Integrating modelling and phenotyping approaches to identify and screen complex traits – Illustration for transpiration efficiency in cereals

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Abstract

Following advances in genetics, genomics, and phenotyping, trait selection in breeding is limited by our ability to understand interactions within the plants and with their environments, and to target traits of most relevance for the target population of environments. We propose an integrated approach that combines insights from crop modelling, physiology, genetics, and breeding to identify traits valuable for yield gain in the target population of environments, develop relevant high-throughput phenotyping platforms, and identify genetic controls and their values in production environments. This paper uses transpiration efficiency (biomass produced per unit of water used) as an example of a complex trait of interest to illustrate how the approach can guide modelling, phenotyping, and selection in a breeding program. We believe that this approach, by integrating insights from diverse disciplines, can increase the resource use efficiency of breeding programs for improving yield gains in target populations of environments.

Keywords: breeding, cereals, crop adaptation, drought, evaporative demand, sorghum, modelling, phenotyping, wheat, transpiration efficiency, vapour pressure deficit, water deficit, maize.

Highlight: Combining crop modelling, physiology and genetics can improve breeding efficiency and enhance the transfer of scientific knowledge to new varieties. This paper illustrates such an integrated approach for a drought-adaptation trait.

Introduction

Following the advances in genetics and genomics, the last decade has seen the development of a multitude of high-throughput phenotyping methods that are taking advantage of rapid developments in computing power and electronic data collection (e.g. Furbank and Tester, 2011; Chapman *et al*., 2014; Potgieter *et al*., 2017). A potential drawback of this development is that the availability of novel technologies may be driving phenotyping, while arguably, in terms of crop improvement, high-throughput phenotyping should be a 'demand-driven' process and collect information addressing a need that emerged from crop improvement in a specific target population of environments.

Many agronomic traits of relevance to growers and plant breeders are complex in nature, and submitted to high genotype × environment × management (G×E×M) interactions in production environments. Successful selection for such complex traits in a crop improvement program requires them to meet a number of criteria, including (1) relevance for the target population of environments (which reflects soil constraints, climatic variations, and local management practices; Chenu, 2015), (2) presence of useful genotypic variation for selection, (3) high heritability, and (4) resource-effective phenotyping and/or genotyping systems. Agronomic traits are usually underpinned by multiple genes, and adequate genetic variation is generally present for such traits, although the effects of individual

genes or quantitative trait loci (QTL) can be small (e.g. Mace *et al*., 2013). In addition, interactions amongst these genes, and with the genetic background and growing conditions, often result in significant G×E×M interactions (Chenu *et al*., 2009; Messina et al., 2011). Such interactions typically adversely affect the rate of selection progress in breeding programs (Podlich *et al*., 1999; Messina *et al*., 2011).

In order to better understand the implications of G×E×M interactions across the adaptation landscape, complex traits can be dissected into underpinning component traits in a manner that can remove context dependencies (Fig. 1; Tardieu, 2003; Hammer *et al*., 2006, 2010; Martre *et al*., 2014; Chenu *et al*., 2017). Component traits are expected to be under simpler genetic control than the complex trait itself, so that their phenotypic expression is likely to be relatively stable across E×M combinations (Reymond *et al*., 2004). The central paradigm of such trait dissection is that G×E×M interactions of the complex trait become an emergent property of interactions between E×M and the component traits, and the interactions amongst those traits (Chenu *et al*., 2008, 2009). Hence, improved understanding of the biological functionality that underpins G×E×M interactions of complex traits can be used to identify improved phenotyping strategies for crop improvement programs (Fig. 1; Hammer *et al*., 2010; Messina *et al*., 2011).

Crop models provide a means to predict the potential value of traits across a wide range of target environments (Fig. 1; e.g. Hammer *et al*., 2014; Casadebaig *et al*., 2016; Messina *et al*., 2015; Zheng *et al*., 2015; Chenu *et al*. 2017). Combined with genetic models, crop models can be used in breeding applications to explore the yield-trait performance landscape (Cooper *et al*., 2002, 2014; Chapman *et al*., 2003; Messina *et al*., 2011). However, this typically requires sufficient biological functionality to allow predictive capabilities in novel parts of the G×E×M landscape where the model has not yet been tested. An aim of the so-called gene-phenotype (G-P) models is to link the phenotypic expression of complex traits to their underpinning genomic regions. For traits for which the gene network is known, the effects of genes can potentially be scaled up to the crop phenotype. This is generally the case for simply inherited traits, although phenology is a rare example of a more complex inherited trait where this could also be achieved (Wilczek *et al*., 2010). For complex traits for which the underpinning gene network is not well known, trait dissection can provide the biological functionality to capture G×E×M interactions and identify environmentally-stable genetic controls (Reymond *et al*., 2003, 2004; Chenu *et al*., 2008, 2009). Hence, a complex trait becomes an emergent property of interactions between simpler traits and E×M, and can be associated to genetic controls related to these simpler traits. For instance, the complex trait of stay-green can result from variations in early canopy development, which allow crops to save water during early development and use it later (Hammer *et al*., 2010; van Oosterom *et al*., 2010). This, in turn, allows crops to photosynthesise (and hence 'stay green') for longer (Borrell *et al*., 2014b) and to yield more (e.g. Borrell *et al*., 2000; Kamara *et al*., 2003; Hong and Kobata, 2009; Christopher *et al*., 2014, 2016). Accordingly, genetic controls related to canopy development traits such as tillering or leaf size have recently been associated to stay-green (Borrell *et al*., 2014b; George-Jaeggli *et al*., 2017).

When looking across traits, a recent study investigating the value of all the traits from a crop model in Australian environments (Casadebaig *et al*., 2016) found that many high-impact traits were related to water capture and usage (root system and transpiration efficiency), light capture and usage (above-ground architecture and photosynthesis), phenology, and biomass partitioning. While most of those traits or processes have been studied to different extents, we focus here on transpiration efficiency (TE), which is defined as the amount of biomass produced per unit of water transpired. The objective of this paper is to present the synergistic benefits of an integrated approach to assist breeding for genotypes better adapted to a target population of environments. The approach combines trait dissection and high-throughput phenotyping with genetics and modelling to provide insights and inform targeted selection for specific adaptation (Fig. 1).

Predictive modelling – Trait value in crop production environments

Water availability is a critical limiting factor for many cropping areas across the globe, and increased TE can potentially assist breeders selecting for germplasm that produces 'more crop per drop' (Marris, 2008) to increase grain yield in water-limited environments. Because the positive effects on grain yield of traits related to drought adaptation (like TE) depend on the timing and intensity of occurrence of drought stress (Tardieu, 2012; Chenu, 2015), the value of such traits can vary greatly depending on the environment and management considered. Crop models provide an avenue to extensively characterise the current and projected target population of environments (e.g. Chenu *et al*., 2011, 2013; Hammer *et al*., 2014; Watson *et al*., 2017), and test the effects of complex traits on grain yield at large scales in a production context (e.g. Veyradier *et al*., 2013; Martre *et al.,* 2015*;* Casadebaig *et al*., 2016). However, predicting the effects of such traits on grain yield in G×E×M conditions that have not been explored experimentally requires a modelling framework that has the biological functionality to capture context dependencies (i.e. interactions with environmental controls) (e.g. Hammer *et al*., 2010; Messina *et al*., 2011; Chenu *et al*., 2017).

