
Lis-2	Lis-2	Sweden	2x
Lisse	Lisse	Netherlands	2x
Lom1-1	Lom1-1	Sweden	2x
Lp2-6	Lp2-6	Czech	2x
Lund	Lund	Sweden	2x
Map-42	Map-42	USA	2x
CS28490	Mc-0	UK	2x
CS76414	Mer-6	Spain	2x
MIB-15	MIB-15	France	2x
MIB-22	MIB-22	France	2x
MIB-28	MIB-28	France	2x
MIB-84	MIB-84	France	2x
MNF-Che-2	MNF-Che-2	USA	2x
MNF-Jac-32	MNF-Jac-32	USA	2x
MNF-Pot-48	MNF-Pot-48	USA	2x
MNF-Pot-68	MNF-Pot-68	USA	2x
MOG-37	MOG-37	France	2x
Mr-0	Mr-0	Italy	2x
Mz-0	Mz-0	Germany	2x
CS28510	N4	Russia	2x
CS28513	N7	Russia	2x
NC-6	NC-6	USA	2x
CS28917	Nemrut-1	Kaukasus	2x
NFA-10	NFA-10	UK	2x
NFA-8	NFA-8	UK	2x
CS28550	NFC-20	UK	2x
CS76402	Nie1-2	Tuebingen	2x
CS28564	No-0	Germany	2x
CS28568	Nok-1	Netherlands	2x
CS28573	Nw-0	Germany	2x
CS28575	Nw-2	Germany	2x
CS28578	Nz1	New Zealand	2x
CS28580	Ob-1	Germany	2x

CS28583	Old-1	Germany	2x
CS28587	Or-0	Germany	2x
CS28849	Ors-2	Romania	2x
Pa-1	Pa-1	Italy	2x
CS28595	Pa-2	Italy	2x
PAR-3	PAR-3	France	2x
PAR-4	PAR-4	France	2x
PAR-5	PAR-5	France	2x
Paw-3	Paw-3	USA	2x
CS76415	Ped-0	Spain	2x
Pent-1	Pent-1	USA	2x
Petergof	Petergof	Russia	2x
CS28610	PHW-10	UK	2x
CS28613	PHW-13	UK	2x
CS28614	PHW-14	UK	2x
CS28620	PHW-20	UK	2x
CS28622	PHW-22	UK	2x
CS28628	PHW-28	UK	2x
CS28631	PHW-31	UK	2x
CS28633	PHW-33	Netherlands	2x
PHW-34	PHW-34	France	2x
CS28635	PHW-35	France	2x
CS28636	PHW-36	France	2x
CS28637	PHW-37	France	2x
CS28645	Pn-0	France	2x
CS28650	Pog-0	Canada	2x
CS28651	Pr-0	Germany	2x
CS76416	Pra-6	Spain	2x
Pro-0	Pro-0	Spain	2x
Pu2-23	Pu2-23	Czech	2x
CS28663	Pu2-24	Czechoslovakia	2x
CS76417	Qui-0	Spain	2x
Rak-2	Rak-2	Czech	2x
Ren-1	Ren-1	France	2x
Rev-2	Rev-2	Sweden	2x
CS28685	Rhen-1	Netherlands	2x
ROM-1	ROM-1	France	2x
CS28692	Rou-0	France	2x
Rsch-4	Rsch-4	Russia	2x
CS76406	Rue3-1-31	Tuebingen	2x
CS28720	S96	Netherlands	2x

Sanna-2	Sanna-2	Sweden	2x
Sap-0	Sap-0	Czech	2x
CS28724	Sapporo-0	Japan	2x
Sav-0	Sav-0	Czech	2x
CS28729	Sei-0	Italy	2x
Sei-0	Sg-1	Germany	2x
CS28734	Sh-0	Germany	2x
CS76382	Sha	Central Asia	2x
CS28739	Si-0	Germany	2x
SLSP-30	SLSP-30	USA	2x
CS28743	Sp-0	Germany	2x
Sparta-1	Sparta-1	Sweden	2x
Sq-8	Sq-8	UK	2x
CS76400	Star-8	Tuebingen	2x
Ste-3	Ste-3	USA	2x
T1040	T1040	Sweden	2x
T1060	T1060	Sweden	2x
T1080	T1080	Sweden	2x
T1110	T1110	Sweden	2x
T510	T510	Sweden	2x
T540	T540	Sweden	2x
T620	T620	Sweden	2x
T690	T690	Sweden	2x
TÅD 01	TÅD 01	Sweden	2x
TDr-1	TDr-1	Sweden	2x
TDr-17	TDr-17	Sweden	2x
TDr-18	TDr-18	Sweden	2x
TDr-3	TDr-3	Sweden	2x
TDr-8	TDr-8	Sweden	2x
CS28759	Ting-1	Sweden	2x
CS28760	Tiv-1	Italy	2x
Tomegap-2	Tomegap-2	Sweden	2x
Tottarp-2	Tottarp-2	Sweden	2x
TOU-A1-115	TOU-A1-115	France	2x
TOU-A1-116	TOU-A1-116	France	2x
TOU-A1-12	TOU-A1-12	France	2x
TOU-A1-43	TOU-A1-43	France	2x
TOU-A1-62	TOU-A1-62	France	2x
TOU-A1-67	TOU-A1-67	France	2x
TOU-A1-96	TOU-A1-96	France	2x
TOU-C-3	TOU-C-3	France	2x

