

Tuning water-use efficiency and drought tolerance in wheat using abscisic acid receptors

Ryosuke Mega¹, Fumitaka Abe², June-Sik Kim³, Yuuri Tsuboi⁴, Keisuke Tanaka⁵, Hisato Kobayashi^{5,11}, Yoichi Sakata⁶, Kousuke Hanada⁷, Hisashi Tsujimoto¹, Jun Kikuchi⁴, Sean R. Cutler⁸ and Masanori Okamoto^{1,9,10*}

Water availability is a key determinant of terrestrial plant productivity. Many climate models predict that water stress will increasingly challenge agricultural yields and exacerbate projected food deficits. To ensure food security and increase agricultural efficiency, crop water productivity must be increased. Research over past decades has established that the phytohormone abscisic acid (ABA) is a central regulator of water use and directly regulates stomatal opening and transpiration. In this study, we investigated whether the water productivity of wheat could be improved by increasing its ABA sensitivity. We show that overexpression of a wheat ABA receptor increases wheat ABA sensitivity, which significantly lowers a plant's lifetime water consumption. Physiological analyses demonstrated that this water-saving trait is a consequence of reduced transpiration and a concomitant increase in photosynthetic activity, which together boost grain production per litre of water and protect productivity during water deficit. Our findings provide a general strategy for increasing water productivity that should be applicable to other crops because of the high conservation of the ABA signalling pathway.

Climate change and population growth have spurred urgent calls to increase crop water productivity (that is, grain yield per litre of consumed water). Rising global temperatures are anticipated to exacerbate crop losses, and agricultural water supplies must be increased by an estimated 17% by 2025 to compensate for global population growth¹. New strategies for improving crop water productivity are thus critical for next-generation agriculture². Most water used in agriculture is driven to the atmosphere by transpiration—a process necessary for CO₂ fixation and growth. Transpiration is controlled by the movements of stomata, which are small epidermal leaf pores formed by a pair of guard cells that regulate gas and water exchange. Abscisic acid (ABA) is a key small molecule responsible for stomatal closure, and ABA receptors (PYR/PYL/RCAR proteins) play a central role in executing ABA's role in water relations^{3,4}. ABA controls the activity of a stress-activated kinase signalling network that regulates guard cell osmotic pressure and stomatal closure. In response to water deficit, ABA levels rise, which directs the formation of a ligand–receptor complex that inhibits the activity of clade A protein phosphatase 2Cs (PP2Cs), which are negative regulators of ABA signalling^{5–7}. ABA-mediated inhibition of PP2Cs enables activation

of sucrose non-fermenting-1 (SNF1)-related protein kinases that elicit stomatal closure by phosphorylation and activation of S-type anion channels to trigger anion flux and stomatal closure^{7–9}.

Because of their pivotal role in transpiration regulation, ABA receptors are excellent targets for manipulating ABA sensitivity and water productivity¹⁰. Moreover, overexpression of ABA receptors has been extensively validated as a means to increase *Arabidopsis* ABA sensitivity¹¹ and biomass produced per litre of water¹². In addition, the same strategies were attempted in rice¹³ and poplar¹⁴. We therefore investigated the effectiveness of ABA receptor overexpression as a strategy for designing water-productive wheat. Wheat is a staple crop of worldwide importance that is particularly vulnerable to future climate conditions, as its yield is predicted to decrease by approximately 6.0% per °C rise in global mean temperature¹⁵.

We exploited the International Wheat Genome Sequencing Consortium sequence for hexaploid wheat¹⁶ (Supplementary Table 1) to identify wheat ABA receptors (TaPYLs) with sequences similar to dicot and monocot ABA receptors. The isolated TaPYLs phylogenetically clustered into three families that are conserved across angiosperms (Fig. 1a, Supplementary Fig. 1a and Supplementary Table 1). We similarly identified six TaPP2Cs (Supplementary Fig. 2a and Supplementary Table 1) and tested TaPYL ABA receptor activity using in vitro phosphatase inhibition assays (Fig. 1b and Supplementary Fig. 3). The isolated TaPYLs, except for TaPYL9, inhibited clade A PP2C activity, with half-maximum inhibitory concentration (IC₅₀) values indicating that these proteins are bona fide ABA receptors. In addition, TaPYL messenger RNA accumulation was suppressed and TaPP2C messenger RNAs were induced by both ABA and abiotic stress treatments, consistent with transcriptional responses observed for orthologues in other species (Fig. 1c,d, Supplementary Fig. 2b and Supplementary Table 3). Next, we investigated whether ABA receptor overexpression could be used to tune the ABA sensitivity of wheat. TaPYL4 was selected as a candidate for two reasons: its expression levels were relatively high under basal conditions and at various developmental stages, and it was determined to be a monomeric-class high-affinity ABA receptor (Fig. 1c,d and Supplementary Figs. 1b and 3). Interestingly, no allelic variations in *TaPYL4* were observed, although we assessed *TaPYL4* using published wheat databases^{17,18} and cloned sequences from the cultivars Cham6, Nesser, Fielder, Roelfs-F2007 and Norin61.

¹Arid Land Research Center, Tottori University, Tottori, Japan. ²NARO Institute of Crop Science, Tsukuba, Japan. ³Gene Discovery Research Group, RIKEN Center for Sustainable Resource Science, Tsukuba, Japan. ⁴Environmental Metabolic Analysis Research Team, RIKEN Center for Sustainable Resource Science, Yokohama, Japan. ⁵NODAI Genome Research Center, Tokyo University of Agriculture, Tokyo, Japan. ⁶Department of Bioscience, Tokyo University of Agriculture, Tokyo, Japan. ⁷Frontier Research Academy for Young Researchers, Kyushu Institute of Technology, Kitakyushu, Japan.