Simulation studies have highlighted a substantial effect of increased TE on grain yield, particularly under drought. Long-term simulations of sorghum (*Sorghum bicolor* (L.) Moench) (Fig. 2; Sinclair *et al*., 2005) and wheat (*Triticum aestivum* L.) (Condon *et al*., 2002) showed that increased TE was generally associated with increased grain yield under post-anthesis drought stress, but not in well-watered environments, at least in parts of Australia. However, this is not necessarily a direct causal relationship because increased TE itself is a consequence of either increased photosynthetic capacity, restricted transpiration rates, or a combination of the two. In the sorghum study of Sinclair *et al*. (2005), the increase in TE was an emergent consequence of a reduction in maximum transpiration rates. Transpiration rates generally increase with increasing vapour pressure deficit (VPD), and hence TE declines with increasing VPD (Kemanian *et al*., 2005). Restricting the maximum transpiration rates thus limits transpiration at high VPD, when water use is least efficient and TE is lowest. This reduces the water use and increases the biomass produced per unit water used (TE). The associated reduction in pre-anthesis water use can then delay the onset of drought stress, particularly in

environments where crops rely on stored soil moisture. Simulations studies for sorghum in Australia (Sinclair *et al*., 2005), soybean (*Glycine max* (L.) Merr.) in the US (Sinclair *et al*., 2010), and maize (*Zea mays* L.) in the US (Messina *et al.*, 2015) all have shown that limiting maximum transpiration rates generally increases grain yield in locations where post-anthesis drought stress is likely to occur. However, because limited maximum transpiration rates are a consequence of reduced stomatal conductance, restricting water loss at high VPD can restrict CO_2 uptake and hence photosynthetic rates, which would explain the yield penalty under well-watered conditions (Sinclair *et al.*, 2005; Messina *et al.,* 2015). Importantly, the development of such biological functionality requires trait dissection in a quantitative manner to determine the dynamics of physiological processes that underpin the phenotypic expression of complex traits in the G×E×M landscape (Fig. 1).

As *in-silico* studies can only be as good as the model used, it is important to evaluate simulation results against some experimental data when feasible. For TE, simulations performed with different models show that TE and its component traits can improve drought adaptation in environments where crops rely heavily on stored soil water (Condon *et al*., 2002, 2004; Sinclair *et al*., 2005). Further, experimental work, based on a surrogate trait for TE, has also highlighted the potential of TE to increase yield in crops like wheat (Rebetzke *et al*., 2002 and 2009). Overall, TE is thus expected to be beneficial for major cropping areas around the globe. Quantitative understanding of TE and its components is required to fine tune simulation results and analyse genotypic variability (Fig. 1).

Trait dissection - Science development to inform both model development and high-throughput phenotyping

Target traits and platform requirements

Dissecting complex traits into underpinning component traits is a way to identify traits that are more environmentally stable and more closely linked to gene expression than the complex trait itself. This allows more robust parameterisation of crop models, and helps narrowing the gap between G and P (Tardieu, 2003; Hammer *et al*., 2006). Trait dissection is thus an integral part of the connection between phenotype and model, and facilitates the connection between phenotype and genotype (Fig. 1).

TE is typically defined at the plant level, and corresponds to the plant dry biomass (with (TE_{plant}) or without (TE_{shoot}) the root system) produced per unit of water transpired. It is generally measured in sealed containers that exclude soil evaporation and deep drainage, and differs from 'water use efficiency' (WUE), which typically refers to field measurements that include soil evaporation, deep drainage and exclude root biomass (Richards *et al*., 2002). Because TE at the plant level is typically measured over a period spanning days to weeks, its integrated nature provides limited insights into the quantitative response to environmental conditions. However, many of the crop physiological processes that determine TE operate at the leaf level. At this level, TE can be defined as the ratio between assimilation (photosynthesis) and the flux of water vapour through the stomata,

measured over a period of a few seconds to a few minutes. Because leaf-level measurements of TE are point measurements in terms of both space (part of a leaf) and time (seconds), they typically are more variable than plant-level measurements of TE. Combined with the time consuming nature of these measurements, they are generally less conducive to large-scale phenotyping than plant-level measurements.

A platform that is suitable for the dissection of TE needs to enable detailed measurements of the response of traits associated with either transpiration or photosynthesis to pedo-climatic conditions. Quantifying the response of transpiration rates to environmental conditions requires regular measurements of water use and environmental conditions. In order to achieve a wide range of environmental conditions, plants can be grown either (i) in environments with minimal environmental control, where diurnal climatic variations can be exploited to develop quantitative responses, (ii) or in a range of controlled or semi-controlled environments designed to develop response curves to specific environmental factors. An advantage of semi-controlled conditions is that they minimise the differences between the growing environment and field conditions, as such differences can markedly affect plant growth (Rebetzke *et al*., 2014; Poorter *et al*., 2016). However, controlled conditions may also allow proper transfer to the field when adequately designed (e.g. Reymond *et al*., 2004). Light conditions and pot size are also important to consider, as reduced light can affect leaf width (Lacube *et al.,* 2017) and small pots can significantly affect plant growth. For example, root-shoot biomass partitioning in sorghum and maize is affected when plants are grown in pots less than *ca.* 30 L and harvested shortly after anthesis (Yang *et al*., 2010). Small pot size can also necessitate frequent irrigation, which increases stomatal conductance per unit of soil moisture (Puértolas *et al*., 2017). Typically, phenotyping platforms recording pot weight over time have relatively small pot size and are generally used in controlled environments (e.g. Granier *et al*., 2006; Pereyra-Irujo *et al*., 2012). A notable exception is the LeasyScan system (Vadez *et al*., 2015) and a facility described in Figure 3 that uses large lysimeters under semi-natural conditions. This latter platform monitors water use per pot and climatic factors in a solarweave enclosure with a time steps of 10 minutes and allows soil water conditions to be customised for each pot. The facility meets the basic requirements for trait dissection in response to atmospheric and edaphic water stress (Figs 3, S1), as it uses large pots (*ca.* 51 L) that allow unrestricted plant and root growth until maturity for most field crops (Yang *et al*., 2010; van Oosterom *et al*., 2011). Investigation of component traits that have to be adjusted for plant size, such as transpiration rates per unit of green leaf area (TR/GLA), necessitate estimation of plant leaf area, either through destructive measurements using a planimeter, or through estimation based on the length and maximum width of fully expanded leaves (see details in the caption of Fig. 3).

Within the context of our integrated approach to crop improvement (Fig. 1), detailed trait dissection experiments have two main objectives. The first one is to develop the scientific insights that underpin the development of crop modelling capacity. To achieve this, the insights from detailed experiments are used to develop modules within a crop model in order to gain the biological functionality needed to predict crop performance in unexplored parts of the G×E×M landscape,

especially for crop improvement benefits (Hammer *et al*., 2010, 2014; Holzworth *et al*., 2014; Chenu *et al*., 2017). Secondly, the insights gained both experimentally and from simulation studies with the improved model, can guide the choice of relevant target traits for phenotyping purposes. Integration with breeding programs is further ensured by using diverse germplasm with relevance to breeding programs, such as parents of mapping populations that are used in high-throughput phenotyping. The next section provides some examples of insights gained from detailed trait dissection experiments.

Phenotyping differences in transpiration efficiency across genotypes

The ability to grow plants for a long duration in large lysimeters reduces the relative size of errors in TE and its component traits, because biomass production and cumulative water use increase with duration. This facilitates the high data quality that is required to develop the quantitative biological relationships that underpin model development and identification of phenotyping traits. The large lysimeters presented in Figures 3 and S1 allow the identification of genotypic differences in TE that are generally well preserved across experiments. This is illustrated for sorghum genotypes grown across three experiments (Table1), where highly significant G and E main effects were found for TE_{shoot} and TE_{plant}, while G×E interactions were not significant. Importantly, the platform provides highly precise data, with a coefficient of variation (CV) for TE typically around 6-7% and root mean square errors for TE around 0.4-0.5 g kg^{-1} for all the cereals tested (Table 2). Low CVs and high precision, combined with repeatability across experiments, increase the capability to detect the physiological mechanisms that underpin genotypic differences in TE.

