

GABA operates upstream of H⁺-ATPase and improves salinity tolerance in Arabidopsis by enabling cytosolic K⁺ retention and Na⁺ exclusion

Nana Su^{1,3,#}, Qi Wu^{2,3#}, Jiahui Chen¹, Lana Shabala³, Axel Mithöfer⁴, Haiyang Wang³,
Mei Qu³, Min Yu², Jin Cui^{1*}, Sergey Shabala^{2,3,*}

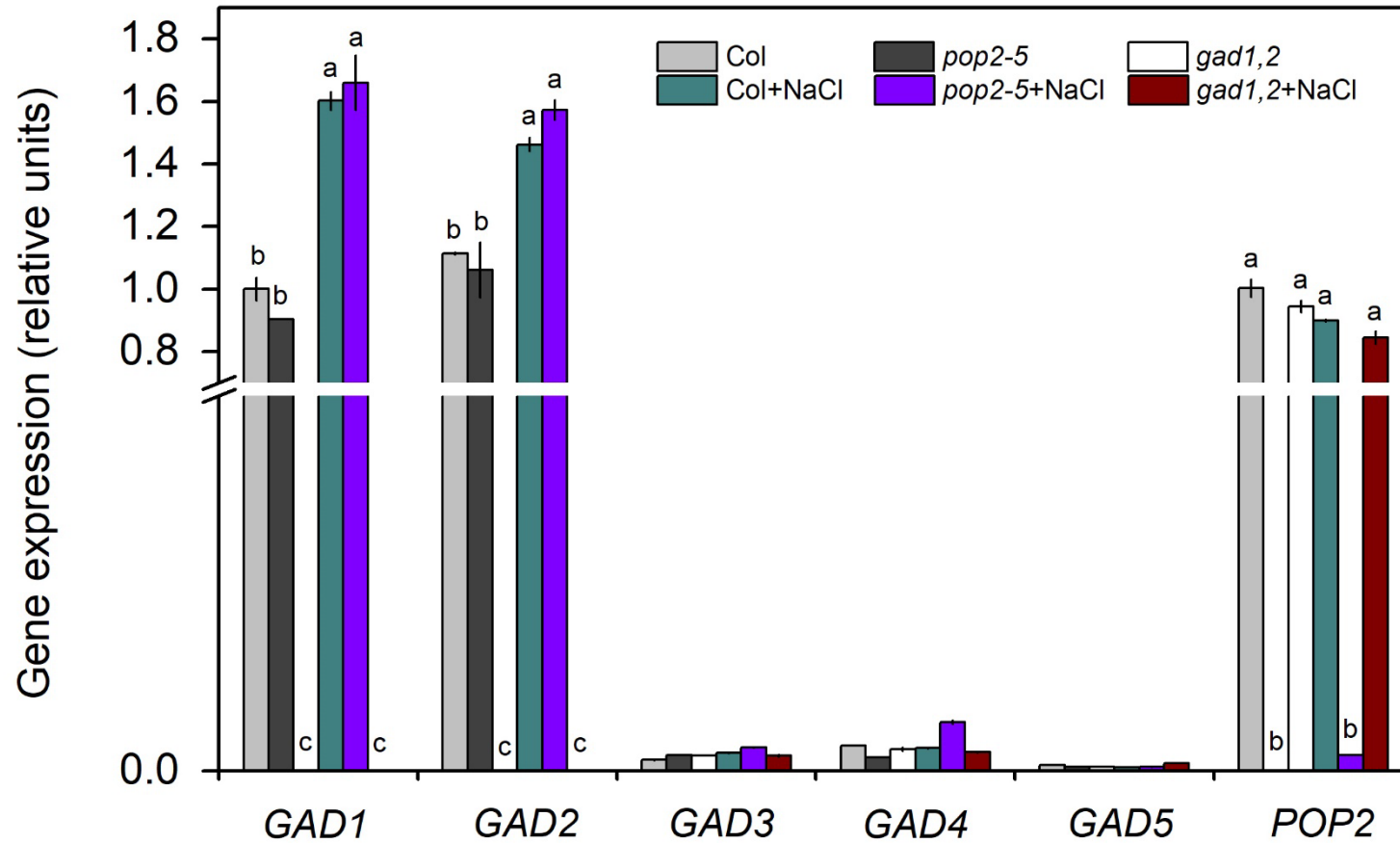


Fig. S1 Relative gene expressions of *GAD1-GAD5* and *POP2* in the roots of 4-week old Col-0, *pop 2-5* and *gad 1,2* seedlings. Plants were first grown in Petri dishes for 1 week in $\frac{1}{2}$ -strength MS medium with 2% w/v phytoigel and then transferred into $\frac{1}{4}$ Hoagland nutrition solution for 3 weeks. Seedlings were then treated with 100 mM NaCl for 12 h under standard condition (day/night cycle, 16/8 hours; light intensity, 100-150 $\text{mmol m}^{-2} \text{s}^{-1}$; temperature day/night, 25/20°C). Data are mean values of three independent experiments, with each containing three replicates. Data labelled with different low case letters is significantly different at $P < 0.05$ level.

