Supplementary Material: Table 1 experiment descriptions

Okamoto *et al*. (2013) cut leaves and measured the rate of water loss, and also stopped the irrigation of Arabidopsis and soybean plants growing in $6 \times 6 \times 5$ cm pots containing 100 g of soil and then assessed wilting (Okamoto *et al.* 2013). This method of complete withholding of irrigation was also used by Ni *et al*. (2013), in potted Arabidopsis plants whose survival rates upon re-watering were examined (Ni *et al.* 2013).

Jeong *et al*. (2013) withheld irrigation of rice in $3 \times 3 \times 5$ pots at the vegetative stage and then measured chlorophyll fluorescence. That group also conferred drought under field conditions in 1-m-deep pots under a rain-off shelter that was used to withhold water for different periods. Those dry periods were followed by resuscitation and yield was measured (Jeong *et al.* 2013).

Nakabayashi *et al*. (2014) used the Arasystem in which pots containing three Arabidopsis plants were exposed to 16 days of no irrigation and then re-watered. That study measured survival (Nakabayashi *et al.* 2014).

Luo *et al*. (2013) used MS plates containing 30% PEG 6000 to simulate drought stress and also halted the irrigation of 3-week-old Arabidopsis plants for 2 weeks followed by resuscitation. That study also measured survival rates (Luo *et al.* 2013).

Martinez *et al*. (2015) halted irrigation of 4-week-old Arabidopsis plants grown in $7 \times 7 \times 6.5$ cm pots for 2 weeks and then measured their fresh weights (Martínez *et al.* 2015).

Ruiz-Lozano *et al*. (2016) conferred drought stress by keeping the soil moisture level at 75% or 55% of that of a well-watered control (moderate and severe stress, respectively). Actual volumetric soil water levels were 14% and 8%, respectively. Plant biomass was measured at different times and transpiration was assessed (Ruiz-Lozano *et al.* 2016).

Ramiro *et al*. (2016) halted irrigation of sugarcane in 10-L pots. As the soil dried, a variety of physiological parameters were examined including leaf lesions, A_N, G_s, RWC and soluble-sugar content. Transgenic sugarcane showed far less of a

reduction in first four variables and an increase in leaf soluble sugar content, suggesting a mechanism for the observed drought tolerance (Ramiro *et al.* 2016).

Ahmad *et al.* (2015) overexpressed the potassium channel OsTPKb in rice. This led to higher K^+ uptake and higher K^+ tissue content and to less of a reduction in the relative growth rate under drought conditions (Ahmad *et al.* 2015).

Metz *et al.* (2015) irrigated *Biscutella didyma* plants in $13 \times 13 \times 13$ pots with 6 different water volumes (from 180 ml to 20 mL) every 3-5 days and confirmed soil water contents were indeed reduced (from 45% soil moisture to around 10%). The number of seeds produced, the survival to reproduction ratio, biomass of survivors, time to first flowering and height at that time, and mass of single-seeded fruit were assessed (Metz *et al.* 2015).

Supplement: Table 2 experiment descriptions

There are many examples of methods used to evaluate drought tolerance. Kang *et al.* (2013) examined the height, root length and fresh and dry biomass of wheat seedlings grown in a hydroponic system. The drought treatment consisted of supplementing growth medium with 15% PEG 6000 (Kang *et al.* 2013).

Timmusk *et al.* (2014) stopped watering wheat at 10 days after germination for 10-14 days (depending on soil), followed by resuscitation. Plants that recovered were counted. Survivors' root and shoot biomass, root number, root hair density and length, ratio between root adhering soil and root biomass, and water-use efficiency – calculated by water use during experiment, were measured. Gas exchange, including Gs, AN and E, was measured by Walz GFS-3000 portable gas exchange system five times between the beginning of the drought treatment and Day 10 of that treatment. Stress-related volatiles were measured by putting samples of gas-exchange chamber air onto a multi-bed stainless steel cartridge. Ethylene emission was assessed using a Picarro G1106 real-time ethylene analyzer (Picarro Inc., CA, USA) (Timmusk *et al.* 2014).

Manthman *et al.* (2013) evaluated drought resistance by measuring plant relative water content 11 days after the start of a drought treatment. In addition,

visible characteristics such as plant vigor and wilting were also examined. Gas-exchange measurements were performed using a portable, open flow, gas-exchange system (LCpro+, Opti-Sciences, Hudson, NH, USA) at 4, 14 and 17 days after drought was treatment was started. However, this was not done to assess drought tolerance, but rather because the silenced genes have direct effects on these parameters through ABA signaling (Manmathan *et al.* 2013).

