Salinity tolerance in chickpea is associated with the ability to 'exclude' Na from leaf mesophyll cells

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Supplementary data: Results S1, Figures S1 – S10, Tables S1 – S14

Supplementary Results S1

Shoot growth

In the controls (non-saline), shoot dry weight (DW) continued to rise in both genotypes (Fig. S3A) with an average shoot RGR for the two genotypes in non-saline conditions during the treatment period of 0.17 g g^{-1} d^{-1} (Fig. S3B). Imposition of 30 mM NaCl did not significantly affect shoot RGR of Genesis836 (0.16 g g^{-1} d^{-1}) but reduced shoot RGR of Rupali to 0.13 g g^{-1} d^{-1}. As a result, for the plants in 30 mM NaCl at the end of treatment period shoot DW was 81% of control in Genesis836 and 28% of control in Rupali (Fig. S3A). At 60 mM NaCl, Genesis836 grew with a shoot RGR of 0.14 g g^{-1} d^{-1} and the final shoot DW at 21 d of treatment was 41% of control. By contrast, for Rupali at 60 mM NaCl leaves showed severe damage and shoot RGR declined to 0.004 g g^{-1} d^{-1} during the last several days and final shoot DW was only 5% of the controls.

Net Na^+, Cl^- and K^+ transport rates from roots to shoots

The net Na^+, Cl^-, and K^+ transport rates were quantified between 0-5, 5-8 and 8-11 d of treatments (Table S8). For non-saline control plants, Na^+ transport rates were low for all periods with 0.2 µmol g^{-1} FW_{root} d^{-1} for both genotypes. The imposition of 30 mM NaCl treatment increased Na^+ transport rate from roots to shoots of both genotypes with an average of 8.7 µmol g^{-1} FW_{root} d^{-1} for 0-5 and 8-11 d, and 1.9 µmol g^{-1} FW_{root} d^{-1} for 5-10 d. In 60 mM NaCl treatment, Na^+ transport rate increased for both genotypes to about 14.8 µmol g^{-1} FW_{root} d^{-1} at 0-5 d, and 4.3 µmol g^{-1} FW_{root} d^{-1} for 5-8 d. At 8-11 d of treatments, Na^+ transport rates significantly increased in Rupali to 31.3 µmol g^{-1} FW_{root} d^{-1}, when compared with 14.5 µmol g^{-1} FW_{root} d^{-1} in Genesis836.

Similarly, to Na^+, Cl^- transport rates for non-saline plants were low in all periods for both genotypes with 1.0 µmol g^{-1} FW_{root} d^{-1}. At 30 mM NaCl, Cl^- transport rates increased in both

genotypes to about 11.7 µmol g^{-1} FW_{root} d^{-1}. In the 60 mM NaCl, the Cl^- transport rate increased to an average of 16.4 and 26.3 µmol g^{-1} FW_{root} d^{-1} in Genesis836 and Rupali, respectively.

Potassium transport rates from roots to shoots for Genesis836 were similar in controls and the two NaCl treatments for all periods with an average 13.2 µmol g^{-1} FW_{root} d^{-1}. In Rupali, K^+ transport rates were on average 20.4 µmol g^{-1} FW_{root} d^{-1} for 0-5 and 5-8 d in controls and the two NaCl treatments, and then 13.9 µmol g^{-1} FW_{root} d^{-1} between 8-11 d.

Supplementary Fig. S1 – S10

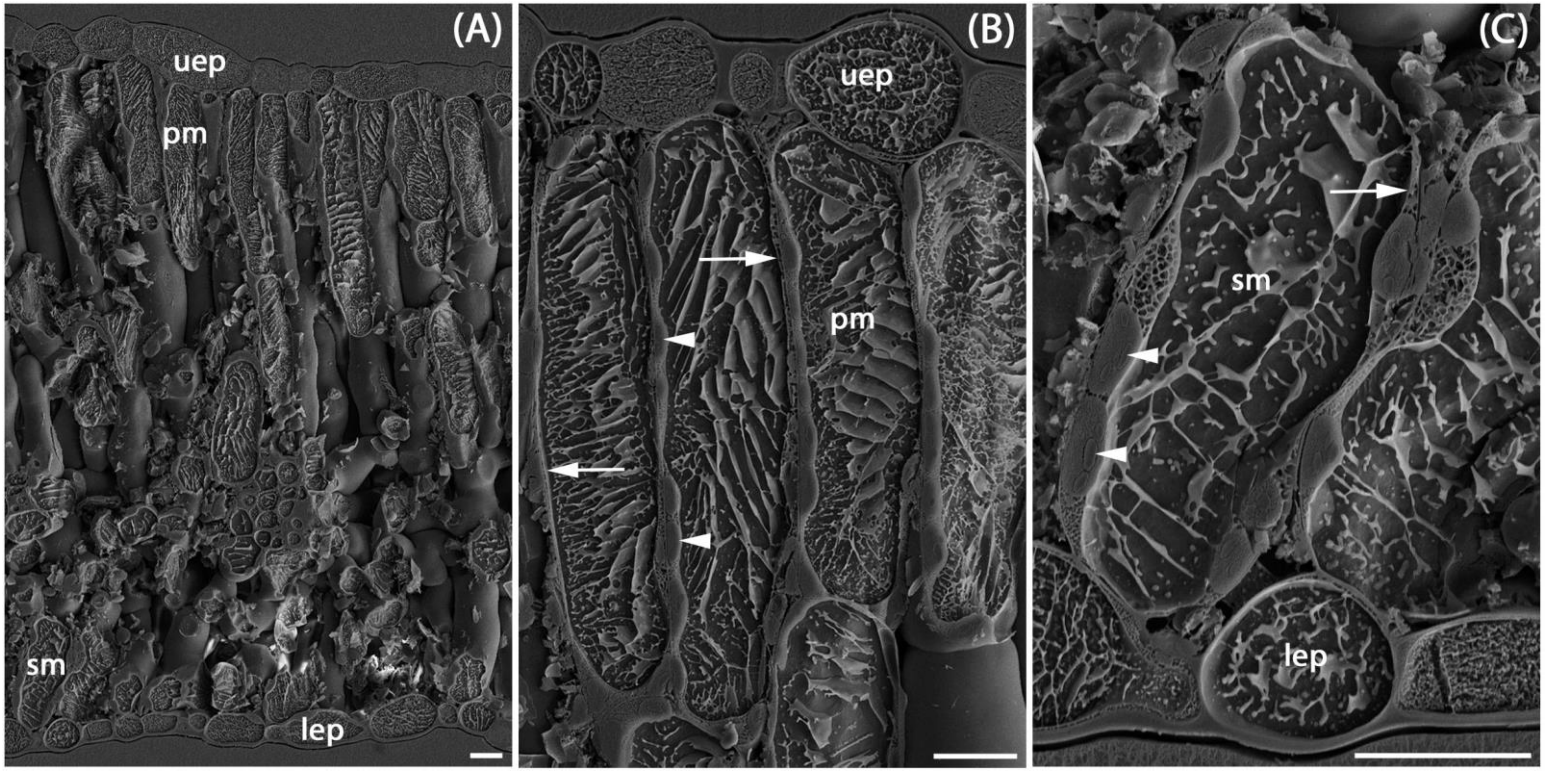

Figure S1. Examples of typical cryo-SEM micrographs of cryo-fixed, cryoplaned and lightly sublimated transverse sections of leaflets of Genesis836 grown with 60 mM NaCl, with labels indicating typical cell types that were analysed for cellular ion concentrations. The central vacuole occupies most of the interior of the cells, thus, the reported measurements of Na, Cl, and K concentrations would largely reflect vacuolar concentrations (see Results). Arrow-heads in the higher magnification micrographs of palisade mesophyll (B) and spongy mesophyll (C) indicate chloroplast 'pushed' by vacuoles to the periphery of the cells. Arrows indicate tonoplasts (vacuolar membrane). uep, upper epidermis; pm, palisade mesophyll; sm, spongy mesophyll; lep, lower epidermis. Scale bars = 10 µm.

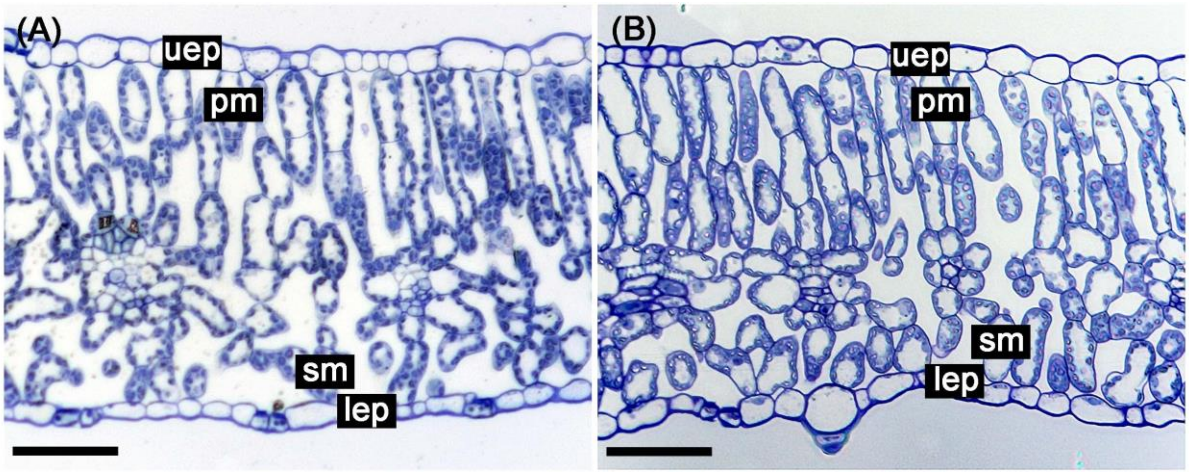

Figure S2. (A, B) Cross-section of the lamina of leaflets of Genesis836 (A) and Rupali (B) indicating typical cell types that were analysed to give cellular concentration profiles. Plants were grown in aerated non-saline nutrient solution for the final 18 d after treatments had been imposed. The sections were stained with toluidine blue O. uep, upper epidermis; pm, palisade mesophyll; sm, spongy mesophyll; lep, lower epidermis. Scale bars = 50 µm.