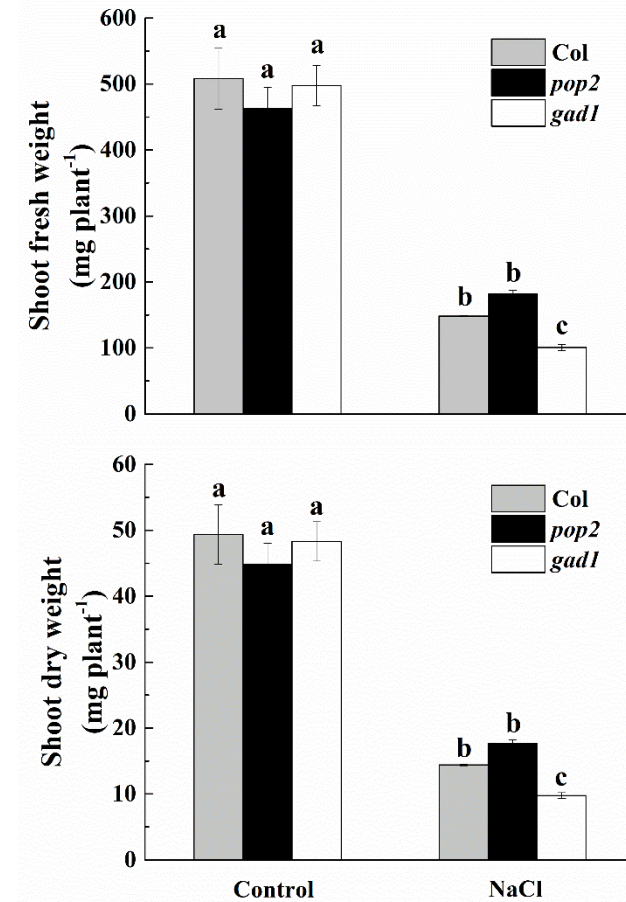
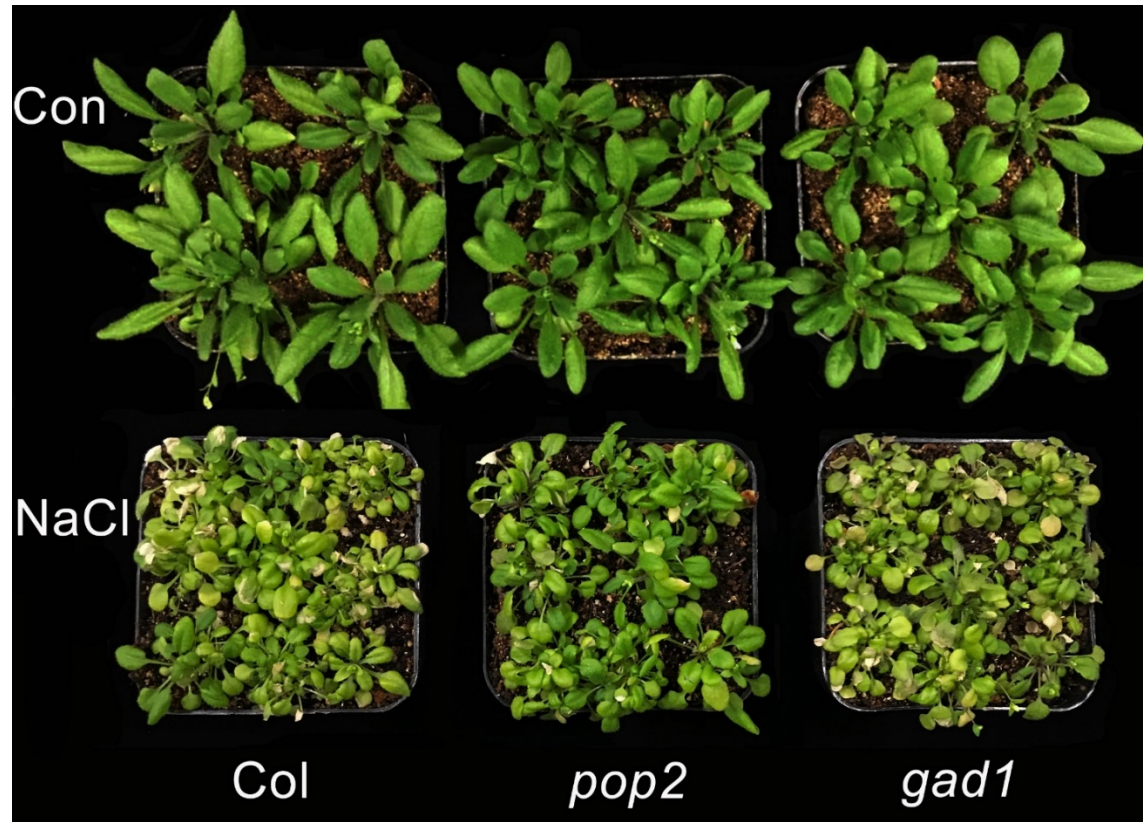