⁸Department of Botany and Plant Sciences, Institute of Integrative Genome Biology, University of California, Riverside, CA, USA. ⁹Center for Bioscience Research and Education, Utsunomiya University, Utsunomiya, Japan. ¹⁰PRESTO, Japan Science and Technology Agency, Kawaguchi, Japan.

¹¹Present address: Department of Embryology, Nara Medical University, Nara, Japan. *e-mail: okamo@cc.utsunomiya-u.ac.jp

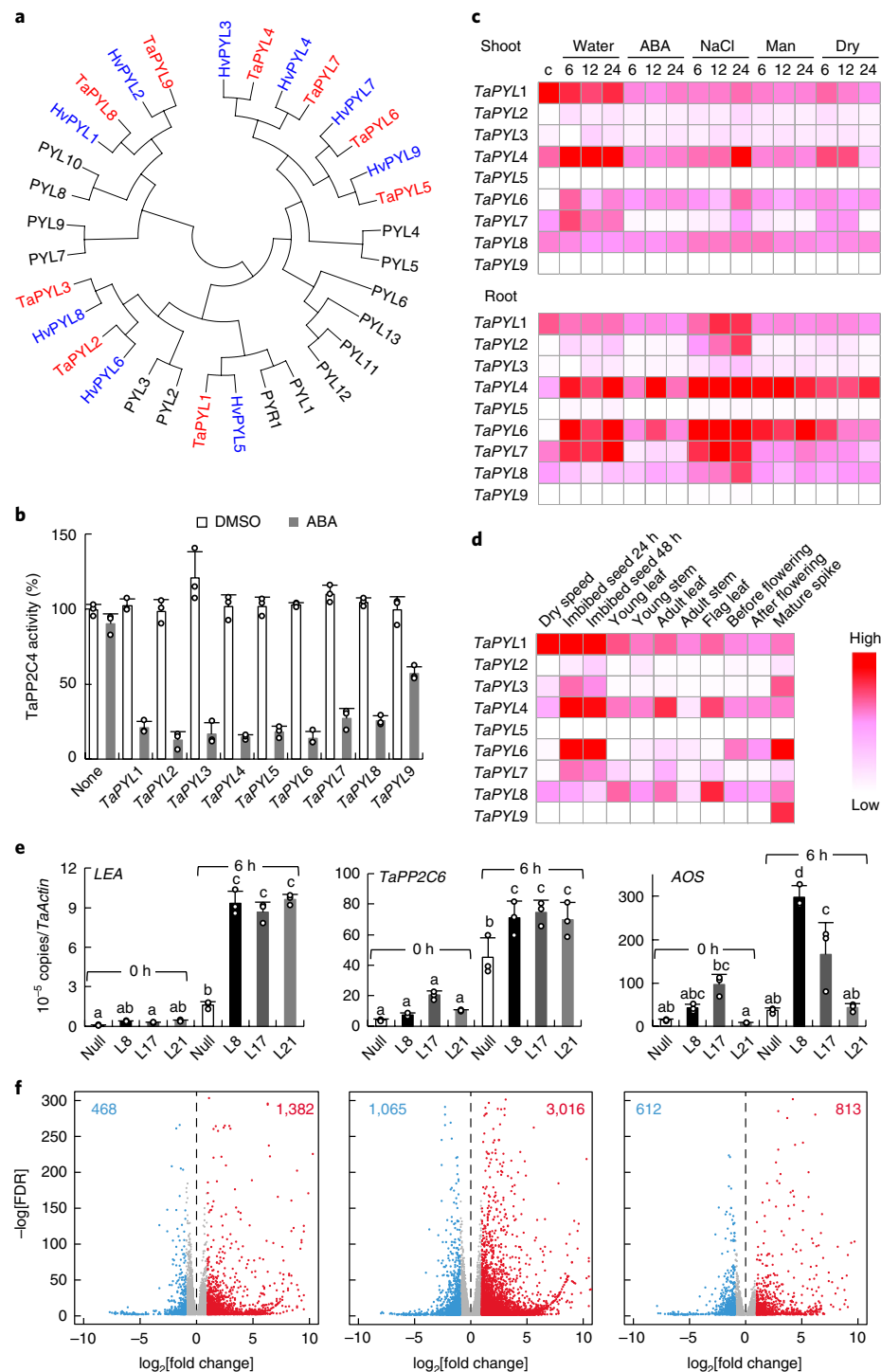


Fig. 1 | Molecular characterization of wheat ABA receptors. **a**, Phylogenetic tree for ABA receptors (black, *Arabidopsis thaliana*; red, *Triticum aestivum* ('Chinese Spring'); blue, *Hordeum vulgare*) constructed using the maximum-likelihood method in MEGA 6.06 followed by bootstrapping with 1,000 replicates. **b**, Inhibition assay of TaPYLs towards TaPP2C4 ($n=3$ biologically independent samples; central values and error bars represent means $\pm$ s.d.). DMSO, dimethyl sulfoxide. **c,d**, Expression of TaPYL genes under various stresses (**c**) and at different developmental stages (**d**). Water was used as a control (**c**). For the stress treatments in **c**, 7-day-old seedlings were subjected to 20 μ M ABA, 150 mM NaCl, 200 mM mannitol (Man) or dehydration (Dry). Numbers are treatment times (h). TaPYL expression levels were evaluated according to copy number and normalized relative to TaActin ($n=3$ biologically independent samples). **e**, Expression of ABA-responsive genes in Null and TaPYLox seedlings. Seven-day-old seedlings were treated without (0 h) or with 0.5 μ M ABA for 6 h ($n=3$ biologically independent samples; central values and bars represent means $\pm$ s.d.). Different letters denote significant differences ($P < 0.05$) based on the Tukey–Kramer test. Gene expression levels measured by qRT-PCR in **c–e** represent the sum of the expression levels of the three homeologues. **f**, Volcano plots of expression comparisons (L8 versus Null) under WWC (left), ABA (middle) and drought treatments (right). Differentially expressed transcripts were defined as those with a fold change ≥ 2 and false discovery rate (FDR) < 0.05 using the likelihood ratio test for multiple comparison ($n=3$ biologically independent samples). Red and blue numbers represent upregulated and downregulated transcripts of L8 compared to Null, respectively.