Honsdorf *et al.* (2014) examined 47 barley introgression lines for drought response, using a high-throughput phenotyping system. Three photographs in RGB were taken of plants every day following stress induction – one photo from above and two from the sides with 90° between the side photos. Pixels were counted and biomass was calculated. This method was used for growth-rate calculations and the assessment of drought tolerance. In addition, caliper length, height, color, shoot area, top view and convex hull area were extracted from images and plant compactness was calculated. At the end of the experiment, plants were harvested and fresh and dry biomass, tiller number and plant height were measured. Water-use efficiency and simple stress indices were calculated, as well as the correlation between pixel number and biomass. Plant images were captured using LemnaTec 3D Scanalyzer (LemnaTec, GmbH, Wuerselen, Germany). In this screening, correlations were noted between several QTLs that affect traits such as plant height and QTLs found in field trials (Honsdorf *et al.* 2014).

Al Abdallat *et al.* (2014) overexpressed the transcription factor hvSNAC1 in barley. To assess drought tolerance, plants were first grown in a greenhouse, in which seedling irrigation was stopped for 10 days, followed by resuscitation. Tolerance was evaluated in terms of survival. Tiller number, RWC, G_s and chlorophyll fluorescence were also recorded. In addition, a field experiment was conducted in an area with an annual rainfall of 200 mm and tiller number, spike number and grain number, grain per spike, plant biomass and yield were measured (Al Abdallat *et al.* 2014).

Kapanigowda *et al.* (2014) examined drought tolerance in 70 sorghum recombinant inbred lines (RILs). Two experiments were performed. In the first, plants in a greenhouse were subjected to drought by reducing soil water content to 40% of field capacity compared to 80% control. Gas exchange was measured using a LI-COR 6400 on four consecutive days starting with plants that were 30 days old. Further

experiments were performed under non-stress field conditions (Kapanigowda *et al.* 2014).

Avramova *et al.* (2016) used eight commercial inbred lines, some of which have been shown to be drought tolerant in field experiments. Drought was imposed by starting experiments at lower soil water content (not followed by re-watering). High-throughput imaging was used to photograph shoots and roots once a day. Visible root length, maximum depth, width, root surface coverage root elongation, projected shoot area and shoot growth rate were calculated from the photographs. The length of the fourth leaf was also recorded daily and the elongation rate was calculated. Plants were harvested, roots were washed and scanned, and then root length was determined as dry biomass and shoot fresh and dry biomass were measured. Gas exchange parameters were measured using a LI-COR 6400. In a second experiment performed in a growth room, the cell production rate, cell division rate, length of mature cells, meristem length and number of cells in the elongation zone were monitored. Of these traits, the data concerning root and shoot biomass correlated nicely with similar data from field trials. Interestingly, tolerant lines had impaired growth under well-watered conditions and may have been drought tolerant due to more conservative water use before the stress appeared (Avramova *et al.* 2016).

Mao *et al.* (2015) overexpressed *zmNAC111* in maize and Arabidopsis. Plants were stressed by reducing water content to 70% or 58% relative water content. Plants were resuscitated after showing a wilting phenotype and the survival rate was evaluated (Mao *et al.* 2015).

Reguera *et a*l. (2013) expressed the gene of an enzyme that mediates the rate limiting step in cytokinin synthesis, *ISOPENTENYLTRANSFERASE* (IPT) in rice, after the senescence-associated SARK promoter. Gas-exchange parameters such as carbon assimilation, stomatal conductance and chlorophyll fluorescence were evaluated. A:Ci curves were constructed from which maximum carboxylation, electron transport and triose phosphate utilization rates were extracted and Rubisco activity, ribulose bisphosphate regeneration and phosphate limitations were assessed. Further measurements were made of ABA levels, sugar content, protein content, enzyme activity and N-related gene expression (Reguera *et al.* 2013).

Tang *et al.* (2012) expressed a transgenic *OsbZIP46CA1* gene that has a deletion in a domain inhibiting its action in rice. Plants were subjected to drought by suspending irrigation for one week at the 4-leaf stage. After one week, plants were resuscitated. Survival rates were recorded, as well as the water loss of detached leaves. Other abiotic stress factors such as salt tolerance and cold tolerance were also evaluated (Tang *et al.* 2012).

Kissel *et al.* (2015) examined the tolerance of different banana species to stress. Drought stress was conferred at two intensities by keeping the relative volumetric soil water content of the soil at 27%-28%, or 19%-25%, as compared to 30%-33% in the control. Biomass was estimated via morphological measurements of the leaf and pseudostem, which were previously found to correlate to biomass with an R^2 of 0.88. TE was calculated from estimated biomass accumulation and transpiration was calculated based on plant weight loss. In addition, carbon isotope composition was also measured. Surprisingly, high TE under drought conditions was correlated with rapid growth under non-stressed conditions (Kissel *et al.* 2015).

