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.

Acknowledgements

The author would like to thank his colleagues M. Stella Grando and Riccardo Velasco for helpful discussions, and Alessandro Cestaro for bioinformatics support.

Journal of Experimental Botany, Vol. 67, No. 3 pp. 555–557, 2016

doi:10.1093/jxb/erw008

Key words: Cytochrome P450, grapevine (Vitis vinifera), guaiene, Pinot Noir, rotundone, sesquiterpene oxidase, sesquiterpene synthase, sesquiterpenoids, Shiraz, wine aroma.

References

Battilana J, Costantini L, Emanuelli F, et al. 2009. The 1-deoxy-d-xylulose 5-phosphate synthase gene co-localizes with a major QTL affecting monoterpene content in grapevine. Theoretical and Applied Genetics 118, 653–669.

Battilana J, Emanuelli F, Gambino G, et al. 2011. Functional effect of grapevine 1-deoxy-D-xylulose 5-phosphate synthase substitution K284N on Muscat flavour formation. Journal of Experimental Botany 62, 5497–5508.

Caputi L, Carlin S, Ghiglieno I, et al. 2011. Relationship of changes in rotundone content during grape ripening and winemaking to manipulation of the ‘peppery’ character of wine. Journal of Agricultural and Food Chemistry 59, 5566–5571.

Drew DP, Andersen TB, Sweetman C, et al. 2016. Two key polymorphisms in a newly discovered allele of the Vitis vinifera TPS24 gene are responsible for the production of the rotundone precursor α-guaiene. Journal of Experimental Botany 67, 799–808.

Dunkel A, Steinhaus M, Kotthoff M, et al. 2014. Nature’s chemical signatures in human olfaction: a foodborne perspective for future biotechnology. Angewandte Chemie International Edition 53, 7124–7143.

Emanuelli F, Battilana J, Costantini L, et al. 2010. A candidate gene association study on muscat flavor in grapevine (Vitis vinifera L.). BMC Plant Biology 10, 241.

Fournier-Level A, Le Cunff L, Gomez C, et al. 2009. Quantitative genetic bases of anthocyanin variation in grape (Vitis vinifera L. ssp. sativa) berry: a quantitative trait locus to quantitative trait nucleotide integrated study. Genetics 183, 1127–1139.

Jaillon O, Aury JM, Noel B, et al. 2007. The grapevine genome sequence suggests ancestral hexaploidization in major angiosperm phyla. Nature 449, 463–467.

Mattivi F, Caputi L, Carlin S, et al. 2011. Effective analysis of rotundone at below-threshold levels in red and white wines using solid-phase microextraction gas chromatography/tandem mass spectrometry. Rapid Communications in Mass Spectrometry 25, 483–488.

Narduzzi L, Stansstrup J, Mattivi F. 2015. Comparing wild American grapes with Vitis vinifera: a metabolomics study of grape composition. Journal of Agricultural and Food Chemistry 63, 6828–6834.

Ocarez N, Mejia N. 2015. Suppression of the D-class MADS-box AGL11 gene triggers seedlessness in fleshy fruits. Plant Cell Reports doi: 10.1007/s00299-015-1882-x.

Schwab W, Wuest M. 2015. Understanding the constitutive and induced biosynthesis of mono- and sesquiterpenes in grapes (Vitis vinifera) – a key to unlocking the biochemical secrets of unique grape aroma profiles. Journal of Agricultural and Food Chemistry doi: 10.1021/acs.jafc.5b04398.

Takase H, Sasaki K, Shinmori H, et al. 2016. Cytochrome P450 CYP71BE5 in grapevine (Vitis vinifera) catalyzes the formation of the spicy aroma compound (→)-rotundone. Journal of Experimental Botany 67, 787–798.

Velasco R, Zharkikh A, Troggio M, et al. 2009. A high quality draft consensus sequence of the genome of a heterozygous grapevine variety. Nature 463, 669–673.

Wood C, Siebert TE, Parker M, et al. 2008. From wine to pepper: rotundone, an obscure sesquiterpene, is a potent spicy aroma compound. Journal of Agricultural and Food Chemistry 56, 3738–3744.