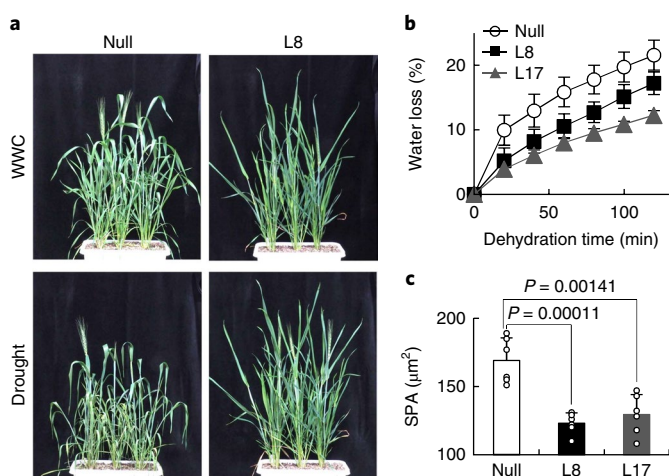


Fig. 2 | ABA receptor overexpression confers drought tolerance.

a, Photographs of Null and TaPYLx L8 lines under WWC and drought. Before photography, plants cultivated for 50 d were subjected to drought stress by withholding water for 13 d. **b**, Results of a water loss assay for Null and TaPYLx. For the assay, leaves of 50-day-old plants were detached and weighed at 20 min intervals ($n=4$ biologically independent samples; central values and error bars represent means $\pm$ s.d.). **c**, TaPYLx stomatal pore area (SPA) ($n=6$ biologically independent samples; central values represent means $\pm$ s.d.). A two-tailed Student's *t*-test was used to compare Null plants and each TaPYLx line.

Thus, without consideration of allelic variation, we generated three TaPYL4-overexpressing wheat (TaPYLx) transgenic lines exhibiting high-level transgene expression (Supplementary Fig. 4). We obtained a non-transgenic 'null-segregant' sibling from the progeny of one of these primary transgenic lines to serve as an isogenic control line for molecular and physiological comparisons. As anticipated, multiple lines of evidence indicated that ABA receptor overexpression in wheat increases ABA sensitivity (Fig. 1e). Transcriptome analyses of wheat plants exposed to well-watered (WWC), ABA-treated (ABA) or water-deficit (drought) conditions revealed that substantially more genes in the TaPYLx lines responded to ABA treatment (Fig. 1f, Supplementary Fig. 5d and Supplementary Table 2), consistent with increased ABA sensitivity. TaPYLx homologue expression appears to be balanced, in contrast with about 40% of homeologous transcripts, where triplets show ternary unbalanced expression (Supplementary Fig. 5a–c and Supplementary Table 1).

Consistent with the increased ABA sensitivity, we also observed an enhanced degree of seed dormancy in TaPYLx seeds—a state that was reversed by stratification (Supplementary Fig. 6). In addition, TaPYLx seedling growth displayed increased ABA sensitivity (Supplementary Fig. 7a–c), and TaPYLx plants were less prone to wilting, their detached leaves exhibited reduced water loss and their stomatal pore apertures were smaller compared with those of the controls (Fig. 2). We also compared the drought sensitivity of TaPYLx lines with three modern wheat cultivars cultivated in arid regions (Cham6, Nesser and Roelfs-F2007) and observed delayed wilting in TaPYLx plants (Supplementary Fig. 8 and Supplementary Video 1). Collectively, these results demonstrate that ABA receptor overexpression increases wheat ABA sensitivity, which in turn reduces the stomatal aperture size and transpiration.

Drought-sensitive wheat cultivars accumulate more ABA during water stress than drought-tolerant cultivars¹⁹. To investigate this relationship, we analysed the ABA levels of TaPYLx and control plants. Drought-inducible ABA levels in TaPYLx lines were lower than in control plants (~17-fold versus ~3–7-fold induction;

Supplementary Fig. 9). Consistent with this result, the number of drought-regulated genes in TaPYLx lines was smaller (2,026) than in control plants (2,968) (Supplementary Fig. 5d).

