

Leveraging abscisic acid receptors for efficient water use in *Arabidopsis*

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Plant growth requires the influx of atmospheric CO₂ through stomatal pores, and this carbon uptake for photosynthesis is inherently associated with a large efflux of water vapor. Under water deficit, plants reduce transpiration and are able to improve carbon for water exchange leading to higher water use efficiency (WUE). Whether increased WUE can be achieved without trade-offs in plant growth is debated. The signals mediating the WUE response under water deficit are not fully elucidated but involve the phytohormone abscisic acid (ABA). ABA is perceived by a family of related receptors known to mediate acclimation responses and to reduce transpiration. We now show that enhanced stimulation of ABA signaling via distinct ABA receptors can result in plants constitutively growing at high WUE in the model species *Arabidopsis*. WUE was assessed by three independent approaches involving gravimetric analyses, ¹³C discrimination studies of shoots and derived cellulose fractions, and by gas exchange measurements of whole plants and individual leaves. Plants expressing the ABA receptors RCAR6/PYL12 combined up to 40% increased WUE with high growth rates, i.e., are water productive. Water productivity was associated with maintenance of net carbon assimilation by compensatory increases of leaf CO₂ gradients, thereby sustaining biomass acquisition. Leaf surface temperatures and growth potentials of plants growing under well-watered conditions were found to be reliable indicators for water productivity. The study shows that ABA receptors can be explored to generate more plant biomass per water transpired, which is a prime goal for a more sustainable water use in agriculture.

carbon assimilation | drought resistance | water deficit | water productivity | water use efficiency

Plants are ferocious consumers of water, and plant transpiration is the dominant vector for water mobilization from terrestrial surfaces to the atmosphere (1). Plant transpiration is sustained by efficient water uptake through the root systems, which can comprise 500 m² of root surface and 500 km in combined length even in a single barley plant. Water is the major factor limiting crop productivity in the field (2). Thus, more than two-thirds of the fresh water resources used globally are channeled into agriculture, thereby contributing to potential social conflicts over water (3).

Whereas the gas exchange of CO₂ and water vapor at the stomatal pore is a physical process controlled by both the ratio in partial pressure gradients and gas diffusivities (4, 5), terrestrial plants are able to capture carbon more efficiently under water deficit. Both short-term leaf gas exchange measurements and ¹³C isotope discrimination analyses revealed increases of the instantaneous water use efficiency (insWUE) and intrinsic WUE (iWUE), respectively, by a factor of 1.5–2.5 in wheat and other species (6, 7). The underlying mechanisms, however, are not fully elucidated. Gains in WUE have been found to be associated with trade-offs in growth potential (8–10). WUE is controlled by genes regulating stomatal density and size (11–13). Stomatal aperture is, in turn, controlled by abscisic acid (ABA) signaling (14).

Water deficit generates a hydraulic signal in plants that is rapidly spread over long distances and induces the stress signal ABA (15).

Subsequently, ABA mediates rapid responses including stomatal closure and long-term adjustments such as the down-regulation of growth and promotion of senescence (16, 17). ABA perception recruits an ABA-binding REGULATORY COMPONENT OF ABA RECEPTOR (RCAR)/PYRABACTIN RESISTANCE 1 (PYR1)/PYR1-LIKE (PYL) and an associated protein phosphatase of type 2C (PP2C) (18, 19). Administration of ABA and ABA agonists (20, 21), ectopic expression of RCARs (17, 18, 22–24), as well as reduced expression of PP2Cs (25) have been shown to minimize plant transpiration and, in some cases, to enhance survival under water deficit. It is clear that plants with strongly reduced transpiration are limited in CO₂ uptake and, hence, in growth and biomass formation. Despite the potential drought resistance of these plants, the growth-restricted trait is not attractive for breeders. A trait providing high WUE without impinging on growth potential would be desirable. Such a trait would confer water productivity by allowing more plant growth and yield per water unit. However, the very existence of such a trait is debated (8, 9).

The central role of ABA in controlling the water status of plants prompted us to investigate whether ABA receptors can be explored to generate water-productive plants. We reasoned that the different facets of ABA responses are likely controlled by specific receptor complexes. The combinatorial complexity of 14 ABA RCAR/PYR/PYL receptors and their interactions with nine PP2C coreceptors hampers the dissection of distinct functions for receptor complexes. Therefore, we examined all ABA receptors of *Arabidopsis* for their potential to mediate a water-productive trait. Plants overexpressing the receptor RCAR6 combined up to 40% increased WUE with high growth rates, i.e., are water productive. Water productivity was associated with maintenance of net carbon assimilation by compensatory increases of leaf CO₂ gradients, thereby sustaining plant growth.