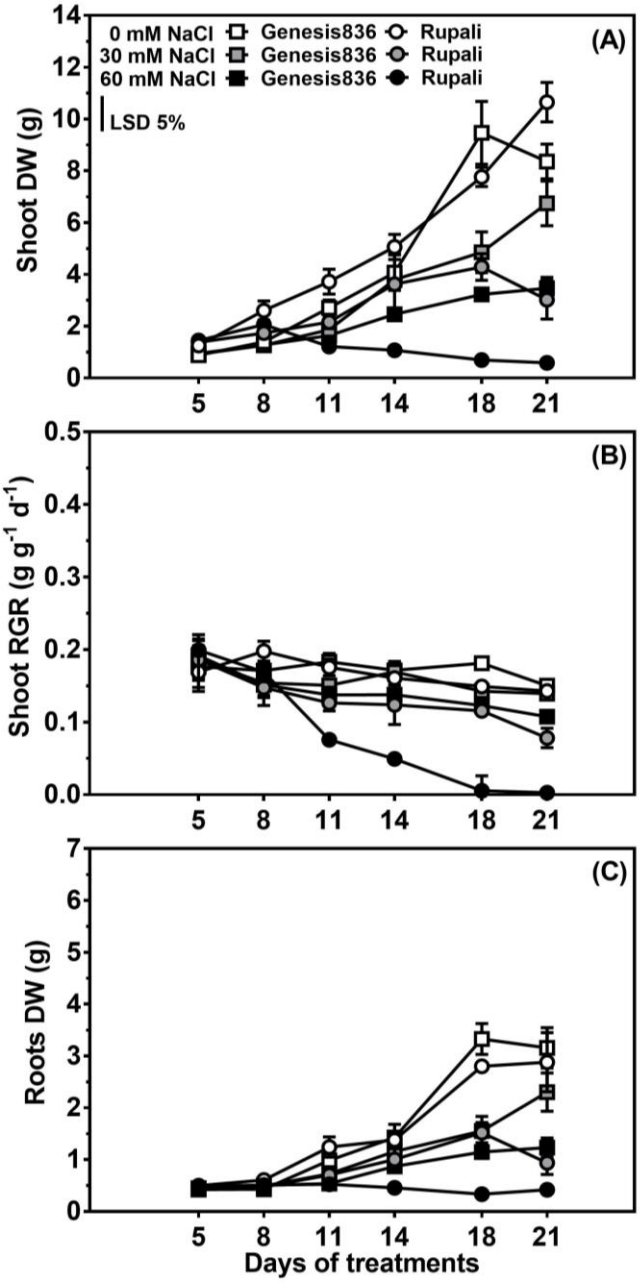

Figure S3. Dry weight of shoot (A), relative growth rate (RGR) of shoot (B) and dry weight of roots (C) of two chickpea genotypes (Genesis836 and Rupali) grown in aerated nutrient solution with 0 (non-saline control), 30 or 60 mM NaCl. Treatments were imposed on 23-d-old plants and samples were taken at 0 (initial harvest), 5, 8, 11, 14, 18 and 21 d of treatments. Data are means ± SE of four replicates. The shoot DW at initial harvest did not differ between genotypes with average value of 0.44 g (two-sample t-test). There was a significant genotype × treatment × days of treatment interaction for shoot dry weight ($P < 0.001$) but the interaction was non-significant for shoot RGR ($P = 0.158$) and roots dry weight ($P = 0.224$; three-way ANOVA). Bars represent least significant difference (LSD) at $P < 0.05$ for genotype × treatment × days of treatment interaction. Additional statistical analyses in Table S2.

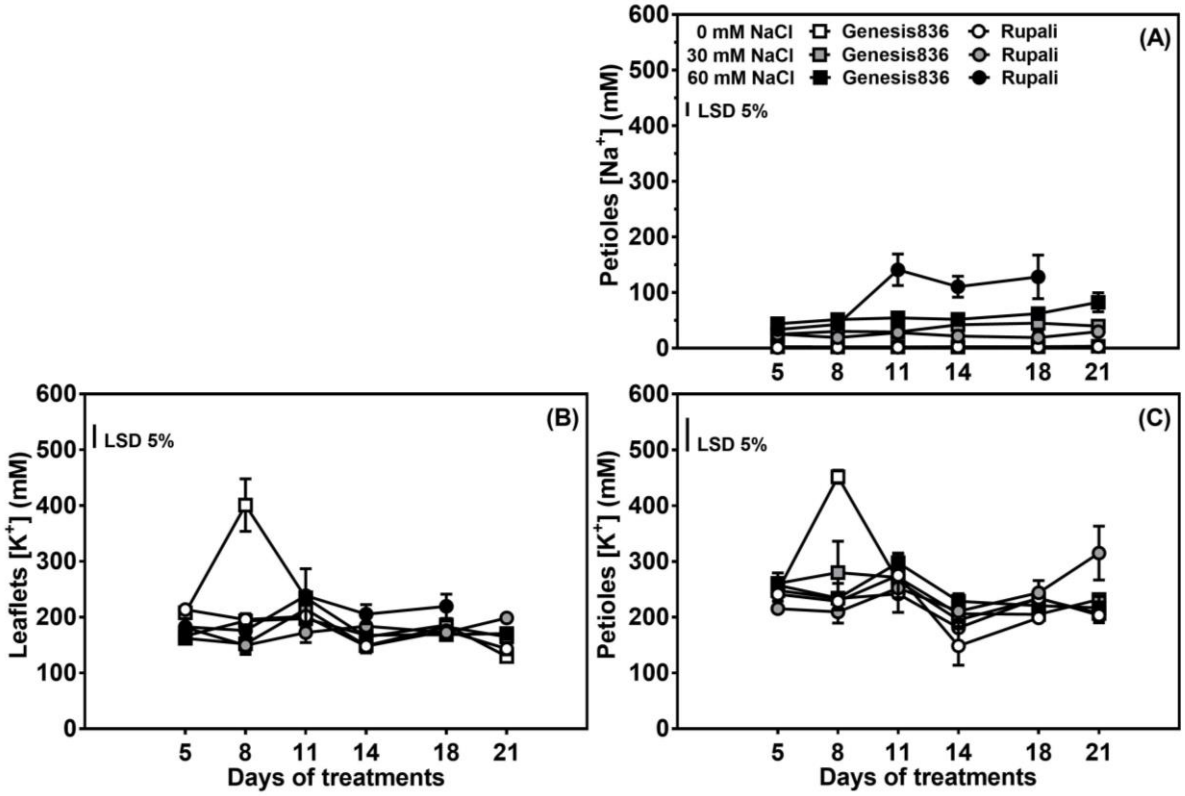

Figure S4. Concentrations (mM, water basis) of Na^+ in petioles (A) and K^+ in leaflets (B) and petioles (C) of the youngest fully-expanded leaves that were measured for gas exchange (see Fig. 5). Concentrations of Na^+ in leaflets are presented in Fig. 1. Leaflets and petioles which were in the LiCor chamber were excised following gas exchange measurements and analysed for Na^+ and K^+. The two chickpea genotypes (Genesis836 and Rupali) were grown in aerated nutrient solution with 0 (non-saline control), 30 or 60 mM NaCl. Treatments were imposed on 23-d-old plants and the measurements were taken at 5, 8, 11, 14, 18 and 21 d of treatments. Data are means ± SE of four replicates. For Rupali at 60 mM NaCl, there were only three replicates at 18 d as the other replicate plant had no green leaves, and no green leaves remaining at 21 d. Three-way ANOVA (data up to 18 d of treatments) showed significant genotype × treatment × days of treatment interaction for concentrations of Na^+ petioles and for K^+ in leaflets and petioles (all at $P < 0.001$). Bars represent least significant difference (LSD) at $P < 0.05$ for genotype × treatment × days of treatment interaction. Additional statistical analyses in Table S3.