Next, we conducted leaf-level gas exchange measurements to better characterize the effects of ABA receptor overexpression on water use and carbon assimilation. Under irradiance levels comparable to the mid-day sun ($>1,000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$), TaPYLx transpiration was suppressed relative to the controls, whereas CO_2 assimilation rates were comparable (Fig. 3a,b), accordingly with 14 and 25% increases in instantaneous water-use efficiency (WUE) obtained for lines L8 and L17, respectively (Fig. 3c,d). Consistent with this result, carbon isotope discrimination ($\delta^{13}\text{C}$) analyses of plant leaves revealed reduced ^{13}C fractionation in the TaPYLx transgenic lines (Fig. 3i). The degree of ^{13}C fractionation by Rubisco decreases as intercellular CO_2 levels decline because of stomatal closure, and, as a result, $\delta^{13}\text{C}$ values are commonly used to estimate differences in WUE between plants²⁰. Examination of the relationship between the CO_2 assimilation rate (*A*) and intercellular CO_2 concentration (C_i) (*A*– C_i curves) revealed that TaPYLx assimilated CO_2 efficiently even though the C_i of TaPYLx was lower than that of control plants at the upper range of CO_2 concentrations (Fig. 3e). These data indicated that the TaPYLx lines possessed increased CO_2 fixation capacity relative to controls, even at ambient CO_2 concentrations (Fig. 3f) where such measurements are more challenging (Fig. 3g). Similar increases in CO_2 assimilation induced by ABA receptor overexpression have also been reported in *Arabidopsis*¹². Analysis of *A*– C_i curves²¹ revealed that maximum rate of carboxylation (V_{cmax}) values in TaPYLx plants were higher than in control plants (Fig. 3h), which suggests that increased Rubisco activity underlies the improvement in carbon assimilation induced by ABA receptor overexpression. Collectively, our data indicate that the TaPYLx lines show improved WUE and achieve this through a combination of reduced stomatal aperture size and increased photosynthetic activity.

ABA receptor overexpression increases wheat WUE. However, to benefit agriculture, this increase must translate to increased grain yield per litre of input water. We therefore measured the total amount of water consumed by TaPYLx strains and control plants over a single generation. Biomass and grain weight were not significantly different between TaPYLx lines and controls under WWC (Fig. 4a–c), although TaPYLx plants varied somewhat in several morphological traits (Supplementary Fig. 10a–e). In contrast, the lifetime water consumption of TaPYLx strains was approximately 20% less than that of the controls and was associated with increased biomass and grain weight per litre of input water in two robust TaPYLx lines (L8 and L17; Fig. 4d–f). These benefits were not associated with significant differences in seed shape or weight (Fig. 4g,h and Supplementary Fig. 10f). Consequently, ABA receptor overexpression does not limit wheat grain production under our growth conditions and improves WUE even in the absence of water stress.

Next, we evaluated grain production under drought conditions. TaPYLx lines and control plants were cultivated under limited water conditions (LWC), which dramatically reduced control grain weight and resulted in small shrunk seeds typical of water-stressed wheat plants. In contrast, the negative effects of water stress were not observed in TaPYLx lines (Fig. 4g,h and Supplementary Figs. 10f and 11a–c). The basis for these improvements was the increased WUE of the transgenic lines, as TaPYLx strains consumed soil water more slowly and retained available soil water for longer than control plants (Supplementary Fig. 11d). In particular, from ~10 d after withholding water supply, TaPYLx lines adopted a more adequate stomatal aperture than control plants. Eventually, this advantage led to enhanced water provision during critical phases of seed maturation in TaPYLx. A similar 'water-banking' or 'water-saving' mechanism has been documented in engineered and