Significance

Water is the major limiting factor for plant productivity in the field, and current water use in agriculture is not sustainable. Under water limitations, plants can ameliorate the carbon for water exchange leading to higher water use efficiencies. The plant hormone abscisic acid is activated in response to drought and regulates the water status and acclimation responses. Here we show in the model species *Arabidopsis* that abscisic acid receptors can be explored to constitutively increase the water use efficiency without impinging on growth potential. The finding may support future efforts to generate “more crop per drop” required for a more sustainable water use in agriculture.

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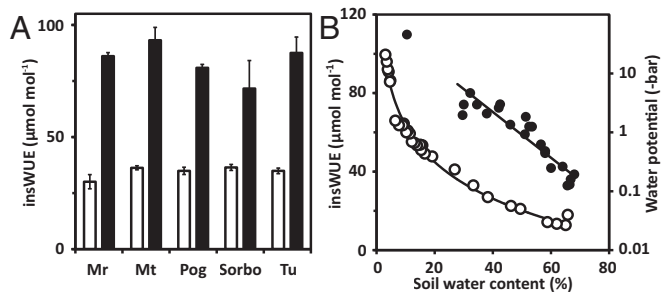


Fig. 1. Water use efficiency of *Arabidopsis* accessions in response to water deficit. (A) The insWUE, i.e., ratio of net CO_2 assimilation and stomatal conductance, were compared among the accession Mr-0 (Italy), Mt-0 (Libya), Pog-0 (Canada), Sorbo (Tadjikistan), and Tu-0 (Italy) under well-watered conditions, $66.7 \pm 1.2\%$ soil water content (vol/vol; white column), and under water deficit ($39 \pm 3\%$ soil water content, black column). Whole leaf rosette measurements at photon flux density $150 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, 50% humidity, $n = 3$ with three technical replicates. (B) insWUE of wild type cv. Columbia (Col-0) affected by soil water content (vol/vol, filled circle). The dependence of the water potential Ψ (open circle, single measurements) on soil water content is given.

Results

Increased WUE of *Arabidopsis* Accessions Under Water Deficit. WUE varies in different accessions of *Arabidopsis thaliana* (26). Under water deficit, an approximately doubling of WUE has been reported in the accessions Columbia (Col-0) and Landsberg (11). We analyzed additional *Arabidopsis* accessions from different continents and climatic environments for this change in WUE by conducting gas exchange measurements on whole plants. Plants growing under well-watered conditions were compared with plants exposed to water deficit. All accessions analyzed responded by an approximately twofold increase in insWUE imposed by $\sim 30\%$ residual water content of the soil (Fig. 1A). The analysis confirms the general WUE response of plants if water becomes limiting. Exposing Col-0 plants to progressively drying soil revealed a continuous rise of insWUE negatively correlated with water availability (Fig. 1B). The question we attempted to address was whether enhanced ABA signaling by ABA receptors can

establish constitutively enhanced WUE without affecting growth performance.

Overexpression of ABA Receptors Affects Growth and Leaf Temperatures. *Arabidopsis* plants with enhanced expression of single RCAR receptors were generated. Three to five independent homozygous lines for each RCAR member were prescreened for their capacity to grow comparably to the parental line while showing elevated leaf surface temperatures as a first indication for reduced transpiration. For each line, four plants were scored under well-watered conditions. In the case that ectopic expression of individual RCARs generated a single plant line that fulfilled the criteria, three independent lines were selected; if not, only one prototype line was chosen. The selected plants lines were again analyzed for confirmation of the phenotypes by thermal imaging of leaves and determination of the rosette size (Fig. 2A and B). In addition, the level of RCAR expression and the ABA sensitivity during seed germination was assessed in these RCAR lines (Fig. S1A and B). The phenotypic assessment revealed increased leaf surface temperatures for the RCAR8 and RCAR9 line associated with severely reduced development of the leaf rosette. Others, such as the RCAR11–RCAR14 lines, showed no significant or marginal elevation of leaf temperatures. However, the *Arabidopsis* lines RCAR1-1, RCAR6-3, and RCAR10-3 combined increased leaf temperatures with no or minor growth trade-offs (Fig. 2B). The maximum leaf expansion rates of most lines examined were in the range of 9.2% growth per day, however, lines overexpressing RCAR4, RCAR8, and RCAR9 showed reduced growth rates (Fig. S1C).