Fig. S2 Phenotypes of *pop2*, *gad1* and Col Arabidopsis seedlings treated with and without 100 mM NaCl. Plants were first grown in Petri dishes for 1 week in ½ -strength MS medium with 2% w/v phytogel and then transferred into soil for 1 week. Seedlings were then treated with or without 100 mM NaCl for 2 weeks. Day/night cycle was 16/8 hours; light intensity was 100-150 mmol m⁻² s⁻¹; temperature day/night was 25/20°C.

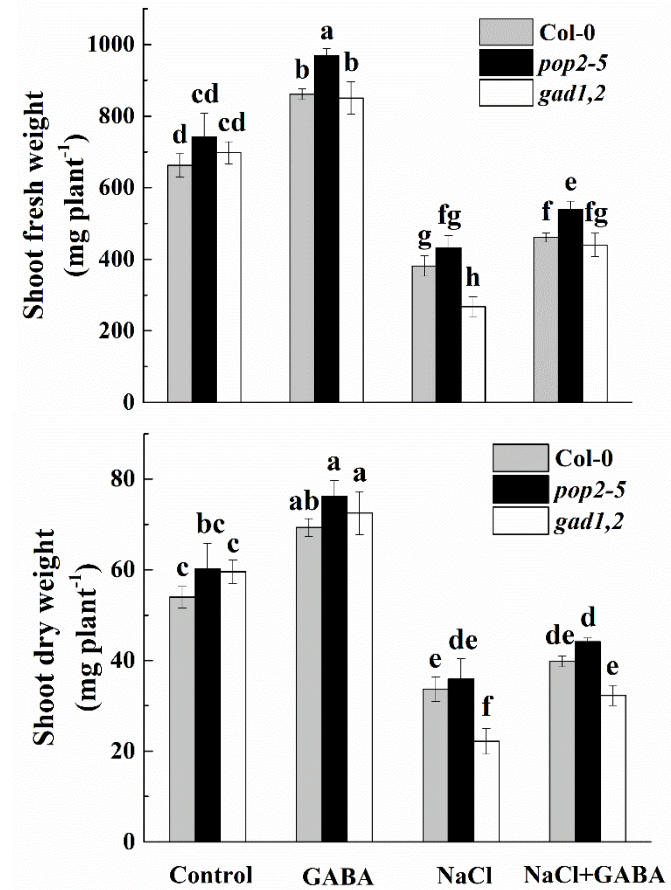
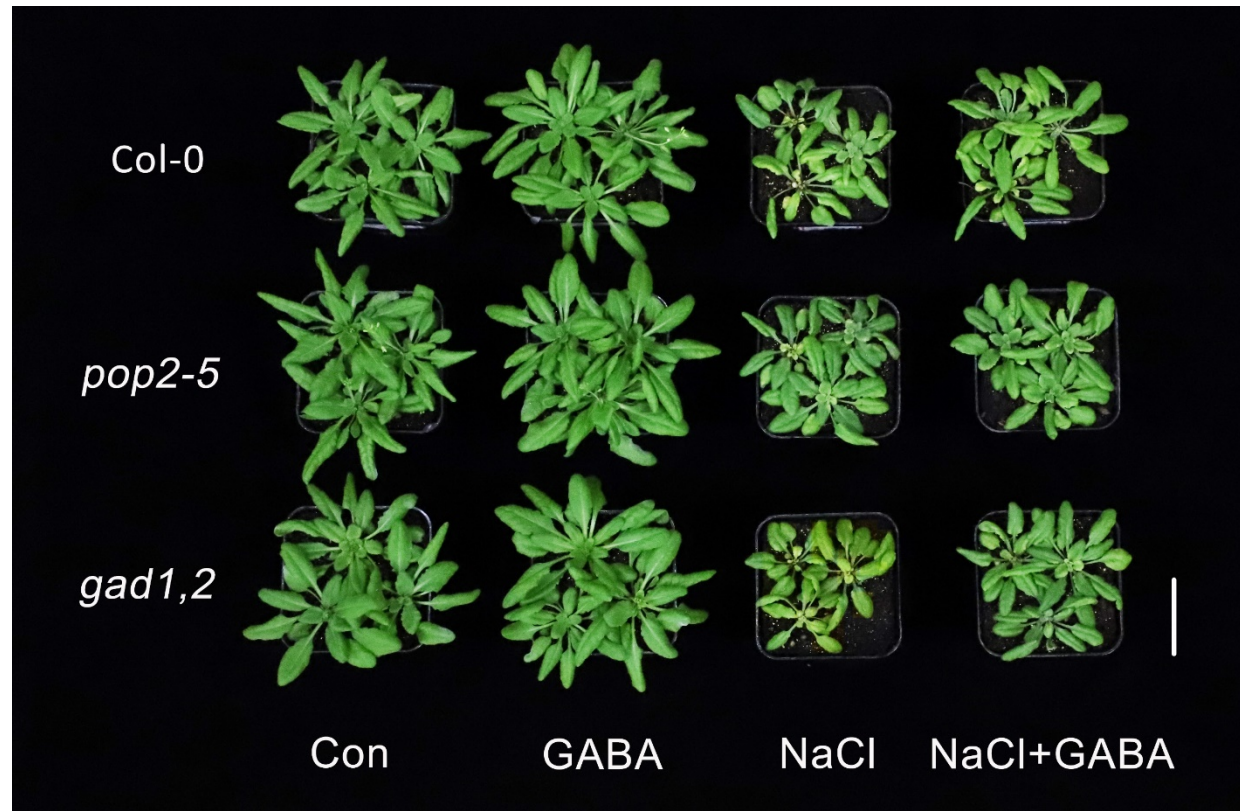


