

## Supplementary Material

### Variation in shoot tolerance mechanisms not related to ion toxicity in barley

*Joanne Tilbrook*<sup>A,B</sup>, *Rhiannon K. Schilling*<sup>A,B</sup>, *Bettina Berger*<sup>B,C</sup>, *Alexandre F. Garcia*<sup>A,B,C</sup>, *Christine Trittermann*<sup>A,B</sup>, *Stewart Coventry*<sup>B</sup>, *Huwaida Rabie*<sup>D,F</sup>, *Chris Brien*<sup>D</sup>, *Martin Nguyen*<sup>D</sup>, *Mark Tester*<sup>E</sup> and *Stuart J. Roy*<sup>A,B,G</sup>

<sup>A</sup>Australian Centre for Plant Functional Genomics, University of Adelaide, PMB1, Glen Osmond, SA 5064, Australia.

<sup>B</sup>School of Agriculture, Food and Wine, University of Adelaide, PMB1, Glen Osmond, SA 5064, Australia.

<sup>C</sup>The Plant Accelerator, Australian Plant Phenomics Facility, University of Adelaide, PMB1, Glen Osmond, SA 5064, Australia.

<sup>D</sup>School of Information Technology and Mathematical Services, University of South Australia, Mawson Lakes, SA 5095, Australia.

<sup>E</sup>King Abdullah University of Science and Technology, Biological and Environmental Sciences and Engineering, Thuwal 23955-6900, Saudi Arabia.

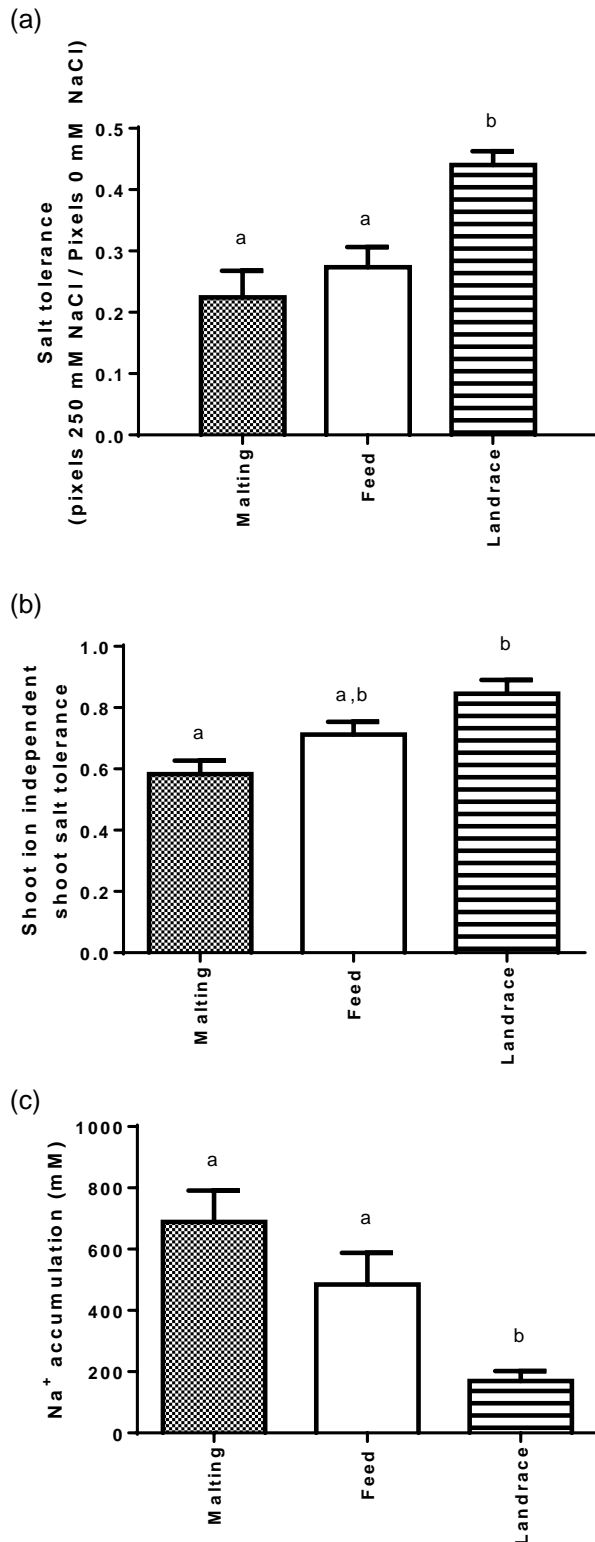
<sup>F</sup>Bethlehem University, Rue des Freres #9, Bethlehem, West Bank, Palestine.