RCAR Lines with Higher Water Use Efficiency. Analysis of three independent RCAR6 lines for water consumption over time indicated a reduced transpiration at similar leaf rosette growth (Fig. 3A) and elevated leaf temperatures of the ABA receptor lines compared with Col-0 (Fig. 3B). The experiment was conducted by withholding watering and minimizing evaporation at an early developmental stage of the plantlets, 18 d after germination with a total leaf area smaller than 0.5 cm^2 . The soil volume and soil water content per planting pot were identical for each single plant. The experiment started under well-watered conditions with a soil water content of 75% (vol/vol) and was

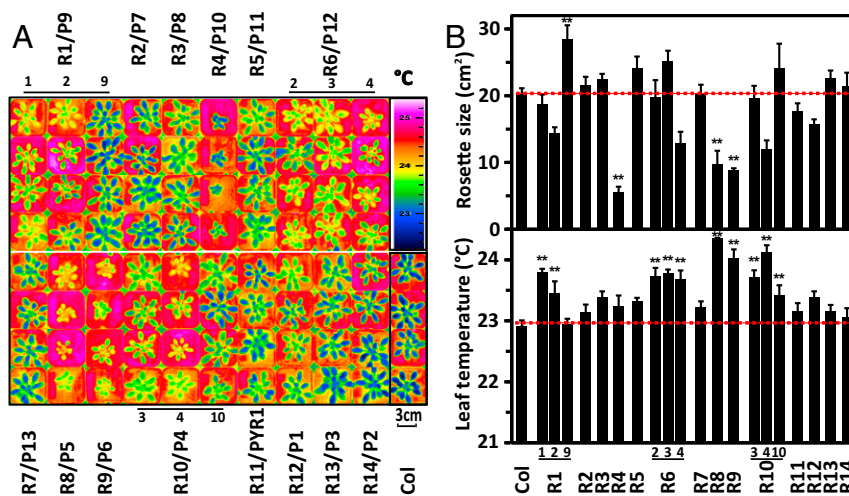


Fig. 2. Overexpression of ABA receptors affects growth and leaf temperatures. *Arabidopsis* Col-0 lines overexpressing the 14 different ABA receptors RCARs (R1–R14)/PYR1/PYLs (P1–P13) under the constitutive viral promoter 35S were homozygous for the transgene. Independent lines are denoted by subscript numbers. (A) Thermogram of the plants grown for 40 d under short day (8 h of light, photon density of $150 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) and under well-watered conditions ($\Psi \geq -0.02$ bar). Four plants were grown per line in separate pots at randomized positions and the thermal pictures were arranged in groups after imaging. (B) Leaf area as projected rosette size and leaf temperatures from data shown in A. The threshold value of Col-0 is indicated by a dotted line. $n = 4$ biological replicates per line, \pm SEM, $***P < 0.001$ compared with wild type.