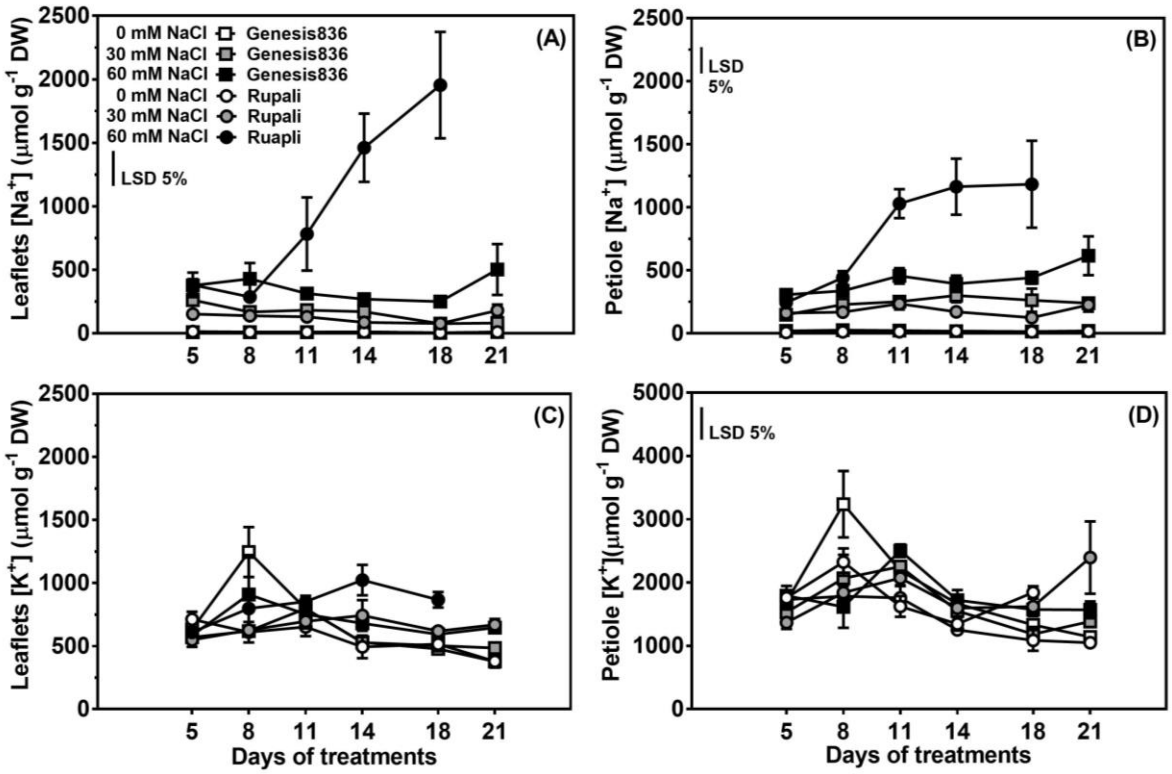

Figure S5. Concentrations (µmol g^{-1} DW) of Na^+ (A, B) and K^+ (C, D) in leaflets (A, C) and petioles (B, D) of the youngest fully-expanded leaves that were measured for gas exchange (see Fig. 5). Concentrations in mM (water basis) are presented in Figs. 1 and S4. Leaflets and petioles which were in the LiCor chamber were excised following gas exchange measurements and analysed for Na^+ and K^+. The two chickpea genotypes (Genesis836 and Rupali) were grown in aerated nutrient solution with 0 (non-saline control), 30 or 60 mM NaCl. Treatments were imposed on 23-d-old plants and the measurements were taken at 5, 8, 11, 14, 18 and 21 d of treatments. Data are means ± SE of four replicates. For Rupali at 60 mM NaCl, there were only two replicates at 18 d as the other replicate plants had no green leaves, and no green leaves remaining at 21 d. Three-way ANOVA (data up to 18 d of treatments) showed significant genotype × treatment × days of treatment interaction for concentrations of Na^+ in leaflets and petioles and K^+ in petioles (all with $P < 0.001$) but the interaction was non-significant for K^+ in leaflets ($P = 0.07$). Bars represent least significant difference (LSD) at $P < 0.05$ for genotype × treatment × days of treatment interaction.

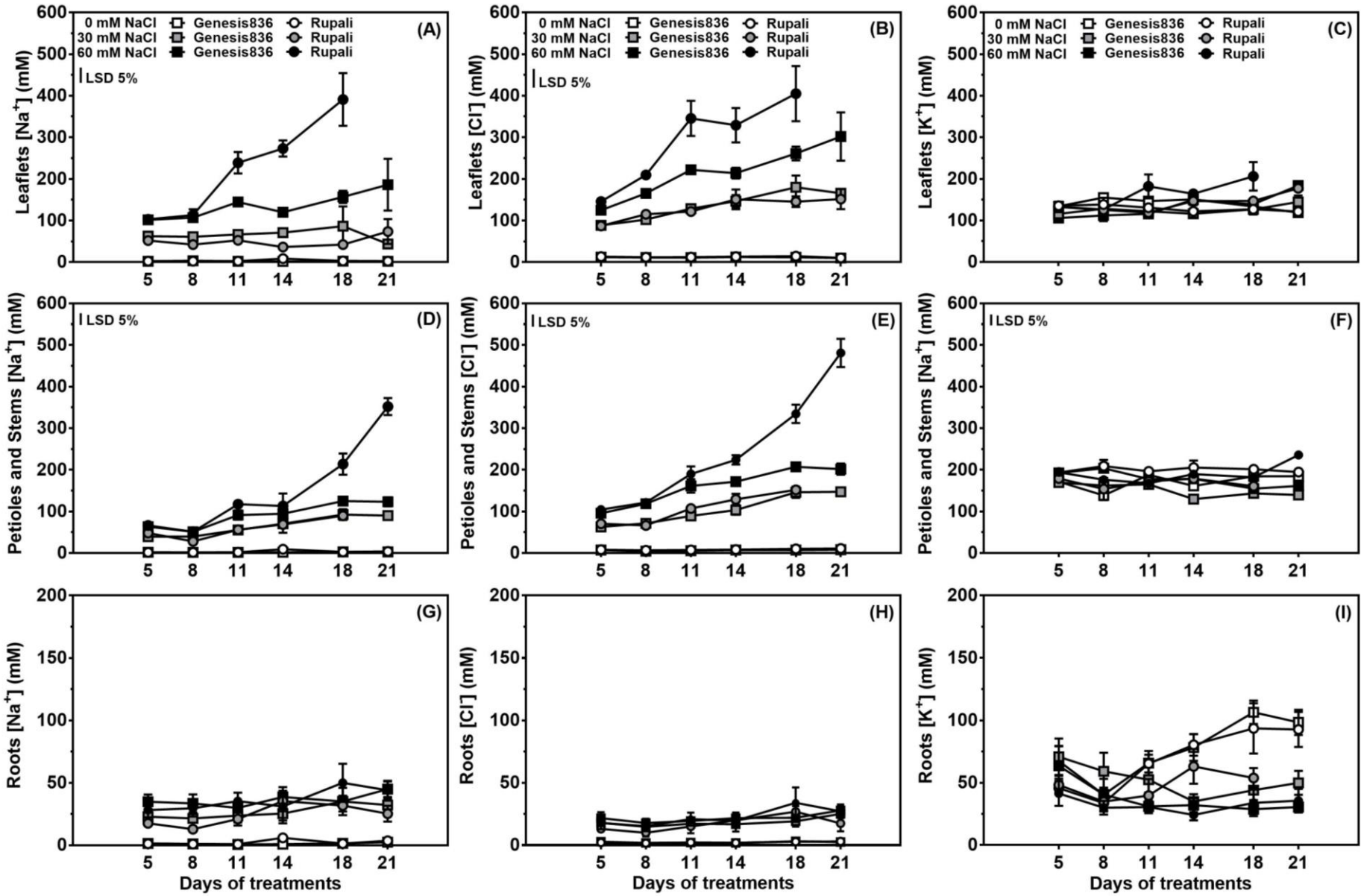
0 mM NaCl Genesis836 Rupali
30 mM NaCl Genesis836 Rupali
60 mM NaCl Genesis836 Rupali
LSD 5%
(A)
(B)
(C)
(D)
(E)
(F)
(G)
(H)
(I)
Leaflets [Na+] (mM)
Leaflets [Cl-] (mM)
Leaflets [K+] (mM)
Petioles and Stems [Na+] (mM)
Petioles and Stems [Cl-] (mM)
Petioles and Stems [Na+] (mM)
Roots [Na+] (mM)
Roots [Cl-] (mM)
Roots [K+] (mM)
Days of treatments

Figure S6. Concentrations (mM, tissue water basis) of Na^+ (A, D, G), Cl^- (B, E, H) and K^+ (C, F, I) in green leaflets of whole shoots (A-C), petioles and stems (D-F), and roots (G-I) of two chickpea genotypes (Genesis836 and Rupali) grown in aerated nutrient solution with 0 (non-saline control), 30 or 60 mM NaCl for 21 d. Measurements were conducted after 5, 8, 11, 14, 18 and 21 d of treatments, which were imposed on 23-d-old plants. Data are means ± SE of four replicates. There were no green leaves on Rupali in 60 mM NaCl after 21 d of treatment. Three-way ANOVA showed a significant genotype × treatment × days of treatment interaction for concentrations of Na^+ ($P < 0.001$) and Cl^- ($P = 0.05$) in leaflets (data up to 18 d of treatments), Na^+ ($P < 0.05$), Cl^- ($P < 0.001$) and K^+ ($P < 0.05$) in petioles and stems but the interaction was not significant for K^+ in leaflets ($P = 0.336$; data up to 18 d of treatments) and for Na^+ ($P = 0.791$), Cl^- ($P = 0.928$) and K^+ ($P = 0.587$) in roots. Bars represent least significant difference (LSD) at $P < 0.05$ for genotype × treatment × days of treatment interaction. Additional statistical analyses in Tables S4, S5 and S6.

