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Water Use Efficiency as a Constraint and Target for Improving the Resilience and Productivity of C_3 and C_4 Crops

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Abstract

The ratio of plant carbon gain to water use, known as water use efficiency (WUE), has long been recognized as a key constraint on crop production and an important target for crop improvement. WUE is a physiologically and genetically complex trait that can be defined at a range of scales. Many component traits directly influence WUE, including photosynthesis, stomatal and mesophyll conductances, and canopy structure. Interactions of carbon and water relations with diverse aspects of the environment and crop development also modulate WUE. As a consequence, enhancing WUE by breeding or biotechnology has proven challenging but not impossible. This review aims to synthesize new knowledge of WUE arising from advances

in phenotyping, modeling, physiology, genetics, and molecular biology in the context of classical theoretical principles. In addition, we discuss how rising atmospheric CO_2 concentration has created and will continue to create opportunities for enhancing WUE by modifying the trade-off between photosynthesis and transpiration.

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INTRODUCTION

The loss of water vapor from aerial tissues of terrestrial plants as they assimilate CO_2 from the atmosphere is biophysically unavoidable. This fundamental trade-off has influenced the evolution of key plant adaptations including cuticles (108), stomata (43), and C_4 (44) and crassulacean acid metabolism photosynthesis (3). Water use efficiency (WUE)—the amount of carbon gain relative to water use—has been studied as a key target for crop improvement for at least a century (17) because water availability is the most important environmental factor limiting crop production (16), even outside of traditionally targeted arid environments. For example, maize yields in the US Midwest have become more sensitive to drought because a tripling of yield and a doubling of planting density since 1930 have increased water use (62, 98). Water limitations have been alleviated in many locations through irrigation, but agriculture already accounts for approximately 70% of freshwater usage worldwide (152). And, with water demand for direct human use increasing, greater irrigation is not a sustainable solution for most growing areas (152). In addition, greater temperatures and atmospheric vapor pressure deficit (VPD) resulting from twenty-first–century climate change will increase crop water use while also increasing variability in the timing and intensity of precipitation (2). This means that water supply limitations will likely impose greater constraints on future crop production across diverse growing regions and crop types, increasing the need to understand and improve WUE.

VPD: water vapor pressure deficit of air

Beyond WUE, crop production under water-limited conditions is influenced by the many factors of management, genetics, and environment, which have been extensively reviewed (see, for example, 60, 120). Reviews have also addressed various aspects of crop physiology that impact WUE (15, 29, 49, 140). While knowledge of the physiological, molecular, and genetic mechanisms controlling WUE has improved substantially, there are still many knowledge gaps and relatively few examples where basic research has translated into more water use–efficient crops growing in farmers' fields (29, 30). This review synthesizes progress in phenotyping, quantitative genetics, molecular physiology, and modeling, with a focus on intrinsic WUE (*iWUE*), i.e., the ratio of the rate of net photosynthetic CO_2 assimilation (A_n) to stomatal conductance (g_s), and the interactions of *iWUE* with other elements of whole-plant WUE. Fundamental questions remain about the coordination of A_n, g_s, and mesophyll conductance (g_m), with significant implications for whether and how *iWUE* can be improved. The two main approaches taken to explore this issue are: (*a*) studies of natural genetic variation and relationships among traits influencing WUE and yield under water limitations, and (*b*) transgenic manipulation of genes that are known to modulate photosynthetic machinery, stomata, g_m, or plant hydraulics. We review these issues in the context of classical physiological and agronomic principles. Finally, we discuss how the rising atmospheric concentration of carbon dioxide, ($[CO_2]$), has improved and will continue to enhance the prospects for improving crop WUE.

Intrinsic water use efficiency (*iWUE*): the ratio of the rate of net photosynthetic CO_2 assimilation to stomatal conductance

A_n: the rate of net photosynthetic CO_2 assimilation

Stomatal conductance (g_s): the inverse of the resistance to water vapor through stomatal pores from the intercellular leaf space to the atmosphere

Mesophyll conductance (g_m): the inverse of the resistance to CO_2 transfer from substomatal cavities to the initial site of fixation in the mesophyll

Leaf water use efficiency (WUE_l): the ratio of the rate of net photosynthetic CO_2 assimilation to the rate of transpiration by leaves

T: the rate of transpiration

c_i: the $[CO_2]$ of the intercellular leaf airspace

c_a: the $[CO_2]$ of the atmosphere

w_i: water vapor concentration of the intercellular leaf airspace

w_a: water vapor concentration of the atmosphere

DEFINING WATER USE EFFICIENCY

WUE is variably defined depending on scale, data availability, and the practitioner, e.g., farmer, agronomist, or physiologist. Derivations of these definitions are widely available and described in detail elsewhere (50, 119, 140), so only a brief summary is provided here.

The leaf is the primary site of gas exchange between the plant and atmosphere. Leaf water use efficiency (WUE_l) is an instantaneous measure of the ratio of A_n to the rate of transpiration (T). It is also often referred to as leaf transpiration efficiency and is defined as:

$$WUE_l = A_n/T = 0.6c_a(1 - c_i/c_a)/(w_i - w_a), \quad 1.$$

where the ratio of CO_2 diffusion to H_2O diffusion is approximately 0.6, c_i is the $[CO_2]$ of the intercellular leaf space, c_a is the $[CO_2]$ of the atmosphere, w_i is the water vapor concentration of the intercellular leaf space, and w_a is the water vapor concentration of the atmosphere. This function reveals that, for a given VPD between the leaf and atmosphere ($w_i - w_a$), WUE_l is controlled by c_i/c_a, reflecting the balance that is achieved between drawdown of $[CO_2]$ by photosynthetic assimilation in the intercellular air spaces and the conductance for CO_2 entry into the leaf through stomatal pores (29). The strong sensitivity of WUE_l to VPD means that evaluating physiological drivers of variation among, for example, crop genotypes can be compromised by environmental differences in time or space. Therefore, it is common to normalize WUE_l to a common VPD or to calculate *iWUE* at the leaf level as the ratio of A_n to g_s (119). For C_3 species, this function can be expressed in a way that demonstrates the importance to WUE_l of both g_m and the drawdown of the $[CO_2]$ in the chloroplast (c_c) by A_n and g_s (50):

$$iWUE = A_n/g_s = (g_m/g_s)/(1 + g_m/g_s) \times (c_a - c_c). \quad 2.$$

No equivalent function has yet been derived for C_4 photosynthesis, but g_m, which for C_4 plants reflects the resistance for CO_2 to reach phosphoenolpyruvate carboxylase in mesophyll cells from the intercellular airspace, as well as the extent of bundle sheath leakiness, could potentially limit *iWUE* in C_4 species (83).

At the whole-plant scale, biomass water use efficiency (WUE_b) is calculated as the ratio of biomass accumulation to water use over some period of time:

Biomass water use efficiency (WUE_b): the ratio of plant biomass accumulation to water use

Grain water use efficiency (WUE_g): the ratio of grain biomass accumulation to water use

$$WUE_b = \text{plant biomass/water use.} \quad 3.$$

As a practical simplification, WUE_b is often calculated using only above-ground biomass even though total above- and below-ground biomass should be used to truly understand how much biomass can be produced per unit of water that is consumed (27). In addition, carbon gain can be estimated from micrometeorological canopy flux measurements (12) or image-based high-throughput phenotyping (47). In some cases, the denominator used to calculate WUE_b is an estimate of whole-plant transpiration. It is increasingly recognized that nocturnal transpiration can contribute significantly, particularly when VPD is high at night (110, 136). This provides information on direct use of water by the plant. However, in other experiments, the denominator used to calculate WUE is canopy evapotranspiration (ET). Water lost from evaporation is wasted in the sense that there is no associated CO_2 uptake. Evaporation drops significantly after canopy closure, but can account for up to 50% of growing season rainfall in some environments (31). Precipitation input is sometimes used as the denominator to calculate WUE_b when measures of water use are not available, particularly in a production setting. Water use can also be estimated by a number of modeling techniques (10, 118). These measures of water use are simpler to estimate than transpiration in a field setting and relate to the overall limitation of production by water supply. In all of these cases, WUE_b is highly sensitive to VPD during the period over which it is calculated, so methods to account for this are well established (140).

