Does trial duration affect transpiration efficiency estimates for wheat?

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Abstract
Wheat productivity is frequently limited by soil-water deficit in rain-fed environments. Increasing the amount of plant biomass produced per unit of water transpired (transpiration efficiency, TE) can lead to gains in crop yields, particularly in water limited environments. Currently, the ability to select for TE is limited by typically low-throughput and labour-intensive screening techniques. To investigate the effects of crop stage and environment on TE, 11 wheat varieties were sown at three dates, and grown for different durations in continuously watered pots in a glasshouse experiment. TE was calculated as the ratio of plant biomass over cumulated water use for plants grown to different stages. Significant genotypic variation for TE was observed when measurements were taken over a sufficient duration. Where genotypic differences were observed, genotype ranking for TE was generally consistent irrespective of the experimental sowing and harvest dates. This result indicates that multiple short trials can be carried out within a season to allow increased throughput of genotypes for TE screening. Increasing throughput to effectively screen for TE at the plant level is essential to make the process of selecting for TE viable at a commercial crop breeding level.

Keywords
Water-use, high-throughput screening, phenotyping, drought adaptation.

Introduction
Bread wheat (*Triticum aestivum* L.) is a staple grain crop of global significance that often suffers yield restriction due to limited soil water availability (Chenu et al. 2013). Predictions for both global population to continue increasing rapidly (Borlaug and Dowswell 2003) and for climate trends to become increasingly variable (IPCC 2014) highlight the necessity to increase crop yields and the efficiency with which essential resources are utilised. Transpiration efficiency (TE) is the efficiency with which plants are able to utilise available soil water to produce biomass. TE at the plant level is commonly expressed as grams of above-ground biomass produced per kilogram of water transpired. We have previously reported that TE has increased in Australian wheat cultivars released in the last five decades, possibly due to indirect or unintentional selection for TE as a by-product of selection for greater yields (Fletcher and Chenu 2015). Breeding crops for greater TE, both now and in the future, is necessary to ensure sustainable food security in a future climate where higher evaporative demand and greater variability in rainfall are likely.

While work has already begun to identify phenotypic and genotypic avenues for increasing TE through selective breeding (Rebetzke et al. 2008; Christopher et al. 2015), improving the methodology to allow accurate and resource efficient screening of TE will help to increase throughput. The aim of this project was to investigate whether meaningful estimates of TE could be assessed without waiting until flowering to be able to discriminate between low- and high-TE wheat genotypes. Reducing trial duration to a shorter period at which significant and repeatable results can be observed could allow multiple experiments to be assessed for TE within a single season.

Methods
Experimental design
Eleven wheat varieties (Figure 1) with known variation for TE were used to investigate the ability to identify and discriminate between varieties for TE at multiple sowing and harvest times. Two plants per pots were sown in eight randomised complete blocks, each consisting in five pots for each genotype (i.e. 55 pots per block). Six of the blocks were sown on the 6th May 2015 and harvested one every two weeks, except for the last block which was harvested just after flowering (Table 1). The last two blocks were sown and harvested at later dates.

Using the 'Pot in Bucket' (PIB) method adapted from Hunter et al. (2012), each pot had a continuous supply of water throughout the trial from an individual water jug. Water use per pot was estimated by measuring the

amount of water used from each jug. A layer of white plastic beads was applied to the soil surface in each pot to reduce moisture lost through evaporation and also to reduce weed growth. Six pots with no plants were placed throughout the trial to measure any background moisture loss (e.g. soil evaporation).

Table 1. Experiment characteristics, including sowing date and harvest dates (treatments), trial duration (TD, days after sowing) and environmental conditions. Mean daily temperature and mean daylight vapour pressure deficit (VPD) were calculated over the period used to estimate TE (i.e. from the beginning to the end of water use measurements in each treatment). Treatments are numbered in the order of harvest date and labelled by timing of sowing (S1, S2 or S3) and trial duration (TD).