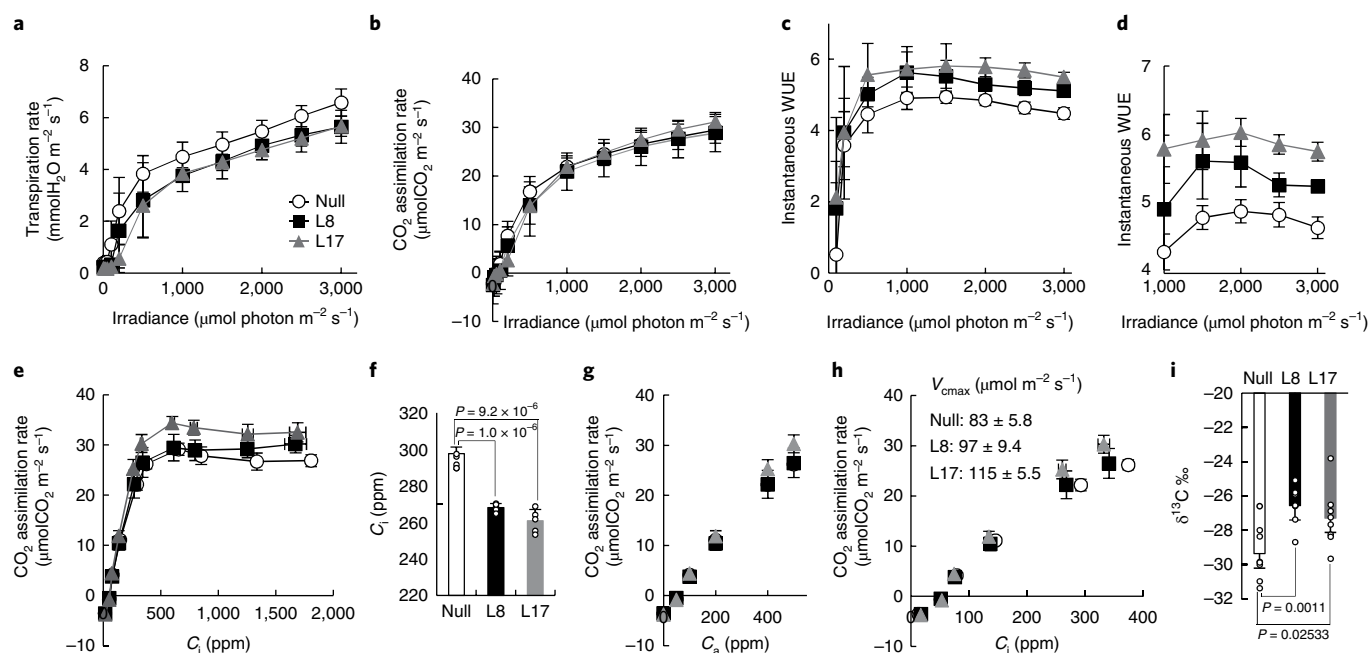


Fig. 3 | ABA receptor overexpression improves WUE in photosynthesis. a, b, Light response of transpiration rate (**a**) and CO_2 assimilation rate (**b**) in Null (open circles), L8 (solid squares) and L17 (solid triangles). **c**, Instantaneous WUE; that is, the ratio of CO_2 assimilation to transpiration rates. **d**, Instantaneous WUE at photon flux density levels $>1,000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$. **e**, Relationship between CO_2 assimilation and intercellular CO_2 concentration (C_i). **f**, C_i values at the ambient CO_2 concentration (C_a) of 400 ppm. **g**, Relationship between CO_2 assimilation and C_i for values of $C_i < 500$ ppm. **h**, Relationship between CO_2 assimilation and C_i for values of $C_i < 400$ ppm. In **a–h**, $n = 5$ biologically independent samples and central values and error bars represent means $\pm$ s.d. **i**, Carbon isotope composition ($\delta^{13}\text{C}$) of Null, L8 and L17 plants. Fully mature leaves similar to those in Fig. 4a were sampled ($n = 8$ biologically independent samples; central values and error bars represent means $\pm$ s.d.). In **f** and **i**, a two-tailed Student's t -test was performed to determine the significance of differences between Null plants and each TaPYLox line.

classically bred drought-tolerant maize lines, suggesting that this approach can be used as a general strategy for crop yield improvement in water-limited environments^{22,23}.

Drought-induced alterations in seed composition can adversely impact grain quality. To investigate seed composition, we performed both one- and two-dimensional NMR analyses to characterize the contents of water-soluble and -insoluble fractions from grains of TaPYLox and control plants. We also compared the samples by principal component analysis (PCA). Control and transgenic lines grown under WWC clustered together in the PCA score plots of both water-soluble and -insoluble fractions, indicating that the TaPYLox transgene had minimal impact on seed metabolite profiles (Fig. 4i and Supplementary Fig. 12a). In contrast, under LWC, TaPYLox lines displayed a profile that was more similar to plants grown under WWC than water-stressed control lines, which displayed a metabolic signature indicative of drought stress. In particular, quantities of drought-inducible amino acids and sugars were elevated in water-stressed controls, whereas arabinoxylan and starch were reduced (Fig. 4j and Supplementary Fig. 12b). Changes in the levels of these compounds are well characterized drought-induced responses in wheat²⁴. These results suggest that TaPYLox lines experience reduced abiotic stress during endosperm development (Fig. 4g,j). Consequently, modulation of ABA sensitivity increases WUE without impacting on grain production or metabolic components under ideal conditions, and increases productivity when water is limited.

Collectively, our findings demonstrate that ABA receptor overexpression in wheat improves grain production under drought and increases WUE. To our surprise, this improvement occurs with negligible effects on final biomass under WWC. The underlying mechanism for the maintenance of high rates of carbon assimilation despite reduced stomatal aperture size is unclear but

involves an increase in carboxylation rates in the TaPYLox lines—a phenomenon also observed in ABA receptor-overexpressing *Arabidopsis* lines¹². Regardless of the mechanism, our study has shown that ABA sensitivity can be rationally tuned by increasing ABA receptor expression levels, with wheat WUE increased in turn. Although we used a transgenic approach to tune water use in this study, altered ABA receptor expression should be readily achievable using new plant breeding techniques through promoter editing or targeted screening for genotypes that increase receptor levels in natural or chemically mutagenized populations^{25–27}. Regardless of the techniques used, new water-banking traits will be essential for engineering futureproof crops that can maintain high productivity in water-limited regions.

Methods

qRT-PCR. For Fig. 1c, wheat Chinese Spring seedlings were hydroponically cultivated at 22/17°C (day/night) and 60% relative humidity under a 16/8 h light/dark photoperiod for 7 d, then treated with 20 μM ABA, 150 mM NaCl, 200 mM mannitol or dehydration for 6, 12 and 24 h. The mannitol and dehydration treatments were used to mimic abiotic stress (a common laboratory approach). For the dehydration treatment, seven-day-old Chinese Spring seedlings were transferred from hydroponic conditions and incubated in a humid box under conditions of gradually imposed drought stress. Shoots and roots were then harvested. For Fig. 1d, Chinese Spring plants were cultivated outdoors in a planter. For Fig. 1e, seedlings were hydroponically cultivated at 22/17°C (day/night) and 60% relative humidity under a 16/8 h light/dark photoperiod for 7 d, then treated without (0 h) or with 0.5 μM ABA for 6 h. Samples were frozen in liquid nitrogen, then ground to a fine powder with a Qiagen TissueLyser II. Total RNA was extracted using a Plant Total RNA purification kit (GMBiolab) from each harvested tissue. Root and aboveground tissues were respectively treated with lysis buffers A and B supplied with the kit, whereas RNA from seeds was extracted after treatment with Plant RNA purification reagent (Thermo Fisher Scientific). Total RNA (500 ng) was reverse-transcribed using ReverTra Ace qPCR RT Master Mix with gDNA Remover (Toyobo). Quantitative reverse-transcriptase PCR (qRT-PCR) was performed on a