<sup>G</sup>Corresponding author. Email: [stuart.roy@adelaide.edu.au](mailto:stuart.roy@adelaide.edu.au)

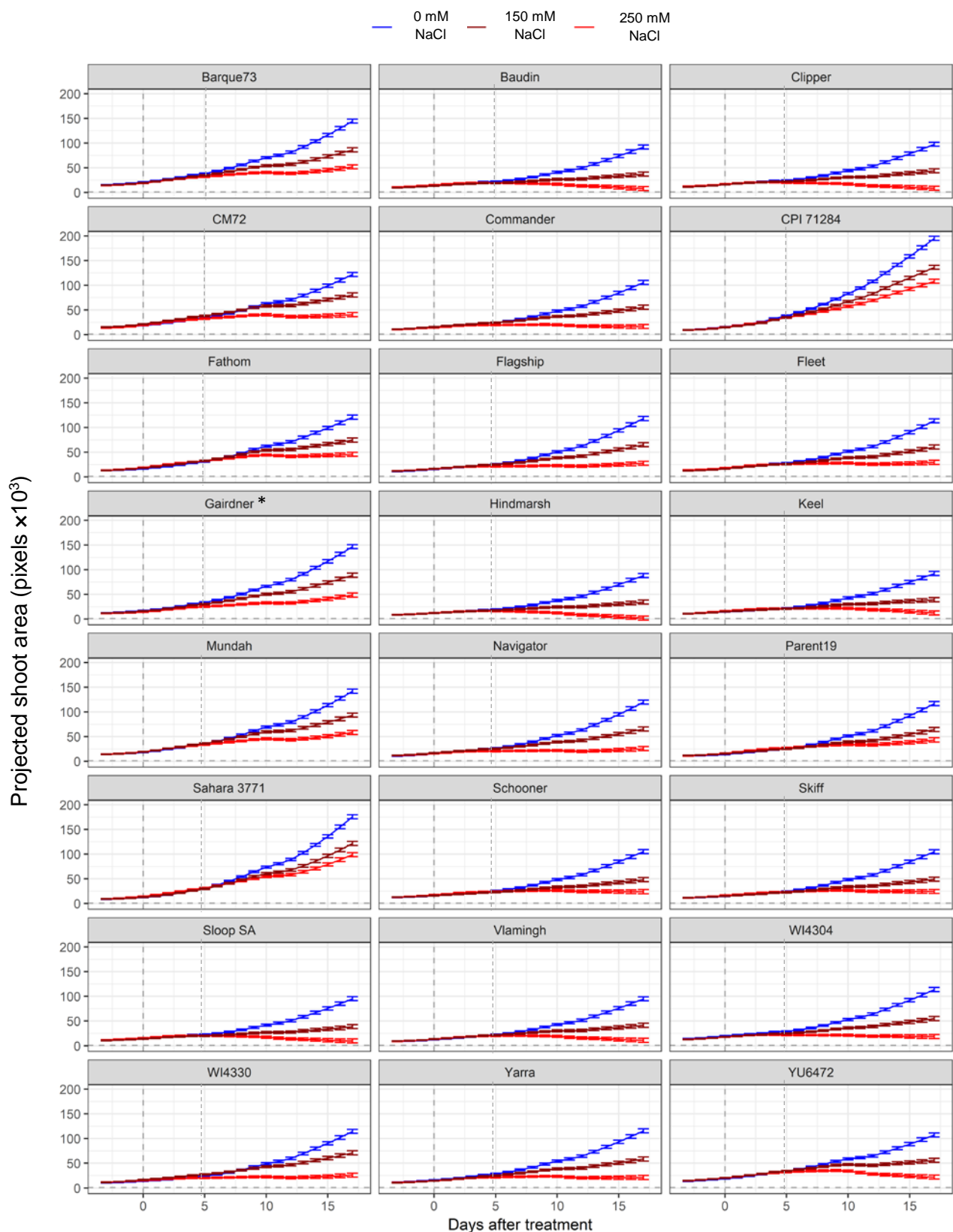
**Table S1. Ranking of the shoot ion independent salt tolerance of barley under 150 mM NaCl treatment, shown in the context of the other traits measured: ion independent tolerance (ratio of the absolute growth rate calculated from days 0 to 5 after treatment), leaf water content (mean at 17 days), leaf health (median green:yellow pixel ratio at day 17), leaf [Na<sup>+</sup>] and [K<sup>+</sup>] (mean, 17 days after salt treatment) and the K<sup>+</sup>/Na<sup>+</sup> ratio**

Accessions WI4304 and WI4330 are genotypes developed as part of the University of Adelaide Barley Breeding Program and have not been rated for malting quality as they are not commercially available. (Mean,  $n = 5-8$ )