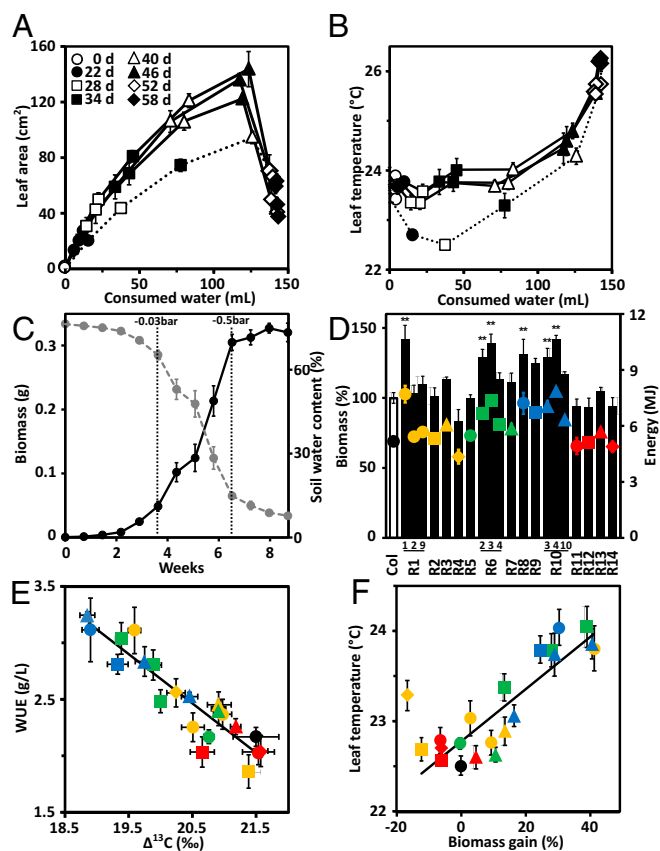


Fig. 3. Efficient water use conferred by ABA receptor expression. Water consumption in relation to (A) growth and (B) leaf surface temperatures of three independent RCAR6-overexpressing lines (solid graphs) compared with Col-0 (dotted line) growing on drying soil. Single plantlets ($n = 4$ per line) were grown for 18 d before the discontinuation of watering at soil water potential $\Psi = -0.02$ bar. At the onset of drought (day 0) water consumption per plant was recorded for 58 consecutive days (58 d). Plants were grown under a short-day light regime (8 h, photon density of $150 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) to keep them at vegetative developmental stage. Growth is expressed as increase in leaf area of the projected rosette. Wilting of leaves caused a reduction in rosette size. (C) Accumulation of biomass (dry weight, $n = 6$) in Col-0 and changes in soil water content (gray line) during a drought experiment as shown in A as mean \pm SEM. The dotted vertical lines indicate threshold Ψ values for mild and severe water deficit, respectively. (D) Above-ground biomass and calorimetric yield of RCAR1–RCAR14 lines (R1–R14) at the end of drought treatment as in A. Biomass of Col-0 was 0.31 ± 0.01 g dry weight per plantlet and set to 100% (column). The energy content of the plants at harvest is indicated by symbols; Col-0 (black filled circle), subclade I comprising members RCAR1–4 lines (yellow), subclade II with RCAR5–7 (green) and RCAR8–10 (blue), and subclade III with RCAR11–14 (red). (E) Association of ^{13}C discrimination in bulk above-ground biomass with WUE (biomass produced per water consumed) as shown in D. (F) Association of biomass gain under drought with leaf surface temperatures of lines under well-watered conditions taken from 7-wk-old plants. Symbols in E and F are as in D. $n = 4$ biological replicates per data point, mean \pm SEM, $**P < 0.001$ compared with wild type (one-way ANOVA).

terminated 8 wk after onset when the plant-available water in the soil was consumed and the residual water content in the soil was below 10%. Under this drought regime, the biomass of wild-type plants increased from 0.001 to 0.32 g and most of the biomass build-up occurred under mild to severe water deficit as a simulation of mounting water deficit in the field (Fig. 3C). Symptoms of mild water deficit were observed at water potentials $\Psi < -0.03$ bar, indicated by changes of stomatal aperture evidenced by increases in leaf surface temperatures. Severe water deficit ($\Psi < -0.5$ bar) resulted in wilting and shrinkage of leaves. All selected

RCAR lines were subjected to this drought regime and analyzed for the maximum accumulation of dry matter and the calorimetric yields (Fig. 3D). The same amount of water was available to every plant; hence, higher biomass values of RCAR lines compared with the wild type reflect higher WUE, i.e., more carbon gain per water unit. Indeed, lines overexpressing RCAR1, RCAR6, RCAR8, RCAR9, and RCAR10 (Fig. 3D) had increased biomass production; some showed increases up to 40% in both dry matter and calorimetric values. Those ABA receptors belong to subclass I and II (18, 19). The plant lines of subclass III receptors, RCAR11–RCAR14, showed no significant gains in biomass.

Stomatal limitation of CO_2 assimilation affects ^{13}C discrimination ($\Delta^{13}\text{C}$), which is an indicator of iWUE (4, 5). Hence, $\Delta^{13}\text{C}$ provides an assessment of WUE independent of the WUE analysis by gravimetry. The values for gravimetrically determined WUE and $\Delta^{13}\text{C}$ of total aboveground biomass (Fig. 3E) showed a clear association ($R \pm 0.92$, $P < 0.001$) for all plant lines. The $\Delta^{13}\text{C}$ -deduced iWUE (Fig. S24) confirmed an approximate 40% increase of iWUE from $69 \mu\text{mol}$ carbon per mole water in wild type to a maximum value of 93 and $99 \mu\text{mol}\cdot\text{mol}^{-1}$ in two RCAR lines, RCAR6-3 and RCAR10-4, respectively, and correlated with biomass-based WUE (Fig. S2B). The $^{13}\text{C}/^{12}\text{C}$ composition of cell wall material integrates water relations during growth, which prevails during periods of less severe water deficit. $\Delta^{13}\text{C}$ of the cellulose fraction indicated an iWUE up to 36% ($\pm 1\%$, $P < 0.001$) higher in RCAR plants than wild type (Fig. S2 C and D). Leaf surface temperatures of RCAR-overexpressing plants under well-watered conditions correlated positively with biomass gain under water-limiting conditions, with the exception of RCAR4 (Fig. 3F, $R = 0.91$, $P < 0.001$). The RCAR4 line had markedly impaired growth and not all plant-accessible water was consumed at harvest. Taken together, the data support an increased WUE conferred by ectopic expression of distinct ABA receptors leading to greater net carbon assimilation per unit of water transpired.