Tugendhaft *et al.* (2016) examined the effects of two levels of drought severity on three olive cultivars. Irrigation was withheld for 11 days until pots reached 33% of field capacity, and then for another 7 days until pots reached 10% field capacity. Stem diameter, leaf water potential (via pressure chamber) and gas exchange, including PS2 ETR (via LI-COR), were measured. Trees that had less reduction in Gs and A_N as well as less reduction in leaf water potential under drought were considered drought tolerant (Tugendhaft *et al.* 2016).

Seghal *et al.* (2015) worked with a pearl millet inbred germplasm association panel (PMiGAP) with 250 lines. Terminal drought treatment was initiated at two different stages either at 50% flowering by withholding irrigation 1 week prior to flowering or initiated during early grain filling by withholding irrigation at 50% flowering. Grain yield, panicle yield, time to 75% flowering, plant height, panicle length, panicle diameter, panicle number, tiller number, biomass yield, 1000-grain weight, the stay-green phenotype and leaf rolling were measured and marker-trait associations were analyzed. The stay-green phenotype during grain filling was correlated positively with yield. Leaf rolling was negatively correlated with yield (Sehgal *et al.* 2015).

Thu *et al.* (2014) evaluated the drought tolerance of 13 Vietnamese soybean cultivars. This was done by measuring survival rates and calculating a drought tolerance index based on monitoring non withered plant ratio along the drought treatment and resuscitation. Root and shoot length, dry biomass and RWC under both non-stressed and drought conditions were also measured. Drought was created by stopping irrigation for 15 days, starting at 12 days after planting, followed by 15 days of resuscitation (Thu *et al.* 2014).

Li *et al.* (2013) expressed the *LOS5/ABA3* gene encoding a molybdenum cofactor sulfurase from Arabidopsis in soybean. Detached leaf water loss, RWC, leaf water potential, transpiration rate, biomass, stomatal aperture and electrolyte leakage were evaluated, as well as yield and pod and seed numbers in field tests. The drought treatment in the growth chamber was applied using two methods: stopping the irrigation of 21-day-old seedlings for 10 days, followed by resuscitation, and for RNA extraction, irrigation water volumes of 60% and 40% of control volumes. Field drought stress was conferred by stopping irrigation and a rain shelter was set up at R1 stage for 21 days followed by irrigation (Li *et al.* 2013).

Varshney *et al.* (2014) searched for correlations between genotypic and phenotypic data using mapping populations. They evaluated 20 drought-related traits, including root length, root length density, root dry weight, rooting depth, root surface area and root volume, plant dry weight, plant height, plant stand, plant width primary branches, secondary branches, days to 50% flowering and days to maturity, 100-seed weight, pods per plant, seeds per pod, yield, biomass, yield index and carbon isotope discrimination. Drought was conferred by planting after the rainy season and using a rainout shelter (Varshney *et al.* 2014).

Singh *et al.* (2013) screened 80 lentil genotypes for drought tolerance. Parameters measured included survivability, growth inhibition and drought score derived from the visual evaluation of wilting phenotype (rated on a scale of 1-5), root and shoot length and fresh and dry biomass. Drought was conferred both in a hydroponic system and in soil. In the hydroponic system, this included exposure of seedling roots to air for 5 h a day for 5 days. For the soil drought treatment, water-saturated pots were let dry for 6 weeks (until approximately 2% field capacity) with no resuscitation or followed by 12 days of resuscitation. The hydroponic drought

treatment proved to be as effective as the soil drought treatment. Tolerant plants were smaller in terms of all parameters, hinting at tolerance through inhibition of growth (Singh *et al.* 2013).

Rolando *et al.* (2015) compared 3 potato variants for drought tolerance, and its correlation with changes in leaf chlorophyll content. Parameters measured included dry plant biomass, dry tuber biomass, leaf chlorophyll content (measured by SPAD), leaf N content, plant coverage, gas exchange measurements including g_s, E and A_{max} where A_{max} was measured according to previously performed light curves. From these, intrinsic WUE was calculated (A_{max}/E) as well as a drought susceptibility index. Drought was conferred by measuring pot water loss every 48 hours, and re-watering with 50% of transpired water volume, compared to 100% in control pots. It was found that plants that had increased greenness under moderate stress were more conservative in their stress response, which led to lower yield compared to risk taking variants. In addition, it was found that genotypes that exhibited lower chlorophyll degradation under mild drought also exhibited higher yield.(Rolando *et al.* 2015).

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