TOU-E-11	TOU-E-11	France	2x
TOU-H-12	TOU-H-12	France	2x
TOU-H-13	TOU-H-13	France	2x
TOU-I-17	TOU-I-17	France	2x
TOU-I-2	TOU-I-2	France	2x
TOU-I-6	TOU-I-6	France	2x
TOU-J-3	TOU-J-3	France	2x
TOU-K-3	TOU-K-3	France	2x
CS28779	Tscha-1	Austria	2x
CS76403	TueSB30-3	Tuebingen	2x
CS76401	Tuescha9	Tuebingen	2x
CS76407	TueV13	Tuebingen	2x
CS76405	TueWa1-2	Tuebingen	2x
CS28788	Uk-2	Germany	2x
UKID101	UKID101	UK	2x
UKID22	UKID22	UK	2x
UKID37	UKID37	UK	2x
UKID48	UKID48	UK	2x
UKID80	UKID80	UK	2x
UKNW06-059	UKNW06-059	UK	2x
UKNW06-060	UKNW06-060	UK	2x
UKNW06-386	UKNW06-386	UK	2x
UKNW06-436	UKNW06-436	UK	2x
UKNW06-460	UKNW06-460	UK	2x
UKSE06-062	UKSE06-062	UK	2x
UKSE06-192	UKSE06-192	UK	2x
UKSE06-272	UKSE06-272	UK	2x
UKSE06-278	UKSE06-278	UK	2x
UKSE06-349	UKSE06-349	UK	2x
UKSE06-351	UKSE06-351	UK	2x
UKSE06-414	UKSE06-414	UK	2x
UKSE06-466	UKSE06-466	UK	2x
UKSE06-482	UKSE06-482	UK	2x
UKSE06-520	UKSE06-520	UK	2x
UKSE06-628	UKSE06-628	UK	2x
UKSW06-202	UKSW06-202	UK	2x
Ull2-3	Ull2-3	Sweden	2x
Ull2-5	Ull2-5	Sweden	2x
Ull3-4	Ull3-4	Sweden	2x
CS28795	Utrecht	Netherlands	2x
Vår2-1	Vår2-1	Sweden	2x

CS22754	Vash-1	Kaukasus	2x
CS28800	Ven-1	Netherlands	2x
CS76418	Vie-0	Spain	2x
VOU-1	VOU-1	France	2x
VOU-2	VOU-2	France	2x
CS28808	Wag-3	Netherlands	2x
CS28809	Wag-4	Netherlands	2x
CS28810	Wag-5	Netherlands	2x
CS76408	WalhaesB4	Tuebingen	2x
CS28814	Wc-2	Germany	2x
CS28823	Ws	Russia	2x
Ws-0	Ws-0	Ukraine	2x
CS28833	Wt-3	Germany	2x
CS22703	Xan-1	Kaukasus	2x
CS22765	Yeg-1	Kaukasus	2x
Zdr-6	Zdr-6	Czech	2x
ZdrI 2-24	ZdrI 2-24	Sweden	2x
ZdrI 2-25	ZdrI 2-25	Sweden	2x
CS28847	Zu-1	Switzerland	2x

Table S2.

Model selection procedure and associated summary statistics used to analyse the seed production data with a lognormal Poisson GLMM. ΔAIC: change in Akaike Information Criterion between nested models, LRT: Likelihood ratio test statistic, DF: difference in degrees of freedom between nested models, P value: p value associated with model term removed. Model hierarchy was respected when considering terms to be removed and therefore all main effects contained in second-order interactions were retained. Nested models with different fixed effects were fitted using maximum likelihood.

Model	ΔAIC	LRT	DF	P value
Full model				
genotype + *cytotype* + *salinity* + *genotype:cytotype* + *cytotype:salinity* + *genotype:salinity* + *genotype:cytotype:salinity*	-	-	-	-
Model terms removed				
genotype:cytotype:salinity	- 3.87	0.14	2	0.934
genotype:cytotype	- 3.46	0.53	2	0.764
genotype:salinity	2.11	6.11	2	0.051
cytotype:salinity	28.03	30.03	1	< 0.001
genotype	22.08	26.08	2	< 0.001
Final model				
genotype + *cytotype* + *salinity* + *cytotype:salinity*	-	-	-	-

Additional Data table S1 (separate file)
ICP-MS data for plant samples described in fig. 1 G and fig. S2.

Additional Data table S2 (separate file)
Survival data for plants described in fig 2 A.

Additional Data table S3 (separate file)
ICP-MS data for plant samples described in fig 2 B & C.

Additional Data table S4 (separate file)
Seed count data for plants described in fig 2 E & F.