Water Productivity Associated with Enhanced CO_2 Gradients. The RCAR6-3 and RCAR10-3 lines combined enhanced water efficiency with no growth penalty (Figs. 2 and 3A), whereas RCAR6-4 and RCAR10-4 lines showed somewhat reduced growth under well-watered conditions. The RCAR10-4 line performed better than RCAR10-3 under drought, which might be attributed to the higher receptor expression in RCAR10-4 (Fig. S14). All four lines showed high ectopic RCAR expression. For analysis of the physiological basis of water productivity, we chose RCAR6-3 and RCAR10-4, the latter as a candidate for higher WUE combined with some penalties in growth potential. Both of these selected *Arabidopsis* lines consistently performed better in reiterated and independently conducted experiments. In comparison with the wild type, biomass gains of 39–46% and 32–69% for the RCAR6 and RCAR10 line, respectively, were found under drought (Fig. S3A). There was no significant growth difference detectable between plants of the RCAR6 line and wild type grown under well-watered conditions (Fig. S3 B and C) and final biomass yield differed by 1% ($\pm 7\%$, $n = 28$, $P = 0.7$).

Under well-watered conditions, gas exchange measurements of whole plants revealed lowered transpiration rates at similar net CO_2 assimilation rates compared with the wild type, leading to higher transpiration efficiencies of both RCAR lines (Fig. 4 A and B). The *Arabidopsis erecta* mutant has increased transpiration rates (11), whereas the farnesyltransferase-deficient *eral-2* mutant is considered drought resistant (27). The *erecta* and *eral* mutants were found to have higher and slightly lower transpiration compared with the wild type, respectively, and inverse transpiration efficiencies (Fig. 4 A and B). The ratio of CO_2 influx and water vapor efflux of plants is determined by the partial pressure differences of both gases at the stomatal pore (4, 5). Hence, increased transpiration efficiencies at comparable net CO_2 assimilation rates require a steeper CO_2