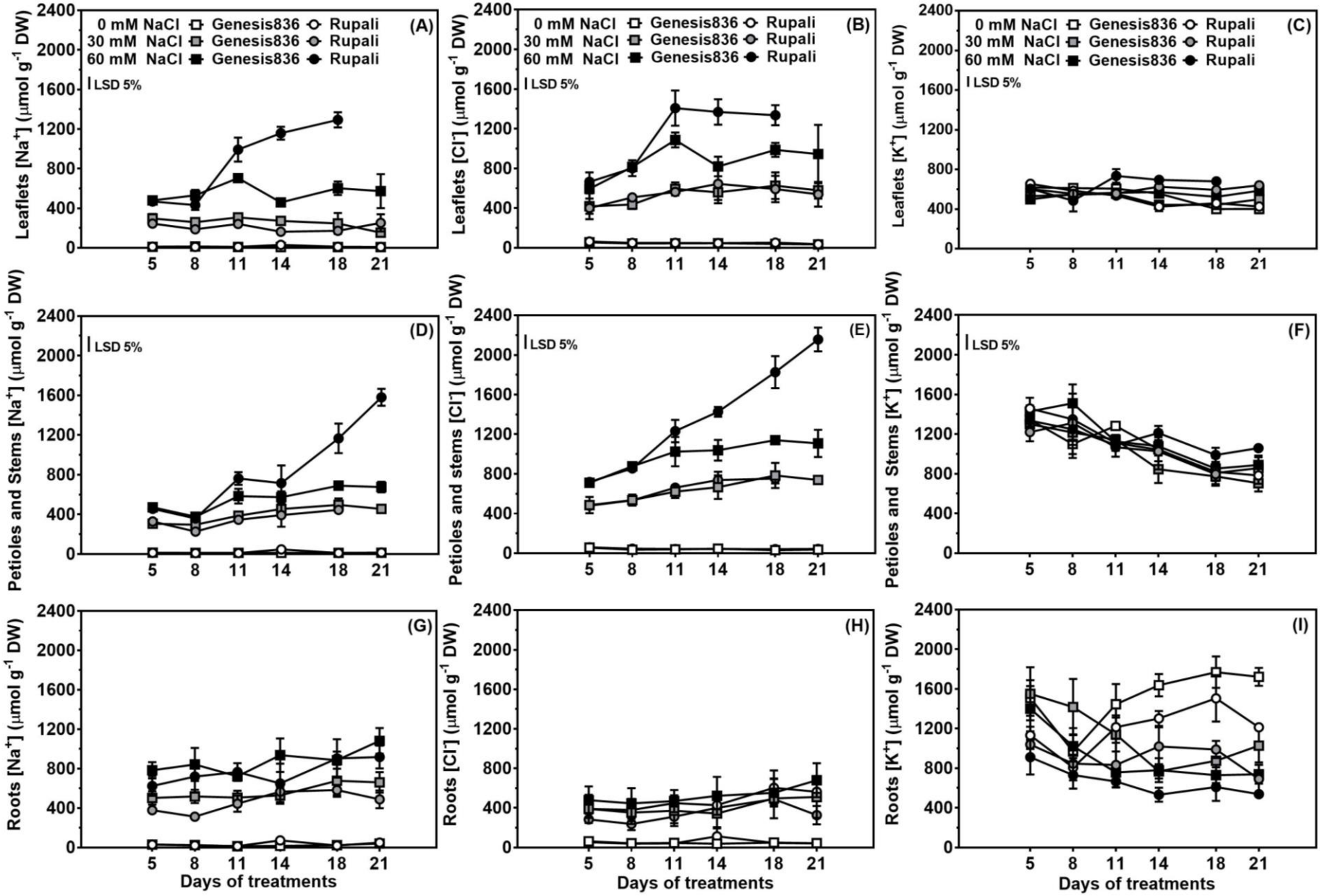
0 mM NaCl Genesis836 Rupali
30 mM NaCl Genesis836 Rupali
60 mM NaCl Genesis836 Rupali
LSD 5%
(A)
(B)
(C)
(D)
(E)
(F)
(G)
(H)
(I)
Leaflets [Na+] (µmol g-1 DW)
Leaflets [Cl-] (µmol g-1 DW)
Leaflets [K+] (µmol g-1 DW)
Petioles and Stems [Na+] (µmol g-1 DW)
Petioles and stems [Cl-] (µmol g-1 DW)
Petioles and Stems [K+] (µmol g-1 DW)
Roots [Na+] (µmol g-1 DW)
Roots [Cl-] (µmol g-1 DW)
Roots [K+] (µmol g-1 DW)
0
400
800
1200
1600
2000
2400
5
8
11
14
18
21
Days of treatments
)

Figure S7. Concentrations (µmol g^{-1} DW) of Na^+ (A, D, G), Cl^- (B, E, H) and K^+ (C, F, I) in green leaflets of whole shoots (A-C), petioles and stems (D-F), and roots (G-I) of two chickpea genotypes (Genesis836 and Rupali) grown in aerated nutrient solution with 0 (non-saline control), 30 or 60 mM NaCl for 21 d. Measurements were conducted after 5, 8, 11, 14, 18 and 21 d of treatments, which were imposed on 23-d-old plants. Data are means ± SE of four replicates. For Rupali at 60 mM NaCl, there were three replicates at 18 d as the other replicate plant had no green leaves, and no green leaves remaining at 21 d. Three-way ANOVA showed a significant genotype × treatment × days of treatment interaction for concentrations of Na^+ ($P < 0.001$), Cl^- ($P < 0.001$) and K^+ ($P < 0.05$) in leaflets (data up to 18 d of treatments), and Na^+ ($P < 0.05$), Cl^- ($P < 0.001$) and K^+ ($P < 0.001$) in stems and petioles but the interaction was not significant for roots (Na^+, $P = 0.893$; Cl^-, $P = 0.996$; K^+, $P = 0.270$). Bars represent least significant difference (LSD) at $P < 0.05$ for genotype × treatment × days of treatment interaction.

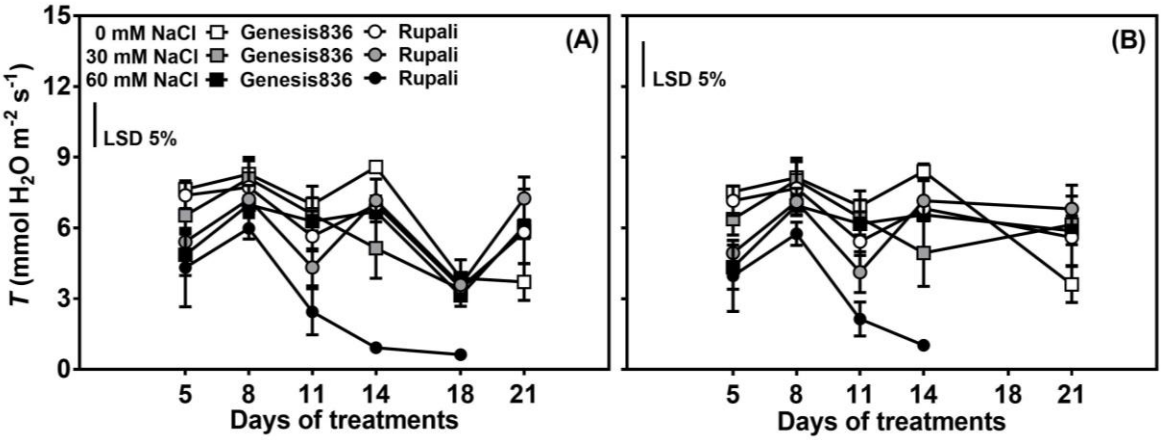

Figure S8. Transpiration rates (*T*) measured on the youngest fully-expanded leaves at a CO_2 concentration of 400 (A) and 800 µmol mol^{-1} (B). Measurements were conducted on two chickpea genotypes (Genesis836 and Rupali) that were grown in aerated nutrient solution with 0 (non-saline control), 30 or 60 mM NaCl. Treatments were imposed on 23-d-old plants and measurements were taken at 5, 8, 11, 14, 18 and 21 d of treatments. Data are means ± SE of four replicates. For Rupali at 60 mM NaCl, only one replicate could be measured at 18 d, and no green leaves were remaining at 21 d. No measurements were conducted at 800 µmol mol^{-1} at 18 d of treatment. Three-way ANOVA showed significant genotype × treatment × days of treatment interaction for *T* at ambient (measured up to 18 d of treatment) and elevated (measured up to 14 d of treatment) levels of CO_2 (both with $P < 0.01$). Bars represent least significant difference (LSD) at $P < 0.05$ for genotype × treatment × days of treatment interaction. Additional statistical analysis in Tables S11 and S12.