For seed crops, grain water use efficiency (WUE_g) is calculated as the ratio of the mass of grain produced to water use, ideally over the entire growing season, in order to provide information about the overall efficiency with which yield is achieved:

$$WUE_g = \text{grain mass/water use.} \quad 4.$$

The measure of water use that is used to calculate WUE_g can vary in the same manner described for WUE_b. Importantly, WUE_g can vary without any change in overall plant carbon gain or water use if the harvest index (*HI*) changes. For example, greater WUE_g in modern versus older hybrids of maize appears to have resulted from similar biomass for a given ET but greater *HI* (30, 115, 132). The framework proposed by Passioura (120), which presents crop yield as a function of *T*, WUE_b, and *HI* has become a very popular way to conceptualize the importance of WUE and the way that it interacts with other key agronomic traits of plants:

$$\text{Yield} = T \times WUE_h \times HI. \quad 5.$$

WUE can even be estimated at the ecosystem and biome scales (12), but these are beyond the scope of this review.

In all cases, careful attention must be paid to timescale. WUE_l and *iWUE* are usually based on instantaneous measurements of leaf gas exchange, which are subject to significant variation across time and environmental conditions (110). Measuring the stable carbon isotope composition ($\delta^{13}C$) of tissue has proven a powerful method to get a measure of WUE_l integrated over time, but results vary depending on the time, environment, and tissues that are sampled (29). WUE_b and WUE_g can be estimated for varying periods of time up to the entire growing season or lifecycle of the plant.

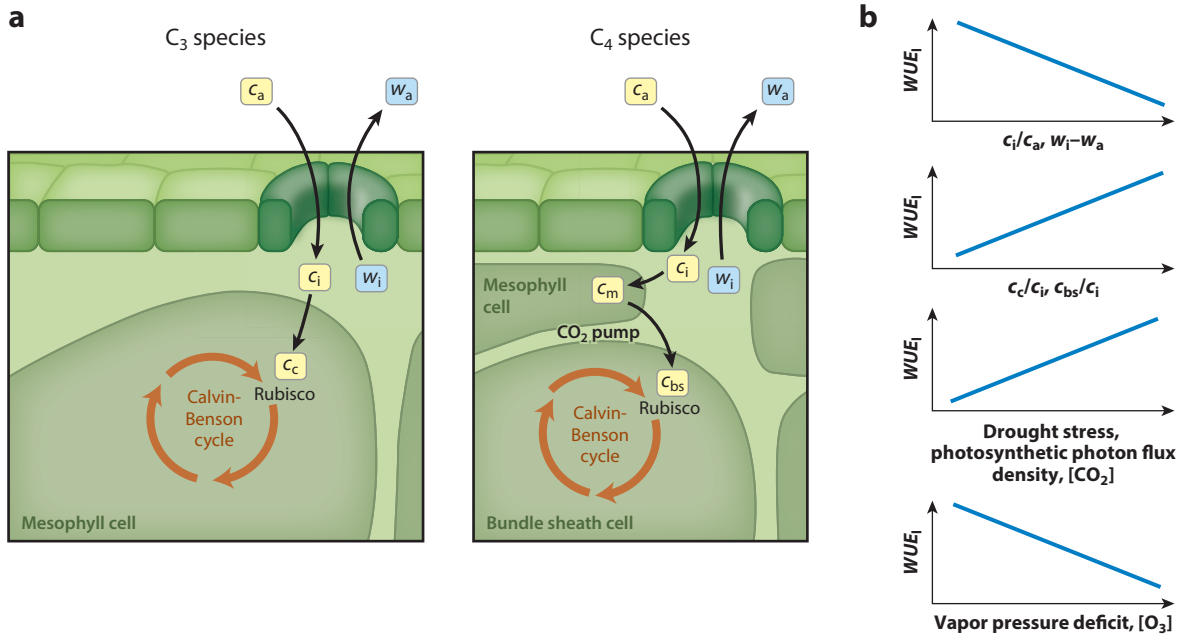


Figure 1

Pathways of carbon and water fluxes that are components of leaf water use efficiency (WUE_l) and environmental interactions of WUE_l. (*a*) Simplified cross section of C_3 and C_4 leaves showing the pathway of photosynthetic CO_2 uptake. Shown here are the concentrations of carbon dioxide ($[CO_2]$) of the atmosphere (c_a), intercellular airspaces (c_i), C_3 chloroplast (c_c), C_4 mesophyll (c_m), and C_4 bundle sheath (c_{bs}) as well as the pathway of transpiration with water vapor concentrations of the intercellular airspaces (w_i) and atmosphere (w_a). Panel *a* adapted from Reference 144. (*b*) Simplified relationships of WUE_l with physiological drivers and environmental factors (see details in the main text).

THE MAJOR DRIVERS OF WATER USE EFFICIENCY AT THE LEAF AND CANOPY SCALES

At the leaf scale in C_3 and C_4 species, WUE_l is greater when c_i/c_a or $w_i - w_a$ is reduced (Equation 1; **Figure 1*a*,*b***). Reducing c_i/c_a can theoretically be achieved by increasing A_n if an equivalent increase in g_s is avoided. This requires an increase in the efficiency or capacity for carboxylation by Rubisco or a reduction in losses to photorespiration. Alternatively, reduced c_i/c_a can be achieved if g_s can be decreased without an equivalent decrease in A_n as a consequence of the reduced supply of CO_2 to the mesophyll. This might be achieved in C_3 species by increasing g_m to increase c_c/c_i (Equation 2) or in C_4 species by increasing the ratio of $[CO_2]$ in the bundle sheath to c_i (c_{bs}/c_i). This will allow greater A_n for the same g_s and water loss, thereby increasing WUE_l. However, as discussed below, A_n, g_s, and g_m are correlated, and it is a key challenge to understand the mechanistic basis for their interdependencies. This includes understanding photosynthesis under variable environments, such as the light environment of canopies in the field, when metabolism and CO_2 transport processes are not at steady state. In addition, respiratory carbon losses may have an important role in driving variation in *iWUE* and WUE_b (110).

Increasing WUE_l by reducing $w_i - w_a$ involves altering the VPD of the atmosphere surrounding the leaf. This can be accomplished either by altering agronomic practices—for example, planting when VPD is low due to cooler temperatures (29)—or by altering plant properties—for example, changing the sensitivity of stomata to environmental conditions and changing canopy

properties (**Figure 1*b***). If g_s and transpiration are restricted under conditions where water loss would be greatest—for example, at high VPD around midday—then disproportionate water savings would be achieved relative to the loss of carbon gain, with overall positive impacts on productivity in situations where drought stress is alleviated (138). This concept can be realized in breeding by exploiting genetic variation in plant hydraulic conductance, which limits transpiration under high VPD (138). Similar results might be achieved with greater sensitivity of stomatal closure to soil drying during drought, a trait that again shows variation within a number of important crop species (138). Lastly, a canopy that is very dense and uniform will have a strong boundary layer and low canopy conductance, such that it is relatively uncoupled from the bulk atmosphere; this leads to lower VPD of subcanopy air. However, dense and uniform canopies generate significant self-shading, which reduces the fraction of carbon fixation performed by shade leaves. This has prompted the suggestion that increasing light penetration into the canopy either between or through leaves could increase WUE (39).

The importance of canopy micrometeorology is highlighted by a well-validated model simulation that found a rice variety, Takanari, with high g_s that has much higher photosynthesis and yields (approximately 30%) with only slightly higher T (5%) (72). The authors argued that the small difference in T can be attributed to the presence of high aerodynamic resistance in the natural field and lower canopy temperatures, in particular from the higher conductance. At the same time, studies using irrigation combined with plastic mulch systems to eliminate evaporation, which achieve very dense and uniform maize canopies with high yields, have found that WUE_g decreases rather than increases at the highest canopy densities (171). Basso & Ritchie (10) have argued that micrometeorological feedbacks allow large yield gains in maize without any increase in ET, but the evidence they present consists only of noting that simulated ET for a scenario of record high yields matches ET values measured in lower-yielding fields. The similarity of these two ET estimates could be caused by many other factors, and the authors do not directly compare simulated and measured ET for the same fields nor do they compare simulated values for normal and record high-yielding fields.

