Sugar Research Australia™

More crop per drop: development of water-efficient and drought tolerant sugarcane cultivars for irrigated and dryland farming

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1. Executive Summary

Water limitation is a major production constraint for sugarcane in Australia. Despite its economic importance, there has been little effort in breeding for water stress (drought) tolerance and water use efficiency (WUE) in sugarcane. This was mainly due to the lack of easy-to-use selection trait for WUE and drought tolerance in sugarcane. This project, building on the findings of its predecessor BSS305, aims i) to understand the genetic association between water use efficiency traits and cane yield, ii) to establish trait modelling capacity for developing varieties with improved yield, WUE and drought tolerance, iii) to identify clones that perform well under different water availability conditions for further trait-yield relationship studies and iv) to develop a selection system for breeding more productive and broadly-adapted varieties.

Field experiments were conducted over three years under fully irrigated and two managed water stress conditions at Dalbeg, Bowen and Brandon, commercial cropping areas with significantly different production conditions. In these experiments a total of 131 genetically diverse sugarcane germplasm were studied for their physiological responses to water deficit, focusing on aspects of stomatal conductance (g_s), photosynthesis, leaf area development and cane yield. Water stress treatments, depending on the severity of water deficit, reduced cane yield (TCH) and total dry matter (TDM) production by 17 to 46% compared to irrigated treatments. There was little genotype × environment interaction variation for TCH, TDM or commercial cane sugar (CCS) under mild to moderate stress conditions, and hence high genetic correlations between irrigated and water stress treatments across test environments. However, there was strong genotype × environment interaction under severe water deficit, especially among unselected clones. In general, commercial clones performed better (higher TCH) than unselected clones under all moisture environments suggesting that intrinsic growth (yield) potential (yield under full irrigation) is a major determinant of crop productivity for both selected and unselected clones in fully irrigated and moderately stressed conditions.

The physiological basis of yield variation in response to water deficit was studied using APSIM sugarcane crop simulation model. These modelling experiments were complemented by appropriately designed field experiments to validate simulation outcomes. Different traits measured in this project were tested in the model to quantify

their impact on clone performance in diverse production scenarios. The initial APSIM yield predictions for water stress conditions were not satisfactory as actual yields were substantially different from the simulated ones for most clones. The issue was resolved by incorporating an additional platform to include hourly variation in temperature, radiation and vapor pressure deficit (VPD) (instead of fixed values in the original APSIM model) for more accurate growth estimations. This significant re-programming greatly improved APSIM sugar module's capacity to simulate transpiration efficiency (TE) and cane yield (TCH) for very diverse crop production conditions.

An important objective of this project is to assess the value of traits associated with WUE as a predictive tool for crop yield under well-watered and water-limited conditions. Extensive research along this line have identified g_s as a potential selection trait for cane yield across different crop production environments. The genetic correlations for g_s between test environments were moderate to high (0.5-0.92) and significant. Further, high heritability estimates from each test environment suggest g_s as a robust and repeatable trait across environments. And, g_s explained up to 40% of variation in cane yield in the introgression population in stress environments. Extensive measurement of g_s in multi-location trials showed moderate correlation with final cane yield. However, when g_s was normalised for the whole canopy (canopy conductance- g_c) the correlation was increased significantly (0.7-0.9) in a range of production environments, further proving its potential as a selection trait for yield. Since g_c can be measured indirectly and rapidly by monitoring canopy temperature, it offers an option for automated aerial screening of large genetic populations for more broadly-adapted and high-yielding clones early in the growth stage.

A genetic model based on the theory of correlated response was applied to compare the genetic gain obtained with the current selection method [2-row plot yield (tch) in clonal assessment trials (CAT)] and the proposed early selection for g_c as a predictor for commercial cane yield (TCH). The genetic correlation between tch in CAT and TCH in final assessment trials (FAT) was estimated to be 0.49 with an average heritability of 0.59 (Jackson et al, 2001). In the current project (BSS334), genetic correlation between g_c and TCH of 4-row plots was 0.72 (up to 0.9 in some environments) with an average heritability of 0.68. Based on the improved yield prediction estimated from the current research trial data, a genetic gain equating to an increase of 8.1 TCH is expected with the inclusion of g_c

in the selection index. The actual economic benefit to the industry however can only be estimated when this genetic model is tested in multi-region breeding trials (CAT & FAT) with a large number of clones.