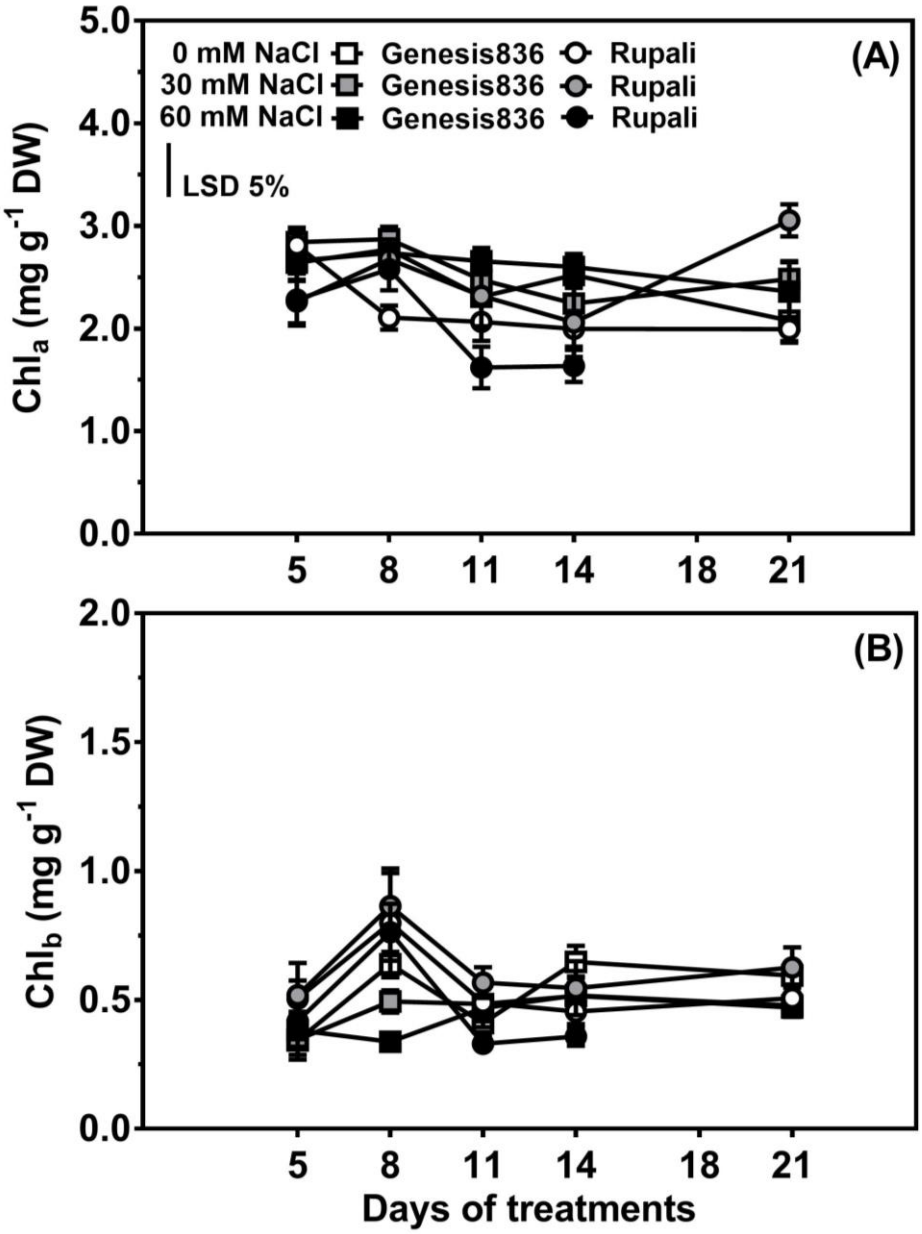

Figure S9. Chlorophyll *a* (Chl_a) (A) and chlorophyll *b* (Chl_b) (B) concentrations in leaflets of the youngest fully-expanded leaves of Genesis836 and Rupali that were previously used for gas exchange measurements (see Fig. 5). The two chickpea genotypes were grown in aerated nutrient solution with 0 (non-saline control), 30 or 60 mM NaCl for 21 d. Measurements were conducted after 5, 8, 11, 14, and 21 d of treatments, which were imposed on 23-d-old plants. Data are means ± SE of four replicates. There were no green leaves on Rupali in 60 mM NaCl after 21 d of treatment. Three-way ANOVA (measured up to 14 d of treatments) showed significant genotype × treatment × days of treatment interaction for concentrations of Chl_a ($P = 0.05$) but the interaction was non-significant for Chl_b ($P = 0.475$). Additional statistical analyses in Table S13.

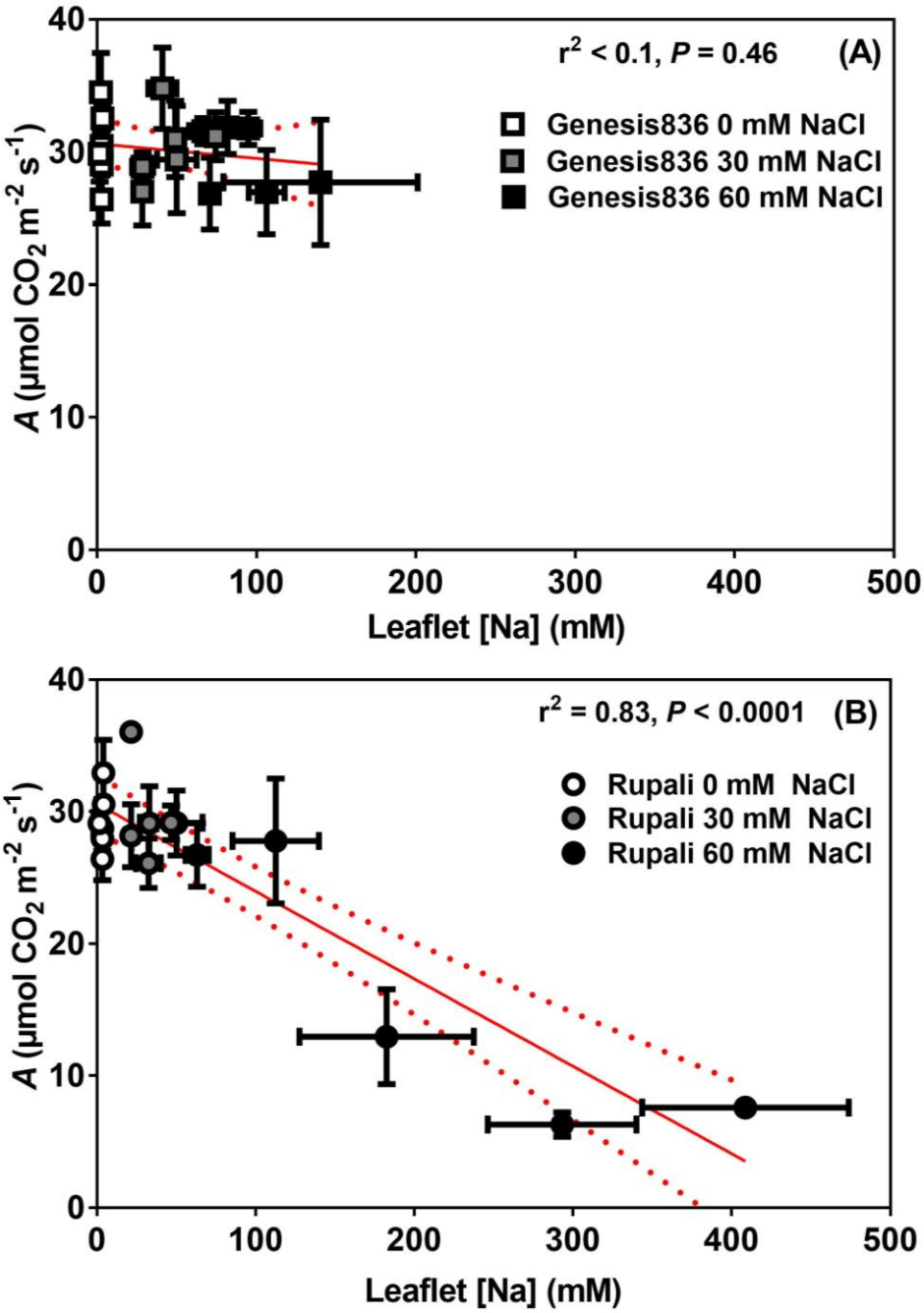

Figure S10. Relationship of net photosynthetic rates (*A*) and concentration of Na^+ in leaflets of the first youngest fully-expanded leaves of Genesis836 (A) and Rupali (B). The two chickpea genotypes were grown in aerated nutrient solution with 0 (non-saline control), 30 or 60 mM NaCl for 21 d. Measurements were conducted after 5, 8, 11, 14, and 21 d of treatments, which were imposed on 23-d-old plants. Data were taken from Figs. 1 and 5A.

Supplementary Tables S1 – S14

Table S1. Composition of nutrient solution used in experiments.

Full strength composition
Macronutrients (mM): Ca^{2+}, 5.0; K^{+}, 5.0; Mg^{2+}, 0.40; NH_4^{+}, 0.625; NO_3^{-}, 4.375; SO_4^{2-}, 5.4; $H_2PO_4^{-}$, 0.20.
Micronutrients (µM): Cl^{-}, 50; B, 25; Mn^{2+}, 2.0; Zn^{2+}, 2.0; Ni^{2+}, 1.0; Mo 1.0; Cu^{2+}, 0.50; Fe-sequestrene, 100.
Solution also contained:
Na^{+}, 0.20; SiO_3^{2-}, 0.10.
1.0 mM MES (2-[*N*-morpholino]ethanesulfonic acid).
pH adjusted to 6.5 using KOH (to give final concentration as above).

Table S2. *F*-values of two-way ANOVA (factors: 'genotypes' and 'salinity treatments') for shoot DW, shoot RGR and root DW of two chickpea genotypes grown in aerated non-saline nutrient solution with 0 (non-saline control), 30 or 60 mM NaCl for 21 d. Plants were sampled after 5, 8, 11, 14, 18 and 21 d of treatments, which were imposed on 23-d-old plants. ^{NS}, $P > 0.05$; *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$. Data are shown in Fig. S3.