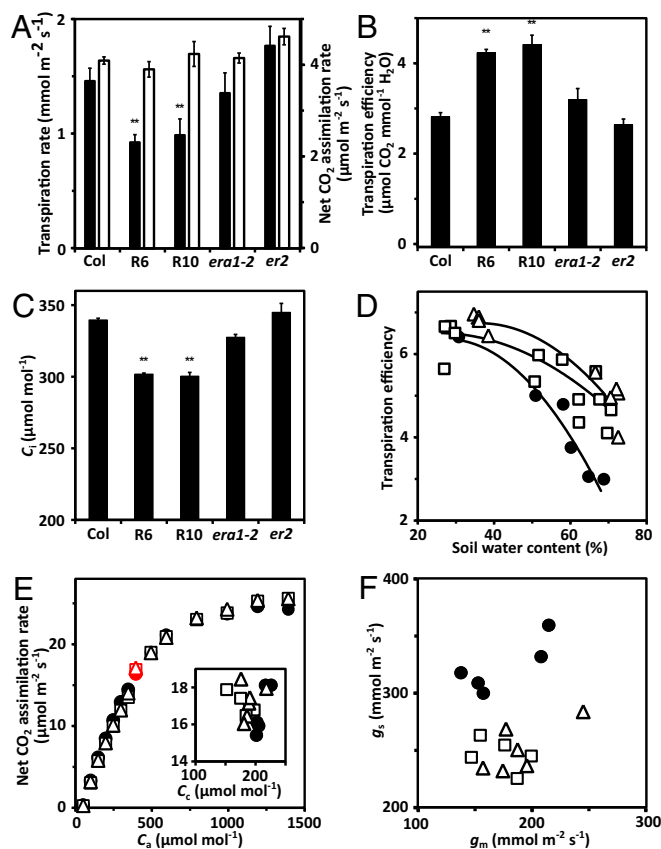


Fig. 4. Reduced transpiration and maintenance of CO_2 assimilation in RCAR6-3 and RCAR10-4 lines. Whole plant gas exchange analysis at well-watered conditions (A–C) and during drought (D). (A) Transpiration (black bars) and net CO_2 assimilation rates (open bars) of RCAR6 (R6) and RCAR10 (R10) lines in comparison with Col-0 and gas exchange mutants *era1-2* and *er2* at water potential $\Psi > -0.03$ bar and external CO_2 concentration C_a of $420 \mu\text{mol}\cdot\text{mol}^{-1}$. (B) Transpiration efficiency, i.e., ratio of rates for net CO_2 assimilation and transpiration ($\text{mmol CO}_2 \text{ mol H}_2\text{O}^{-1}$) and (C) intercellular CO_2 concentration C_i of the same plants as in A. (D) Transpiration efficiency affected by soil water content in RCAR6 (open squares), RCAR10 (open triangles), and wild type (filled circles); single whole plant measurements with 10 technical replicates per data point (D), $**P < 0.001$ compared with wild type. (E) Analysis of net CO_2 assimilation rates in response to external CO_2 concentrations (C_a , ambient C_a in red) and (F) mesophyll (g_m) and stomatal conductance (g_s) in single leaves at high photosynthetic photon flux density ($1,500 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) and soil $\Psi > -0.03$ bar. The CO_2 concentration in chloroplasts (C_c ; Inset) and conductance rates were analyzed at ambient C_a ($400 \mu\text{mol}\cdot\text{mol}^{-1}$). Labeling of plant lines is as in D. (E) $n \geq 5$ with six technical replicates. (Inset and F) Data of single measurements.

gradient at a given water pressure difference. Indeed, both RCAR lines had intercellular CO_2 levels lowered by about $38 \mu\text{mol}\cdot\text{mol}^{-1}$ leading to $\sim 59\%$ enhanced CO_2 gradients at the stomata (Fig. 4C and D). As a consequence, the transpiration efficiency was higher in the RCAR6 and RCAR10 lines by $\sim 49\%$ and 55% ($P < 0.001$), respectively (Fig. 4B). Higher transpiration efficiencies of RCAR lines were found irrespective of the soil water content (Fig. 4D). The ratio of net CO_2 assimilation to stomatal conductance g_s , which reflects the partial CO_2 pressure gradient at the stomatal pore, increased with reduced soil water content in wild type but remained high and fairly constant in the RCAR6-3 line (Fig. S4A).

Similarly, g_s in wild-type plants was lowered to one-half by a reduction of soil water content from $\sim 70\%$ to 50% , whereas g_s in the RCAR6-3 line was largely unaffected but consistently lower than wild type, possibly reflecting an enhanced ABA response (Fig. S4B).

The gas exchange analyses were conducted on entire plants at ambient CO_2 level and photon density of $150 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$; both parameters are limiting photosynthesis (Fig. S4C and D).

At saturating light and modulating different CO_2 concentrations of the air, the net CO_2 assimilation rates of leaves from RCAR6, RCAR10, and wild-type plants were quite similar (Fig. 4E). At ambient CO_2 levels ($400 \mu\text{mol}\cdot\text{mol}^{-1}$) there was no significant difference ($P > 0.3$), which supports a comparable CO_2 flux from air to chloroplasts under these conditions. The CO_2 diffusion path from air to the site of photosynthesis, the chloroplast stroma, is controlled by the stomatal conductance g_s and mesophyll conductance g_m that control CO_2 entry from the atmosphere into the intercellular space and the passage from there into chloroplasts, respectively. Changes in mesophyll conductance for CO_2 affect the CO_2 flux and a higher mesophyll conductance may sustain photosynthesis under CO_2 limiting conditions (28). The net CO_2 assimilation was similar among the plant lines in response to variation of the intercellular CO_2 level (Fig. S5) even under CO_2 starvation conditions, as generated by artificially low CO_2 concentrations in the air ($< 150 \mu\text{mol}\cdot\text{mol}^{-1}$). Analysis of the mesophyll conductance revealed variable values among leaves of a single line (Fig. 4F) but did not detectably differ among the lines ($P > 0.9$). The mesophyll conductance was somewhat lower than the stomatal conductance. The calculated CO_2 concentration in chloroplasts of the RCAR lines was indistinguishable (Fig. 4E). The plastidic CO_2 level in leaves of the RCAR6 line was $\sim 177 \pm 8 \mu\text{mol}\cdot\text{mol}^{-1}$, whereas the levels were higher by $\sim 30 \mu\text{mol}\cdot\text{mol}^{-1}$ in the wild type. As a consequence, the CO_2 gradient between ambient air and chloroplasts differed by 16% ($P = 0.01$) between leaves in RCAR6 and wild type: $\sim 217 \mu\text{mol}\cdot\text{mol}^{-1}$ versus $189 \mu\text{mol}\cdot\text{mol}^{-1}$ in Col-0 leaves. This increase was sufficient to counterbalance the 24% reduction in stomatal conductance and thereby sustaining net CO_2 influx (Fig. 4E and F).

