Intriguing correlations between leaf architecture and intrinsic water-use efficiency enable selective breeding to mitigate climate challenges

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Growing crops in a changing climate requires drought tolerance and strong recovery ability from drought spells. Scientists, growers and policymakers are faced with the challenge to create crops and cropping conditions that allow more crop per drop, whether that drop comes from rainfall or irrigation water. For crop scientists, this translates into creating crops with a higher water-use efficiency of crop productivity (more biomass or yield per unit of water received either by rainfall or irrigation) or into a higher transpiration efficiency (more dry matter or yield produced per amount of water transpired by the crop) (Leakey et al., 2019). For plant scientists, this translates into more net photosynthesis per unit of water transpired at leaf level. This is called intrinsic water-use efficiency (iWUE in $\mu$mol CO$_2$ (mol H$_2$O)$^{-1}$), commonly quantified as the net photosynthesis ($A_n$ in $\mu$mol CO$_2$ m$^{-2}$ s$^{-1}$) divided by stomatal conductance for water vapour ($g_{sw}$ in mol H$_2$O m$^{-2}$ s$^{-1}$) (Medlyn et al., 2017). Note that iWUE only includes the water loss through the stomata and not the water loss through the epidermis of the leaf. iWUE is affected by climate change through the combined action of a multitude of factors: elevated CO$_2$ concentrations in the ambient air, elevated temperature, increases in variation in soil moisture and precipitation (e.g., delays of monsoon, variable rainfall, increasing frequency and duration of drought spells), increases in variation in relative humidity (Hatfield & Dold, 2019) and incoming radiation and clarity of the sky (Gao et al., 2018). In particular, fluctuating light plays an important role in iWUE at leaf level because of the relatively slow response of stomata to light in comparison with photosynthetic processes (Lawson & Viale-Rabine, 2019).

1 | TOWARDS AN INCREASE IN INTRINSIC WATER-USE EFFICIENCY

One important way to produce more crop per drop is to make use of the interspecific and intraspecific variation in iWUE, either for C$_3$ or for C$_4$ crops. There is abundant variation in iWUE among (Yi et al., 2019) and within (Hatfield & Dold, 2019) C$_3$ crop species. C$_4$ crop species have a higher iWUE than C$_3$ plants. The main reason for this difference between photosynthesis types is that the CO$_2$ concentrating mechanism of C$_4$ species allows them to operate efficiently at lower stomatal conductance (Leakey et al., 2019). Therefore, C$_4$ crops could be very useful in sustainable food systems under future climates with increasing chances of drought spells, increasing temperatures associated with enhanced transpiration, and therefore increasing water needs. C$_4$ crop species also demonstrate variation in iWUE, both between C$_4$ grasses (Cano et al., 2019; Pathare et al., 2020) and within important C$_4$ crop species (Cruz de Carvalho et al., 2011; Feldman et al., 2018; Li et al., 2017). The mechanisms behind that variation in C$_4$ crops are less clear than for C$_3$ crops.

iWUE is difficult to assess on large numbers of genotypes, is time-consuming, and requires sophisticated equipment and advanced skills. Both components of iWUE (photosynthesis and stomatal conductance) are very sensitive to fluctuations in environmental factors, such as light, temperature, and relative humidity, although Gu et al. (2012) demonstrated that statistical or physiological modelling approaches might help to account for those fluctuations. Overall, this makes iWUE a much-needed, but difficult trait to select for in...
breeding. There is an urgent need for robust iwue selection methods that can unlock the inter- and intraspecific variation for breeding better crops.

2 | INDIRECT SELECTION FOR ENHANCED INTRINSIC WATER-USE EFFICIENCY

For C3 crops, it has been shown that indirect selection based on relations between leaf width (LW) or vein characteristics and iwue might be helpful (e.g., Feldman et al., 2017). But does this also work for C4 crop species with their CO2 concentrating mechanism? Could LW be an indirect selection criterion for drought tolerance and high water-use efficiency in C4 crops? Answers come from an intriguing paper by Pan et al. (2021) published in this issue of Plant, Cell & Environment.

Previously, Cano et al. (2019) demonstrated that LW correlates strongly with gs and iwue across C4 grass species and that iwue depends more on gs than on A. Pathare et al. (2020) showed that vein density correlates negatively with iwue, while Ogle (2003) showed that vein density positively correlates with a quantum yield of photosynthesis across C4 grasses. Pan et al. (2021) investigated whether such correlations between morphological/anatomical traits and iwue can also be found within a C4 crop species and what the mechanisms are behind them in sorghum (Sorghum bicolor (L.) Moench.), a suitable crop for these research questions because of its huge variation in and high heritability of LW.

Pan et al. (2021) grew 48 sorghum genotypes with large variation in LW and different genetic backgrounds under rain-fed conditions, to assess whether LW correlated with iwue and what the mechanism behind such a relationship was. They carried out leaf gas exchange measurements on fully expanded, but young leaves during the morning, mid-day, and afternoon and measured leaf morphological and anatomic traits, including stomata and vein traits. Particularly, they elegantly assessed the % stomatal aperture as the ratio of the operational stomatal pore area to the mean stomatal pore area when fully open. Vein traits measured included leaf vein density (also called total vein length per leaf area; VD), including both longitudinal and transversal veins, and interveinal distances. Finally, the authors estimated the leaf boundary layer conductance and the leaf energy balance.