2. Background

Sugarcane is a crop of major economic importance in Australia and the rest of the world, and has been expanding due to incremental increase in demand for sucrose and bioenergy (electricity from biomass and ethanol). Productivity improvement is essential for long-term sustainability and the profitability of the sugar industry, and is usually the key objective of breeding programs worldwide. Efficient water use is important in both rainfed and irrigated production regions and is becoming increasingly important due to reduced water availability, recurring drought and increasing costs in many regions.

A biological understanding of factors underpinning sugarcane adaptation to variable moisture environments is required for defining selection traits for breeding. Among many morphological attributes, stomata play a central role in regulating plant growth and water loss through simultaneously affecting rates of diffusion of both CO_2 and water vapor into and out of leaves. The potential impacts of stomatal limitation *via* CO_2 diffusion on rate of photosynthesis and on plant water use efficiency has been extensively discussed (Jarvis and Mansfield, 1981; Farquhar and Sharkey, 1982; Grantz, 1990; Grantz *et al.*, 1987). Despite the importance of stomata and many decades of study, our understanding of the apparently complex control mechanisms of stomata is still only fragmentary (Roelfsema and Rainer Hedrich, 2010). However, major factors affecting stomatal conductance (g_s) include internal CO_2 level (C_i) and plant water status. One of the most well understood roles for stomata is the restriction of water loss during periods of low water availability..

Photosynthesis and g_s are usually found to be approximately linearly related giving rise to relatively constant C_i, although this relationship can be disrupted under such as high temperatures which may induce stomatal opening (Zeiger, 1983; Feller, 2006). Conductance among different genotypes has been correlated to their relative growth and yield in different environments. A number of studies have shown a generally positive correlation across genotypes between stomatal conductance (or negative correlation with leaf temperature) and yield (Blum, 1983 and 1989; Reynolds *et al.*, 1992 and 1994; Fischer *et al.*, 1998; Araus *et al.*, 2002; Olivares-Villegas *et al.*, 2007). Some studies have found a high (negative) genetic

correlation between canopy temperature in a population of wheat genotypes under water stress, but a weaker (still negative) correlation under irrigated conditions (Olivares *et al*., 2007) while others have found correlations relatively unaffected by irrigation (Reynolds *et al*., 1994). Studies have also indicated that some crop breeding programs have indirectly selected for high stomatal conductance through selection pressure for yield. There is evidence for this in wheat (Fischer *et al*., 1998; Jiang *et al*., 2003), cotton (Lu *et al*., 1998), rice (Takai *et al*., 2010) and sugar beet (Ober *et al*., 2004), with more recent and higher yielding cultivars having higher conductance. However, no such relationship was found in the C4 species of sweet corn (Bunce *et al*., 2011).

It has been suggested that the ability of genotypes to extract water from the soil under increasing soil water deficit was a major attribute of drought adaptation and this is reflected in greater conductance under these conditions. Under well-watered conditions, increased stomatal conductance in high yielding genotypes may be driven simply by higher photosynthetic demand. However, it is conceivable that such relationships may be complicated by other factors in certain environments, such as genotypes with high conductance (and fast rates of water loss) depleting water when there is insufficient water in the soil profile particularly at depth, and stomatal responses to high temperature.

Selection for yield is difficult in sugarcane breeding programs, with estimates of yield in early stages of selection in small plots being subject to higher inter-plot competition effects (Jackson and McRae, 1998 and 2001) and sometimes higher error variance. It is possible that measures of stomatal conductance may add value in selection indices in these stages if these provide predictive capacity for yield. Stomatal conductance or measures related to stomatal conductance such as leaf or canopy temperature have been suggested as potential selection criteria in breeding programs for predicting both general yielding performance and GxE interactions across environments with limited water availability (Condon *et al*., 2004). In this project genetic variation in stomatal conductance and its relationship with yield under conditions of varying moisture availability were examined.

3. Outputs and Achievement of Project Objectives

3.1. Project objectives

The long-term objective of this project is to reduce the impact of recurring drought and the rising cost of water on industry profitability and competitiveness through more water use-efficient and drought tolerant sugarcane cultivars. This is the second phase of the More Crop Per Drop (MCPD) project. An integrated physiological-genetic approach to identify and understand traits underpinning water use efficiency (WUE) and crop yield across different production conditions forms the basis of this project. Consequently it involves studying a relatively large genetically diverse germplasm in glasshouse and field conditions, complemented by targeted trait modelling experiments.

The specific objectives of this project were:

1) Test and validate the value of key water use efficiency and drought tolerance traits in different production environments (modelling and targeted field trials in different locations)
2) Evaluate the results of trait validation experiments in breeding program
3) Identify elite water use efficient and drought tolerant clones with high productivity .

