Supplementary Material

Plant ionic relation and whole-plant physiological responses to waterlogging, salinity and their combination in barley

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Fig. 1. Salinity (A) and waterlogging (B) stress tolerance of selected barley varieties (based on Wu *et al.* 2015 and Huang *et al.* 2015, respectively). For quantitative estimation of tolerance index, see Materials and Methods.



Fig. S2. Quantification of salinity tolerance index in barley. The extent of salt damage to plant is quantified on 0 to 10 scale (0 = no visual symptoms; 10 = completely dead plants) as illustrated in photos above based on the percentage of chlorotic and necrotic leaves. Salinity treatment (320 mM NaCl) was applied when plants reach 3-leaf stage and lasted for 5 weeks, before the visual assessment was conducted. Reproduced from Xu R, Wang J, Li C, Johnson P, Lu C, *et al.* (2012) A single locus is responsible for salinity tolerance in a Chinese landrace barley (*Hordeum vulgare* L.). *PLoS ONE* 7(8), e43079. doi:10.1371/journal.pone.0043079, with some modifications.



Fig. S3. Correlation between shoot fresh (SFW) and dry (SDW) weight (in grams per plant) of 12 barley varieties grown under saline, waterlogged and control conditions with SFW and SDW of plants grown under combined salinity and waterlogging stress. Each point represents an individual plant.



Fig. 4. Effects of separate and combined salinity and waterlogging stresses on chlorophyll content (SPAD values) of selected 12 barley varieties relative to their control (%). SPAD value measurements were taken 10 and 15 days after the treatment onset.

| 10 Days Treatment (all varieties) | | | | 10 Days Treatment (tolerant varieties only) | | |
|-----------------------------------|--|--|--|--|---|--|
| Con | trol NaCl | WL | | Control | NaCl | WL |
| 0.45 | 5** | | NaCl | 0.280 | · · | |
| 0.49 | 5** 0.458* | * | WL | 0.380 | 0.085 | |
| 0.0 | 57 0.099 | 0.234 | WL/NaCl | 0.324 | 0.033 | 0.629** |
| Days Treat | ment (sensitive | varieties only) | | 15 Days Tre | atment (toler: | ant varieties only) |
| ntrol | NaCl | WL | | Control | NaCl | WL |
| 0.487** | | | NaCl | 0.086 | | |
| 0.523** | 0.537** | | WL | 0.489* | 0.559** | |
| -0.018 | -0.020 | 0.005 | WL/NaCl | 0.447* | 0.541* | 0.746** |
| | Com 0.45: 0.49: 0.0: Days Treat ntrol 0.487** 0.523** | Control NaCl 0.455** 0.495** 0.495** 0.458* 0.057 0.099 Days Treatment (sensitive mtrol NaCl 0.487** 0.523** 0.537** 0.018 0.020 | Control NaCl WL 0.455** 0.495** 0.458** 0.095 0.099 0.234 Days Treatment (sensitive varieties only) wL 0.487** 0.523** 0.537** 0.018 0.020 0.005 | Control NaCl WL 0.455** NaCl 0.495** 0.458** 0.057 0.099 0.234 WL/NaCl Days Treatment (sensitive varieties only) WL ntrol NaCl WL 0.487** 0.537** WL 0.018 0.020 0.005 WL/NaCl | Control NaCl WL Control 0.455** NaCl 0.280 0.495** 0.458** WL 0.380 0.057 0.099 0.234 WL/NaCl 0.324 Days Treatment (sensitive varieties only) 15 Days Tree ntrol NaCl WL 0.086 0.523** 0.537** WL 0.489* 0.018 0.020 0.005 WL/NaCl 0.447* | Control NaCl WL Control NaCl 0.455** 0.455** NaCl 0.280 0.495** 0.458** WL 0.380 0.085 0.057 0.099 0.234 WL/NaCl 0.324 0.033 Days Treatment (sensitive varieties only) 15 Days Treatment (toler: ntrol NaCl WL 0.086 0.487** 0.537** WL 0.489* 0.559** 0.018 0.020 0.005 WL/NaCl 0.447* 0.541* |

Fig. 5. Correlation (r values) between chlorophyll content (SPAD value) in barley leaves exposed to different stresses and their combinations. Plants were divided into two groups (sensitive and tolerant) according to their overall salinity stress tolerance, and r values have been calculated for chlorophyll data measured after 10 and 15 days of appropriate stress.