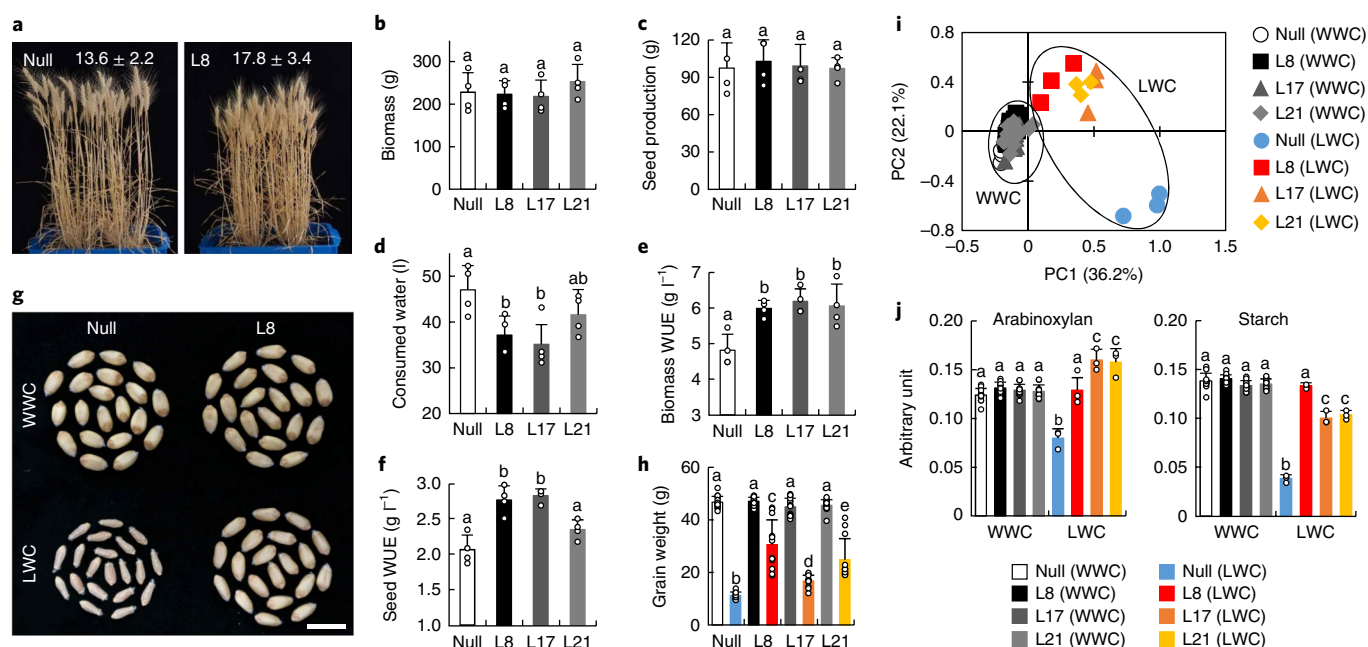


Fig. 4 | Improvement of biomass production, seed production and water conservation by ABA receptor overexpression. **a**, Photographs represent 120-day-old whole plants. The values are the average productive tiller numbers $\pm$ s.d. ($n=16$). **b,c**, Total biomass (dry weight; **b**) and seed production (**c**) per experiment. **d**, Water consumption volume per experiment. **e,f**, Total biomass (**e**) and grain weight production (**f**) from 1 l water. In **b–f**, $n=4$ biologically independent experiments. **g**, Seed shape under WWC and LWC. Scale bar, 1 cm. **h**, Grain weight per 1,000 seeds ($n=16$ under WWC; $n=12$ under LWC). White and grey bars correspond to values under WWC, while coloured bars represent values under LWC. PC, principal component. **i**, PCA score plot of water-insoluble seed components under WWC ($n=12$) and LWC ($n=3$). **j**, Amounts of arabinosylan and starch in seeds ($n=12$ under WWC; $n=3$ under LWC). In **h–j**, n represents biologically independent samples. In **h** and **j**, central values and error bars represent means $\pm$ s.d. In **b–f**, **h** and **j**, different letters denote significant differences ($P < 0.05$) according to the Tukey–Kramer test.

StepOnePlus Real Time PCR system (Life Technologies) using KOD SYBR qPCR Mix (Toyobo) and the gene-specific primer sets shown in Supplementary Table 4. The PCR programme consisted of an initial temperature of 98°C for 2 min, followed by 40 cycles of 98°C for 10 s, 60°C for 10 s and 68°C for 30 s. A melting curve was constructed by increasing the temperature from 68 to 99°C at a rate of 0.05°C s⁻¹. The copy number was calculated by generating a standard curve of the pMD20 plasmid containing the target gene DNA sequence. Three biological replicates were performed. For normalization of data, *TaActin* was used as an internal standard. The primer sequences used in this study are shown in Supplementary Table 4. qRT-PCR-based gene expression was evaluated as the sum of the expression levels of the three homeologues, as distinguishing each transcript was difficult because of the paucity of single-nucleotide polymorphisms among the three homeologous complementary DNAs of TaPYL, TaPP2C and ABA-responsive genes.

Transcriptome analysis. The transcriptome was analysed using three biological replicates of RNA samples from the leaves of 40-day-old plants after exposure to 1 of 3 stress conditions. The three treatments were as follows: WWC, corresponding to normal conditions (soil water potential (SWP) > -110 kPa); ABA (namely, incubation for 24 h after spraying with 25 μ M ABA); and drought, involving incubation for 24 h after withholding water to maintain SWP < -390 kPa. Each total RNA was isolated using the RNeasy Plant Mini Kit (Qiagen) and evaluated for quality and quantity with an Agilent 2100 Bioanalyzer (Agilent Technologies) and the Agilent RNA 6000 Nano Kit (Agilent Technologies). Library preparation was performed according to the TruSeq RNA Sample Preparation version 2 guide (Illumina). Sequencing on an Illumina HiSeq 2500 system generated an average of 91.3 million paired-end reads (2×101 nucleotides) per library. The data analysis is detailed in the Supplementary Information.