	Days of treatments					
	5	**8**	**11**	**14**	**18**	**21**
Shoot DW						
Genotype	12.99^{**}	19.45^{***}	1.78^{NS}	0.14^{NS}	9.07^{*}	7.35^{*}
Treatment	0.38^{NS}	2.31^{NS}	22.44^{***}	11.13^{***}	52.95^{***}	67.65^{***}
Genotype × Treatment	0.08^{NS}	1.25^{NS}	3.45^{NS}	1.92^{NS}	1.14^{NS}	12.59^{***}
Shoot RGR						
Genotype	0.00^{NS}	0.83^{NS}	15.55^{***}	19.61^{***}	47.05^{***}	85.35^{***}
Treatment	0.36^{NS}	2.07^{NS}	28.48^{***}	15.87^{***}	47.66^{***}	71.86^{***}
Genotype × Treatment	0.04^{NS}	0.51^{NS}	4.13^{*}	4.28^{*}	11.68^{***}	20.37^{***}
Roots DW						
Genotype	0.96^{NS}	0.77^{NS}	0.62^{NS}	2.62^{NS}	11.78^{**}	8.55^{**}
Treatment	0.13^{NS}	0.10^{NS}	13.95^{***}	11.35^{NS}	90.26^{***}	21.03^{***}
Genotype × Treatment	0.10^{NS}	0.84^{NS}	0.98^{NS}	0.74^{NS}	2.63^{NS}	1.26^{NS}

Table S3. F-values of two-way ANOVA (factors: 'genotypes' and 'salinity treatments') for concentrations (mM) of Na^+ and K^+ leaflets and petioles of the youngest fully expanded leaves that were previously used for gas exchange measurements. The two chickpea genotypes (Genesis836 and Rupali) grown in aerated non-saline nutrient solution with 0 (non-saline control), 30 and 60 mM NaCl for 21 d. Plants were harvested after 5, 8, 11, 14, 18 and 21 d of treatments, which were imposed on 23-d-old plants. ^{NS}, $P > 0.05$; *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$. Data are shown in Figs. 1 and S4.

	Days of treatments					
	5	**8**	**11**	**14**	**18**	**21**
[Na^+] - leaflets						
Genotype	0.31^{NS}	6.52^{*}	2.27^{NS}	14.41^{***}	21.85^{***}	NA
Treatment	37.74^{***}	74.23^{***}	16.78^{***}	40.68^{***}	38.41^{***}	NA
Genotype × Treatment	0.889^{NS}	3.76^{*}	3.82^{*}	21.99^{***}	24.24^{***}	NA
[Na^+] - petioles						
Genotype	2.65^{NS}	8.68^{***}	12.50^{***}	2.45^{NS}	1.38^{NS}	NA
Treatment	83.68^{***}	118.0^{***}	49.25^{***}	32.44^{***}	22.10^{***}	NA
Genotype × Treatment	2.04^{NS}	1.69^{NS}	12.74^{***}	8.12^{***}	5.56^{*}	NA
[K^+] - leaflets						
Genotype	4.55^{*}	23.76^{***}	0.35^{NS}	3.11^{NS}	1.75^{NS}	NA
Treatment	15.18^{***}	32.08^{***}	0.72^{NS}	2.82^{NS}	1.64^{NS}	NA
Genotype × Treatment	0.42^{NS}	17.12^{***}	1.86^{NS}	2.76^{NS}	6.80^{**}	NA
[K^+] - petioles						
Genotype	2.44^{NS}	16.74^{***}	4.40^{NS}	1.88^{NS}	0.21^{NS}	NA
Treatment	0.48^{NS}	8.15^{**}	0.22^{NS}	1.84^{NS}	1.14^{NS}	NA
Genotype × Treatment	2.52^{NS}	6.59^{**}	0.70^{NS}	4.06^{NS}	1.84^{NS}	NA

Table S4. *F*-values of two-way ANOVA (factors: 'genotypes' and 'salinity treatments') for concentrations (mM) of Na^+, Cl^- and K^+ in leaflets of whole shoots of two chickpea genotypes (Genesis836 and Rupali) grown in aerated non-saline nutrient solution with 0 (non-saline control), 30 or 60 mM NaCl for 21 d. Plants were sampled after 5, 8, 11, 14, 18 and 21 d of treatments, which were imposed on 23-d-old plants. ^{NS}, $P > 0.05$; *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$. Data are shown in Fig. S6.

	Days of treatments					
	5	**8**	**11**	**14**	**18**	**21**
[Na^+] - leaflets						
Genotype	0.62^{NS}	0.48^{NS}	6.37^{*}	31.40^{***}	8.66^{*}	NA
Treatment	235.9^{***}	90.97^{***}	116.8^{***}	248.2^{***}	57.59^{***}	NA
Genotype × Treatment	1.03^{NS}	1.48^{NS}	10.86^{***}	58.94^{***}	15.41^{***}	NA
[Cl^-] - leaflets						
Genotype	2.61^{NS}	16.32^{***}	6.10^{*}	5.48^{*}	2.70^{NS}	NA
Treatment	280.4^{***}	430.1^{***}	102.3^{***}	75.46^{***}	62.25^{***}	NA
Genotype × Treatment	2.73^{NS}	7.50^{**}	7.44^{**}	4.75^{*}	$5,55^{*}$	NA
[K^+] - leaflets						
Genotype	4.50^{*}	0.02^{NS}	$1,93^{NS}$	0.29^{NS}	5.70^{*}	NA
Treatment	1.65^{NS}	1.90^{NS}	2.33^{NS}	2.51^{NS}	4.95^{*}	NA
Genotype × Treatment	1.09^{NS}	0.60^{NS}	5.16^{*}	3.21^{NS}	3.42^{NS}	NA

Table S5. F-values of two-way ANOVA (factors: 'genotypes' and 'salinity treatments') for concentrations (mM) of Na^+, Cl^- and K^+ in petioles and stems of two chickpea genotypes (Genesis836 and Rupali) grown in aerated non-saline nutrient solution with 0 (non-saline control), 30 and 60 mM NaCl for 21 d. Plants were harvested after 5, 8, 11, 14, 18 and 21 d of treatments, which were imposed on 23-d-old plants. ^{NS}, $P > 0.05$; *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$. Data are shown in Fig. S6.

	Days of treatments					
	5	**8**	**11**	**14**	**18**	**21**
[Na^+] - petioles and stems						
Genotype	2.70^{NS}	1.32^{NS}	2.41^{NS}	0.41^{NS}	8.25^{**}	NA
Treatment	253.4^{***}	70.00^{***}	110.4^{***}	20.83^{NS}	93.08^{***}	NA
Genotype × Treatment	1.27^{NS}	1.38^{NS}	2.42^{NS}	0.22^{NS}	9.07^{**}	NA
[Cl^-] - petioles and stems						
Genotype	6.53^{*}	0.00^{NS}	2.97^{NS}	13.53^{**}	23.24^{***}	NA
Treatment	590.0^{***}	171.1^{***}	111.2^{***}	234.3^{***}	259.3^{***}	NA
Genotype × Treatment	1.23^{NS}	0.28^{NS}	0.71^{NS}	4.24^{*}	18.9^{***}	NA
[K^+] - petioles and stems						
Genotype	4.73^{*}	0.74^{NS}	0.07^{NS}	23.75^{***}	9.99^{**}	NA
Treatment	4.71^{*}	2.07^{NS}	3.37^{NS}	7.41^{**}	13.32^{***}	NA
Genotype × Treatment	1.33^{NS}	4.55^{*}	0.61^{NS}	2.71^{NS}	0.26^{NS}	NA

Table S6. F-values of two-way ANOVA (factors: 'genotypes' and 'salinity treatments') for concentrations (mM) of Na^+, Cl^- and K^+ in roots of two chickpea genotypes (Genesis836 and Rupali) grown in aerated non-saline nutrient solution with 0 (non-saline control), 30 and 60 mM NaCl for 21 d. Plants were harvested after 5, 8, 11, 14, 18 and 21 d of treatments, which were imposed on 23-d-old plants. ^{NS}, $P > 0.05$; *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$. Data are shown in Fig. S6.

	Days of treatments					
	5	**8**	**11**	**14**	**18**	**21**
[Na^+] - roots						
Genotype	1.72^{NS}	1.12^{NS}	0.08^{NS}	0.17^{NS}	0.30^{NS}	0.25^{NS}
Treatment	33.39^{***}	18.83^{***}	29.18^{***}	10.90^{***}	12.03^{***}	39.52^{***}
Genotype × Treatment	0.41^{NS}	0.36^{NS}	0.48^{NS}	0.78^{NS}	0.66^{NS}	0.30^{NS}
[Cl^-] - roots						
Genotype	1.33^{NS}	0.97^{NS}	0.00^{NS}	0.01^{NS}	1.58^{NS}	0.53^{NS}
Treatment	15.01^{***}	11.28^{***}	13.74^{***}	11.18^{***}	8.66^{**}	16.86^{***}
Genotype × Treatment	0.21^{NS}	0.27^{NS}	0.12^{NS}	0.18^{NS}	0.49^{NS}	0.49^{NS}
[K^+] - roots						
Genotype	5.85^{*}	3.14^{NS}	0.40^{NS}	1.98^{NS}	0.09^{NS}	1.21^{NS}
Treatment	0.20^{NS}	0.95^{NS}	7.93^{**}	16.93^{***}	20.66^{***}	29.90^{***}
Genotype × Treatment	0.00^{NS}	0.48^{NS}	0.32^{NS}	2.21^{NS}	1.41^{NS}	0.16^{NS}

Table S7. Na^+, Cl^- and K^+ net uptake rates by roots of Genesis836 and Rupali grown in aerated nutrient solution with 0 (non-saline control), 30 or 60 mM NaCl. Treatments were imposed on 23-d-old plants. Values are calculated for periods of 0-5, 5-8 and 8-11 days of treatments. The ion uptake rates were not calculated for the later time periods owing to leaf drop as the plants became older or grew longer in the NaCl treatments. Data are means ± SE. Three-way ANOVA showed non-significant genotype × treatment × time interaction for Na^+ (P = 0.058), Cl^- (P = 0.756) and K^+ (P = 0.248). Additional statistical analyses in Table S9.