Fig. S3 Exogenous ABA application rescues salt-sensitive *gad1,2* phenotype. Col, *pop2-5* and *gad1,2* Arabidopsis seedlings were grown in soil (turf : nutrient soil=1:1) for 4 week. Seedlings were then treated with 100 mM NaCl, 1 mM GABA or 100 mM NaCl + 1 mM GABA for 10 days. Day/night cycle was 14/10 hours; light intensity was 100-150 $\mu\text{mol m}^{-2} \text{s}^{-1}$; temperature day/night was 25/20°C. Bar=3 cm.

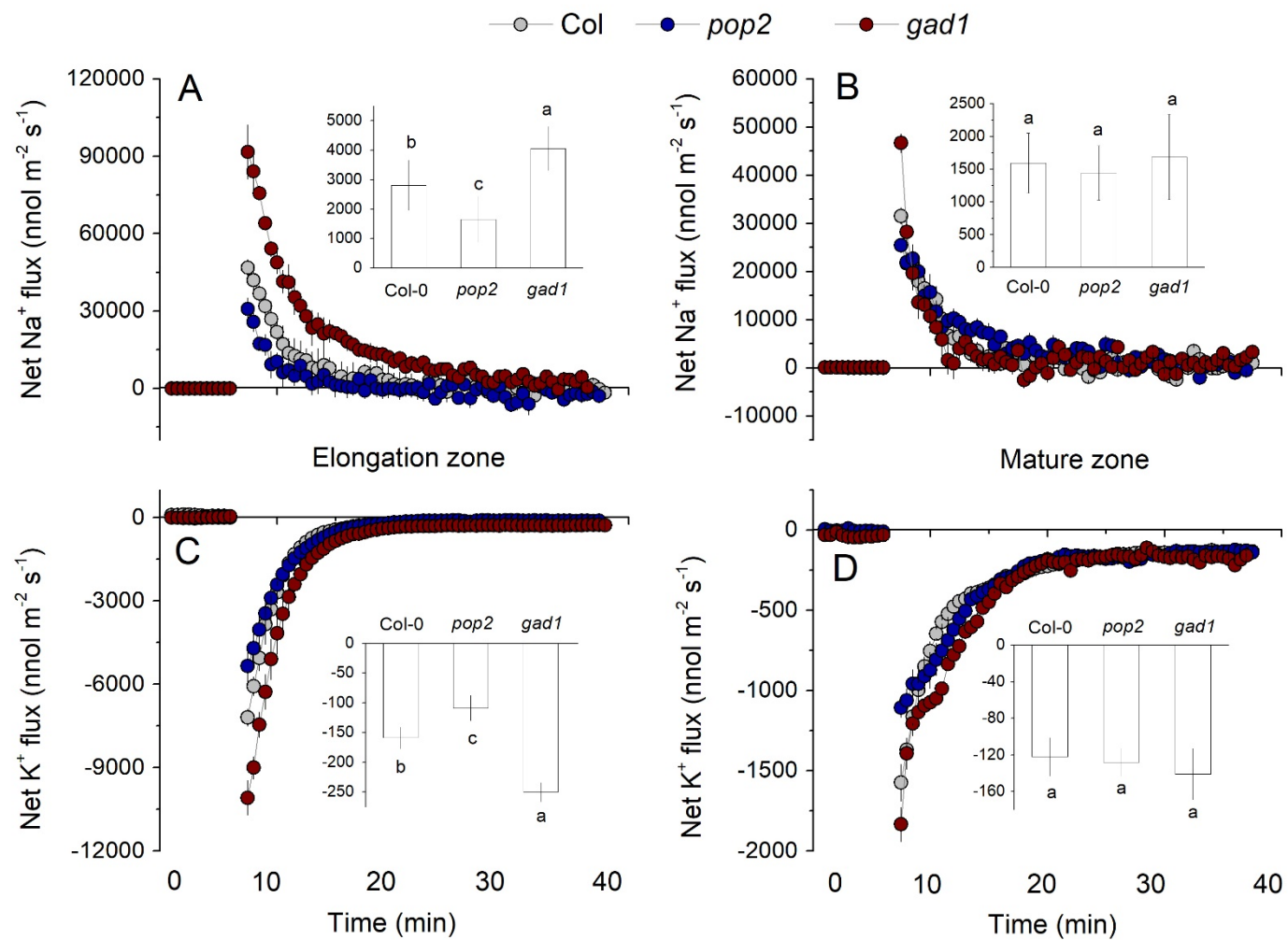