3.1.1. Objective 1. Test and validate the value of key water use efficiency and drought tolerance traits in different production environments (modelling and targeted field trials in different locations)

The APSIM sugar model has been used to validate the association between key drought tolerance traits and yield prediction across the test environments, and step-wise incursion was done to identify the contribution of each trait for the accuracy of prediction. Addition of stomatal conductance (g_s) as an indicator of root water extraction (RWE) under drought and radiation use efficiency (RUE) in irrigated conditions showed an improvement in predicting yield in the mild stress, semi-irrigated and irrigated environments. However, the prediction was weak when the stress was severe and yield reduction was more than 50%. A few major limitations of the APSIM sugar simulation model were identified. The use of a single value for transpiration efficiency (*TE*) across varieties caused some errors in yield prediction. To address this issue, the APSIM program was modified to capture hourly variation of vapor pressure deficit (VPD) by predicting hourly temperature and humidity. With this change a

significant improvement in the yield prediction was observed for different varieties under different test environments.

3.1.2. Objective 2. Evaluate the results of trait validation experiments in breeding program

A plant crop (PC) established in 2009 were ratooned and maintained for 2 ratoon crops (1R and 2R) at Dalbeg to test and validate the trait (g_s)-yield (TCH) relationship under different water availability conditions. Simulated and actual yields under fully irrigated (IR), half-irrigated (HI) and rainfed (RF) conditions were compared. In addition, a new field experiment was established in Bowen in 2011. A sub-set of 20 clones used in Dalbeg trial was planted in Bowen with the same 3 water treatments (IR, HR RF) as in Dalbeg trial. Stomatal conductance , photosynthesis, early biomass, leaf area at 6 and 12 months and final yield data were collected. For yield simulation and trait validation, three different water treatments in these experiments represented variable moisture environments naturally occurring at the production environments. The variable water availability in the field made trait validation effort more relevant to breeding and selection process.

Genetic parameters and genetic correlations between yield at harvest and the early stage g_s or canopy conductance (g_c) was estimated to test the hypothesis that early estimation of g_s is a reliable predictor of yield (TCH) in sugarcane. The g_s was measured in Bowen and Dalbeg trials in 20 and 40 clones, respectively, during the early stress period (Winter-Spring) and final cane yield was taken after harvest at 12 months (Summer). The g_s –TCH genetic correlation was high and strong when g_s was measured at certain environmental conditions, but the optimal measurement conditions for different production environments are not clear at this stage.

3.1.3. Objective 3. Identify elite water use efficient and drought tolerant clones with high productivity

To further test the hypothesis that stomatal conductance (g_s) as a significant contributor to yield, another field trial was established in Brandon with two different populations. They were nine clones from the final stage of the breeding program (FAT) and eleven clones with different levels of g_s identified in the current project. Clones of FAT trials were selected for high sugar and yield in favorable moisture environments, while lines from this project were

selected for varying levels of g_s. Using the leaf level g_s, whole-plot conductance was estimated as canopy conductance (g_c) by multiplying g_s by total leaf area of canopy.

The heritability and genetic correlation between g_c and TCH was high and stable across a number of g_s measurements under variable environmental conditions. The economic value of the trait was estimated using the genetic principles of correlated response of multiple trait selection (Kempthorne, 1969). The trait-based selection and the current conventional selection methods were compared using the principles of genetic analysis (Jackson and McRae 1998). An 8 t ha^{-1} cane yield advantage was estimated when early selection was made based on g_c in four test plots.

3.2. Methodology

3.2.1. Test and validate predictions about the value of key water efficiency and drought tolerance traits in different production environments (modelling and targeted field trials in different locations)

3.2.1.1. Production Environment characterization

APSIM sugarcane (Keating *et al.,* 1999) version 7.3 was used to determine the timing and level of water stress in the different environments sampled in the field experiments (Home Hill, Crystal Creek and Dalbeg). The inputs included local weather data collected from all the sites and soil physical properties from soil types considered to be of closest matches as those in the experimental field. The model estimated soil moisture content, root water supply and crop water demand (water balance). Water stress was determined as the fraction (0 to 1) of water demand that could not be met by the roots.Stress index, the ratio of water supply by the root to water demand (mostly transpirational) by the plant calculated on a daily basis was used to determine the extent of water stress experienced by the crop for a given period. .