	Ion uptake rate (µmol g_{root} fresh weight^{-1} d^{-1})								
	Na			**Cl**			**K**		
Genesis836	**0**	**30**	**60**	**0**	**30**	**60**	**0**	**30**	**60**
0-5 d	0.3 ± 0.1	13.9 ± 1.6	22.1 ± 2.1	1.7 ± 0.1	16.3 ± 2.2	22.5 ± 1.9	19.7 ± 2.5	20.0 ± 3.9	18.4 ± 3.6
5-8 d	0.3 ± 0.0	3.3 ± 0.9	9.3 ± 4.2	0.2 ± 0.1	8.0 ± 2.4	12.1 ± 5.4	18.2 ± 6.7	17.1 ± 7.0	14.7 ± 3.5
8-11 d	0.4 ± 0.2	10.4 ± 3.5	13.8 ± 6.2	1.3 ± 0.5	15.5 ± 4.2	21.2 ± 5.8	39.8 ± 13	22.3 ± 7.1	11.1 ± 10
Rupali									
0-5 d	0.3 ± 0.0	14.5 ± 1.8	24.5 ± 1.5	1.7± 0.1	18.4 ± 0.6	29.5 ± 2.3	20.1 ± 3.8	18.2 ± 3.2	20.0 ± 2.8
5-8 d	0.2 ± 0.0	2.3 ± 1.9	5.8 ± 2.8	1.2 ± 0.3	13.7 ± 5.6	18.9 ± 2.3	30.8 ± 4.8	15.6 ± 7.9	12.2 ± 5.0
8-11 d	0.2 ± 0.1	16.1 ± 4.9	32.3 ± 4.9	1.0 ± 0.2	16.2 ± 6.2	36.5 ± 7.8	18.5 ± 6.5	17.3 ± 6.3	14.6 ± 2.5

Table S8. Na^+, Cl^- and K^+ net transport rates to shoots in Genesis836 and Rupali grown in aerated nutrient solution with 0 (non-saline control), 30 or 60 mM NaCl. Treatments were imposed on 23-d-old plants. Values are calculated for periods of 0-5, 5-8 and 8-11 days of treatments. The ion transport rates were not calculated for the later time periods owing to leaf drop as the plants became older or grew longer in the NaCl treatments. Data are means ± SE. Three-way ANOVA showed significant genotype × treatment × time interaction for Na^+ ($P < 0.05$) and K^+ ($P < 0.05$) but the interaction was non-significant for Cl^- ($P = 0.817$). Least significant difference (LSD) values at $P < 0.05$ for genotype × treatment × days of treatment interaction for Na^+, Cl^- and K^+ are 7.5, 9.2 and 10.8, respectively. Additional statistical analyses in Table S9.

	Ion transport rate from root to shoot (μmol g^{-1} FW_{root} d^{-1})								
	Na^+			**Cl^-**			**K^+**		
Genesis836	**0**	**30**	**60**	**0**	**30**	**60**	**0**	**30**	**60**
0-5 d	0.2 ± 0.0	7.7 ± 0.7	12.4 ± 0.7	1.1 ± 0.1	11.5 ± 1.2	16.7 ± 1.3	13.9 ± 3.4	13.1 ± 2.3	13.5 ± 2.4
5-8 d	0.2 ± 0.0	2.0 ± 1.0	4.8 ± 1.5	0.6 ± 0.4	5.5 ± 1.3	12.2 ± 4.1	13.3 ± 7.7	7.6 ± 4.1	10.3 ± 4.1
8-11 d	0.2 ± 0.1	7.4 ± 1.7	14.5 ± 4.2	0.9 ± 0.3	13.1 ± 3.6	20.3 ± 4.7	25.0 ± 6.4	16.7 ± 5.9	8.4 ± 5.8
Rupali									
0-5 d	0.2 ± 0.0	10.2 ± 1.7	17.1 ± 2.4	1.3 ± 0.1	15.2 ± 0.5	25.0 ± 3.2	17.6 ± 1.2	18.3 ± 2.2	20.6 ± 4.6
5-8 d	0.3 ± 0.0	1.5 ± 1.0	3.9 ± 2.0	1.3 ± 0.3	10.4 ± 4.9	18.8 ± 3.6	32.5 ± 1.1	19.7 ± 5.1	15.5 ± 2.8
8-11 d	0.1 ± 0.1	9.6 ± 3.0	31.3 ± 4.2	0.6 ± 0.2	15.9 ± 4.8	35.2 ± 7.3	6.1 ± 2.1	17.1 ± 5.9	16.0 ± 1.2

Table S9. F-values of two-way ANOVA (factors: 'genotypes' and 'salinity treatments') for Na^+, Cl^- and K^+ net uptake rates and net transport rates of two chickpea genotypes (Genesis836 and Rupali) grown in aerated non-saline nutrient solution with 0 (non-saline control), 30 and 60 mM NaCl for 21 d. Values are calculated for periods of 0-5, 5-8 and 8-11 days of treatments. ^{NS}, $P > 0.05$; *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$. Data are shown in Tables S7 and S8.

	Times (days)		
	0-5	**5-8**	**8-11**
Na^+ net uptake rates by roots			
Genotype	0.71^{NS}	0.63^{NS}	3.28^{NS}
Treatment	125.2^{***}	5.31^{*}	14.82^{***}
Genotype × Treatment	0.37^{NS}	0.31^{NS}	3.22^{NS}
Na^+ net transport rates to shoots			
Genotype	5.27^{*}	0.15^{NS}	6.13^{*}
Treatment	65.54^{***}	4.79^{*}	27.49^{***}
Genotype × Treatment	1.65^{NS}	0.06^{NS}	4.48^{*}

	Times (days)		
	0-5	**5-8**	**8-11**
Cl^- net uptake rates by roots			
Genotype	5.99^{*}	1.83^{NS}	1.33^{NS}
Treatment	132.9^{***}	6.53^{*}	12.49^{***}
Genotype × Treatment	2.84^{NS}	0.26^{NS}	1.33^{NS}
Cl^- net transport rates to shoots			
Genotype	10.99^{**}	1.84^{NS}	2.17^{NS}
Treatment	86.93^{***}	7.37^{*}	15.74^{***}
Genotype × Treatment	3.68^{*}	0.31^{NS}	1.46^{NS}

	Times (days)		
	0-5	**5-8**	**8-11**
K^+ net uptake rates by roots			
Genotype	0.00^{NS}	0.33^{NS}	1.24^{NS}
Treatment	0.03^{NS}	1.68^{NS}	1.79^{NS}
Genotype × Treatment	0.13^{NS}	0.89^{NS}	1.08^{NS}
K^+ net transport rates to shoots			
Genotype	4.99^{*}	10.45^{**}	0.68^{NS}
Treatment	0.14^{NS}	2.78^{NS}	0.44^{NS}
Genotype × Treatment	0.18^{NS}	1.14^{NS}	3.04^{NS}

Table S10. F-values of two-way ANOVA (factors: 'genotypes' and 'salinity treatments') for concentratins of Na, Cl and K and K/Na ratio in various cell types of leaflets of the youngest fully-expanded leaves of Genesis836 and Rupali grown in aerated nutrient solution with 0 (non-saline control), 30 or 60 mM NaCl for 18 d. Treatments were imposed on 23-d-old plants. Elemental concentrations were measured by cryo-SEM X-ray microanalysis. ^{NS}, $P > 0.05$; *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$. Data are shown in Fig. 3.

	Element			
	Na	**Cl**	**K**	**K/Na**
Upper epidermis				
Genotype	56.11^{***}	10.76^{***}	20.08^{***}	3.52^{NS}
Treatment	297.23^{***}	490.45^{***}	101.20^{***}	72.67^{***}
Genotype × Treatment	141.81^{***}	106.03^{***}	24.55^{***}	9.89^{***}
Palisade mesophyll				
Genotype	137.28^{***}	0.11^{NS}	15.57^{***}	12.39^{***}
Treatment	188.90^{***}	793.52^{***}	49.56^{***}	39.15^{***}
Genotype × Treatment	186.13^{***}	19.15^{***}	87.15^{***}	5.56^{***}
Spongy mesophyll				
Genotype	148.21^{***}	8.12^{**}	3.17^{NS}	3.87^{NS}
Treatment	212.69^{***}	509.43^{***}	2.17^{NS}	25.66^{***}
Genotype × Treatment	165.91^{***}	27.05^{***}	5.08^{**}	1.56^{NS}
Lower epidermis				
Genotype	49.68^{***}	70.96^{***}	20.00^{***}	0.50^{NS}
Treatment	233.45^{***}	793.20^{***}	114.86^{***}	71.18^{***}
Genotype × Treatment	117.71^{***}	196.52^{***}	29.13^{***}	2.50^{NS}

Table S11. F-values of two-way ANOVA (factors: 'genotypes' and 'salinity treatments') for gas exchange parameters measured on the youngest fully-expanded leaves at a CO_2 concentration of 400 µmol mol^{-1}. Measurements were conducted on two chickpea genotypes (Genesis836 and Rupali) that were grown in aerated nutrient solution with 0 (non-saline control), 30 or 60 mM NaCl. Measurements were conducted after 5, 8, 11, 14 and 18 d of treatments, which were imposed on 23-d-old plants. ^{NS}, $P > 0.05$; *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$. Data are shown in Figs. 5 and S8.

