

known that the expression of rotundone in the berry is highly dependent on environmental parameters, with cooler seasons leading to much higher accumulation and a high degree of variability observed within the same vineyard (Caputi *et al.*, 2011). Further investigations of environment-dependent enzymes and transcription factors promoting the accumulation of rotundone in the berry are needed to complete the picture. I hope that the globally recognised teams of researchers carrying out these brilliant experiments can make rapid progress, inspiring others to use our new biochemical understanding for comparative studies of the origin of rotundone among common herbs and spices, such as basil, marjoram, oregano, rosemary and thyme (Wood *et al.*, 2008). From spicy wine back to spices proper.

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## Insight

# How can we breed for more water use-efficient sugarcane?

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**Selection on the basis of physiological traits is hedged with obstacles in conventional breeding programmes – it is a little-explored concept. However, in this issue of *Journal of Experimental Botany* (pages 861–872), Jackson *et al.* present research in which the broad-sense heritability of leaf- and crop-level transpiration efficiency was tested within the framework of Australia’s main sugarcane breeding programme.**

Conventional breeding mostly consists of large-scale crosses followed by quick selection methods. To date, most breeding programmes do not use physiological indices, while some rely on experienced breeders walking through field or nursery trials and visually selecting the winners for the following stages. Further, breeders mostly select for vigour and disease resistance. Therefore, selecting for physiological

traits, particularly something as complex as transpiration efficiency (TE), is deemed unworkable. The main obstacles include physiological traits often being complex, time-consuming to measure, subject to significant genotype–environment interactions, not clearly linked to genetic markers, and with their broad or narrow sense heritability weak or untested.

The key contribution of the study by Jackson *et al.* (2016) stems from the authors' attempt to devise the least number of leaf gas exchange measurements required to infer statistically meaningful conclusions about variation and heritability in leaf TE, and the link with plant TE and yield in sugarcane. The main findings were significant genetic variations in plant TE and intrinsic leaf TE as measured by leaf intercellular CO<sub>2</sub> concentration (C<sub>i</sub>); high broad-sense heritability for mean C<sub>i</sub> (0.81); and C<sub>i</sub> having a strong genetic correlation (−0.92) with plant TE at mid-range stomatal conductance (g<sub>s</sub>).

## Physiological definitions and variations of leaf transpiration efficiency

According to Fick's law,

$$A = g_{s\_CO_2}(C_a - C_i) \text{ and } E = g_{s\_H_2O}(e_i - e_a) \quad (1)$$

where  $A$  and  $E$  are the rates of leaf CO<sub>2</sub> assimilation and transpiration (H<sub>2</sub>O), C<sub>i</sub> and C<sub>a</sub> are the leaf intercellular and ambient CO<sub>2</sub> partial pressures, and e<sub>i</sub> and e<sub>a</sub> are the water vapour pressures inside the leaf and in the surrounding air, respectively. In addition,  $g_{s\_H_2O} = 1.6g_{s\_CO_2}$ , where  $g_{s\_CO_2}$  and  $g_{s\_H_2O}$  refer to the stomatal conductance for CO<sub>2</sub> and water vapour, respectively; and 1.6 is the ratio of binary diffusivity of water vapour to that of CO<sub>2</sub> in air (Farquhar *et al.*, 1989).

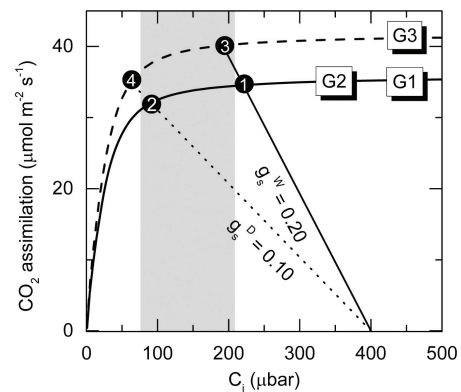
Accordingly, leaf-level TE (TE<sub>L</sub>) is given by:

$$TE_L = \frac{A}{E} = \frac{C_a(1 - C_i/C_a)}{1.6(e_i - e_a)} \quad (2)$$

Assimilation rates depend on both g<sub>s</sub> and photosynthetic biochemistry, while transpiration rates depend on boundary layer conductance, g<sub>s</sub> and the leaf-to-air vapour pressure difference, which in turn depends on leaf temperature and the relative humidity of the surrounding air. Hence, this expression of TE is not ideal in screening for genetic differences because it is highly dependent on environmental conditions. A better expression that reflects a genotype-level trait is intrinsic TE (TE<sub>i</sub>), given by:

$$TE_i = \frac{A}{g_s} = 1 - \frac{C_i}{C_a} \quad (3)$$

Reduced g<sub>s</sub> leads to lower C<sub>i</sub> and C<sub>i</sub>/C<sub>a</sub>, which represents an integrative parameter of TE<sub>i</sub>, reflecting changes in both  $A$  and g<sub>s</sub> (equation 3). The contrasting influence of improved photosynthesis and reduced stomatal conductance on TE<sub>i</sub> is illustrated in Fig. 1.



| Intrinsic leaf TE <sub>i</sub> (μmol m <sup>-2</sup> s <sup>-1</sup> ) |                                  |                                 |
|--|----------------------------------|---------------------------------|
| Genotype   | high g <sub>s</sub> <sup>W</sup> | low g <sub>s</sub> <sup>D</sup> |
| G1, Reference  | 175                              |                                 |
| G2, Low-g <sub>s</sub>   |                                  | 300                             |
| G3, High-A   | 200                              | 350                             |

**Fig. 1.** Modelled responses of C<sub>4</sub> photosynthesis to changes in C<sub>i</sub> at 25°C and saturating irradiance according to von Caemmerer *et al.* (2000). The modelling depicts three hypothetical genotypes. G1 (continuous line) and G3 (broken line) possess different photosynthetic capacity. G2 (continuous line) has similar photosynthetic capacity to G1, but operates with lower g<sub>s</sub>. Within each scenario, reduced g<sub>s</sub> (due to low-g<sub>s</sub> genotype or dry soil or air) increases TE<sub>i</sub> at the expense of reduced  $A$ . Accordingly, TE<sub>i</sub> increases by 73% while  $A$  decreases by 13%, when moving from points 2 to 1 (filled circles) in G1 and from 3 to 4 (filled circles) in G3; g<sub>s</sub> decreases by 50%. Greater photosynthetic capacity in G3, relative to G1 and G2 genotypes, leads to increased TE<sub>i</sub> at any given g<sub>s</sub>. G3 is the desirable genotype because it can potentially fix more CO<sub>2</sub> in wet (e.g. high g<sub>s</sub><sup>W</sup>) or dry (e.g. low g<sub>s</sub><sup>D</sup>) conditions. The shaded area represents the ideal (C<sub>i</sub>) conditions under which genotypic screening gives the best population estimates of TE<sub>i</sub> according to Jackson *et al.* (2016). At higher C<sub>i</sub>,  $A$  is no longer sensitive to changes in g<sub>s</sub>. At lower C<sub>i</sub>,  $A$  is highly sensitive to C<sub>i</sub> giving erroneous estimates of TE<sub>i</sub>; or reduced g<sub>s</sub> may be due to water stress, in which case C<sub>i</sub> rises due to photosynthetic inhibition (Ghannoum, 2009), rendering TE<sub>i</sub> estimates unreliable.

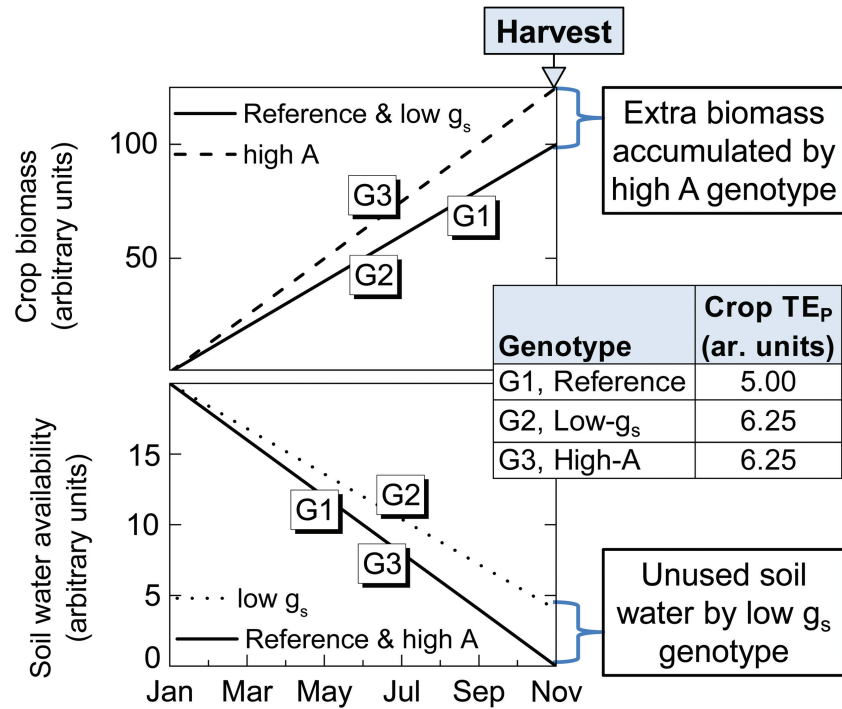
## Paradoxical relationship between crop yield and transpiration efficiency