Insight

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

Oula Ghannoun

ARC Centre of Excellence for Translational Photosynthesis, Hawkesbury Institute for the Environment, Western Sydney University, Locked Bag 1797, Penrith, NSW 2751, Australia

ghannoun@westernsydney.edu.au

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 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 CO₂ concentration (Cᵢ); high broad-sense heritability for mean Cᵢ (0.81); and Cᵢ having a strong genetic correlation (~0.92) with plant TE at mid-range stomatal conductance (gₛ).

**Physiological definitions and variations of leaf transpiration efficiency**

According to Fick’s law,

\[ A = gₛCO₂(C_a – Cᵢ) \] and \[ E = gₛH₂O(eᵢ – e_a) \]

where \( A \) and \( E \) are the rates of leaf CO₂ assimilation and transpiration (H₂O), \( C_a \) and \( Cᵢ \) are the leaf intercellular and ambient CO₂ partial pressures, and \( eᵢ \) and \( e_a \) are the water vapour pressures inside the leaf and in the surrounding air, respectively. In addition, \( gₛH₂O = 1.6gₛCO₂ \), where \( gₛCO₂ \) and \( gₛH₂O \) refer to the stomatal conductance for CO₂ and water vapour, respectively; and 1.6 is the ratio of binary diffusivity of water vapour to that of CO₂ in air (Farquhar et al., 1989).

Accordingly, leaf-level TE (TEₐ) is given by:

\[ TEₐ = \frac{A}{E} = \frac{C_a(1 – Cᵢ/C_a)}{1.6(eᵢ – e_a)} \]  (2)

Assimilation rates depend on both \( gₛ \) and photosynthetic biochemistry, while transpiration rates depend on boundary layer conductance, \( gₛ \) 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 (TEᵢ), given by:

\[ TEᵢ = \frac{A}{gₛ} = 1 – \frac{Cᵢ}{C_a} \]  (3)

Reduced \( gₛ \) leads to lower \( Cᵢ \) and \( C_a/Cᵢ \), which represents an integrative parameter of TEᵢ, reflecting changes in both \( A \) and \( gₛ \) (equation 3). The contrasting influence of improved photosynthesis and reduced stomatal conductance on TEᵢ is illustrated in Fig. 1.

**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 (TEₚ), 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:

\[ Y = TEₚ \times \text{Water use} \times \text{Harvest index} \]  (4)

Greater TEₚ may potentially lead to greater crop yield only if improved TEₚ does not entail reduced water use. This is the case when improved TEₚ results from improved \( A \) rather than reduced \( gₛ \). 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 TEₚ, water use and crop yield. For example, grain crops that flower early may not
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 with plant TE and yield (Jackson et al., 2016) is surprising, but good news for breeders and crop improvement.

Improved TE 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, 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 $C_i$ experienced by leaves in gas exchange cuvettes varies depending on photosynthetic capacity, amongst other factors, I suggest that $C/C_a$ is a more suitable screening index than $C_i$ (equation 3). Selecting for lowered $C/C_a$ 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.

References

Farquhar GD, Ehleringer JR, Hubick KT. 1989. Carbon isotope discrimination and photosynthesis. Annual Review of Plant Physiology and Plant Molecular Biology 40, 503–537.

Ghannoum O. 2009. $C_4$ photosynthesis and water stress. Annals of Botany 103, 635–644.

Jackson P, Basnayake J, Inman-Bamber G, Lakshmanan P, Natarajan S, Stokes C. 2016. Genetic variation in transpiration efficiency and relationships between whole plant and leaf gas exchange measurements in Saccharum spp. and related germplasm. Journal of Experimental Botany 67, 861–871.

Passioura JB. 1977. Grain yield, harvest index, and water use of wheat. Journal of the Australian Institute of Agricultural Science 43, 117–120.

Passioura JB. 2002. Environmental biology and crop improvement. Functional Plant Biology 29, 537–546.

von Caemmerer S. 2000. Biochemical models of leaf photosynthesis . Melbourne: CSIRO Publishing.