LW varied 2.6-fold among the 48 sorghum genotypes, while gs (3-fold), A (2.4-fold), and iwue (1.6-fold) also varied widely among genotypes and throughout the day. Figures 1 and 2 illustrate the correlations identified in the paper by Pan et al. LW correlated strongly (positively, linearly) with gs and A, but negatively with iwue (Figure 1). Interestingly, these linear correlations all disappeared in the afternoon. iwue was mainly associated with changes in gs (negative correlation) rather than with changes in A (Figure 1). There was a negative correlation between LW and VD, a positive correlation between LW and interveinal distance of longitudinal veins (IVDL), and total number of longitudinal veins, a negative correlation between LW and stomatal density, and no correlation between LW and stomatal size. VD was positively correlated with stomatal density, but negatively with IVDL, gs, % stomatal opening and A (Figure 2).

There were no correlations of gs or A with anatomical stomatal traits, strong, positive correlations of both gs and A with % stomatal opening (Figure 2), and a negative correlation between iwue and % stomatal opening (Figure 1). Most interestingly, however, was the positive correlation between LW and % stomatal aperture during the morning and at midday (Figure 1), suggesting that, at least during part of the day, genotypes with wider leaves have their stomata more open than those with narrower leaves, resulting in a lower iwue and indicating that % stomatal opening was a crucial factor in determining variation in iwue.

LW was also associated with the boundary layer thickness and the associated boundary layer conductance for water vapour was strongly correlated with iwue (Figure 1). Leaf energy balances at different levels of wind speed, various differences between leaf and

**FIGURE 1** Relationships between anatomical, morphological and physical characteristics contributing to variation in leaf width and intrinsic water-use efficiency (iwue) in the C4 crop sorghum
air temperatures and during different parts of the day showed that LW correlated positively with gsw because wider leaves had a wider stomatal opening, and this associated well with the energy balance of the leaves in the morning and at midday.

The lack of correlations during the afternoon was perhaps associated with the mild stress that occurred at that time of the day as the experiment was rainfed. This mild stress was visible by occasional leaf rolling, occurring earlier with narrower leaves, thus saving water.

### 3 | SOME ASPECTS REQUIRING FURTHER RESEARCH

While the detailed analysis of the correlations between leaf anatomy and morphology with iWUE is intriguing, the analysis of the temporal dynamics is very insightful, the leaf energy balance provides additional triangulation and the paper contains an interesting discussion on scale issues in water-use efficiency. Still, more quantitative research is required to scale up to canopy level (Table 1). Hammer et al. (2020) showed that

#### TABLE 1  Emerging research questions

| Scaling up research questions: |
|-----------------------------|
| Does iWUE scale up to crop WUE? |

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|-----------------------------|
| How does LW affect microanatomy and 3D pathways of water, CO₂ and O₂ inside leaves? |
| With low stomatal opening, how does epidermal conductance affect iWUE? |
| Are differential dynamics of stomatal behaviour and photosynthesis under fluctuating conditions also affected by LW? |

| Specific questions on relation between vein structure and iWUE aspects: |
|-----------------------------|
| How does vein structure relate to stomatal density, size and behaviour? |
| How does vein structure relate to net photosynthesis? |

Abbreviations: 3D, three-dimensional; iWUE, intrinsic water-use efficiency; LW, leaf width.
genetic variation in crop level water-use efficiency was rather small, although not insignificant, in contrast to the findings at leaf level reported by Pan et al. (2021). On the other hand, Condon et al. (2004) stated that iWUE does scale with crop water-use efficiency under field conditions.

Scaling down, LW determines the three-dimensional (3D) tissue arrangement of a leaf to a considerable extent, which is relevant for gas exchange. There is more research necessary on the 3D pathways of water vapour, CO₂, and O₂ inside the microanatomy of the leaves, including water loss through the epidermis (Table 1). Precise measurements of multidirectional movement of gases inside the leaves, combined with innovative approaches of measuring tissue geometries (Earles et al., 2019) and microscale modelling of gas exchange in C₄ (Retta et al., 2022) are relevant to validate all kinds of traditional assumptions about the true conditions inside a leaf and thus mechanisms controlling iWUE. Recently, the conductance of the epidermis to water vapour was identified as a relevant factor when % opening of the stomata is very small and it, therefore, affects iWUE under such conditions (Márquez et al., 2021).

In the field, conditions are dynamic and can fluctuate at relatively small time steps. Relative response time of the various processes associated with iWUE then becomes relevant (Table 1). Stomatal opening requires energy which can come from photosynthesis or respiration. Therefore, there is a direct link between photosynthesis and stomatal opening (Viallet-Chabrand et al., 2021). The light spectrum is important: blue light is more effective in opening the stomata than red light (Viallet-Chabrand et al., 2021). At the same time, photosynthesis responds an order of magnitude faster to fluctuating light than stomata can (McAusland et al., 2016; Taylor and Long, 2017), even though Taylor and Long (2017) proved that fluctuating light also impedes photosynthesis. When environmental conditions are very dynamic, Aᵣ and Eᵣ may be temporally decoupled, causing a reduction in iWUE. This decoupling should ideally be prevented, as highlighted by for example, Lawson and Viallet-Chabrand (2019), who demonstrated that ‘speedy stomata’ can enhance iWUE.

In short: LW is an intriguing and promising variable with important genetic variation and a large impact on many physiological aspects relevant for iWUE that deserve to be unravelled in more detail at different temporal and spatial scales for proper use in breeding for drought tolerance.

CONFLICTS OF INTEREST
The authors declare that there are no conflicts of interest.

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