Importantly, WUE is not a fixed parameter for a given genotype but varies in response to many environmental factors and biotic interactions and through the progression of plant development. Important relationships include negative correlations between WUE_l and ozone (155) or VPD (**Figure 1*b***) and positive correlations between WUE and [CO_2] (114), the photosynthetic photon flux density incident on a leaf (109), or mild to moderate drought stress (110, 172) (**Figure 1*b***). In addition to impacting water use via effects on VPD, temperature influences photosynthesis, respiration, allocation, and growth with complex consequences for WUE. For these reasons, genetic variation in any traits (e.g., rooting, growth rate) that influence plant-environment interactions—including the risk or severity of drought stress—may influence observed WUE and obscure variation in traits directly influencing WUE.

THE RELATIONSHIP BETWEEN DROUGHT STRESS AND WATER USE EFFICIENCY

Drought stress results from the supply of water failing to meet crop water demand. Depending on the timing and magnitude of water deficit, this leads to varying degrees of impairment in physiological and agronomic performance relative to well-watered conditions for a given genotype. The negative impacts of drought stress can be reduced by either drought stress tolerance or drought stress avoidance (95). Drought tolerance generally involves enhanced capacity to maintain cellular and physiological functions, including growth, when the plant is experiencing drought stress (24). In contrast, drought stress avoidance is characterized by improved acquisition of water or reduced

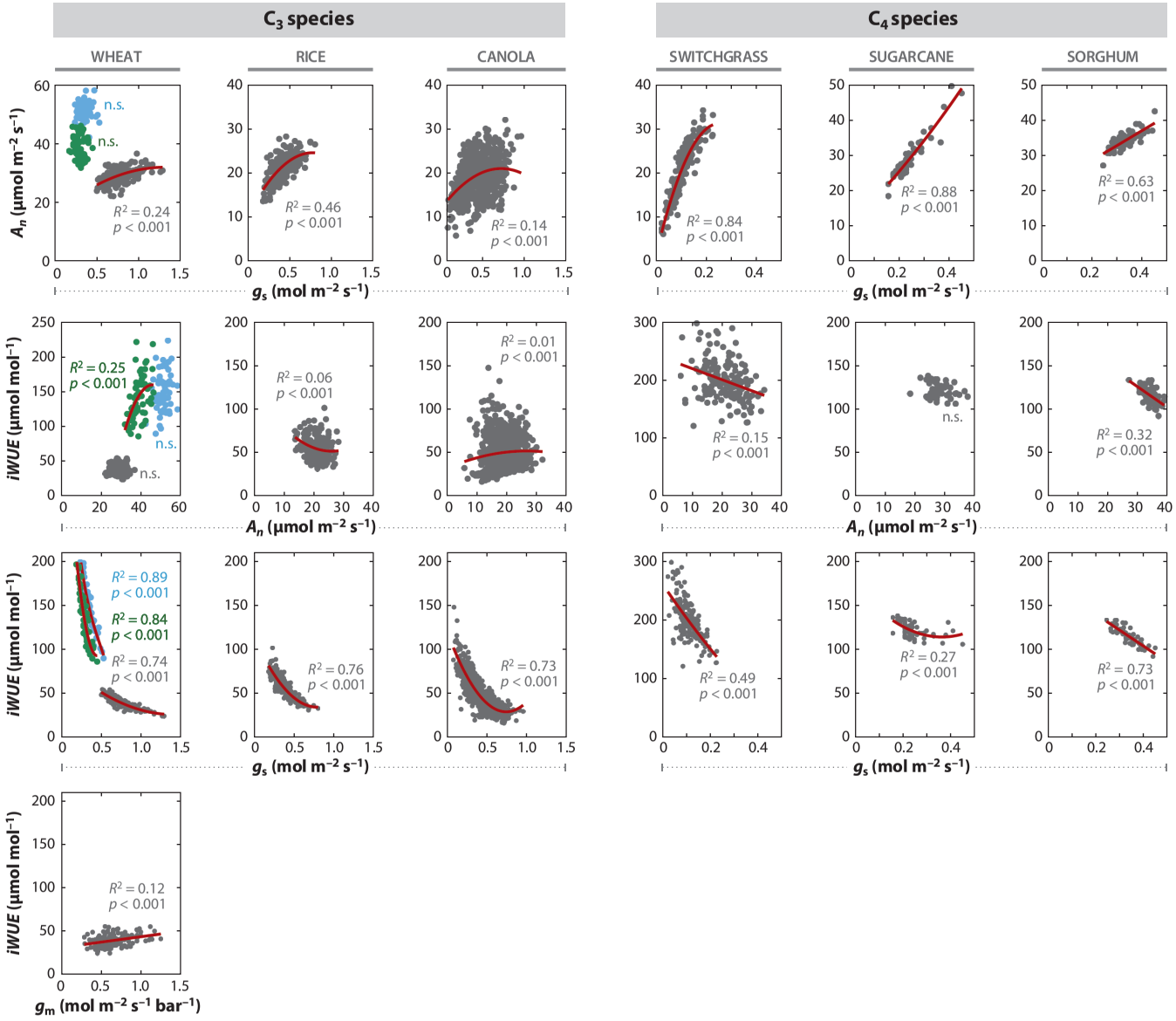
water use. Reduced use of water is often associated with slower growth by smaller plants, with the undesirable result of lower yields. The benefits of maximizing acquisition and use of available water to maximize yield under water-limited conditions have been clearly articulated (120). At the same time, greater WUE is notable in that it has the potential to support greater carbon gain and productivity relative to the amount of water used by the crop.

The interaction between WUE and drought stress depends on whether greater WUE is associated with equivalent carbon gain while using less water or greater carbon gain while using equivalent amounts of water (or a combination of the two). Maintaining equivalent carbon gain while using less water can create the potential for drought stress to be avoided if the unused water remains stored in the soil for subsequent access and use. Achieving greater carbon gain while using equivalent amounts of water can create the potential for greater productivity without increasing the risk of drought stress. However, as others have been at pains to point out (15, 29), the potential for greater WUE to lead to enhanced productivity and yield is not always met. Two distinct syndromes of whole-plant response downstream of having greater WUE can negate the potential benefits of greater WUE. First, greater carbon gain—resulting directly from greater photosynthesis as the driver of improved WUE or indirectly from stress avoidance—can support greater growth to produce greater canopy leaf area, which in turn will compound into greater growth and greater water use, which can increase the risk of drought stress. The likelihood of positive or negative outcomes in this scenario is a function of the timing of drought stress relative to the development of the crop. Second, reduced water use as a driver of greater WUE can be associated with generally conservative growth and allocation strategies that are undesirable for highly productive crops. As a result, two key research challenges have emerged: (*a*) understanding and manipulating leaf physiology to achieve greater *iWUE* through breeding or biotechnology and (*b*) understanding and manipulating whole-plant performance in a manner that allows greater *iWUE* to be tailored to drive greater productivity and yield in a given growing environment. The context of the growing environment, the domestication history (or genetics), and the physiology of any given crop will influence the manner and extent to which these goals might be achieved.

PHENOTYPING TO ASSESS WATER USE EFFICIENCY

To ensure maximal genetic gains in WUE, rapid phenotyping of WUE-related traits is needed. Direct assessment of g_s and A_n via leaf gas exchange systems is relatively slow, and data from individual timepoints do not always scale across days, weeks, or growing seasons (110). However, the use of more gas exchange equipment to collect data from multiple leaves in parallel and the application of carefully designed, controlled-measurement conditions are allowing larger scale analyses (**Figure 2**). Gravimetric assessments of WUE_b or WUE_g are also particularly labor and time intensive (75, 116). However, new high-throughput phenotyping techniques relevant to assessing WUE at various scales are being developed and tested.

Measuring the stable carbon isotope composition of plant tissues was the original high-throughput method for assessing WUE because it is closely associated with both WUE_b and WUE_g in C_3 species (29). Variation in $\delta^{13}C$ and A_n/g_s (*iWUE*) correlate due to independent associations of these parameters with the ratio of intracellular to ambient CO_2 concentrations (c_i/c_a), but $\delta^{13}C$ has the advantage of reflecting the integral of A_n/g_s over the period of time in which the carbon forming the tissue was fixed (29). $\delta^{13}C$ also satisfies the breeding requirement of having high broad sense heritability in important C_3 crop species including wheat (0.45) (131), canola (0.63) (42), barley (0.80) (25), and rice (0.64) (74). But, $\delta^{13}C$ is sensitive to interactions with environmental conditions, including drought stress, and varies among plant tissues and developmental stages. So, as with other techniques, choices in sampling strategy and experimental design can



(Caption appears on following page)

Figure 2 (*Figure appears on preceding page*)

The associations of g_s and A_n, A_n and *iWUE*, and g_s and *iWUE* in the C_3 species wheat (7, 23), rice (127), and canola (42) and the C_4 species switchgrass (148), sugarcane (73), and sorghum (79). Data for wheat (23) are for the preanthesis period (*blue points*) and postanthesis period (*green points*). The association of g_m and *iWUE* is also provided for wheat (8). For all associations and where significant, a quadratic model (*red fit line*) and associated R^2 and *p*-value designations are provided. Abbreviations: A_n, the rate of net photosynthetic CO_2 assimilation; g_m, mesophyll conductance; g_s, stomatal conductance; *iWUE*, intrinsic water use efficiency; n.s., nonsignificant association.

significantly impact the information gathered by assessing $\delta^{13}C$ or its value as a target for selection in breeding programs (29).