3.2.1.2. APSIM sugar model to improve the yield prediction based on variable TE and hourly temperature

At first, the APSIM sugar model was used for the trait-based simulation with the existing capacity. The physiological parameters for the" sim" files were taken from the field experiments as well as the pot experiments conducted during the project period. The g_s, root water extraction and leaf elongation rates were taken from the field trials while transpiration efficiency (*TE*) was considered to be constant for the first stage prediction. However, the yield

prediction improved significantly with the APSIM modification enabling simulation for variable VPD and *TE*.

1. Use of variable TE for yield prediction

Step 1. *Intrinsic water use efficiency (k) = transpiration efficiency (TE) = 'transp_eff_cf'*

The intrinsic water use efficiency depends on the growth stage in the old APSIM sugar model. Our research suggested that there is a significant genetic variation among clones for *TE* under well-watered and water-limited conditions and the modification is needed to re-define the Intrinsic water use efficiency (k) which is depending on the water stress (S) defined as the ratio of water supply to water demand (D). The re-defined model is,

S = root water supply/ sw_demand = W/D (1)

Daily transpirational demand (D) is derived thus:

$D = R(1-\exp(-E.LAI)) * RUE * VPD / k$ (2)

where, *R* = Daily radiation (MJ/m^2), *E* = extinction_coefficent, *k* = Y, transpiration_effficiency_co- efficient (g kPa/Kg), *RUE* = radiation use efficiency (g/MJ), *LAI* = leaf area index, VPD = Mean daily vapor pressure deficit

$W = RWU \text{ (Root water supply)} = \Sigma(KL_n * ESW_n)$ $n = 1 \text{ to } n \text{ layers}$ (3)

where, *KL* = root water extraction coefficient in 1…n layer, ESW = extractable soil water in the layer 1…n layer

Step 2. *Develop hourly temperature, radiation and VPD values from daily data*

APSIM generally under-estimates VPD by about 25% probably because the assumption that minimum and dew point temperatures are equal which is not always true. An improvement to the VPD estimate would be to allow the user to select functions of the other daily climate variables as the dew point. This could be a simple deduction from the minimum temperature or something more complex requiring some thought and modelling on the part of the user.

Project developed the procedure for determining hourly values from daily values in a folder labelled Script(C#). The user can modify the way VPD is determined for example by changing the estimate of dew point temperature. The user can also supply VPD as from the SILO data base.

This initial check was done with a data set from Kalamia closest to Brandon, and Ord covering a very wide range of conditions. In the automatic weather station (AWS), data is compared to hourly data generated in the new version of APSIM. The rainfall data are daily totals from the

AWS, and APSIM just to confirm that the same data source was used. In the case of Kalamia, temperature was estimated well with R^2>0.9 as we required in the agreement.

Step 3. *Derive maximum hourly transpiration rate from hourly radiation, and VPD estimates*

The potential hourly transpiration was estimated as,

Potential hourly transpiration (T_{Oi}) for the i^{th} hour

$$T_{Oi} = R_i(1\text{-}exp(\text{-}E.LAI)) * RUE * VPD_i / k_a \quad (4)$$

where, Ri = hourly radiation (MJ/m^2) = 1.8 for plant and 1.65 for ratoon crops, VPDi = hourly vapour pressure deficit, k_a = as derived in step 1.

APSIM derives potential biomass gain first, then potential transpiration (demand) after as described in equation 2. However, potential gain of biomass was limited by temperature, N supply, oxygen supply and lodging before this calculation is done. In the new version, RUE is also affected if the user invokes leaf number and CO_2 responses. So when demand is calculated on an hourly basis, all these limitations and effects have been accounted for in the hourly biomass gain (*dWi*) value. The aim is to initiate the diurnal pattern of transpiration to influence transpiration efficiency which is common in the Australian production environments. In the new version, constrictions on hourly demand and transpiration after all these effects have been accounted for the hourly biomass gain.

3.2.1.3. Simulating clonal differences based on trait contribution

The TCH in each of the 40 clones in the Dalbeg experiment was simulated rather than groups of clones as was originally planned. The reason for this change was that it was difficult to classify clones into groups based on observed traits as we could not estimate how much weighting to allocate to each trait in the classification. Tools were developed to allow for rapid simulation of all 40 clones as well as rapid modification of their traits in the model.