	Days of treatments					
	5	**8**	**11**	**14**	**18**	**21**
Net photosynthetic rates (*A*)						
Genotype	0.00^{NS}	9.86^{**}	14.35^{***}	26.64^{***}	7.24^{*}	NA
Treatment	1.27^{NS}	0.62^{NS}	3.73^{*}	34.03^{***}	8.23^{**}	NA
Genotype × Treatment	0.17^{NS}	0.21^{NS}	6.63^{**}	40.08^{***}	6.07^{*}	NA
Intercellular CO_2 concentration (*Ci*)						
Genotype	3.41^{NS}	0.94^{NS}	5.75^{*}	2.62^{NS}	0.69^{NS}	NA
Treatment	8.31^{**}	2.72^{NS}	0.95^{NS}	12.48^{***}	1.18^{NS}	NA
Genotype × Treatment	0.58^{NS}	0.26^{NS}	0.18^{NS}	11.69^{***}	1.12^{NS}	NA
Stomatal conductance (g_s)						
Genotype	1.83^{NS}	0.01^{NS}	13.85^{**}	0.44^{NS}	1.54^{NS}	NA
Treatment	7.61^{**}	2.73^{NS}	1.90^{NS}	5.12^{*}	2.31^{NS}	NA
Genotype × Treatment	0.31^{NS}	0.18^{NS}	0.26^{NS}	5.36^{*}	0.88^{NS}	NA
Transpiration (*T*)						
Genotype	0.91^{NS}	1.88^{NS}	17.92^{***}	14.26^{**}	2.55^{NS}	NA
Treatment	6.03^{*}	2.37^{NS}	3.62^{*}	25.85^{***}	3.09^{NS}	NA
Genotype × Treatment	0.15^{NS}	0.05^{NS}	1.49^{NS}	23.03^{***}	1.68^{NS}	NA

Table S12. F-values of two-way ANOVA (factors: 'genotypes' and 'salinity treatments') for gas exchange parameters measured on the youngest fully-expanded leaves at a CO_2 concentration of 800 µmol mol^{-1}. Measurements were conducted on two chickpea genotypes (Genesis836 and Rupali) that were grown in aerated nutrient solution with 0 (non-saline control), 30 or 60 mM NaCl for 21 d. Measurements were conducted after 5, 8, 11, 14 and18 d of treatments, which were imposed on 23-d-old plants. ^{NS}, $P > 0.05$; *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$. Data are shown in Figs. 5 and S8.

	Days of treatments					
	5	**8**	**11**	**14**	**18**	**21**
Net photosynthetic rates (*A*)						
Genotype	1.78^{NS}	6.96^{*}	6.19^{*}	21.63^{***}	NA	NA
Treatment	0.63^{NS}	0.54^{NS}	4.53^{*}	36.73^{***}	NA	NA
Genotype × Treatment	0.48^{NS}	0.73^{NS}	6.56^{**}	35.45^{***}	NA	NA
Intercellular CO_2 concentration (*Ci*)						
Genotype	4.66^{*}	0.02^{NS}	12.39^{**}	1.20^{NS}	NA	NA
Treatment	8.46^{**}	1.09^{NS}	1.42^{NS}	4.55^{*}	NA	NA
Genotype × Treatment	0.42^{NS}	0.44^{NS}	0.69^{NS}	5.95^{*}	NA	NA
Stomatal conductance (g_s)						
Genotype	1.87^{NS}	1.06^{NS}	15.30^{***}	0.50^{NS}	NA	NA
Treatment	8.07^{**}	2.47^{NS}	2.06^{NS}	5.43^{*}	NA	NA
Genotype × Treatment	0.34^{NS}	0.30^{NS}	0.40^{NS}	6.03^{*}	NA	NA
Transpiration (*T*)						
Genotype	1.27^{NS}	1.95^{NS}	21.43^{***}	10.83^{**}	NA	NA
Treatment	7.68^{**}	2.37^{NS}	4.12^{*}	21.77^{***}	NA	NA
Genotype × Treatment	0.31^{NS}	0.12^{NS}	$1.67^{N}S$	20.58^{***}	NA	NA

Table S13. F-values of two-way ANOVA (factors: 'genotypes' and 'salinity treatments') for concentrations of chlorophylls *a* and *b* in the leaflets of the first youngest fully-expanded leaves of two chickpea genotypes (Genesis836 and Rupali) that were grown in aerated nutrient solution with 0 (non-saline control), 30 or 60 mM NaCl for 21 d. Measurements were conducted after 5, 8, 11, 14, 18 and 21 d of treatments, which were imposed on 23-d-old plants. ^{NS}, $P > 0.05$; *, $P \leq 0.05$; **, $P \leq 0.01$. Data are shown in Fig. S9.

	Days of treatments					
	5	**8**	**11**	**14**	**18**	**21**
Chlorophyll *a*						
Genotype	3.21^{NS}	7.45^{*}	8.36^{*}	10.97^{**}	NA	NA
Treatment	1.06^{NS}	2.48^{NS}	0.92^{NS}	0.26^{NS}	NA	NA
Genotype × Treatment	2.34^{NS}	1.72^{NS}	2.83^{NS}	1.81^{NS}	NA	NA
Chlorophyll *b*						
Genotype	3.89^{NS}	11.59^{**}	0.03^{NS}	4.13^{NS}	NA	NA
Treatment	0.09^{NS}	1.18^{NS}	2.38^{NS}	1.74^{NS}	NA	NA
Genotype × Treatment	0.50^{NS}	0.74^{NS}	2.35^{NS}	1.73^{NS}	NA	NA

Table S14. Number of stomata, number of secretory trichomes and volume of a single secretory trichomes on adaxial and abaxial surfaces of youngest fully-expanded leaves of Genesis836 and Rupali grown with 0, 30 or 60 mM NaCl for 18 d.

	Genesis836			Rupali		
Number of stomata per mm^2	**0 mM NaCl**	**30 mM NaCl**	**60 mM NaCl**	**0 mM NaCl**	**30 mM NaCl**	**60 mM NaCl**
Adaxial	218 ± 5	219 ± 7	227 ± 7	234 ± 15	249 ± 6	248 ± 4
Abaxial	400 ± 21	406 ± 7	384 ± 11	333 ± 22	346 ± 14	407 ± 17
Number of secretory trichomes per mm^2						
Adaxial	4.3 ± 0.3	4.8 ± 0.4	5.1 ± 0.5	3.0 ± 0.3	2.4 ± 0.2	4.9 ± 0.4
Abaxial	5.6 ± 0.6	6.8 ± 0.6	5.6 ± 0.2	4.4 ± 0.2	4.3 ± 0.4	6.9 ± 0.7
Total volume of single secretory trichome (10^{-5} mm^3)						
Adaxial	11.4 ± 1.2	11.5 ± 1.4	12.6 ± 0.9	16.1 ± 2.0	18.0 ± 2.6	13.0 ± 0.5
Abaxial	14.5 ± 0.9	8.7 ± 0.5	9.7 ± 0.7	14.7 ± 1.4	16.2 ± 1.1	12.2 ± 0.5
Volume of head cells of trichome (10^{-5} mm^3)						
Adaxial	4.8 ± 0.5	4.7 ± 0.6	5.2 ± 0.4	5.8 ± 0.4	6.2 ± 0.6	5.1 ± 0.2
Abaxial	5.3 ± 0.1	4.0 ± 0.2	4.0 ± 0.3	5.2 ± 0.3	5.5 ± 0.3	4.9 ± 0.3
Volume of stalk cells of trichome (10^{-5} mm^3)						
Adaxial	6.6 ± 0.7	6.8 ± 0.8	7.4 ± 0.5	10.2 ± 1.5	11.7 ± 2.1	7.9 ± 0.3
Abaxial	9.2 ± 0.8	4.8 ± 0.4	5.7 ± 0.5	9.4 ± 1.2	10.6 ± 0.8	7.3 ± 0.3

Plants were grown in non-saline nutrient solution for 23 d before treatments had been imposed. Data are means ± SE. Number of stomata were determined on 2 regions on both sides of the main vein (excluding main vein) on adaxial and abaxial surfaces of leaflets from 3 replicates leaves. Number of secretory trichomes were determined on both sides of main vein (excluding main) on adaxial and abaxial surfaces of leaflets from 2 to 3 replicate leaves. Volume of secretory trichomes were determined from 2 to 3 trichomes on adaxial and abaxial surfaces of leaflets from 2 to 3 replicate leaves. The volume of trichomes was obtained by summing the volume of head cells and stalk cells. The volume of head and stalk cells were obtained by using the formula for calculating the volume of a cylinder. All analyses were done from SEM images of leaf segments that were preserved in 2.5% glutaraldehyde, dehydrated in a graded series of ethanol, critical point dried and sputter-coated with gold. Three-way ANOVA showed significant genotype × treatment × leaf side interaction for number of stomata ($P < 0.05$) but the interaction was not significant for number of secretory trichomes ($P = 0.341$), total volume of single secretory trichome ($P = 0.163$), volume of head cells of secretory trichome ($P = 0.175$) and volume of stalk cells of secretory trichome ($P = 0.193$).