Importance of roots for phenotyping TE differences across genotypes

Significant genotypic differences in biomass partitioning to roots have been observed under well-watered conditions in the large lysimeters for sorghum (Table 1), maize, and wheat (data not shown). Hence, exclusion of roots can affect the TE ranking of genotypes, in particular for genotypes with extremely high or low root biomass allocation. For instance, sorghum line R931945-2-2 had a low TE_{shoot} but average TE_{plant} (Table 1). Similarly, wheat cultivar Babax had a high TE_{plant} but a low TE_{shoot}, due to its high root:shoot ratio (Fig. 4A). The potential importance of including roots in the calculation of TE was also highlighted by Xin *et al*. (2009), who observed that G×E interactions were significant for TE_{shoot}, but not for TE_{plant} among 25 sorghum genotypes grown in two experiments. Nonetheless, the association between TE_{shoot} and TE_{plant} is generally highly significant (Fig. 4B). As extraction of roots from soil is a labour intensive process, the current results from detailed studies in large lysimeters imply that the costs involved in root extraction are unlikely to justify the additional insights obtained, especially when dealing with large number of plants such as in a genetic study.

Night-time transpiration

Recent studies have reported genotypic variations in night-time transpiration under very high night VPD of 2.2 kPa, with reports such transpiration could account for up to 55% of the total daily transpiration rate in wheat (Schoppach *et al*., 2014a). In semi-controlled conditions, genotypic

variation was also found for night-time transpiration, but night-time transpiration rates were close to zero for both wheat grown in winter and sorghum grown in summer (Fig. 5). Here, night-time transpiration only accounted for a small fraction of the total daily transpiration. This discrepancy with the results of Schoppach *et al*. (2014a) is likely due to the lower VPD recorded in the semi-natural setup used (Figs 3, S1), where night-time VPD was <1 kPa, even on hot sunny days in summer. Similar results have been reported under natural and semi-natural conditions in India and in France (sorghum and pearl-millet, Vadez *et al*., 2015; maize; Alvarez Prado *et al*., 2018). In grapevine, night-time transpiration rates were less than 10% of day-time transpiration rates in an outdoor experiment in France (Coupel-Ledru *et al*., 2016). Overall, selection for genotypic variation in night-time transpiration does not appear to be the highest priority to improve drought adaptation.

Response of transpiration efficiency and transpiration rate to VPD

TE is inversely related to VPD (Kemanian *et al*., 2005) given that transpiration rates per plant, when adjusted for green leaf area to account for differences in plant size (TR/GLA), increase with VPD (Fig. 6A). Accordingly, genotypes with high TE often have lower TR/LA at high VPD than those with low TE (Fig. 6B), although both low and high TE genotypes may or may not have a pronounced breakpoint in the response of TR/GLA to VPD (Ryan *et al.*, 2016). This TR/GLA vs VPD relationship is consistent for data within and across experiments, which indicates that both the plant developmental stage and the time of the year have no substantial effect on the response (Fig. 6A). The generic nature of this response makes it suitable for use in a simulation model as a component process that underpins TE. However, the diurnal patterns of transpiration rates also indicate that in the afternoon, transpiration rates declined rapidly, even though VPD remains high, meaning that transpiration rates were also associated with factors other than VPD (Figs 5B, 5D for wheat and sorghum, respectively). Accordingly, the response of transpiration rates to VPD has been established in some studies by excluding afternoon data (e.g. Schoppach *et al*., 2016). Possible other pedo-climatic factors affecting transpiration rates and their response to VPD include (i) radiation (Alvarez Prado *et al.,* 2018), (ii) temperature, which can modify the breakpoint and the slope of the response of transpiration rates to VPD (Yang *et al.,* 2012; Shekoofa *et al.,* 2015), and (iii) soil drying around the root surface, which could trigger a drought response.

Diurnal and day-to-day variations in VPD can be used to quantify genotypic and species differences in the response of transpiration rates to VPD. Results for sorghum from such semi-controlled conditions (Fig. 6B) are comparable to those obtained for sorghum in controlled environments with regulated VPD (e.g. Gholipoor *et al.*, 2010; Choudhary and Sinclair, 2014). Similar genotypic differences have been observed for other crops, including soybean (Fletcher *et al*., 2007; Sadok and Sinclair, 2009a,b), peanut (*Arachis hypogea* L.) (Jyostna Devi *et al*., 2010), pearl millet (*Pennisetum glaucum* (L.) R.Br.) (Kholová *et al*., 2010b, 2016), chickpea (*Cicer arietinum* L.) (Zaman-Allah *et al*., 2011), wheat (Schoppach and Sadok, 2012), cowpea (*Vigna unguiculata* (L.) Walp.) (Belko *et al*., 2013), and maize (Gholipoor *et al*., 2013; Sunita *et al*., 2014).

Because hourly plant-level transpiration rates are highly correlated to leaf stomatal conductance (Fig. 7; Alvarez Prado *et al*., 2018), it is possible that genotype and species differences in plant-level transpiration rates relate to differences in leaf stomatal conductance. A reduction in transpiration rates per unit leaf area under high VPD has been linked to low stomatal conductance, low stomata number, decreased root metaxylem diameter and endodermis cell size, and restricted hydraulic conductance, which can limit water flow from xylem to guard cells (Sadok and Sinclair 2009a,b; Kholová *et al*., 2010b, 2016; Borrell *et al*., 2014a; Schoppach *et al.,* 2014b). In addition, the gene MPK12, which reduces TE in *Arabidopsis*, has been linked to increased guard cell size (Des Marais *et al*., 2014). Results from platforms like the one presented in Figures 3 and S1 can provide insights into how the response of component traits like TR/GLA to environmental conditions can affect TE.

Response of transpiration efficiency and transpiration rate to soil water deficit

A number of studies have looked at genotypic differences of response of transpiration rates to soil water deficit in pots. Commonly, the level of water deficit is expressed in terms of fraction of transpirable soil water (FTSW). Relative transpiration rates (i.e. transpiration rates of stressed plants normalised by transpiration rates of well-watered plants, and adjusted for any difference in plant size) typically decreases linearly with FTSW below a certain threshold of FTSW. This FTSW threshold at which transpiration starts to decline varies across genotypes and crops (e.g. sunflower (*Helianthus annuus* L.), Casadebaig *et al*., 2008; peanut, Jyostna Devi *et al*., 2009; pearl millet, Kholová *et al*., 2010a; sorghum, Gholipoor *et al*., 2012; and wheat, Schoppach and Sadok, 2012). Overall, genotypes with a high threshold FTSW tend to have high TE under drought, because they reduce TR/GLA earlier in the dry-down cycle than genotypes with a low threshold (Sinclair *et al*., 2005; Sinclair, 2012; Jyostna Devi *et al*., 2009). Such a response can delay the onset of the drought stress and increase post-anthesis water availability. Platforms with large pots and controlled watering of individual pots, such as the one presented in Figures 3 and S1, can be used to investigate relationships between TE and component traits such as TR/GLA under dry down or controlled drought conditions.

Genetic dissection - High-throughput phenotyping and genetic analysis

Target traits and platform requirements

The purpose of trait dissection in the context of crop improvement is to identify component traits of complex traits that are associated with targeted complex traits, and are more suitable for selection by virtue of a reduced E dependency, reduced G×E interactions, and closer alignment to underpinning genetics (Tardieu, 2003; Hammer *et al*., 2006 and 2010). Accordingly, Sinclair (2012) proposed that TE is not a suitable trait for high-throughput phenotyping, as it is an integrated measure over time that provides limited insights into responses to environmental conditions. Instead, Sinclair (2012)

advocated the use in breeding programs of component traits that contribute to TE. Component traits of interest that have shown genotypic differences include:

(1) the ability to limit transpiration rates per unit leaf area under high VPD (Fletcher *et al*., 2007; Sadok and Sinclair, 2009b; Jyostna Devi *et al*., 2010; Gholipoor *et al*., 2010; Kholová *et al*., 2010b; Zaman-Allah *et al*., 2011). The advantage of phenotyping for transpiration rates is that it does not require biomass sampling. However, an obvious prerequisite is an ability for continuous measurement of both water use and leaf area development. Solid progress has been made in estimating leaf area in a high-throughput manner through imaging, even in high-tillering plants with overlapping leaves (Fanourakis *et al*., 2014; Vadez *et al*., 2015). Although imaging of individual plants has its restrictions in terms of the maximum plant size for which leaf area can be accurately imaged, methods have been developed that appear sufficiently accurate for large-scale estimation of transpiration rates per unit leaf area (Vadez *et al*., 2015; Alvarez Prado *et al.,* 2018).