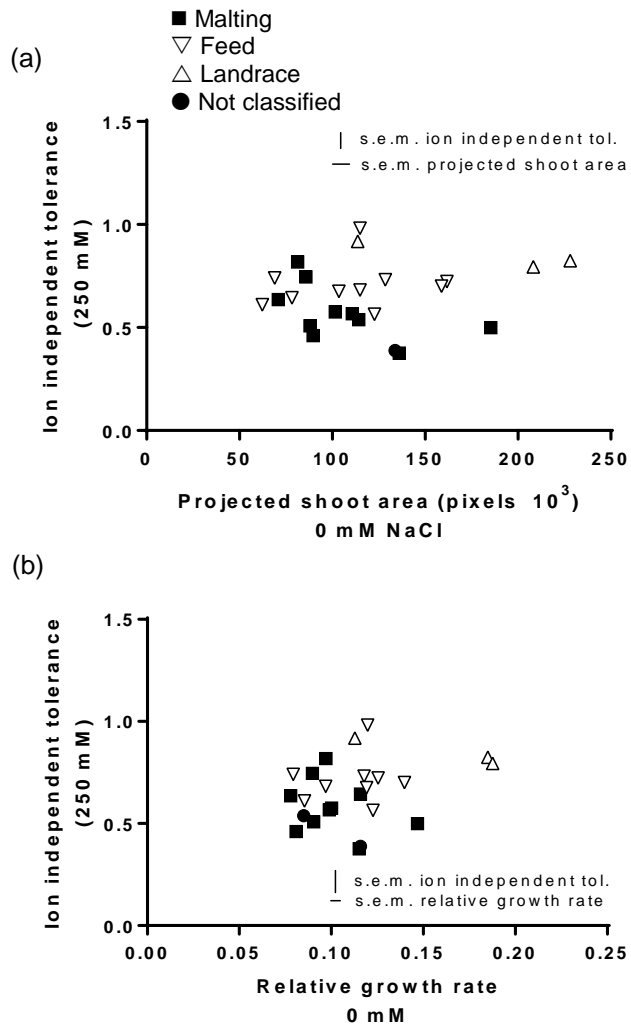
Genotype	Grain Use	Ion independent tolerance	Na <sup>+</sup> accumulation (mM)	K <sup>+</sup> accumulation (mM)	K <sup>+</sup> /Na <sup>+</sup>	Leaf water content (g)	Leaf health	Salt tolerance
Fathom	Feed	1.027	93.7	253.8	2.71	0.055	0.994	0.694
CM72	Feed	1.000	169.5	172.5	1.02	0.322	0.993	0.657
Skiff	Malting	0.866	113.4	225.3	1.99	0.092	0.991	0.638
Mundah	Feed	0.869	99.2	240.4	2.42	0.436	0.992	0.636
Sahara 3771	Landrace	0.894	98.9	237.1	2.40	0.076	0.990	0.623
Keel	Feed	0.810	128.5	212.4	1.65	0.040	0.984	0.615
Schooner	Malting	0.841	139.8	186.0	1.33	0.055	0.992	0.610
YU6472	Feed	0.859	99.7	255.0	2.56	0.181	0.992	0.601
Flagship	Malting	0.933	115.4	217.6	1.88	0.086	0.991	0.598
Hindmarsh	Feed	0.874	126.6	197.8	1.56	0.049	0.987	0.582
Baudin	Malting	0.886	107.0	215.3	2.01	0.050	0.990	0.569
Vlamingh	Malting	0.825	154.6	159.9	1.03	0.089	0.982	0.568
Commander	Malting	0.881	163.2	186.3	1.14	0.068	0.988	0.562
Barque73	Feed	0.832	106.7	242.0	2.27	0.323	0.991	0.552
Parent19	Landrace	0.698	176.7	323.4	1.83	0.138	0.989	0.540
CPI 71284-48	<i>Spontaneum</i>	0.855	118.0	225.6	1.91	0.507	0.994	0.520
Gairdner	Malting	0.873	103.8	213.2	2.05	0.384	0.989	0.518
WI4330	Undetermined	0.954	195.6	169.1	0.86	0.111	0.981	0.492
Clipper	Malting	0.806	193.3	200.8	1.04	0.061	0.978	0.471
Fleet	Feed	0.931	100.5	272.2	2.71	0.135	0.992	0.469
Yarra	Feed	0.863	144.6	183.8	1.27	0.141	0.987	0.462
Navigator	Malting	0.795	173.0	175.2	1.01	0.096	0.984	0.444
WI4304	Undetermined	0.796	116.1	189.0	1.63	0.019	0.965	0.439
Sloop SA	Malting	0.722	360.7	303.3	0.84	0.043	0.850	0.323



**Fig. 1.** Performance of barley lines when group by their malting classification for (a) salinity tolerance (size of plant in 250 mM NaCl ÷ size of plant in 0 mM NaCl), (b) shoot ion independent tolerance and (c) accumulation of Na<sup>+</sup> in the leaf. Results which are significantly different  $P \leq 0.05$  (One-way ANOVA) are indicated with different letters. Recent landrace derived lines were included in the landrace classification. Varieties were classified according to malting or feed characteristics. However, most malting varieties can also be used as feed barley. The landrace derived lines are significantly different to the malting and feed varieties for all three traits.



**Fig. S2.** Growth of barley over a period of 21 days with NaCl treatment of 0 (blue), 150 (dark red) or 250 (light red) mM applied at day 0. The period between 0 and 5 days after treatment (indicated with the vertical broken lines) was used to calculate the shoot ion independent tolerance, before ions were able to accumulate to high concentrations in the shoot and significantly affect shoot function. Values plotted are the mean and s.e.m. of the mean ( $n = 5-8$  \*Gairdner  $n=1$  at 250 mM as all other plants died).



**Fig. S3.** Relationship between mean relative shoot growth rate for each cultivar at 0 mM NaCl and the cultivar's ion independent tolerance at 250 mM NaCl.