Shoot Versus Root Contribution to Water Use Efficiency. Water deficit triggers a plethora of physiological changes, in which ABA plays a key role by mediating osmoregulation at the whole plant level, photosynthetic adjustments, and root-specific responses such as the stimulation of root growth for exploring soil moisture (29). Reciprocal grafts of above- and below-ground organs between the RCAR10 line and wild type were performed to decipher which organ part contributes to WUE. Analysis of leaf temperatures indicated a shoot-prone reduction in transpirational cooling of the ABA receptor-expressing plants consistent with enhanced ABA signaling in guard cells (Fig. 5A). The ^{13}C discrimination analysis of above-ground biomass from the various grafted plants grown for 10 wk also supported a major effect

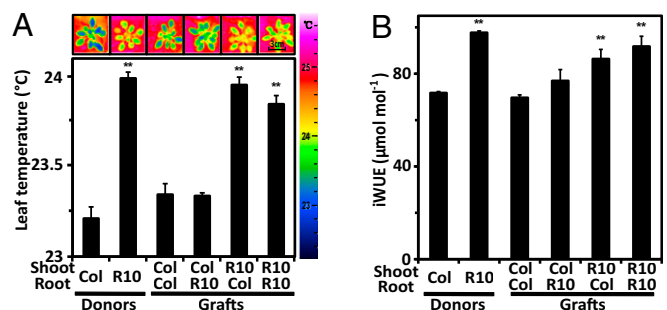


Fig. 5. Organ-mediated changes in WUE analyzed by grafting of the RCAR10-4 line and wild type. (A) Leaf surface temperature of donor lines, self-grafted donor lines, and reciprocal graftings of roots and shoots by thermal imaging of 6-wk-old plants at soil water potential $\Psi \geq -0.03$ bar. (B) iWUE based on ^{13}C analysis of above-ground biomass collected from 10-wk-old plants exposed to progressive drought as described in Fig. 3. $n \geq 4$, two technical replicates, mean \pm SEM; $**P < 0.001$ compared with wild type.

by RCAR10 shoots for increasing iWUE. The carbon isotopic signatures also tentatively supported a contribution of the root system to enhance WUE (Fig. 5B). Taken together, the findings emphasize the dominant role of the shoot in ameliorating carbon for water exchange.

Discussion

It is well established that terrestrial plants can improve WUE under water-restricted conditions. Whether this response is necessarily linked to reduced biomass production and which signals increase WUE under these conditions has not been fully elucidated. In this study, we analyzed the ABA receptor family of *Arabidopsis* for its potential to provide a water-productive trait by mediating a constitutively increased WUE with no or marginal impacts on growth potentials.

Our findings support the conclusion that activation of ABA signaling is able to increase WUE. Because ABA is induced under drought, ABA and concomitantly triggered ABA responses are likely sufficient to mediate a more efficient carbon capture under water deficit. In our analysis, we were able to separate increased WUE from trade-offs in growth potentials and showed that ABA receptors can be used to generate water-productive plants. An *Arabidopsis* line overexpressing the ABA receptor RCAR6, and analyzed in detail, showed a constitutively elevated WUE without significant reduction in growth under well-watered conditions. Water productivity was associated with reduced transpiration and maintenance of carbon assimilation as well as growth. Stomatal size and densities were not different in the RCAR6 line from the wild type, indicating that the observed changes in transpiration reflect ABA signaling-mediated changes in apertures of stomata. This conclusion is in line with other studies in which ectopic ABA receptor expression caused ABA-hypersensitive stomatal responses (18, 22–24).