(2) the threshold soil water content at which transpiration rates respond to soil drying (e.g. Jyostna Devi *et al*., 2009; Gholipoor *et al*., 2013). However, a disadvantage of this trait is that phenotyping can be cumbersome as it requires soil drying in relatively large lysimeters that are not conducive to high-throughput phenotyping. High-throughput estimation of leaf area may also be required for this trait.

(3) photosynthetic capacity (Kidambi *et al*., 1990; Balota *et al*., 2008; Gilbert *et al*., 2011a,b). Hyperspectral imagery shows promises for high-throughput phenotyping of photosynthetic activity. Solar-induced chlorophyll fluorescence from airborne hyperspectral imagery has been significantly associated with leaf-level measurements of CO_2 assimilation obtained in the field (Zarco-Tejada *et al.*, 2016) and has been used for large-scale monitoring of crop photosynthesis (Guanter *et al*., 2014). However, although chlorophyll fluorescence may now be a means for high-throughput screening of photosynthetic activity, genotypic differences in photosynthetic capacity are likely to be partly a consequence of differences in stomatal conductance. They could thus be indirectly associated with differences in transpiration rates, which has already been identified as a major component to explain genotypic differences in TE.

Despite the dependence of TE on environmental conditions and its complex nature, TE itself can be used as a trait for large-scale phenotyping. Although the integrated nature of TE does not provide quantitative insights into responses to environmental conditions, its measurement does not require continuous monitoring of water use and leaf area development, and may thus be more suitable for breeding programs with limited resources. Moreover, despite the complex nature of TE, its G×E interactions are often not significant under well-watered conditions (Haefele *et al*., 2009 for rice (*Oryza sativa* L.); Mortlock and Hammer, 1999 and Vadez *et al*., 2011a for sorghum; Fletcher *et al*., 2017 for wheat). Even in studies where significant G×E interactions for TE were observed, these interactions were still smaller than the genotypic main effect (in sorghum, Xin *et al*., 2009 and Vadez *et al*., 2011b; in peanut, Krishnamurthy *et al*., 2007). Moreover, a significant ($P<0.01$) positive correlation between TE in well-watered and drought stressed conditions have been reported in wheat (Condon *et al*., 1990).

High-throughput measurements of TE has been done using Carbon-13 Isotype Discrimination (CID, Farquhar and Richards, 1984) as a surrogate trait for TE. CID in leaf dry biomass generally has a high heritability in both C_3 (Rebetzke *et al*., 2002 for wheat) and C_4 cereals (Gresset *et al*., 2014 for maize) and is significantly negatively correlated to TE in C_3 cereals like wheat (Farquhar and Richards, 1984; Condon *et al*., 1990) and rice (Impa *et al*., 2005). High-throughput measurements of CID have been used to produce a low-CID (high-TE) wheat cultivar, Drysdale, which generally yield better than its closely related high-CID (low-TE) parent, Hartog (Rebetzke *et al*., 2009). However, CID is expensive to measure. Moreover, the association of CID with TE is more complex in C_4 species (Farquhar, 1983; Henderson et al., 1996).

Automated lysimeter platforms can be used to measure plant-level TE. Figure 8 presents a high-throughput lysimetry platform, where plants grow under well-watered conditions in up to 560 pots simultaneously. The platform allows continuous monitoring of water use and a high turnover of experiments through short experiment cycles (Fletcher *et al*., 2017). An imaging system for leaf area would also allow high-throughput phenotyping of transpiration rates per unit leaf area, similar to the platform described by Vadez *et al*. (2015). The facility presented in Figure 8 is currently used for the phenotyping of TE_{shoot}, as the exclusion of roots appears to have only a limited effect on genotype ranking for TE within species (Fig. 4).

Linking phenotype to genotype

Phenotyping of mapping populations allows high-throughput platforms to be the link between genotype and phenotype (Fig. 1). Use of partially replicated spatial designs, where only part of the genotypes are replicated within an experiment, provides a balance between maximising the number of genotypes tested in an experiment, and an ability to capture spatial variation in environmental conditions. In addition, the use of common genotypes with known variation in TE across experiments (i.e. probe genotypes) is essential in genetic trials to allow meta-analyses across those experiments that have experienced different environmental conditions.

Preliminary analyses of two sorghum experiments conducted in the facility presented in Figure 8 indicated high correlations for TE_{shoot} across 42 common genotypes and wide-sense heritabilities in the range of 32-51%. These results highlight the repeatability of the setup across (high correlation) and within (moderate heritability) experiments, allowing the identification of a number of significant quantitative trait loci (QTL) for TE. The presence and effects of some of these QTL were highly dependent upon the population considered, which indicates the likely presence of multiple alleles across populations.

Modelling the value of genetic controls in production environments

Linking physiology and genetics insights into crop and genetic models opens avenues to assess the values of genetic controls across production environments (Chenu *et al*., 2009), and to develop gene-trait-yield landscapes that can not be fully explored experimentally (Messina *et al.* 2009, 2011; Zheng

et al., 2016). While TE and its component traits have been found important in drought-prone environments where crops rely heavily on stored soil water (e.g. Condon *et al*., 2002; Sinclair *et al*., 2005), Hammer *et al*. (2005) incorporated preliminary knowledge on the genetic variation for TE in a crop model. In this study, they assumed that TE was regulated by five genes with two additive alleles each, resulting in 11 different phenotypes (presence of 0-10 positive alleles). They found that under severe end-of-season drought stress, positive alleles for TE were associated with higher yields, while under mild end-of-season drought stress the value of positive alleles for TE only became evident when combined with positive alleles for other traits.

A modelling platform linking crop modelling and whole-genome prediction models can add substantial value to breeding programs if it has the biological functionality required to capture the impact of genetic manipulation on G×E×M interactions for yield in the target population of environments. Such an approach was demonstrated as considerably more accurate than the benchmark method of genomic best linear unbiased prediction (GBLUP; Meuwissen *et al.*, 2001) in a proof-of-concept study on simulated data (Technow *et al.*, 2015). Applied to an empirical dataset of a maize population in two drought environments, this new method gave an average prediction accuracy similar to the benchmark GBLUP method (Cooper *et al.*, 2016). While the method can be improved (see discussion from Cooper *et al.*, 2016), such results appear promising and demonstrate that the method can already be applied to breeding programs to generate useful predictions of yield. While such an approach requires more inputs (e.g. soil and climate information) than most other whole-genome prediction methods, it permits to deal with non-additive genetic effects such as physiological epistasis and G×E×M interactions associated with complex traits such as yield. However, the approach can only be as good as the crop model used. New and upcoming insights gained from trait dissection and large-scale phenotyping of mapping populations are crucial to develop the physiological and genetics knowledge necessary for the development of a relevant modelling platform (Messina *et al*., 2011). Overall, the approach presented in Figure 1 proposes a framework to develop, integrate, apply and test our current understanding, thus offering a foundation for defining priorities in research (physiology, genetics, and modelling) and for assisting the design of efficient breeding strategies.