Observed differences between clones were added to the model one trait at a time to assess the relative importance of each trait for determining biomass or cane yield. The biomass yields of 40 clones (selected for Dalbeg trial) from the Home Hill site was used initially when all traits were added step-wise for simulation. Secondly, simulations were conducted to test the validity of first hypothesis against largely independent datasets at Dalbeg and Home Hill. The traits include for the simulation of TCH in different environments are as follows:

1. **Leaf area and leaf size**: The canopy cover (light interception) of the 40 clones were measured at Dalbeg plant crop at 6-month stage. The standard variety Q183^A (available in

APSIM sugar) was used to standardize the leaf area of other 40 clones. For each of the 40 clones, maximum area per leaf in the model's library of clones characteristics ('.ini file') was multiplied by the ratio of the canopy cover for that test clone to the canopy cover of Q183 observed at Dalbeg in the fully irrigated treatment.

2. Simulations with root water extraction coefficient (Kl) modified to reflect mean differences in stomatal conductance in dry conditions: The mean stomatal conductance (g_s) of 40 clones in the dry regime at Home Hill was used for the simulation. The g_s of 40 clones were expressed relative to the mean of Q117 (162 mmol $m^{-2}s^{-1}$). The relative value was then amplified by multiplying 2, because it takes a 40% reduction in Kl to reduced water uptake by 20% on average.

3. Simulations with radiation use efficiency (RUE) modified to reflect differences in stomatal conductance (g_s) under well-watered conditions: The evidence here is that photosynthesis is proportional to g_s and that when g_s is measured under non-limiting water conditions, it reflects radiation use efficiency. Another approach would be to vary RUE based on final biomass yields but this gets close to a circular argument where we introduce the same variation to the model that we are trying to account for. As in the 2 above, mean g_s of Q117 in well-irrigated condition was used to estimate the relative value for other 40 clones. Standard RUE for the plant crop was 0.85, 1.8 and 1.8 g/MJ/m^2 for growth stages 3, 4 and 5 months, respectively, and for the ratoon crop, standard RUE was 1.6 g/MJ/m^2 for all growth stages.

4. Simulations with the lower limit of soil water extraction modified to reflect differences in root water extraction determined for 10 clones with neutron attenuation (NMM – neutron moisture meter): Neutron moisture meter readings (NMM) were taken to a depth of 3.3 m on four occasions in the dry treatment for 10 of the 40 clones in the Dalbeg experiment. Means of 3 replicate plots were obtained for each occasion and for each depth. Maximum and minimum values were obtained from these means over all sampling occasions and these values were assumed to be close to the drained upper limit (DUL) and the lower limit (LL) of soil water availability, given the extensive dry period prior to readings taken in the month of November.

5. **Simulations with the transpiration efficiency (*TE*) modified to reflect differences in *TE* determined for 10 clones in a glasshouse experiment**: Transpiration efficiency (*TE*) was determined for the same 10 clones identified for root water extraction at Dalbeg, in a glasshouse experiment with well-watered (wet) and dry (watered to 50% of field capacity) treatments. In APSIM Sugar, a constant *TE* value of 8.7 g DM/ Kg water at 1 kPa vapour pressure deficit was used for standard varieties. When *TE* was measured in the dry regime of the glasshouse experiment, this was decreased as much as 9% or increased as much as 52% depending on the *TE* of the particular clone relative to that of Q183. The *TE* estimated for 10 clones (Basnayake *et al.,* 2012b) from this experiments were used to simulate yields of those 10 clones grown under test environments, and validate and check the accuracy of prediction.

3.2.2. Evaluate the results of trait validation experiments in breeding program

The methodology used to conduct field experiments are presented in this section. Some physiological and agronomic measurements were used for the simulation exercise described in Objective 1. The data was used for genetic analysis and to quantify the genetic association between trait and TCH for trait validation purpose. Finally, the genetic parameters were used to a) quantify the correlated response of the trait when it is used as a selection trait for TCH and b) develop a selection index based on the indirect selection for stomatal conductance.

3.2.2.1. Trait validation experiments with selected and unselected (introgression) lines

A random genetic population (a mixture of clones including introgression lines, parental lines and commercial varieties) was used for this analysis. The data obtained from 3 different production environments and the constructed water environments were used for trait validation.