In C_4 species, the enzyme initially fixing CO_2 (phosphoenolpyruvate carboxylase) discriminates more in favor of ^{13}C, so that the above-described associations should theoretically be reversed compared to C_3 species or be nonexistent (20). Early evaluations of this approach demonstrated significant genetic variation in $\delta^{13}C$ among genotypes of C_4 crops as well as correlations between $\delta^{13}C$ and WUE_b (63, 67, 70). But, uncertainty about interpretation of variation in $\delta^{13}C$ in C_4 species has limited its application. However, recent work in *Setaria* has revisited this topic to demonstrate that a negative association exists between $\delta^{13}C$ and WUE_b, which was partially driven by WUE_l (45, 47). This is consistent with $\delta^{13}C$ in maize, which was also negatively correlated with WUE_b and moderately heritable across diverse inbred lines grown in the field across environments (151). Overlap in quantitative trait loci (QTLs) for $\delta^{13}C$ and WUE_b suggests that this phenotypic link may be due to a common genetic basis (45), which highlights the potential value of QTLs identified in maize (58).

New methods are available to predict physiological and anatomical traits related to WUE_l based on hyperspectral reflectance (168), thermal and chlorophyll fluorescence imaging (106, 122), microscopy of leaf epidermal structures coupled with image analysis enabled by computer vision (35), and leaf gas exchange (11). Each of these has the potential to allow screening and selection for component traits of WUE_l, as advocated by Sinclair (138). Likewise, at the whole-plant and canopy scales, automation or remote sensing-based estimation of proxies for biomass and water use are now becoming routine. For example, in controlled environment conditions, WUE_b can be measured based on the imaging of above-ground biomass coupled to automatic lysimeters (26, 47, 153). One advantage of large, pot-based methods is that including measures of below-ground biomass in estimates of WUE_b can significantly alter genotype rankings (27). In the field, photosynthetic activity can be assessed by remote sensing (170) and above-ground biomass can be estimated from sensors deployed above (124) or below (6) the canopy. Aerial and ground vehicles can use thermal remote sensing to measure canopy temperature as a proxy for water use (100, 121). However, better strategies are still needed to gather data at high frequency over large germplasm trials and correct for environmental noise in time and space.

To varying degrees, all of the phenotyping approaches and traits described above are impacted by environmental interactions, which make it harder to characterize genetically driven variation in WUE. In particular, screening of WUE at any scale is made harder by changes in the carbon and water relations of crops that occur in response to drought stress. There has been some notable success when screening WUE under conditions of low-drought stress (27, 29), which is consistent with the notion that baseline WUE under nonstressed conditions is driven primarily by the direct controls of WUE described above. A broader and more complex array of traits, such as rooting depth or hydraulic limitations, may indirectly influence WUE through their effects on drought stress when water becomes limiting. Controlling and characterizing the response to water availability can open up additional opportunities for improving WUE (138). Increasingly, models are recognized as an important tool for integrating data to match favorable traits with appropriate

target environments in the breeding process (27). The potential to capture genotype-to-phenotype relationships and genotype-to-phenotype interactions with the environment and management allows the value of genetic controls to be modeled, saving both time and effort.

NATURAL GENETIC VARIATION IN WATER USE EFFICIENCY

Studies of natural genetic variation have generally reported that A_n increases in a manner best fit with a nonrectangular hyperbola as g_s or g_m increases (50). g_s and g_m are positively correlated. However, the goodness-of-fit for these relationships are often low, making interpretation difficult. If variability around the line of best fit for the A_n/g_s or A_n/g_m relationship reflects heritable, genetic variation within a species, then it increases the likelihood that breeding for high *iWUE* is possible. But, photosynthetic gas exchange data can be noisy. The slow speed of data acquisition means that meta-analyses have often been performed on data pooled across species, growing conditions, and measurement conditions (50) or that analyses have been limited to small data sets with limited inference space relative to the genetic diversity within a species (53, 157). This has made it difficult to determine the true nature of these trait relationships. In particular, the extent to which variation in *iWUE* is associated with variation in A_n or g_s has been ambiguous (50). However, a recent focus on large-scale phenotyping has led to the publication of a number of new data sets where photosynthetic gas exchange has been measured on 50–753 genotypes per species in a given study. Compiling those results reveals that *iWUE* is significantly negatively correlated with g_s for rice, wheat, canola, switchgrass, sugarcane, and sorghum. However, the relationship between *iWUE* and A_n is much weaker, especially in C_3 species. This implies that there are genotypes that can combine relatively high A_n and low g_s to achieve high *iWUE*. The data represent both biparental mapping populations and natural diversity (**Figure 2**), indicating that substantial recombination does not significantly alter the trait relationships. This implies that physiological trade-offs, rather than genetic associations, may be key. A more complex analysis of 11 soybean genotypes also concluded that g_s was the dominant driver of variation in *iWUE* and was independent of variation in photosynthetic capacity (53). While a similar approach applied to 20 genotypes of sugarcane did not find that photosynthetic capacity and g_s were independent drivers of *iWUE*, it identified genotypes with high *iWUE* as a result of high photosynthetic capacity (96). For both soybean and sugarcane, this was interpreted to suggest that breeding for greater *iWUE* could be achieved without undesirable reductions in productivity. This differs from the outcome of past selection, at least in soybean, where modern lines have greater photosynthetic carbon fixation under wet conditions. Such conditions are associated with greater g_s without any apparent improvement in *iWUE* (81). In contrast, the effectiveness of $\delta^{13}C$ as a proxy for WUE and its tractability for breeding programs has been exploited successfully in the production of the Drysdale and Rees bread wheat varieties in Australia (29).

Only one data set is available to evaluate large-scale genetic variation in g_m, and it indicates that *iWUE* is significantly correlated with g_m in wheat. In that case, variation in g_m was sufficiently driven by genetic factors to allow QTLs to be mapped (8). In soybean, it has been suggested that the correlation between g_m and g_s is such that selecting for greater g_m would result in greater g_s and no increase in *iWUE* (149). In contrast, there may be enough uncoupling of g_m and g_s in rice and grape to support selection for greater *iWUE* via greater g_m/g_s (50).

Model simulations indicate that increasing the sensitivity of g_s to high VPD, and thereby reducing maximum T, increases WUE in a manner that would improve sorghum yields by up to 13% in dry sorghum-growing regions in Australia, although not in the more productive areas (139). Significant genetic variation in the sensitivity of g_s to VPD—associated with differences

in hydraulic conductance—have been described in a broad range of species, including soybean, peanut, sorghum, pearl millet, chickpea, and maize (138). This creates significant potential for breeding, although screening large populations for this trait may be challenging.

The wealth of genomic resources available to the *Arabidopsis* community has enabled high-throughput assessment of $\delta^{13}C$ by many independent groups to reveal the genetic basis of WUE (77, 105, 112). Through these assessments, the importance of stomatal characteristics for determining WUE has become apparent. *ERECTA* was identified as the gene responsible for variation in WUE due primarily to its effect on stomatal density but also because of its effects on epidermis expansion and g_m to CO_2 (105). Similarly, a single naturally occurring amino acid substitution in *MITOGEN-ACTIVATED PROTEIN KINASE 12* substantially reduced WUE in *Arabidopsis* by reducing stomatal responsiveness to abscisic acid (ABA) and increasing overall stomatal size. These mechanisms result in an overall increase in g_s, which reduces WUE at the whole-plant level (22, 33). This leaves the challenge of translating knowledge about gene networks underlying stomatal density into improvements for crop WUE.