Conclusion

Selection for component traits should be preferred over selection for complex traits. However, identification of optimum target traits for selection requires in particular a sound understanding of both (i) the crop physiology that underpins the phenotypic expression of complex traits, and (ii) the implications of manipulation of this phenotypic expression on grain yield. To achieve this, we advocate an approach that integrates crop simulation modelling, physiology, genetics and breeding (Fig. 1). Such integration requires (i) a model that can integrate the necessary level of biological functionality (e.g. Holzworth *et al*., 2014; Chenu *et al*., 2017), (ii) experimental setups that can accommodate detailed experimentation for trait dissection, and/or have the high-throughput capability

required for genetic studies and crop selection, and (iii) diverse germplasm with relevance to breeding programs. We use transpiration efficiency (TE) as an example of this approach, given its importance for crop production, particularly in environments where crops rely on stored soil water. The key to the integrated approach advocated here is a very close interaction among crop physiologists, programmers, biometricians, molecular biologists and breeders to capitalise on interdisciplinary synergies. Extending such interactions across crops can capitalise on insights gained previously and techniques developed elsewhere. Such an integrated approach is most likely to maximise return on investment with respect to selection for complex traits, including grain yield.

Supplementary data

Fig. S1. Detailed description of the large lysimeter facility introduced in Figure 3.

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Table 1. Average values and analysis of variance for shoot transpiration efficiency (TE_{shoot}), root:plant biomass ratio, and whole-plant transpiration efficiency (TE_{plant}) for 13 sorghum genotypes grown in three experiments in the large lysimeter platform presented in Figures 3 and S1. Plants were grown under well-watered conditions in spring 2011, autumn 2011 and spring 2013. They were harvested five days after anthesis of the main shoot. Genotypes are sorted by TE_{shoot}; their rank for TE_{plant} is indicated in brackets (from 1: highest to 13: lowest). Italic underlined values correspond to lowest three averages for each trait; bold underlined values correspond to highest three averages for each trait. ns, non-significant (P>0.10); **, P<0.01; ***, P<0.001.

Genotype	**TE_{shoot}** ($g\ kg^{-1}$)	**Root:plant biomass ratio** (%)	**TE_{plant}** ($g\ kg^{-1}$)
QL12	*6.12*	**21.42**	*7.66 (12)*
RTAM422	*6.64*	15.94	*7.63 (13)*
R931945-2-2	*6.68*	**22.71**	8.40 (8)
BTx642 (B35)	6.72	16.61	*7.83 (11)*
RTx7000	6.79	16.45	7.99 (10)
R9403463-2-1	7.14	**17.78**	8.42 (7)
R9188	7.21	14.03	8.46 (6)
B923296	7.45	16.40	8.53 (5)
IS8525	7.47	*12.98*	8.07 (9)
SC170-6-8	7.54	15.19	**8.73 (3)**
A1*F_B963676/R931945-2-2	**7.66**	15.95	**8.84 (2)**
PI391652	**7.75**	*12.11*	8.66 (4)
B963676	**7.96**	*12.19*	**8.86 (1)**

Effect	**Degrees of freedom**	**TE_{shoot}**	**Root:plant biomass ratio**	**TE_{plant}**
Genotype (G)	12	***	***	***
Experiment (E)	2	***	***	***
G×E interaction	24	ns	**	ns

Table 2. Mean, root mean square error (RMSE) and coefficient of variation (CV) for shoot transpiration efficiency (TE_{shoot}) and whole-plant transpiration efficiency (TE_{plant}) in well-watered experiments conducted in the automated large lysimetry system presented in Figures 3 and S1. Maize and sorghum plants were harvested five days after flowering, wheat plants were harvested at flowering.

Exp	Genotype number				TE_{shoot}			TE_{plant}		
Sow date	maize	sorghum	wheat	Replications	Mean (g kg^{-1})	RMSE (g kg^{-1})	CV (%)	Mean (g kg^{-1})	RMSE (g kg^{-1})	CV (%)
May 2011			15	3	5.66	0.29	4.2	6.61	0.32	4.0
Sept 2011	8	22		4	5.92	0.29	4.8	7.13	0.28	3.9

Figure legends

Fig. 1. Schematic of a trans-disciplinary integrated approach to breeding systems highlighting integration and roles of physiology and modelling with genetics to understand and take advantage of G×E×M interactions.

Fig. 2. Simulated gains in relative yield when increasing transpiration efficiency by restricting maximum transpiration rates. Simulations were conducted with maximum transpiration rates limited to 0.6, 0.4 mm h^{-1} or no restriction (default) in sorghum. The gains are presented for 115 growing seasons at Dalby, Queensland, Australia. Relative yield corresponds to gain or loss related to the change in maximum transpiration rates compared to the default (no restriction). Figure from Sinclair *et al*. (2005).

Fig. 3. Setup of a large lysimeter system with a general view of an experiment with sorghum (short and intermediate plants on the picture) and maize (tall plants) (A) and with wheat (B); the watering system (C-D); and a screenshot of the software interface (E) that shows the weight of eight lysimeters on a trolley for a selected period of time. The platform has a capacity of 128 lysimeters placed on 16 trolleys. Positioned on a load call, each lysimeter consists of a large pot (*ca*. 51L), which is well above the threshold of *ca*. 30L below which biomass partitioning is affected in crops like sorghum (Yang *et a*l., 2010). The system mimics a field canopy, with border pots, and plants grown at densities similar to field conditions (the number of plants per pot varies from 1 to 10 depending on the species). The system is installed in a shade house, which has solarweave covers that exclude rain and some direct radiation (20-30% reduction in total radiation, which did not affect leaf length, width, stem length, nor biomass allocation across organs (data not shown)), and has temperature and vapour pressure deficit (VPD) slightly greater than ambient values. Each lysimeter is weighed and watered automatically at regular intervals. Irrigation is applied to meet a target weight specific to each lysimeter, thus allowing controlled water deficit. Water is distributed through a flexible pipe (blue pipe in (C-D)) that feeds into a narrow PVC access tube (white tube in (C-D)) that is embedded in the soil for better diffusion of water through the pot. The soil of each pot is covered with plastic sheets (C) or beads (D) to minimise soil evaporation. Every 10 minutes, the average weight of each lysimeter since the last reading is recorded in a CSV file. A user interface (E) that is remotely accessible via the internet provides a real-time view of the weights of all the lysimeters. In (E), each coloured line represents the weight of an individual lysimeter. A gradual decline in lysimeter weights represents water use during the day, a constant weight represents lack of transpiration during night time, and a sudden increase in weight corresponds to a watering event. The constantly decreasing blue line corresponds to the dry down of a lysimeter to a target weight below its current weight. The interface provides a quick and easy way to check for any problems in the watering regime. See Fig S1 for more information.

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Plants are grown under well-watered conditions, as the system combines the concept of a constant water table (as developed by Hunter et al., 2018) with the automatic weighing and watering. To ensure a continuous supply of water, each lysimeter is positioned on a reservoir that holds approximately 600 mL of water. The pots used for the lysimeters are ANOVApot® (www.anovapot.com), which are specifically designed to minimise escape of roots through a circular elevation at the base of the pot that surrounds a central drainage hole (B). A wick made of capillary tape is glued to the bottom of each lysimeter to cover the drainage hole and capillary action ensures transfer of water from the reservoir into the growing medium (in white in B). Reservoirs are rewatered through a small plastic pipe (A) with two rows of 14 lysimeters controlled by a single solanoid. Watering occurs at user-defined times, from once a week (e.g. soon after emergence) to twice daily (e.g. prior to harvest). Each reservoir has an overflow (B), and rewatering is stopped once all 28 reservoirs on a single solanoid are overflowing and the software no longer detects any change in weight in any of the lysimeters. Lysimeters connected to different solanoids are watered successively. Plastic sleeves are used to cover the soil after emergence (C) to minimise soil evaporation.