3.2.2.1.1. Dalbeg experiment with 40 clones

The field experiment established in 2009 at Dalbeg under the 1st phase of the MCPD project was ratooned and maintained for further evaluation of clone x water treatment interactions and trait validation based on the APSIM model predictions for Dalbeg environment. In summary, there were 40 genetically diverse clones representing a wider genetic background. This population used for trait validation composed of 20 non-selected introgression lines

(hybrids, F1, back crosses BC1 and BC2), 4 parental lines and 14 commercial cultivars recommended for a range of production environments. Rainfed, half-irrigation and full irrigation treatments were used as three different water environments in this study. The Dalbeg site had irrigation water restrictions, hence the full irrigation treatment of 1R and 2R crops experienced some degree of water stress. Half-irrigation treatment received only 50% of the amount of water that full irrigation treatment received. Irrigation and weather data were recorded continuously during the crop season. There were 3 main blocks with 3 water treatments (main treatment), and the 40 clones were allocated randomly in each main treatment. The physiological measurements including stomatal conductance, relative water content, photosynthesis, transpiration, internal CO_2 level, leaf elongation and root water extraction were made during Spring (September - December). Biomass sampling was done at the end of the water stress period, just before the Summer rainfall. Data on crop recovery after drought, and yield and sugar content at 12 months were also recorded.

Weather data collected from an automated weather station were used for APSIM simulation. However, the ratoon crops in Dalbeg were badly affected by cyclone Yasi, hence some data, such as biomass at 6 months, was not available for 1R crop.

3.2.2.1.2. Clones selected for the experiment in Bowen

The model prediction indicated that adding another location in Burdekin would not provide additional information on clone adaptation to drought. Therefore, a new site in Bowen was identified as the most appropriate location for further experimentation, and a trial was planted with 20 clones selected from the Dalbeg experiment. List of clones selected for the experiment and their characteristics are presented in Table 1. Soil nutrients were tested before planting and appropriate amount of macro and micro nutrients were applied.

As in the Dalbeg experiment (2009), full irrigation, half-irrigation (50% of water supplied to full irrigation treatment) and no irrigation (rain-fed) were the three main water treatments. A split-plot design (3 replications) was used with water treatment as the main factor and clones as sub-factor. The 20 clones were randomly allocated within each sub-block. The unit plot size was 10 m x 4 rows, with 1.8 m row spacing. Three buffer rows of the commercial variety Q208 were maintained between two water treatments to prevent the lateral moisture movement between treatments.

Ten clones (as used in Dalbeg trial) were selected for detailed moisture measurements from deep soil layers during the crop growth period, and aluminium access tubes (3 m, 2 m and 1

m tubes in rainfed, half-irrigated and fully irrigated plots, respectively) were installed for the neutron moisture meter measurements. In addition, nine EnviroSCAN probes were installed to profile the daily soil moisture variations in replicates of main treatment. These probes recorded moisture variation in different depths at hourly intervals during crop growth. The early non-stress measurements on conductance were taken on 5 October 2011.

Table 1. Clones selected for further experimentation in 2011 (Bowen) and their contrasting patterns of adaptation to test environments in Home Hill (HH) and Dalbeg (DB)

High TCH reduction (%) in all locations						
	TCH Reduction %		Physiological attributes* under rainfed			
Clone	Home Hill	Dalbeg	g_s -HH	RWC-HH	g_s - DB	RWC-DB
R570	75.0	43.0	135.8	89.1	74.7	90.1
Q183	66.0	43.0	97.0	86.4	57.8	86.5
Q229	65.0	42.0	133.7	88.4	98.9	88.4
CT04-69	65.0	59.0	60.6	87.1	74.4	92.3
QS95-6004	56.0	57.0	104.9	87.2	96.9	85.3
CT04-61	64.0	46.0	65.1	86.9	88.4	92.0
QN91-3028	54.0	57.0	135.9	92.2	94.0	84.5
Average	63.6	49.6	104.7	88.2	83.6	88.4
Low reduction (%) in all locations						
CT05-50	16.0	31.0	100.1	91.1	96.9	92.9
CT04-33	32.0	28.0	112.0	86.9	83.3	90.9
CT04-450	32.0	28.0	77.7	89.5	48.4	87.2
CT05-853	38.0	39.0	153.9	91.9	70.4	84.5
CT05-594	18.0	35.0	149.6	90.3	97.9	87.3
Average	27.2	32.2	118.7	89.9	79.4	88.6
High in Home Hill, low in Dalbeg - Interaction						
Q200	56.0	31.0	100.0	91.0	109.3	92.7
QN66-2008	75.0	16.0	88.6	80.5	81.0	91.0
KQ228	61.0	36.0	119.3	91.1	97.9	92.9
QN80-3425	64.0	35.0	115.9	80.7	101.9	88.6
MQ93-538	68.0	39.0	144.9	96.0	111.0	89.5
Average	64.8	31.4	113.7	87.9	100.2	90.9
Low in Home Hill, High in Dalbeg - Interaction						
QC91-580	39.0	53.0	196.7	91.0	123.6	90.1
QC93-1863	39.0	43.0	74.1	88.8	52.5	88.3
QB01-5	39.0	45.0	139.1	89.1	72.0	88.3
Group average	39.0	47.0	136.6	89.6	82.7	88.9
Average	0.52	0.40	109.5	88.8	91.2	89.3
Range	16-75%	16-59%	55-197	80-96	48-158	84-94