TRANSGENIC MANIPULATION OF WATER USE EFFICIENCY

Transgenic approaches to manipulating WUE have produced a wide range of results: from uncoupling of the A_n/g_s relationship when photosynthetic processes are impaired (9, 128, 158) to plasticity that maintains A_n/g_s such that gains in A_n or reductions in g_s do not confer improvements to *iWUE* (49, 86). There are also examples where coordination between A_n and g_s or g_m has been altered to improve *iWUE*, although demonstrations of efficacy under field conditions are rare, and the mechanistic basis of response is often not fully understood. Indeed, in the last decade there have been at least 20 studies that report improved WUE through transgenic modification of a crop species (based on a PubMed search for: [transgenic] AND [water use efficiency] AND [publication year >2008]). Of these, six have directly targeted stomatal developmental genes, and a further six have targeted genes that mediate reductions in g_s (**Table 1**), e.g., elevated guard cell sensitivity to ABA accumulation (66). The remainder have targeted diverse genes including those involved in hormone signaling, water transport, antioxidant metabolism, photosynthetic metabolism, and leaf or root development.

Stomatal patterning has been effectively targeted to improve WUE in multiple crop plant species. The overexpression of one stomatal development gene, *EPIDERMAL PATTERNING FACTOR 1* (*EPF1*), has been particularly noteworthy. *EPF1* regulates asymmetric cell division and is produced by stomatal precursor cells as a secretory peptide to ensure apposite spacing between stomatal complexes (65). Through molecular phylogenies, the most identical homologs to *Arabidopsis EPF1* have been identified in barley and poplar. In both species, overexpression of the native *EPF1* gene significantly reduces stomatal density (71, 159). Despite the potential for divergence between dicotyledonous and monocotyledonous species in regulation of leaf anatomy and physiology (68), the significant reduction in stomatal density resulted in greater WUE and drought avoidance in both species. However, we are not aware of any reports of transgenic manipulation of stomatal patterning enhancing WUE in crop species that have evolved C_4 photosynthesis. The greater *iWUE* observed when overexpressing EPF1 was dependent on reductions in g_s that were not matched by decreases in A_n. Notably, similar effects were reported when greater *iWUE* was achieved by overexpressing photosystem II subunit S in field-grown tobacco (54).

Brugière et al. (19) reduced g_s in maize not through modified stomatal density but rather increased sensitivity to the ABA hormone via overexpression of the RING domain E3 ligase, Xerico, achieving 40% greater leaf-level *iWUE* and 20% greater yield in plants subjected to drought

Table 1 Studies from the past decade where transgenic plants displayed greater water use efficiency

Gene target(s)	Species	Proposed mechanism(s)	Reference
AtHDG11	Peanut	Upregulation of stress responsive genes and reduced stomatal density	7
PeCHYR1	Poplar (*Populus alba* × *P. glandulosa*)	Increased abscisic acid (ABA) sensitivity	66
OsGA2	Rice	Gibberellin-mediated plant architecture modifications	99
Hv-miR827	Barley	Maintenance of photosynthesis during drought	48
HvEFP1	Barley	Reduced stomatal density	71
ZmXerico1 and ZmXerico2	Maize	Increased ABA sensitivity	19
MoHrip1 and MoHrip2	Rice	Increased ABA sensitivity	161
SoCyt*Sod* and PsCyt*Apx*	Plum	Improved enzymatic antioxidant capacity	34
PdEFP1	Poplar	Reduced stomatal density	159
PaAQUA1	Poplar	Improved regulation of water homeostasis	4
ZmNAC111	Maize	Increased ABA sensitivity	103
OsHVA1	Rice	Lateral root initiation promotion and maintenance of cell metabolism under drought	26
ZmSDD1	Maize	Reduced stomatal density	97
AtERECTA	Tomato and rice	Reduced stomatal density	137
AtAREB1	Soybean	Reduced transpiring leaf surface area and reduced stomatal conductance	94
AtEDT1/HDG11	Rice	Reduced stomatal density and more extensive root system	169
AtDREB1A	Wheat	Mechanism not elucidated	135
vgb, SacB, JERF36, BtCry3A, and OC-I	Poplar	Elevated fructan and proline accumulation and increase maximum quantum yield of photosystem II	143
AtDREB1A	Peanut	Improved antioxidative performance	13
LeNCED1	Tomato	Increased ABA sensitivity	150

stress. Overexpression of the ubiquitin E3 ligase, PeCHYR1, elicited very similar responses in poplar (66). Modulation of a transcription factor, protein elicitors from fungal pathogens, and ABA biosynthetic genes have also been reported to improve WUE via effects on ABA signaling (103, 161).

Improvements to WUE have been effectively achieved through targeting key aspects of plant architecture and morphology. Overexpressing site-directed mutant forms of gibberellin 2-oxidase in rice reduced gibberellins, resulting in reduced height, more productive tillers, and an expanded root system (99). A combination of these traits resulted in an increase in WUE_b of 42–74%. Similarly, overexpression of the late embryogenesis–abundant HVA1 protein substantially increased the length, density, and number of crown and seminal roots in rice (26). In turn, this contributed to a more than 100% increase in WUE_b. For this particular study, it is worth noting that a marked increase in water use is masked by an even greater increase in biomass, which hints at the previously described impact of variation in traits that influence drought stress avoidance (improved water acquisition in this case) directly impacting WUE.

Considerable effort is being focused on engineering improved photosynthesis in crops. A number of approaches could theoretically improve *iWUE* by increasing the efficiency with which

CO_2 is fixed in the mesophyll. These include: (*a*) introducing carbon concentrating mechanisms (CCMs) or C_4-like photosynthesis to C_3 species (93, 125), (*b*) enhancing the kinetic properties of key photosynthetic enzymes (52, 129, 163), (*c*) bypassing photorespiration (166), and (*d*) speeding up the rate of decline of nonphotochemical quenching (NPQ) in dynamic environments (134). However, the theoretical benefits might not be realized if there are unintended consequences to g_s because of manipulating mesophyll structure and function. Improving CO_2-limited A_n in *Arabidopsis* and tobacco through the introduction of the hydrogen carbonate accumulation gene, *ictB*, from cyanobacterium did not elicit an increase in g_s, suggesting an improvement in WUE (40). The expression of the *SBPase* Calvin-Benson cycle enzyme has been targeted in both rice (55) and wheat (41). In both instances, A_n was significantly improved as was biomass production; however, g_s was simultaneously increased to a point where *iWUE* did not differ from the wild type. Transgenic introduction of the glycolate oxidative cycle (101) and the glycolate catabolic pathway (80) to *Arabidopsis* have improved photorespiratory bypasses with consequential photosynthetic and biomass accumulation gains. The impact on g_s and WUE were not directly addressed in these studies; however, it should be noted that the use of $\delta^{13}C$ to determine changes to CCMs in a study by Maier et al. (101) demonstrated a nonsignificant difference in carbon isotope discrimination. This in turn suggests that g_s may have concurrently increased with A_n, since $\delta^{13}C$, a proxy for WUE, did not significantly increase. From a sustainability perspective, it is crucial that attempts to improve A_n to this end are characterized in the context of WUE. We encourage those working in this area to report g_s and WUE responses.

WATER USE EFFICIENCY UNDER FLUCTUATING LIGHT

In crop canopies, light fluctuates due to leaf movement, passing clouds, and the daily movement of the sun (141, 162). McAusland et al. (107) provide a template to gauge dynamic *iWUE* and the underlying A_n and g_s in increasing and decreasing light (**Figure 3**). 15 species varied in dynamic A_n and g_s, often overshooting or undershooting the steady state at the new light intensity. Overall, g_s always responds much more slowly than A_n to decreasing light intensity and usually responds more slowly than A_n to increasing light intensity (85, 87, 107, 162). This can cause dynamic A_n and g_s to become desynchronized, (88, 107); notably, when A_n is low but g_s is high—e.g., on transfer from high to low light—a surplus in transpiration occurs, reducing *iWUE*. This suggests that *iWUE* could be improved by accelerating the rate of change in g_s (85).

Leaves with faster change in g_s often have numerous, smaller stomata, possibly due to a greater surface-to-volume ratio enabling faster transport of solutes for guard cell turgor manipulation (1, 38). This is relevant in light of successful efforts to reduce stomatal density to lower g_s and to improve *iWUE* at steady state (71). There is a consistent, naturally occurring trade-off between stomatal density and size (69). This trade-off is maintained following transgenic manipulation of stomatal density in peanut (7) and rice (169), but it is reversed in barley; i.e., transgenic plants displayed reductions in both stomatal density and size (71). If reduced stomatal density is accompanied by larger, slower stomata, tests are needed to determine if a penalty to dynamic *iWUE* in the field occurs, cancelling out benefits to steady-state *iWUE*.