Comparison of drought tolerance between Null and TaPYLox. T₃ plants were cultivated in a glasshouse for 50 d. As a drought stress treatment, water supply was withheld for 13 d. The plants were then photographed.

Water loss assay. T₃ plants were cultivated at 22/15°C (day/night) and 60% relative humidity under a 14/10 h light/dark photoperiod for 50 d in a bulk growth chamber (internal dimensions: 170 cm $\times$ 260 cm $\times$ 200 cm high). Ten leaves per plant were detached per experiment, incubated at room temperature and weighed

at 20 min intervals. Water loss (%) was calculated by dividing the post-incubation weight by the initial leaf weight. Four replicates were performed.

Measurement of stomatal pore area. Leaf epidermal impressions of Null, L8 and L17 plants were obtained according to Suzuki's universal micro-printing (SUMP) method using SUMP impression solution and SUMP B plates (Kenis). The copied stomatal images were observed by optical microscopy (BX41; Olympus). The stomatal aperture area was measured using ImageJ version 1.48 software (<https://imagej.nih.gov/ij/>). Six individual plants were used for measurements of the stomatal aperture, with 30 stomata measured per plant.

Plant WUE assay. T₄ wheat plants (Null, L8, L17 and L21) were cultivated in planters (56.8 cm $\times$ 37.9 cm $\times$ 22.0 cm; containing 19.5 kg soil) lacking drainage holes at 22/15°C (day/night) and 60% relative humidity under a 14/10 h light/dark photoperiod. To reduce evaporative water loss from the soil surface to a negligible level, the soil surface was completely covered with aluminium foil except for the plant-growing position. Plants were grown under two environments (WWC and LWC) in a bulk growth chamber. Plants grown under WWC (SWP > -110 kPa) were cultivated until grain ripening. Four plants were cultivated in one planter; this was regarded as one replicate. Four sets were prepared, with a total of 16 plants cultivated for this experiment. The volume of consumed water was measured on a balance. After harvesting, biomass and grain weight were measured, and their values were divided by the volume of consumed water to determine the biomass and grain weight per litre of water. Plants subjected to LWC were first maintained under WWC before withholding water supply, which was halted one week after flowering. Under LWC, 21 l water were supplied to each planter (19.3 cm $\times$ 34.2 cm $\times$ 16.9 cm; containing 5.0 kg soil) containing 3 plants. Soil water content (SWC) was measured using a 10HS soil moisture sensor and an Em5b data logger (Decagon). For use in Supplementary Fig. 11d, SWC was converted to SWP using the following equation: $\text{SWP} = 1,606.8 \times e^{(-17.69 \times \text{SWC})}$. Simultaneously with SWC to SWP conversion, SWP was monitored with a TEROS-21 SWP sensor and an Em50 data logger. Finally, seeds were harvested to determine the grain weight, seed number and grain weight per 1,000 seeds. Seed number was counted with a Multiauto counter (Ohya Tanzo Seisakusho), with the grain weight from 1 l water calculated in the same manner as under WWC. The harvested seeds were also used for NMR analysis.

Gas exchange measurements. Gas exchange measurements were performed using a LI-6400 instrument (LI-COR). Parameters were calculated with the software supplied by the manufacturer. Fully developed flag leaves were used from plants that had been cultivated in a bulk growth chamber at 22/15 °C (day/night) under a 14/10 h light/dark photoperiod and 60% relative humidity for 50 d. The lights were set to turn on at 06:00 and shut off at 20:00. Gas exchange measurements were started at 10:00. The CO₂ flow rate and leaf temperature were kept constant at 500 µmol s⁻¹ and 25 °C, respectively. To obtain light response curves, measurements were conducted at a CO₂ concentration of 400 ppm. After an initial 1 h of dark adaptation, the photon flux density of red and blue light (9:1) was increased incrementally to 20, 50, 100, 200, 500, 1,000, 1,500, 2,000, 2,500 and 3,000 µmol m⁻² s⁻¹. To obtain CO₂ response curves, leaves were first acclimatized for 1 h at 400 ppm CO₂ and 2,000 µmol m⁻² s⁻¹ of red and blue light. The CO₂ concentration was then adjusted incrementally from 0 to 50, 100, 200, 400, 500, 800, 1,000, 1,500 and 2,000 ppm. CO₂ response curves were constructed using a LI-6800 instrument.

¹³C analysis. The carbon isotope composition (δ¹³C) of wheat leaves was analysed using an Elemental Analyzer interfaced with a continuous-flow isotope ratio mass spectrometer (EA/IRMS; Thermo Fisher Scientific). The samples to be analysed were harvested from approximately four-month-old plants and dried in a desiccator. Each sample was measured against standard CO₂ calibrated with an isotope standard (accuracy of calibration ± 0.066‰ s.d.). ¹³C discrimination was calculated as δ¹³C = (R_{sample}/R_{standard} - 1) × 1,000, where R is the ¹³C/¹²C isotope ratio of samples and standards.

Seed component analysis by NMR. Seed water-soluble fractions were extracted by gentle shaking with 100 mM potassium phosphate buffer containing 90% deuterium oxide, together with 1 mM sodium 2,2-dimethyl-2-silapentane-5-sulfonate as the chemical shift reference (δ = 0 ppm)²⁸. The remaining insoluble fraction was washed with methanol, and the residual macromolecular fraction (mainly starch, hemicellulose and proteins) was solubilized with deuterated dimethyl sulfoxide and pyridine (4:1, v/v), as described previously²⁹. Both water-soluble and -insoluble seed components were analysed by 1D-¹H NMR and 2D-¹H, ¹³C-NMR. The normalized NMR dataset was imported into the R software programme³⁰, following which the PCA package was used to compute the variance and regression of the principal component scores.