Fig. S1. A large lysimeter facility in semi-natural conditions placed in a shade house (A) include 128 lysimeter (B) and sensors to measure climatic factors (C). The lysimetery facility is set up in a 12 x 10 m^2 shade house at Gatton in southeast Queensland, Australia (27°33’ S, 152°20’ E). The shade house has solarweave covers that exclude rain and some direct radiation, but also increase diffuse radiation, resulting in an overall reduction in total radiation of 20-30%. This, however, does not result in any observable shade response in terms of organ expansion and biomass allocation across organs compared to field grown plants (van Oosterom, unpublished data). The sides of the shade house contain mesh that allows adequate airflow, but also have solarweave panels that can be rolled down for temporary protection against heavy rain, strong winds, or low night temperatures. In addition, gable fans at opposite sides of the shade house can extract hot air during the summer months. In general, mean temperatures inside the shade house are 2-3˚C higher than ambient, with the greatest increase in minimum temperatures. As a result, the vapour pressure deficit (VPD) inside the shade

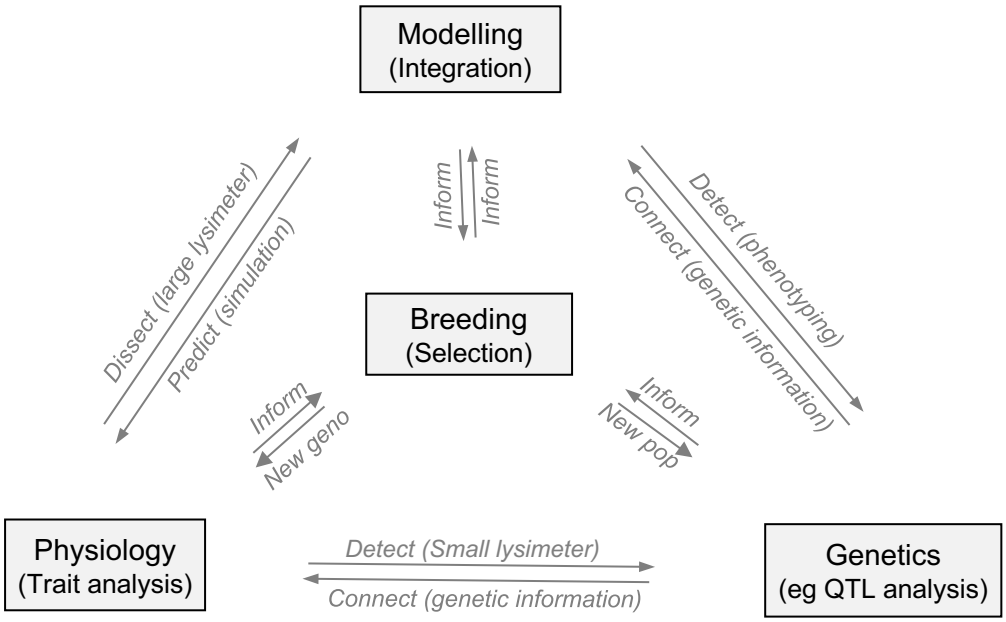
house is slightly greater than ambient and on hot summer days, maximum VPD can exceed 5 kPa. This, however, causes no visible wilting in well-watered plants of C_4 cereals.

The platform has a capacity of 128 lysimeters, located on 16 trolleys that hold two rows of four lysimeters each (B). Within a trolley, lysimeters are spaced 50 cm center-to-center, such that if trolleys are butted up, a single plant per lysimeter allows a density of up to four plants m^{-2}, which is similar to low densities for large field crops like sorghum and maize. For smaller crops like wheat, multiple plants can be grown as a mini canopy inside a single lysimeter. Growing 10 plants per lysimeter corresponds to a density of ~100 plants m^{-2} within each lysimeter, which is similar to field densities in the region. The use of borders further helps to mimic field canopies. Each lysimeter has a volume of *ca.* 51 litres, which is well above the 30 litre threshold below which root-shoot partitioning of maize and sorghum plants is affected (Yang et al., 2010). This allows unrestricted plant and root growth until maturity of most field crops, which is particularly important if drought stress is imposed, as that can significantly affect biomass partitioning to roots and result in differences in grain set (van Oosterom et al., 2011).

The platform is computer controlled, with each lysimeter being weighed and watered automatically. Lysimeters are positioned on their own individual load cell (B) and weights are monitored continuously (Fig. 3E). Every 10 minutes, the average weight of each lysimeter since the last reading is recorded and appended to a CSV file. A user interface that is remotely accessible via the internet provides a real-time view of the weights of all lysimeters (Fig. 3E) and provides a quick and easy check for any problems in the watering regime. As plants grow and use water, the weight of a lysimeter gradually declines (Fig. 3E) and once the recorded weight drops below a user-defined target weight, a user-defined amount of water (typically *ca.* 250 mL) is applied through a flexible pipe that feeds into a narrow PVC access tube that is embedded in the soil to minimise evaporation (Fig. 3C-3D). This results in a sudden increase in the lysimeter weights, which is visible in the interface (Fig. 3E). The volume of the PVC pipes is typically around twice the volume of water added per rewatering, to provide some buffer against overflow in case of slow infiltration rates and high rates of water use. To prevent water logging associated with uneven distribution of water inside a lysimeter, target weights for well-watered experiments are typically set to *ca.* 1-2 kg below the drained upper limited (DUL). This leaves sufficient plant available water, as total plant available water per lysimeter exceeds 10 litres when using local black vertisols. Water use per plant is calculated from the change in lysimeter weight, adjusted for any watering events. With data recorded at 10 minute intervals, water use per plant can be calculated at resolutions of 10 minutes, or aggregated to hourly or daily periods. A weather station located in the centre of the shade house (C) and additional temperature probes are used to monitor environmental conditions. These are recorded in a CSV file every 10 minutes, along with the lysimeter weights.

At the start of each experiment, lysimeters are typically filled to a constant weight using soil that is relatively homogenous in terms of soil water content. After emerged seedlings have established and are thinned to the desired density, the soil of each pot is covered with plastic sheets or beads to minimise soil evaporation. Target weights for watering are based on the amount of dry soil in each lysimeter, which is calculated from the soil water content at the time filling and from DUL and the lower limit (LL) for the soil used. DUL is determined

gravimetrically from a reference lysimeter with holes in the bottom that is left to drain. Similarly, LL of each soil for each crop is determined gravimetrically from lysimeters with plants that are well watered until a late vegetative stage to ensure the root system has explored the entire lysimeter, and are subsequently left to extract all available water from the lysimeter until complete senescence. In addition, control lysimeters without plants are used in each experiment to measure any background evaporation.



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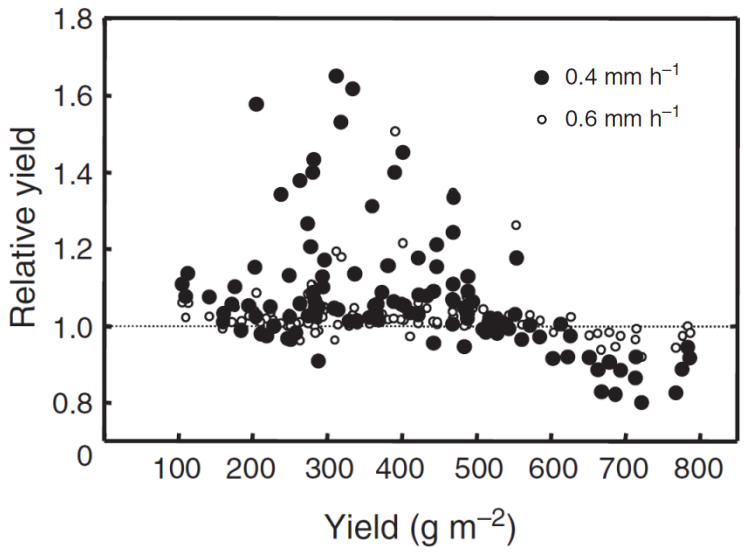