Treatment	Sowing timing	Harvest Date	TD (days)	TD (GDD*)	Mean Temperature (°C)	Mean VPD (kPa)
S1TD40	Sowing 1 *(06/05/2015)*	15/06/2015	40	677	21.7	1.70
S1TD54		29/06/2015	54	912	21.4	1.58
S1TD68		13/07/2015	68	1127	21.2	1.54
S1TD83		28/07/2015	83	1352	20.6	1.46
S1TD98		12/08/2015	98	1572	20.9	1.54
S1TD104		18/08/2015	104	1664	21.1	1.59
S2TD70	Sowing 2 *(16/06/2015)*	25/08/2015	70	1079	21.3	1.68
S3TD58	Sowing 3 *(14/07/2015)*	10/09/2015	58	905	23.9	2.18

*Growing degree days with base 0°C.

Measurements and analysis

Measurements of water use were made weekly from 25 days after sowing until harvest. Water loss through transpiration prior to 25 days was too little for accurate and reliable measurement. Zadoks scores were recorded each week for the main stem of one plant per pot for the block of Sowing 1 harvested the latest (i.e. S1TD104) and for all pots of sowings 2 and 3 (i.e. S2TD70 and S3TD58).

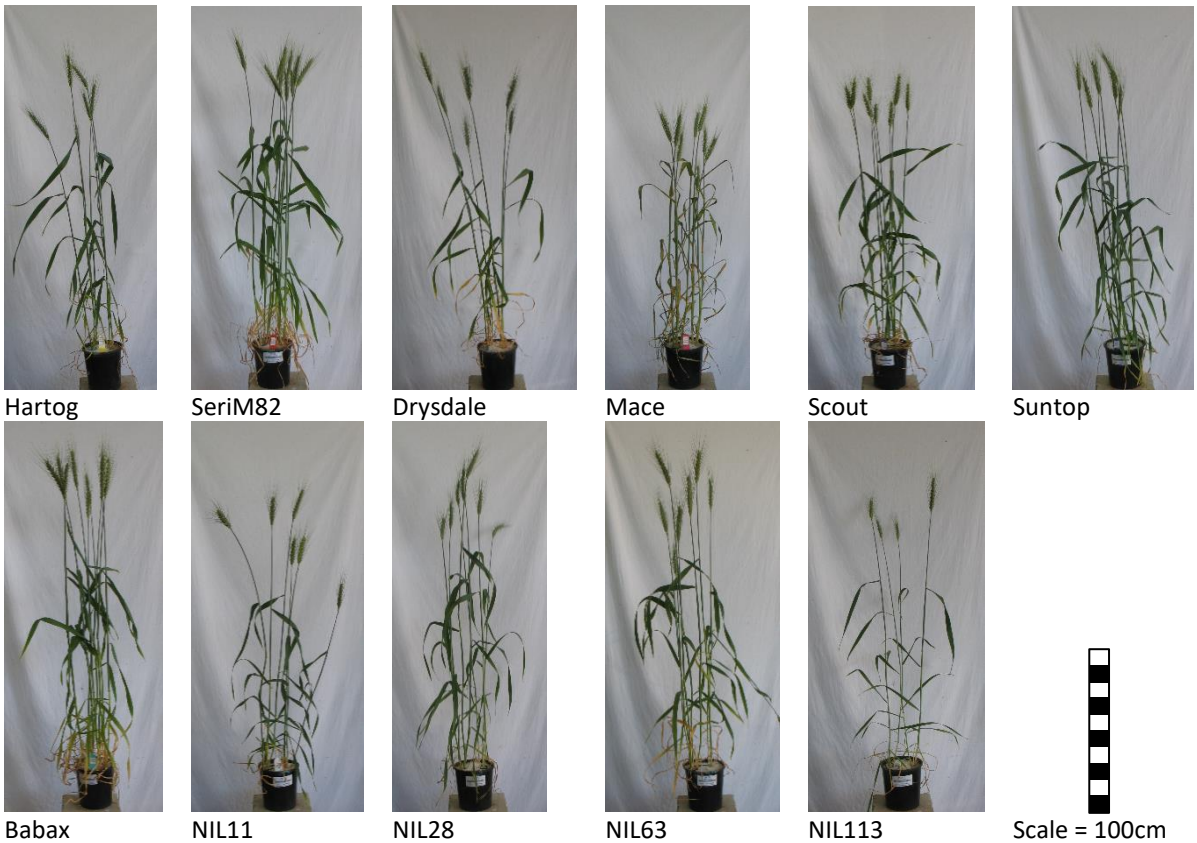

Figure 1. Photographs of wheat varieties examined.