Statistical information. Significance was determined according to a two-tailed Student's *t*-test for comparisons between Null and TaPYLx. The Tukey–Kramer test was used for multiple comparisons. Error bars represent s.d. in all figures.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

Newly identified wheat ABA receptors (TaPYLs) were deposited in NCBI databases: TaPYL1, MG273651; TaPYL3, MG273653; TaPYL6, MG273656; TaPYL7, MG273657; TaPYL8, MG273658; and TaPYL9, MG273659. To update TaPYL2, TaPYL4 and TaPYL5 information, MG273652, MG273654 and MG273655, respectively, were also deposited. The wheat reference gene annotation and sequence (International Wheat Genome Sequencing Consortium version 1.1) are available at <https://wheat-urgi.versailles.inra.fr/Seq-Repository/Annotations>. The raw sequence and processed data for our RNA-Seq analysis were deposited in the NCBI Gene Expression Omnibus database under the specific accession number GSE79522.

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Author contributions

R.M. and M.O. conceived the project and planned the experiments. F.A. generated the TaPYLx lines. K.T., H.K. and Y.S. performed the RNA-Seq experiments. J.-S.K. analysed the data. Y.T. and J.K. performed the NMR experiments and data analysis. H.T. provided wheat materials and supervised the research. J.-S.K. and K.H. searched for fragment sequences of TaPYLs in the draft wheat genome. R.M. and M.O. performed all of the other experiments. R.M., S.R.C. and M.O. wrote the manuscript. All authors commented on the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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Correspondence and requests for materials should be addressed to M.O.

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Software used for data collection in this study are as follows: qRT-PCR, StepOne Software ver. 2.3 (Applied Biosystems); RNA-seq analysis, HiSeq Control Software ver. 2.2.58 (HiSeq 2500, illumina); protein phosphatase 2C assay, SF6 ver. 4.7.11 (CORONA ELECTRIC); soil water potential and contents, ECH2O ver. 1.82 (METER); gas-exchange measurements, OPEN ver. 6.1 (LI-6400, Li-Cor) and Bluestem ver. 1.2.2 (LI-6800, Li-Cor); $\delta^{13}\text{C}$ analysis, Isodat Acquisition ver. 3.0 (Thermo); Size exclusion chromatography analysis, LabSolutions ver. 5.81 (Shimadzu); ABA measurement, Analyst ver. 1.6.2 (AB Sciex); NMR analysis, TopSpin ver. 3.0 (Bruker BioSpin).

Data analysis

Software used for data analysis in this study are as follows: phylogenetic analysis, MEGA ver. 6.06 (Build#: 6140226); $\delta^{13}\text{C}$ analysis, Isodat Workspace ver. 3.0 (Thermo); measurement of stomatal area and seedling length, ImageJ ver. 1.48 (<https://imagej.nih.gov/ij/>); measurement of seed area, SmartGRAIN ver. 1.2 (<http://www.naro.affrc.go.jp/archive/nias/ql/SmartGrain/>); statistical analysis, Microsoft Excel 2016 MSO (ver. 16.0.10730.20127) and R statistical software (ver. 3.4.4; <https://www.r-project.org/>); quality trimming of the NGS (mRNA-seq) resultant sequence reads, Trim Galore (ver. 0.5.0; https://www.bioinformatics.babraham.ac.uk/projects/trim_galore/); read mapping, Kallisto-Sleuth (ver. 0.44.0; <https://pachterlab.github.io/kallisto/about/>); transcriptional analysis and drawing heatmap and ternary plot, gplots (ver. 3.0.1; <https://cran.r-project.org/web/packages/gplots/index.html>) and ade4 (ver. 1.7-4; <https://pbil.univ-lyon1.fr/ade4/ade4-html/00Index.html>); ABA measurement, MultiQuant ver. 3.01 (AB Sciex); principal component analysis, R statistical software with package library prcomp (ver. 3.4.4).

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Newly identified wheat ABA receptors (TaPYLs) were deposited in NCBI databases: TaPYL1, MG273651; TaPYL3, MG273653; TaPYL6, MG273656; TaPYL7, MG273657; TaPYL8, MG273658; and TaPYL9, MG273659. To update TaPYL2, TaPYL4 and TaPYL5 information, MG273652, MG273654 and MG273655 were also deposited. The wheat reference gene annotation and sequence (IWGSC v1.1) are available at <https://wheat-urgi.versailles.inra.fr/Seq-Repository/Annotations>. The raw sequence and processed data for our RNA-seq analysis were deposited in the NCBI Gene Expression Omnibus (GEO) database under the specific accession number GSE79522.

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Sample size	Sample size was chosen based on previous studies and experience. We analyzed three transgenic wheat lines, which were isolated from independent callus. For the gene expression, seed component (limited water condition) and protein phosphatase analyses, a minimum number of three independent replicates was used. For soil water content analysis using soil moisture sensor and movie analysis using video camera, the experiments were repeated twice with similar results. Data other than those were obtained from at least more than four independent samples, and the detail information are described in our manuscript.
Data exclusions	No data were excluded from analyses.
Replication	All experiments were repeated at least twice with similar results. The number of biological replicates is noted in each figure legend.
Randomization	We sometimes changed the positions of planters under well watered condition in plant water use efficiency assay. In limited watered condition assay, plants were moved constantly on automatic turntable. In other analyses, samples were collected randomly.
Blinding	RNA-seq and seed component analyses were performed without material information. In other analyses, we did not apply blinding because the process of sample collection and analysis was carried out by the same person.

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Materials & experimental systems

n/a	Involved in the study
☒	☒ Unique biological materials
☒	☐ Antibodies
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Methods

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