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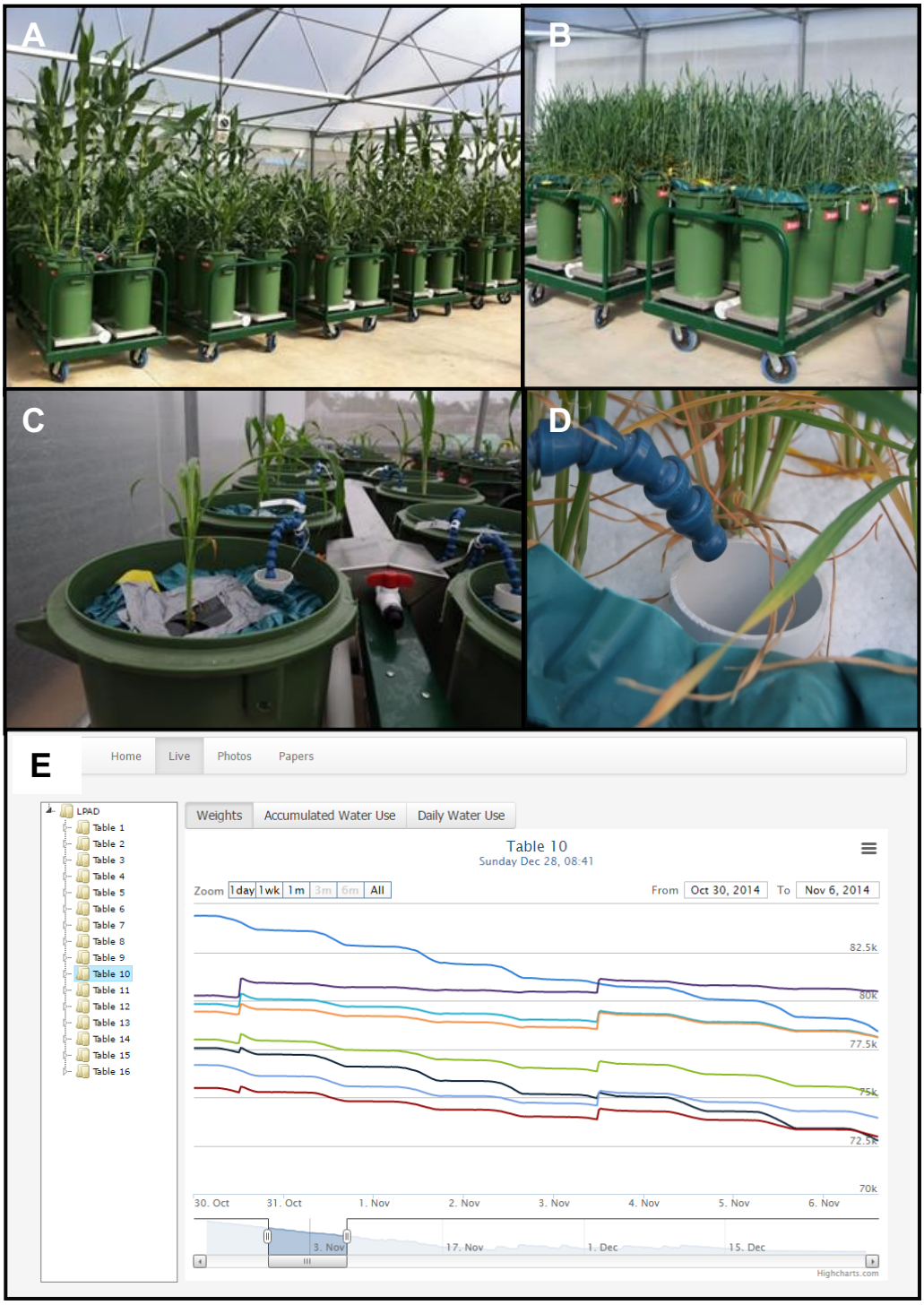

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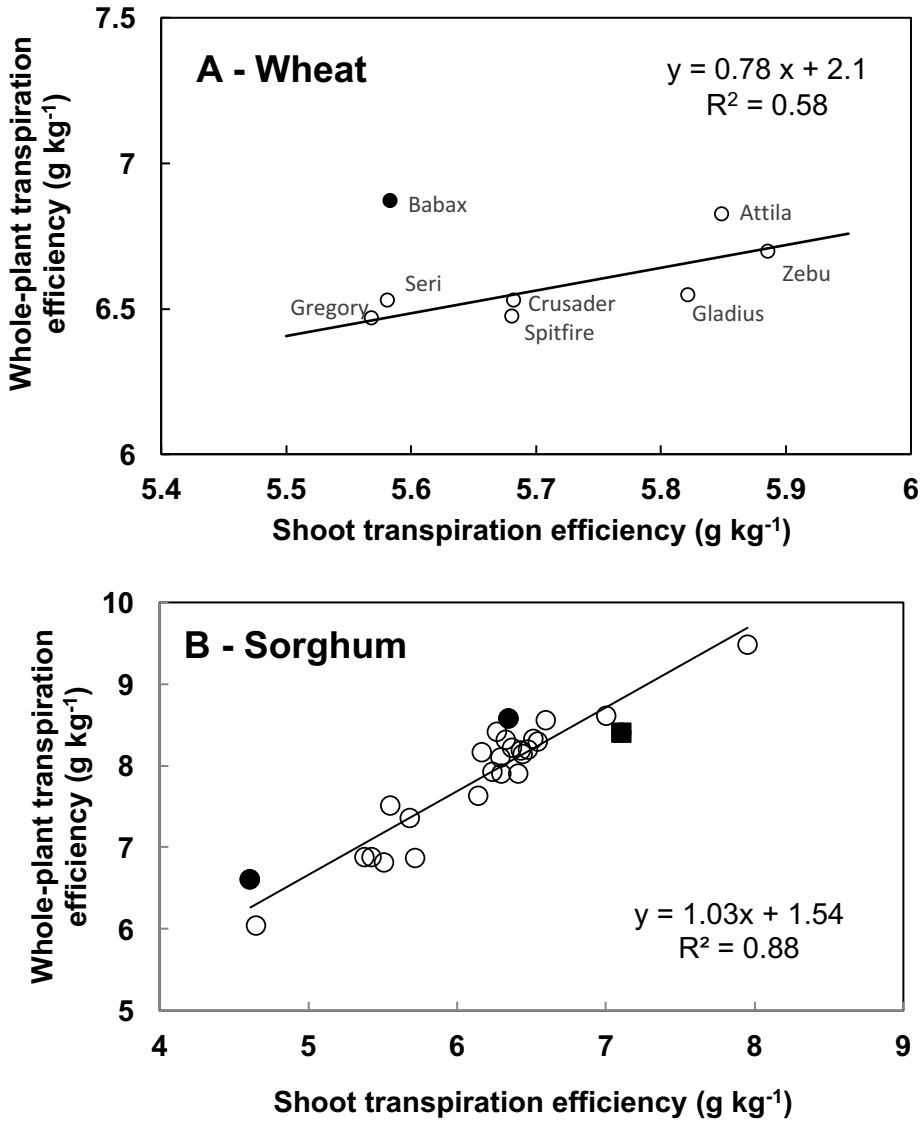