There are diverse stomatal morphologies; most notably, kidney-shaped guard cells of dicots versus the dumbbell-shaped guard cells that are flanked by subsidiary cells in grasses. Grass stomata typically open and close more quickly (21) and improve coordination between A_n and g_s (107). Many grass stomatal genes are homologous to those controlling dicot stomatal development, but others are rewired for additional functions such as subsidiary cell differentiation (21, 130). g_s responsiveness also declines with leaf age and stress history (156), and fluctuating light can exacerbate damage from stressors such as temperature (91). This is important because while rapid

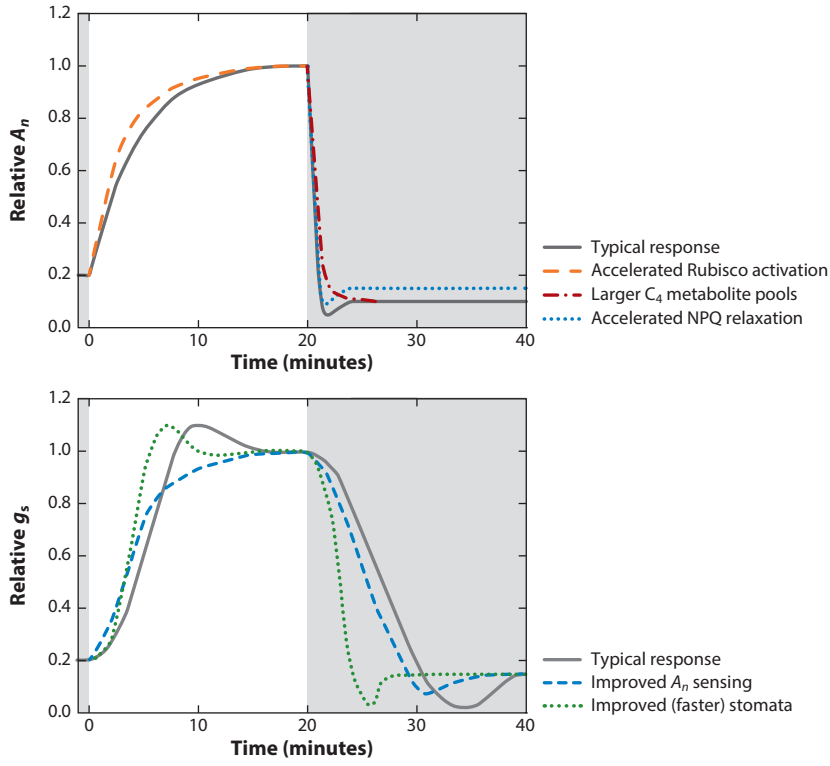


Figure 3

Approaches to improving *iWUE* by altering the speed of A_n and g_s responses to transitions between high and low PPFD. Theoretical time series are drawn for transitions between low (*gray background*) and high (*white background*) PPFD. A_n and g_s are plotted as relative values, with 1.0 being the steady-state value reached at high PPFD. A typical response (*solid gray line*) highlights key inefficiencies in observed responses of dynamic A_n and g_s. Dotted blue, dashed orange, dotted green, dashed blue, and dash-dot red lines show achieved or theoretical methods to increase coordination of dynamic A_n and g_s and improve dynamic *iWUE*. Abbreviations: A_n, the rate of net photosynthetic CO_2 assimilation; g_s, stomatal conductance; *iWUE*, intrinsic water use efficiency; NPQ, nonphotochemical quenching; PPFD, photosynthetic photon flux density.

stomata would be useful under the fluctuating light dominant in the lower canopy, leaves there may rely on old, slow stomata that are ill-equipped to adjust to rapid light changes.

Stomata open and close by modulating water efflux in and out of guard cells to adjust turgor. They do so via inorganic and organic ion transport across the plasma membrane and tonoplast coupled with organic anion synthesis (85). Interactions between classes of ion transporters make it difficult to accelerate stomatal dynamics and improve *iWUE* through single-target modifications: for instance, overexpression of guard cell plasma membrane H^+-ATPases enhanced light-induced stomatal opening but not *iWUE* (160). Instead of single-gene manipulations, breeding for dynamic g_s and *iWUE* may prove effective, as there is significant genotypic variability in these traits within sorghum (122) and rice (126).

Coordination of A_n and g_s may be impaired not only by slow stomatal movement but also if stomata are slow to detect and respond to changing light and/or A_n (**Figure 3**). In species such as *Vicia faba* (85) and barley (107), g_s either took several minutes to begin to respond to decreasing light intensity, or, when light was increased, continued to rise after A_n had reached a maximum *iWUE*. Both responses resulted in temporary decoupling of g_s from A_n, resulting in depression of

iWUE that resorbed over several minutes (**Figure 3**). This suggests poor coordination between A_n and g_s due to imperfect signaling between mesophyll and stomatal processes. Guard cell light sensory systems were reviewed by Assmann & Jegla (5) and consist of blue light sensing intrinsic to guard cells and red light sensing, which may result indirectly from sensing of A_n. Enzymes such as carbonic anhydrase (46) and phosphoenolpyruvate carboxylase (32) appear to have a role in the photosynthesis-to-stomata signaling pathway, possibly via the effect of A_n on intercellular CO_2 levels. In maize, transgenic knockdown of carbonic anhydrase impaired A_n-g_s coordination (82), but no transgenic manipulation has yet improved coordination of these processes for greater dynamic *iWUE*.

Accelerating g_s responses is a promising means to improve *iWUE* under fluctuating light (85). Developments in dynamic A_n are also worth highlighting and were reviewed by Slattery et al. (141). Once engaged in high light, the photoprotective mechanism NPQ takes time to relax upon transfer to shade and continues to dissipate energy even though light is now limiting and not saturating. Transgenic acceleration of NPQ adjustment in tobacco improved canopy CO_2 uptake and biomass by 15% (84) (**Figure 3**). C_4 plants may accumulate large pools of metabolites and energetic intermediates in high light, providing a buffer to enable more gradual decline in A_n during transition to low light (**Figure 3**) (141). Although the specific mechanism is unknown, there is substantial genotypic variation in sorghum for the speed of decline in A_n after a drop in light (122). In wheat (147) and soybean (142), photosynthetic induction, i.e., the rate of increase in A_n from low to high light, was primarily restricted by the speed of Rubisco activation, costing up to 21% of total assimilation. Induction was accelerated by overexpression of Rubisco activase in rice (51, 167) (**Figure 3**). The improvements described here, whether theoretical or already achieved, all permit an improvement to A_n independent of g_s, i.e., greater dynamic *iWUE*.

RISING ATMOSPHERIC CO_2 AS CONTEXT FOR UNDERSTANDING AND IMPROVING WATER USE EFFICIENCY

Concentrations of [CO_2] increased from 302 parts per million (ppm) in 1917 to 404 ppm in 2017 (36) and are projected to continue rising significantly this century. This is important to understanding and improving WUE in two ways: First, the large literature on plant responses to growth at elevated [CO_2] provides valuable evidence on how greater *iWUE* scales to alter whole-plant performance across a range of environmental conditions. These findings can be used to understand and guide the development of crops with greater *iWUE* as a result of breeding or biotechnology. Second, greater concentrations of [CO_2] alter the trade-offs between carbon gain and water loss by leaves in important ways, which might be exploited as new pathways for improving WUE today and in the future.

Arguably, greater *iWUE* is the most universal response of plants to rising [CO_2] and is a result of lower g_s (C_3 and C_4 plants) and greater A_n (C_3 plants) (90). Elevated CO_2 also reduces the time needed for stomatal opening, increasing the efficiency of photosynthesis under dynamic irradiance (91). These steady-state and dynamic responses have the potential to stimulate biomass production and yield through greater carbon fixation and avoidance of drought stress. The contrasting response of C_3 and C_4 plants is very informative. In C_4 species, photosynthesis is already saturated at present-day [CO_2], so there is no direct stimulation of photosynthetic carbon gain, biomass production or yield (89). But, elevated CO_2 leads to a decline in g_s of approximately 20%, which reduces whole-plant water use, conserves soil moisture, and prevents the onset of drought stress that would otherwise impair leaf physiology and yield (89, 102, 104). This indicates that the potential benefits of greater *iWUE* associated with lower g_s and equivalent A_n can be realized at the whole-plant scale in highly productive crops.