Most rain-fed crops experience periods of water stress during the growing season. Hence, traits related to water use are critical for crop productivity and survival. Whole plant TE (TE<sub>p</sub>), the ratio of biomass produced to water used, is an important determinant of crop yield (Passioura, 1977), and crop yield (Y) can be expressed as:

$$Y = TE_p \times \text{Water use} \times \text{Harvest index} \quad (4)$$

Greater TE<sub>p</sub> may potentially lead to greater crop yield only if improved TE<sub>p</sub> does not entail reduced water use. This is the case when improved TE<sub>p</sub> results from improved  $A$  rather than reduced g<sub>s</sub>. These contrasting scenarios are illustrated in Fig. 2.

Sugarcane is a largely biomass crop, where harvest index is a fixed proportion of final biomass at harvest. This is not the case for grain crops, where traits and environmental conditions regulating the time of flowering and grain filling complicate the relationship between TE<sub>p</sub>, water use and crop yield. For example, grain crops that flower early may not



**Fig. 2.** Illustration of sugarcane biomass accumulation and soil water availability (or use) between transplanting and harvest dates in three hypothetical genotypes. Within each scenario, reduced  $g_s$  (due to low- $g_s$  genotype or dry soil or air) potentially increases  $TE_p$  without contributing to increased biomass accumulation at harvest relative to the reference genotype, G1. Most inefficiently for both rain-fed or irrigated crops, low- $g_s$  genotypes or conditions imply that crops reach maturity without exhausting all available soil water at harvest, which translates into lower farm-level TE and productivity. Greater photosynthetic capacity in G3 relative to G1 and G2 genotypes potentially leads to increased  $TE_p$  and crop productivity at any stage during crop growth, including at harvest. G3 is the desirable genotype because it theoretically leads to greater productivity and  $TE_p$  in wet (high  $g_s$ ) or dry (low  $g_s$ ) conditions. In addition, the G3 genotype consumes all available soil water by harvest.

have built enough biomass to fill lots of grains, while late-flowering crops may have too little water left in the soil during grain filling (Passioura, 2002). Hence, sugarcane is a crop where improved photosynthetic capacity will probably lead to greater potential crop yield.

## Perspectives

For most crops, and particularly for biomass crops such as sugarcane, improved TE is a desirable trait as long as it does not compromise total crop water use, which ultimately drives crop productivity in water-limited environments. Water-use is determined by a myriad of traits, including TE, root architecture, biomass partitioning and tissue respiration, amongst others (Farquhar *et al.*, 1989). Therefore, reporting good genetic correlations of leaf-level  $TE_i$  with plant TE and yield (Jackson *et al.*, 2016) is surprising, but good news for breeders and crop improvement.

Improved  $TE_i$  without compromising productivity is essentially a quest for improved photosynthetic capacity. Jackson *et al.* (2016) honed in on  $C_i$  as both an integrator of  $TE_i$  and a screening index, and have proposed that reduced  $C_i$  at any given stomatal conductance may result in improved yields in water-limited environments without compromising rates of crop water use and growth.

Finally, a word of caution. Given that atmospheric  $CO_2$  is rising and that  $C_a$  experienced by leaves in gas exchange cuvettes varies depending on photosynthetic capacity,

amongst other factors, I suggest that  $C_i/C_a$  is a more suitable screening index than  $C_i$  (equation 3). Selecting for lowered  $C_i/C_a$  per stomatal conductance via breeding is highly desirable, especially for water-limited environments, and research should focus on developing low-cost, high-throughput screening tools that can be enticing for breeders.

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**Key words:** Conductance, crop productivity, germplasm, photosynthesis, sugarcane, transpiration efficiency, water-use efficiency.

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