S2TD70 and S3TD58 were harvested at approximately 1000 growing degree days (GDD), at 70 days and 58 days after sowing respectively, in order to analyse TE for plants grown in different environmental conditions, particularly with greater VPD for S3TD58. At harvest, above-ground plant material was dried at 70ºC for 5 days and then weighed for dry biomass. TE was calculated as the ratio of above-ground dry biomass produced per cumulative gram of water transpired from 25 days after sowing until harvest. Cumulative transpiration was calculated as the total amount of water drawn from each water jug, minus the average cumulative water lost from the six pots measuring soil evaporation. Note that biomass accumulation as well as cumulative water use during the 25 days following sowing were negligible compared to values at harvest. All data analysis was performed using ASReml-R (Butler et al. 2009) in the R software environment. Predictions for each genotype at each treatment were generated as both empirical Best Linear Unbiased Predictions (BLUPs) and empirical Best Linear Unbiased Estimators (BLUEs) for different comparative purposes. Significance of genotype differences were assessed using a Wald test and the Fisher's protected least significant difference test was used to perform pair-wise comparisons between genotype BLUEs.

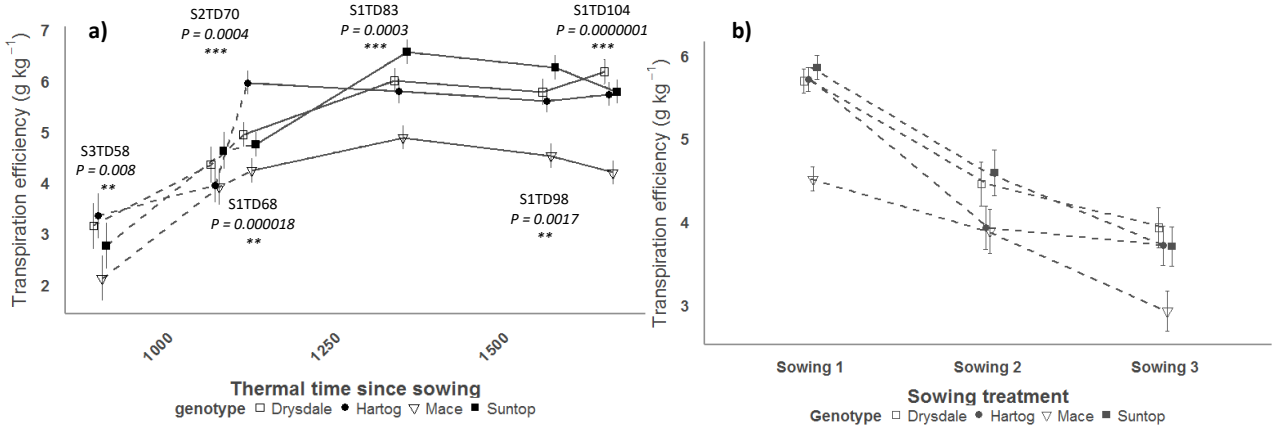

Figure 2. (a) Transpiration efficiency (BLUPS) for various trial durations (shown in thermal time, GDD) for 4 of 11 studied genotypes. Error bars correspond to standard errors. P-values indicate the likelihood of significant TE differences among all 11 genotypes in each treatment. (b) Transpiration efficiency (BLUPs) for all sowing dates and all trial durations (excluding S1TD40 and S1TD54). Only four contrasting genotypes out of the 11 were presented in each panel for clarity. As no significant genotypic differences in TE were detected in S1TD40 and S1TD54, those treatments were not presented. Statistical significance *, P < 0.05; **, P < 0.05; ***, P < 0.001.