In C_3 species, greater *iWUE* at elevated [CO_2] is generally associated with greater A_n and lower g_s (14). This typically results in greater leaf area, root mass, and sugar and hormonal signals associated with transcriptional reprogramming of metabolism (92, 113). In recent free-air CO_2 enrichment (FACE) experiments, these indirect effects of elevated CO_2 on canopy micrometeorology, root-to-shoot signaling of drought stress, and nitrogen dynamics counteracted greater *iWUE*, in an environmentally dependent fashion, in both soybean and wheat (56, 57, 117, 154). This highlights that the consequences of achieving greater *iWUE* in different ways can interact strongly with the amount and timing of drought stress experienced by the crop. Fortunately, it appears that existing crop models can simulate the complex mechanisms that determine how greater *iWUE* translates to different impacts on yield depending on the nature of drought stress (76). Overall, while greater *iWUE* is clearly not a magic formula for greater crop yields in all growing environments and in all years, this large body of data does indicate that yield is generally enhanced and rarely—if ever—reduced when g_s is reduced while A_n is maintained or improved (14, 89, 102). This provides an optimistic counterpoint to reports of the historical challenges involved in successfully producing and deploying crops with improved *iWUE* (49, 60).

In addition to naturally inducing stomatal and photosynthetic responses that result in greater *iWUE*, rising [CO_2] also alters the potential benefits and trade-offs that would result from improving *iWUE* by using breeding or biotechnology to decrease g_s and/or increase A_n. This results from the nonlinear response of A_n to c_i in C_3 and C_4 plants (**Figure 4*a***). C_4 crops have received less attention as a target for improving *iWUE* because they were perceived to have limited capacity for improvement due to: (*a*) already high *iWUE* (140) and (*b*) low genetic variability for *iWUE* (37). C_4 plants have high *iWUE* because of their ability to concentrate CO_2 around Rubisco in the bundle sheath cells (78). Increases in [CO_2] over recent decades mean that C_4 photosynthesis is now operating at or very close to saturating c_i, i.e., above the inflection point of the A_n/c_i curve (**Figure 4*a***). As a consequence, the limitation to photosynthesis imposed by resistance to diffusion of CO_2 through stomata has declined close to zero at the modern [CO_2] of 400 ppm (**Figure 4*b***). This suggests that if we can reduce g_s in C_4 crops, then water use could be substantially reduced with little to no reduction in photosynthetic carbon gain. The potential for this strategy to

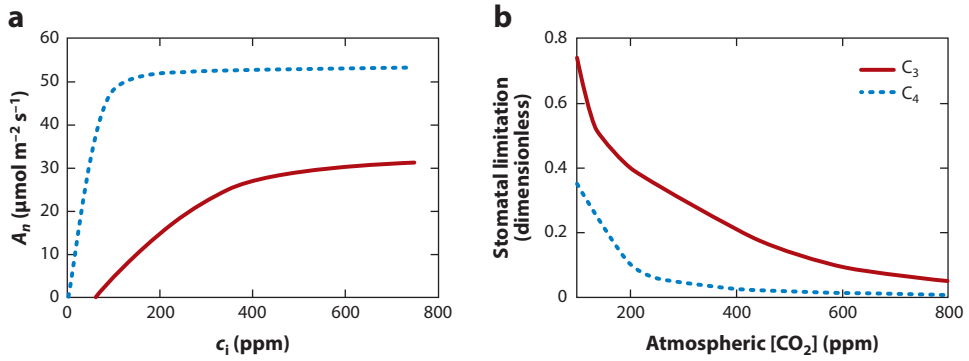


Figure 4

Examples of A_n and stomatal limitation in C_3 and C_4 crop plants under past, present, and future atmospheres. (*a*) Response of A_n to c_i and (*b*) stomatal limitation to A_n derived from the A_n/c_i curves as a function of the growth of [CO_2] in C_3 soybean (*solid red line*) and C_4 maize (*dotted blue line*). The atmospheric [CO_2] corresponding to a given c_i was obtained by assuming that the A_n/c_i curve is unaffected by the [CO_2] at which plants are grown, and c_i/c_a remains constant at 0.7 for C_3 and 0.4 for C_4 plants. Abbreviations: A_n, the rate of net photosynthetic CO_2 assimilation; c_a, the [CO_2] of the atmosphere; c_i, the [CO_2] of the intercellular leaf airspace; [CO_2], atmospheric concentration of carbon dioxide; ppm, parts per million.

improve *iWUE* and confer on C_4 crops the ability to avoid drought stress will only become greater as $[CO_2]$ continues to rise in the coming decades. In fact, given the timescale required for development of new crop varieties by biotechnological or breeding approaches (60), there appears to be a strong argument for a C_4 crop ideotype that has lower g_s to optimize carbon gain and water use at CO_2 concentrations of greater than 500 ppm, which will likely occur in approximately 20 years.

The concept that as $[CO_2]$ increases a given reduction in g_s will cause the same decrease in water loss while imposing increasingly less of a penalty to photosynthesis also applies to C_3 species. However, the nonlinearity of the A_n/c_i curve for C_3 species is less dramatic than for C_4 species (**Figure 4*a***). Therefore, greater *iWUE* as a result of reducing g_s in C_3 species should, in theory, always involve some trade-off of reduced leaf A_n. Recently, the complex consequences of interactions between these leaf-level processes and whole-plant physiology at elevated $[CO_2]$ have been investigated with a crop model (28). The yield advantage of the high-*iWUE* wheat cultivar, Drysdale, over its near-isogenic low-*iWUE* parent, Hartog, was significantly greater and distributed over a broader range of growing conditions under elevated $[CO_2]$ than under ambient $[CO_2]$. Having greater *iWUE* only failed to be an advantage in locations that were extremely dry or wet. Notably, under elevated $[CO_2]$, the greatest projected advantage of the high-*iWUE* cultivar versus the low-*iWUE* cultivar was in locations with greater rainfall; i.e., the greatest yields are achieved when plentiful water resources are used in an efficient manner. These findings reinforce previous experimental findings that the high *iWUE* of Drysdale resulted in greater outperformance of Hartog under elevated $[CO_2]$ rather than under ambient $[CO_2]$ (145, 146). Overall, it is clear that efforts to improve *iWUE* should explicitly consider rising $[CO_2]$ as a factor changing how the trade-off between plant carbon gain and water use operates from the past to the present and to the future. Additional opportunities to improve *iWUE* appear to open up as $[CO_2]$ rises.

MODEL SIMULATIONS OF CROP PERFORMANCE TO EXPLORE THE EFFECTS OF WATER USE EFFICIENCY IMPROVEMENT THROUGH STOMATAL AND PHOTOSYNTHETIC MANIPULATION IN A DIVERSE RANGE OF ENVIRONMENTS UNDER AMBIENT AND ELEVATED ATMOSPHERIC CO_2

Process-based mechanistic crop models that simulate interactions between genotype and environment have been used to help navigate the biological complexity of crop improvement (61), including the photosynthetic manipulations discussed previously (59, 111, 133, 165). To explore the impacts on crop yield of increasing WUE by manipulating g_s and A_n, we conducted a simulation study using the crop models in the Agricultural Production Systems sIMulator (APSIM 7.8) (18, 64) that had been enhanced to incorporate a diurnal canopy, photosynthesis-conductance model (164; A. Wu, G.L. Hammer, A. Doherty, S. von Caemmerer & G.D. Farquhar, unpublished manuscript). Wheat (C_3) and sorghum (C_4) crops were simulated for a xeric Australian site (Dalby, Queensland) and a mesic US site (SoyFACE, Champaign, Illinois) under current (400 ppm) and elevated (550 ppm) $[CO_2]$. Three scenarios were tested: (*a*) increasing A_n by 20%, (*b*) increasing A_n by 10% while reducing g_s by 10%, and (*c*) reducing g_s by 20%. Our hypothesis was that greatest yield gains will come from increasing A_n in mesic locations and decreasing g_s in xeric locations without irrigation.