Fig. S4 Transient Na⁺, K⁺ and H⁺ fluxes measured from the elongation zone (A-C) and mature (D-F) root zone of 4-d old Col-0, *pop 2* and *gad 1* seedlings in response to 100 mM NaCl treatment. The insets show steady fluxes after 30 min of salinity exposure. Data are mean \pm SE (n = 6). The sign convention is “efflux negative”. Data labelled with different low case letters is significantly different at $P < 0.05$ level.

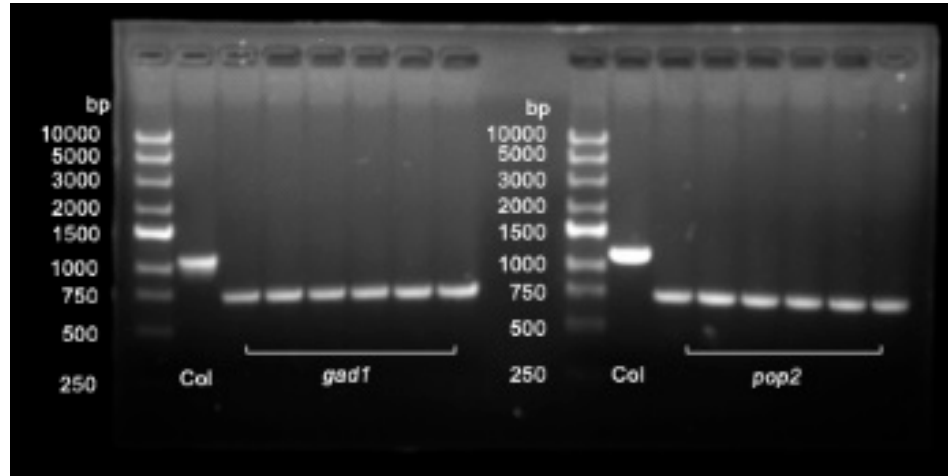


Fig. S5 The homozygosis of *gad1* and *pop2*. The primers (LBb1.3+LP+RP) were designed by T-DNA Primer Design Tool (<http://signal.salk.edu/tdnaprimers.2.html>).