Reduced stomatal conductance found in the RCAR6 and RCAR10 lines was compensated by increased CO₂ gradients across the stomatal pore that allowed for maintenance of the CO₂ influx. As a consequence, whole plant transpiration efficiencies were increased in the high WUE lines under well-watered conditions to levels induced in *Arabidopsis* accessions under water deficit. The mesophyll conductance did not differ among the RCAR6-3 and RCAR10-4 lines and wild type under light saturating conditions. Light saturation ensures maximal photosynthetic CO₂ demand and may uncover differences in net CO₂ assimilation. However, such differences were not observed as reported for gas-exchange-restricted plants (30) despite the lower chloroplastic CO₂ levels in the water-productive plants. Even at lowered environmental CO₂ concentrations that exacerbate CO₂ deficiencies for the carboxylation reaction, leaves of the RCAR lines showed comparable net CO₂ assimilation rates. Hence, the water-productive RCAR lines were able to sustain net CO₂ assimilation by a slightly (10%) increased CO₂ gradient between ambient air and the chloroplast. The physiological adjustments of water-productive plants might also involve specific long-term adjustments of shoot and root organs (29, 31) for optimizing water use. The finding of higher WUE conferred by the root system overexpressing RCAR10 tentatively supports such a role.

Constitutively increased transpiration efficiencies mediated by reduced transpiration of RCAR overexpressing lines requires ABA receptors capable of modulating stomatal apertures in response even to low ABA levels in the nanomolar range, as present under well-watered conditions (32). However, ABA receptors that are exceedingly sensitive to ABA and too efficient in mediating stomatal closure would curb net CO₂ assimilation and growth. The dynamics in water status within a plant's life and concomitant changes in endogenous ABA concentrations by a factor of 40 and more (32) may add to the selectivity of ABA receptors capable of mediating water productivity. Hence, the delicate balance of net CO₂ assimilation versus transpiration at varying ABA

levels, and water status of plants likely imposes major constraints on the suitability of ABA receptors to confer water productivity. In addition, the transfer DNA (T-DNA) insertion site and posttranscriptional silencing induced by the high ectopic RCAR expression levels could influence RCAR abundance, the regulation of its abundance under water deficit, and thus affect stomatal aperture and trade-offs in photosynthesis. Hence, our analysis provides a proof of concept that certain RCARs are suitable to improve water productivity of plants but the study is not exhaustive enough to clearly exclude other RCAR members.

Subclade I receptors showed a variable result; the RCAR1 overexpressing plants were more water productive in one of three lines. The variability in growth might be attributed to RCAR1/PYL9-mediated induction of leaf senescence found in a recent study by Zhao et al. (17). Such a phenotype was not observed in an earlier characterization of RCAR1 overexpressing plants (18). Zhao et al. (17) had been using overexpression of the *Arabidopsis* ABA receptors and identified RCAR1 to mediate drought resistance. Whereas that study focused on severe drought scenarios and senescence-related processes, our analysis has addressed the contribution of RCAR members to water productivity. For instance, expression of the high ABA affinity receptor RCAR4, also a subclade I member, resulted in stunted growth. Such a trait might be beneficial to provide drought resilience but the water productivity of the RCAR4 line was found to be low in our study. However, most ABA subclade II receptors, comprising RCAR5–RCAR10, contributed to water productivity under drought. In contrast, subclade III receptors, which are considered to be dimeric and less affine receptors (33) that include the prototype PYR1/RCAR11 (19), did not.

Water shortage is an environmental challenge encountered by most perennial plants even in the tropics (34, 35). Several plant species have developed specialized water-storing organs, as exemplified by succulents. Others, such as resurrection plants, are truly drought tolerant (36). However, most plants do not conserve water and initiate adaptive responses only when water becomes scarce. This physiological response is possibly due to selection forces in nature, where a species would not benefit from water productivity but face trade-offs associated with reduced transpiration, including higher leaf temperatures and possible constraints on nutrient and CO₂ uptake. This situation would change, however, for crop plants where water-saving strategies in monocultures increase water productivity and sustain biomass production under water-limiting conditions.

Both the general response of terrestrial plants to increase WUE under water deficit and the conservation of ABA receptor components in dicots and monocots (37, 38) imply that water-productive plants can be generated with crops. Such crops would combine elevated WUE and high yields similar to plants cultivated under deficit irrigation (39). The plants engineered for water productivity, however, would offer the advantage that no technical equipment is necessary for establishing enhanced WUE and saving soil-borne water to better withstand periods of drought. The identification of ABA receptors for providing water productivity will aid in breeding programs and biotechnical efforts to reduce unsustainable water withdrawal (40) and to generate “more crop per drop” (35, 39, 41).

Methods

Details on materials and methods, including the plant material and chemicals, drought assay, analysis of ¹³C discrimination and intrinsic water use efficiency, as well as other physiological analyses and statistical tools used are provided in *SI Methods*.

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