Simulations were conducted using historic (1900–2016) weather data at the Dalby site and 2004–2015 data at the SoyFACE site. The long-term weather data at Dalby included a wide range of environmental conditions, whereas at the SoyFACE site conditions were mostly high yielding. Simulations for the SoyFACE site should be treated as conceptual because that region grows mainly soybean and maize rather than wheat and sorghum. Detailed descriptions of the study sites,

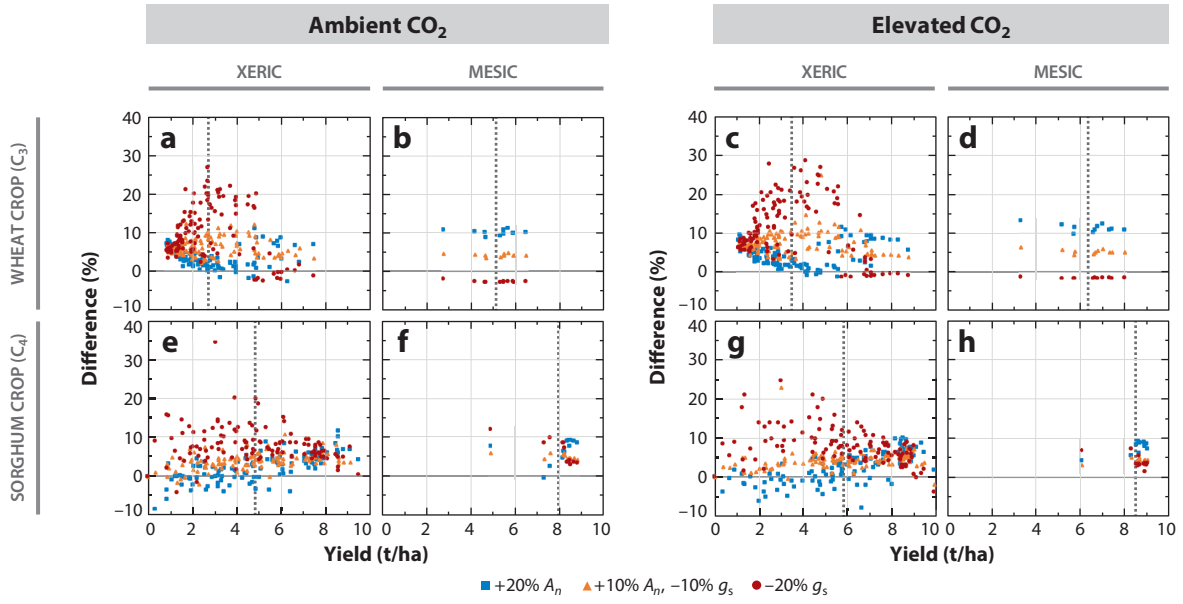


Figure 5

Simulation of wheat and sorghum yield response to *iWUE* manipulation. (*a–b*) Yield responses at Dalby, Australia (xeric) and SoyFACE, United States (mesic) sites shown under ambient CO_2 (400 ppm) and elevated CO_2 (550 ppm) levels. Results are relative to the control simulation at each site and [CO_2]. Vertical lines show average yield in each panel. Abbreviations: A_n, the rate of net photosynthetic CO_2 assimilation; [CO_2], atmospheric concentration of carbon dioxide; g_s, stomatal conductance; *iWUE*, intrinsic water use efficiency; ppm, parts per million; t/ha, tonnes per hectare.

Supplemental Material >

photosynthesis routines in the model, and simulation setups are included in the **Supplemental Text**.

At ambient CO_2, simulated yields with default A_n and g_s at the Dalby site ranged from 1 to 7 tonnes per hectare (t/ha) for wheat and 1 to 9 t/ha for sorghum (**Figure 5*a,e***). At SoyFACE, default A_n and g_s resulted in higher average yields than Dalby for both crops (**Figure 5*b,f***). The three A_n and g_s manipulation scenarios differed in yield responses (−10% to 30%) depending on the crop and growing environment. Overall, the data supported the hypothesis that increasing A_n was of greater benefit under more favorable higher-yielding conditions, and reducing g_s was of greater benefit under lower-yielding, drier conditions. A 20% increase in photosynthetic capacities resulted in 10% yield gain at high-yield levels for both crops at both sites (**Figure 5*a,b,e,f***). However, this was not maintained at the lower yields levels at the Dalby site, where there were few (wheat) or negative (sorghum) consequences. In contrast, reducing g_s by 20% resulted in 10–20% yield gains at lower yield levels, and this result was diminished (sorghum) or reversed (wheat) at high-yield levels. The combined scenario of a 10% increase in A_n with a 10% decrease in g_s generated an intermediate result across all yield levels. The results are consistent with the importance of potential growth (increased A_n) in high-yielding situations with adequate moisture, along with the need for conservation of soil moisture (reduced g_s) for late-season use in low-yielding, water-limited situations (111).

With elevated CO_2 (550 ppm), average yields of wheat and sorghum were increased significantly in all situations [29% and 21%, respectively, at Dalby (**Figure 5*c,g***) and 24% and 8%, respectively, at the SoyFACE site (**Figure 5*d,h***). These differences in the yield response of C_3 and C_4 species are consistent with observations in FACE studies (14, 89, 102). Responses to the three

A_n and g_s manipulation scenarios were similar to those at ambient CO_2 but shifted to higher yield levels, again reflecting the inherent increase in background WUE. The simulations show that targeting reduction in g_s results in a slightly enhanced yield response in lower yielding environments than that found for ambient [CO_2] for both wheat and sorghum (**Figure 5*c*,*g***). This supports the theoretical assertions made above; increasing benefits are expected as [CO_2] rises beyond 550 ppm later this century.

Overall, this simulation analysis suggests that the greatest yield gains for C_3 and C_4 crop species will come from increased A_n in mesic locations and decreased g_s in dry locations without irrigation, but either scenario can have adverse consequences in other environmental contexts. Further, elevated [CO_2] is likely to enhance these yield effects. While these findings show general patterns that reflect the understanding of WUE, the derived yield responses were context dependent and should not be used for extrapolating broadly, either spatially or into future climates. To better understand the global-scale implications of WUE improvement efforts that would manipulate stomata and photosynthesis and, potentially, aim to deliver irrigation strategically, further modeling is needed throughout different agroecological zones that account for known biological linkages to key drivers and interactions. However, data availability with respect to soil, weather, and genotypic traits remains as a significant impediment to such studies (123).

CONCLUSION

WUE has long been recognized as an important target for crop improvement due to the central importance of water limitations to crop yield. Yet WUE is a complex trait that is subject to physiological trade-offs and that is sensitive to genotype-by-environment-by-management interactions. These qualities traditionally made WUE a challenging target for improvement by breeding or biotechnology. Integration of recent improvements in phenotyping and modeling capabilities, along with advances in the mechanistic understanding of the physiological, molecular, and genetic controls of WUE in crop species, suggest that WUE can now be improved in C_3 and C_4 species.

SUMMARY POINTS

1. Water use efficiency (WUE) is a physiologically and genetically complex trait that also varies with crop development and environmental variables.
2. Greater WUE can enhance crop productivity when water supply is limiting, but WUE and drought tolerance are nonsynonymous and interacting traits.
3. Major developments in high-throughput phenotyping and modeling of the component traits of WUE are creating new opportunity to understand genotype-to-phenotype relationships in the context of environmental interactions.
4. There is significant genetic variation in WUE within C_3 and C_4 crop species.
5. Genetic variation in intrinsic WUE (*iWUE*) in C_3 and C_4 species is primarily driven by variation in stomatal conductance (g_s), creating the opportunity to identify genotypes with high *iWUE* and a high rate of net photosynthetic CO_2 assimilation (A_n).
6. A growing number of transgenic studies are providing proof of concept for the biotechnological improvement of crop WUE by targeting photosynthetic and stomatal processes, but demonstrations of consistent efficacy in field trials are needed.

7. Experiments that grow C_3 and C_4 plants under elevated CO_2 have demonstrated that greater leaf WUE does generally translate to improved agronomic performance, but understanding the interactions between plant growth and development dynamics and water supply is key to maximizing the benefits of improving WUE by increasing A_n rather than decreasing g_s.
8. Where and when water is limiting for crop productivity, improving *iWUE* by reducing g_s may lead to greater yield gains than improving A_n.
9. Rising atmospheric concentration of carbon dioxide ($[CO_2]$) has and will continue to create opportunities for enhancing WUE by modifying the trade-off between photosynthesis and transpiration.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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