*g_s - Stomatal conductance; RWC - relative water content

Detailed measurements were collected during the stress period from October to December 2011, and photosynthesis (A), stomatal conductance (g_s) internal CO_2 (C_i), transpiration, and intrinsic transpiration efficiency were estimated at 3-5 month stage.

3.2.2.1.3. *Trait validation with breeding lines in FAT trials*

A site with minimal soil variation was identified at the SRA farm in Brandon, Burdekin, and a field experiment was established on 15 May 2013. The soil type was brown dermosol (Australian soil classification) with light clay. The organic carbon content was 0.68% and the soil pH ranged from 6.8 (1:5, $CaCl_2$-based) to 7.1 (1:5, water-based). Average annual rainfall at the site was 840 mm and irrigation was available during the crop season.

A total of 20 clones were used in this trial. Eleven clones were selected from the populations used in the previous experiments at Bowen (Table 2). Ten of them exhibited variable stomatal conductance (g_s), photosynthesis (A) and biomass production, and one was a low biomass clone (QB01-5) with moderate g_s and A. Remaining nine were commercial varieties and advanced clones from different regional selection programs of the SRA breeding program (QA01-5267, QA04-1448, Q240, Q252, Q256, QN04-121, QN04-1643, QS00-486 and QS01-1078).

The trial area was 1.82 ha and the three water treatments are full irrigation, half irrigation and rainfed (drought). The experimental plot was divided into 3 blocks, and the lower and middle blocks were allocated for well-irrigated and half irrigated treatments, and the upper block was maintained as rainfed treatment.

Table 2. Average TCH (yield data from 5 field experiments), stomatal conductance (g_s) and photosynthesis (A) (average of 2R in Dalbeg and plant crop in Bowen) of 11 selected clones with varying water use patterns

MCPD Clones	Cane yield (t ha^{-1})			Stomatal connductance $H_2Ommolm^{-2}s^{-1}$			Photosynthesis $CO_2mic\ molm^{-2}s^{-1}$		
	Rainfed	Half irrigate	Irrigated	Rainfed	Half irrigate	Irrigated	Rainfed	Half irrigate	Irrigated
CT05-853	94.2	114.9	131.1	197.6	252.3	292.2	13.2	21.2	35.2
CT05-735	93.9	95.4	95.9	174.6	234.3	308.3	15.9	21.2	33.1
KQ228	93.8	92.0	121.3	206.2	265.1	324.0	14.7	19.4	40.5
MQ239	93.0	109.2	111.0	191.4	258.4	330.9	12.4	22.9	39.3
N29	92.9	108.6	118.9	230.6	245.2	340.2	14.2	22.3	37.6
Q183	92.3	116.3	130.6	180.0	218.1	311.7	6.6	24.8	40.8
Q208	91.0	113.6	125.9	180.1	244.5	343.7	7.4	20.0	34.4
Q229	88.1	110.8	120.6	213.1	252.2	338.4	10.7	17.3	44.2
QB01-5	55.4	61.5	67.8	161.5	221.7	302.3	9.7	12.9	33.0
QC91-580	79.0	106.7	103.6	233.2	259.8	330.6	12.3	24.9	44.7
QN66-2008	85.6	97.1	115.0	184.5	207.2	308.1	12.2	18.3	42.3

Field experiment was based on a split-plot design with 3 replications, using water supply as the main treatment and clones as the sub-treatment. Clones were randomly allocated within each replication in the three water treatments. For the analyses of variance, treatment and replicate effects were treated as fixed while the sample and clone effects were as random.

As in Bowen experiment, three buffer rows were maintained between blocks to prevent water seepage between treatments. Irrigation frequency was determined by the crop water demand and it varied between treatments during the cropping cycle. The rainfed treatment was irrigated until 5 August 2013 for proper crop establishment and thereafter allowed to grow under rainfed conditions. Soil moisture variation was monitored using EnviroSCAN probes installed in Q208 plots in each replication. Soil moisture profiles were determined from EnviroSCAN data, and evapotranspiration (ET_0) was estimated using the weather data. Between dry periods, moisture levels at 13 depths, reaching up to 3 m deep, in all clones in the drought and 1 m in well-watered treatments (for 10 clones) were monitored using neutron moisture meter. Crops were irrigated based on crop water demand.