Results and Discussion

For the first sowing date (S1) TE was relatively stable over most of the trial durations tested (Figure 2a). However, trials sown later exhibited lower TE (Figure 2b), which was at least partly due to warmer temperatures and higher VPD. Genotypic differences for TE were not significant at the earliest two harvest dates S1TD40 and S1TD54 which were harvested 677 and 912 GDD after sowing, respectively. This is likely due to the small plant size and small water use values that makes differences in TE hard to capture.

Table 2. Mean transpiration efficiency values (BLUPs) and least significant difference (LSD) classes for all genotypes. In Sowing 1, mean values are presented for the combined predictions for S1TD68, S1TD83, S1TD98 & S1TD104. Differences between mean values with the same letter within each sowing group are not significantly different (*P* > 0.05).

Genotype	Sowing 1 Mean TE	Sowing 1 LSD	Sowing 2 Mean TE	Sowing 2 LSD	Sowing 3 Mean TE	Sowing 3 LSD
NIL28	6.01	A	4.42	A	3.93	AB
Suntop	5.86	AB	4.30	ABC	3.71	ABC
Drysdale	5.69	ABC	4.47	A	3.93	AB
Hartog	5.71	ABC	3.93	BC	3.67	ABC
NIL11	5.73	ABC	4.23	ABC	3.95	A
Scout	5.70	ABC	4.33	AB	3.79	ABC
NIL113	5.55	BC	3.89	BC	3.71	ABC
NIL63	5.66	BC	4.11	ABC	3.87	ABC
SeriM82	5.45	C	4.07	ABC	3.45	BCD
Babax	4.97	D	3.08	D	3.38	CD
Mace	4.52	E	3.86	C	2.96	D

Data for these two treatments were excluded from the ASReml analysis and, for simplicity, are not presented in Figure 2a. In S1TD68 (harvested at1127 GDD), significant differences in TE among genotypes were detected (Figure 2a). Measurements taken at the two later sowing dates (S2TD70 and S3TD58) also showed significant genotypic differences, and interestingly the duration of these crops in thermal time was between that of the non-significant trial durations (SD1TD40 and SD1TD54) and the first significant trial duration in sowing 1 (SD1TD68, Figure 2b, Table 2). This indicates that significant differences between genotypes can be identified for TE using trial durations above approximately 900 GDD (thermal time), particularly at later sowing dates. Interestingly, while S1TD54 had a thermal time of 912 GDD and did not produce significant results, S3TD58 had a shorter thermal duration (905 GDD) but still produced significant results which were consistent with the other treatments and most likely due to environmental factors. Differences in the actual TE values between experiments were likely influenced by differences in environmental factors such as VPD. Significant differences ($P \leq 0.05$) among genotypes were detected within each treatment except in S1TD40 and S1TD54 with no significant change in the rankings of these genotypes between trial durations ($P > 0.05$). Therefore, it was possible to pool data from S1TD68, S1TD83, S1TD98 and S1TD104 for analysis (Table 2). While ranking of TE estimates for genotypes varied slightly between sowing dates (Table 2), there was a general trend in TE rankings where NIL28, Drysdale, Hartog and Suntop are consistently ranked higher than Babax and Mace with the other six lines intermediate between these two groups (Table 2).

Conclusion

Estimating TE during early plant development did not allow discrimination among genotypes, as low values for plant mass and water use led to large variations in TE estimates. However, it is not necessary to grow plants to flowering (e.g. Fletcher and Chenu 2015) to compare genotypes for TE. When harvesting at approximately 900 GDD and in the studied conditions (VPD over 1.6 kPa), significant variations in TE were measured with a relatively consistent genotype ranking. Such a result suggests that multiple trials can be conducted in a single season to effectively double (or more) the number of genotypes which can be screened for TE in a season. This is one of the many challenges that must be overcome before screening for TE can become more widely adopted in breeding programs. Such screening protocols are essential if TE and other complex traits are to be incorporated into commercial breeding programs.

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