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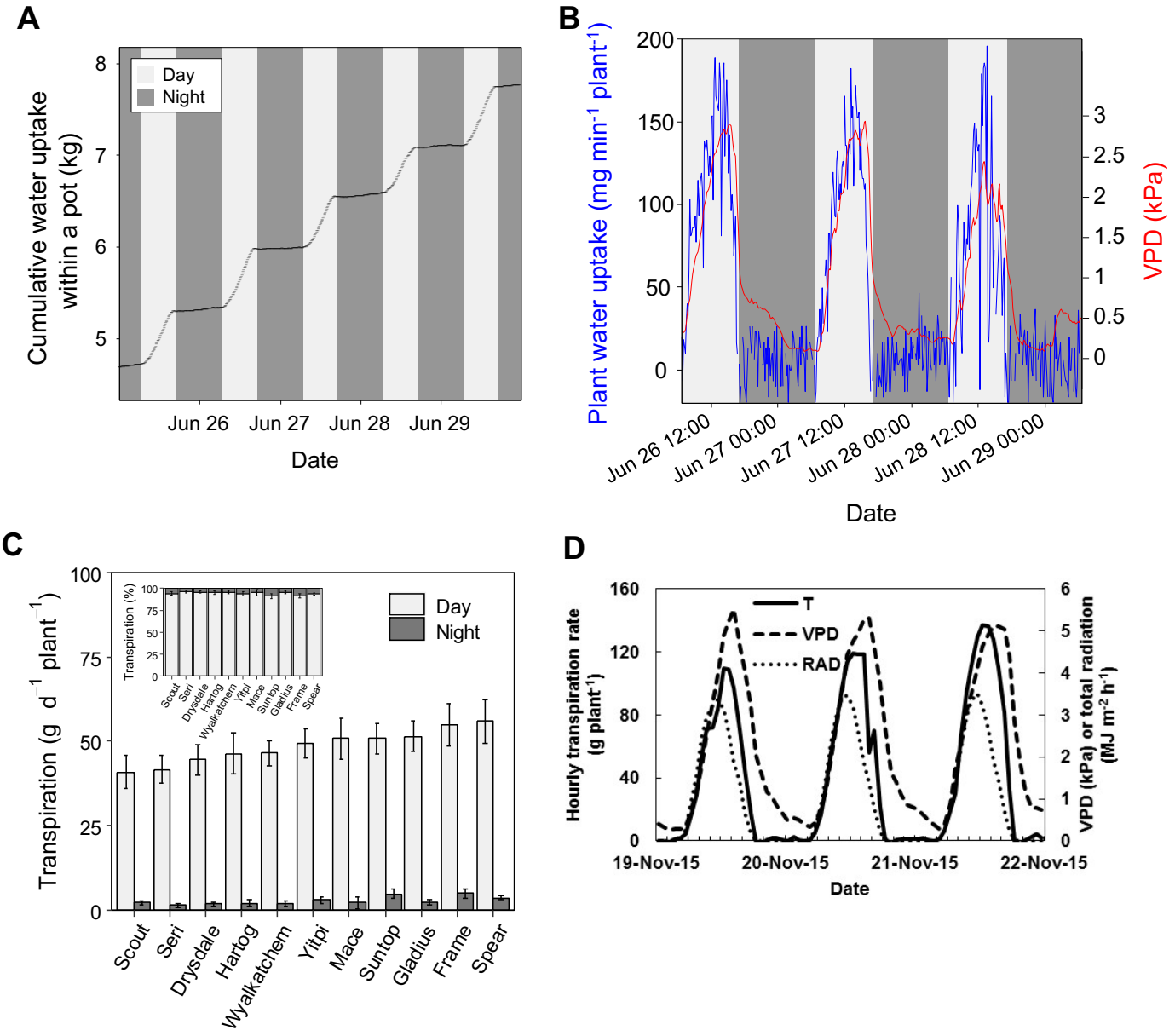

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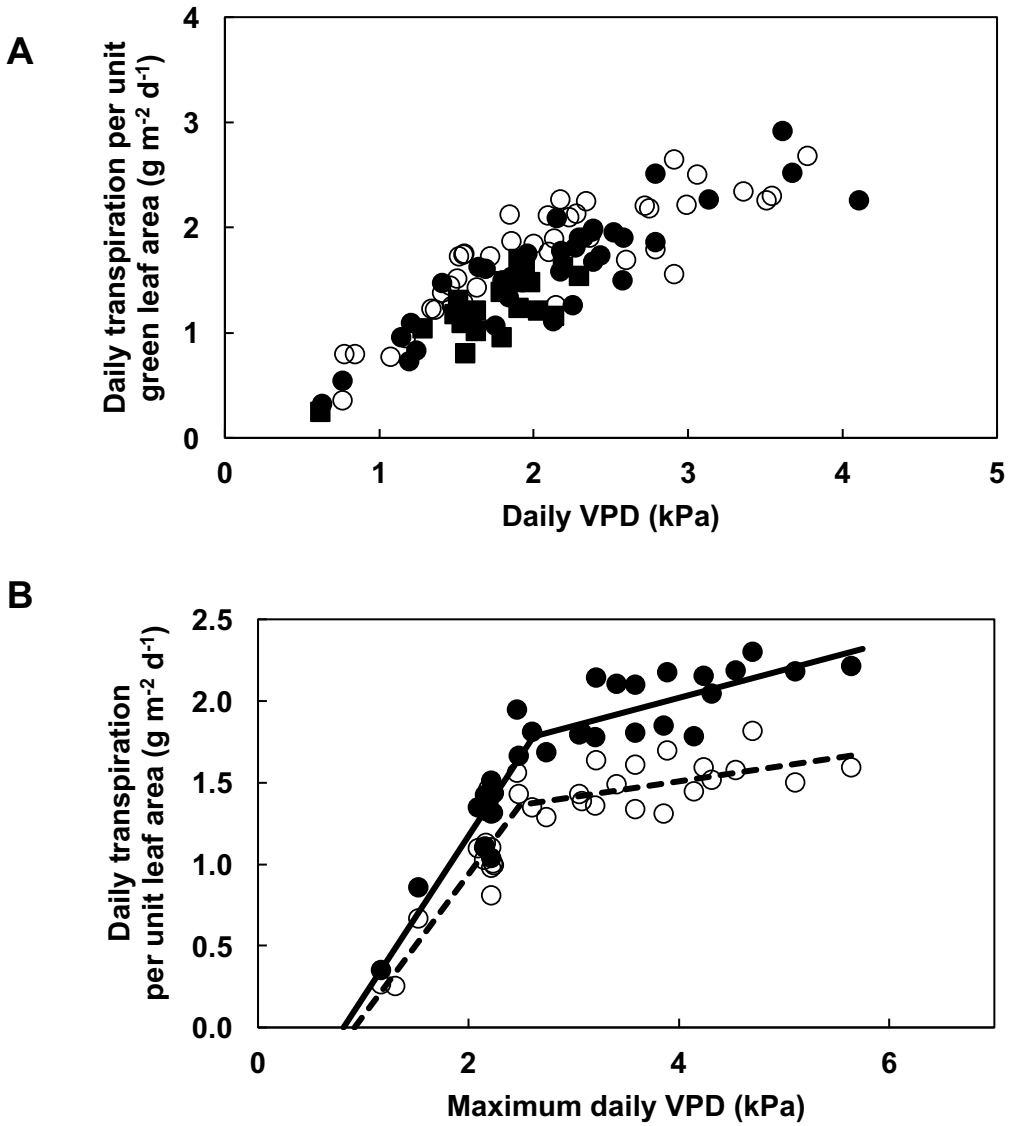

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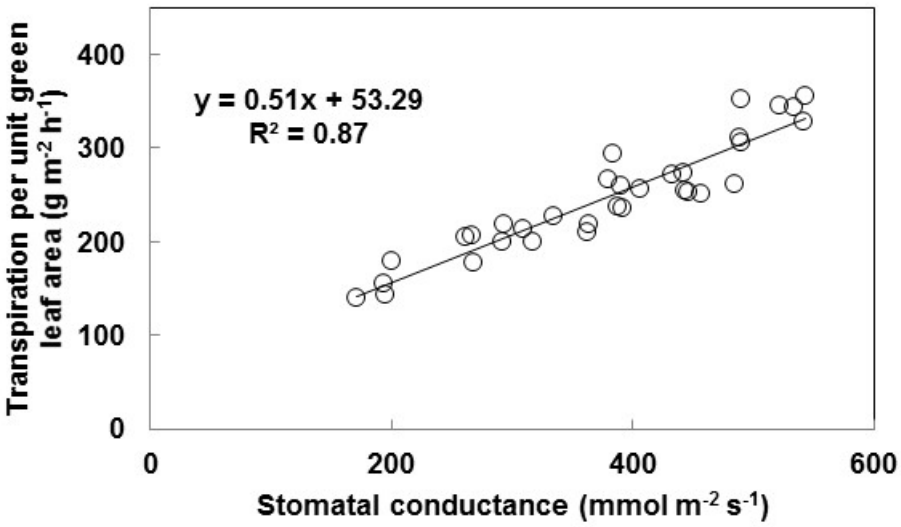

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