During Spring, photosynthesis (A), stomatal conductance (g_s), leaf water potential (Ψ_L), relative water content of leaf tissue (RWC), leaf area/canopy development, leaf area index (*LAI*), leaf elongation and canopy temperature (using Arducrop sensors) and soil moisture extraction were measured. Observations on crop establishment, leaf development and tillering were made at 3-month stage, biomass at 6 months, and all other crop phenotypic data, cane and sugar yields at harvest. The root water extraction was monitored in the drought treatment from day 1 of the imposition of drought (through withdrawal of irrigation) until harvest.

3.2.3. Identification of elite clones with desired water use efficiency and drought tolerance attributes

Following the consultative committee recommendations detailed genetic analyses was conducted using all the stomatal conductance **(g_s)** and TCH data collected in the current and its predecessor stage 1 More Crop Per Drop (MCPD) project. These analyses suggested that g_s in sugarcane is an important traits associated with adaptation to variable moisture environments. These results are comparable with cotton, maize, wheat and some other agricultural crops. Detailed genetic analyses were conducted to quantify the genetic correlation between g_s that measured under highly variable environments (temperature, VPD, radiation and water stress) at 3-6 months stage and final yield (TCH). Widely used genetic models were used for the analysis, and findings were collated for a manuscript submitted to the Journal of Experimental Botany (Basnayake *et* al., 2014, currently under review).

3.2.3.1. Estimation of genetic parameters, heritability and genetic correlation between TCH and stomatal conductance

The stomatal conductance **(g_s)** and cane yield (TCH) data from MCPD stage 1 and the current projects were used to investigate the genetic association between the trait **(g_s)** and TCH, and also to evaluate the clone performance across a range of moisture environments. The test environments were characterized using the local weather data collected from the automated weather stations.

3.2.3.1.1. Estimation of genetic parameters

An analysis of variance (ANOVA) was initially performed for each of the measurements taken from MCPD stage 1 (with large number of clones) to estimate the error and genetic variances. The following statistical model was used to partition the genetic variance as described by Cockerham (1963).

$Yij = \mu + gi + b_j + (gb)/ij_,$

where, Y_{ij} = observed g_s of the *i*th genotype in *j*th block (average of three samples per plot), μ = mean of all observations, *gi* = effect of the *i*th genotype, *i* = 1 to 89, 99, 40, 20 (Home Hill, Crystal Creek, Dalbeg, Bowen and Brandon experiments, respectively), b_j = effect of *j*th block, *(gb)ij* = interaction effect between the *i*th genotype and the *j*th block (referred to as experimental error).

Genetic and error variances were estimated, and broad sense heritability (h^2_b) on the basis of genotype means was estimated for each of the 51 measurements as described by Fehr (1987):

$$h_b^2 = \frac{\sigma_g^2}{\sigma_g^2 + \frac{\sigma_e^2}{n_r}}$$

where, σ^2_g and σ^2_e are genetic and error variances and n_r is the number of replications.

Analyses were also conducted across multiple treatments for particular dates of measurements and the variance components of genetic and genotype x treatment interactions were estimated. Measurements of g_s in different water treatments made on the same dates were used for the combined analysis. Genotypes (or clones) were considered as random effects while water treatments were treated as fixed effects for the variance component analysis within the following model (Cockerham, 1963):

$Y_{ijk} = \mu + tj + bkj + gi + (gt)ij + (gb)ijk,$

where Y_{ijk} = observed g_s of the *i*th genotype in the *j*th water treatment in the *k*th block (mean of three samples per plot), μ = mean of all g_s observations, t_j = the effect of j^{th} water treatments, j=1,2 or 3, b_{kj} = effect of j^{th} treatment within k^{th} block the; k=1,2 or 3 (error 1), g_i = effect of the i^{th} genotype; i = 1 to 89 (Home Hill experiment), 1 to 40 (Dalbeg experiment), $(gt)_{ij}$ = interaction effect between the i^{th} genotype and the j^{th} water treatment and $(gb)_{ijk}$ = interaction effect between the i^{th} genotype and the k^{th} *block* within the j^{th} treatment (error 2).

The genetic (σ^2_g) and phenotypic (σ^2_p) variance components were estimated from the combined analysis, and the heritability was estimated on the experiment mean. The broad sense heritability for g_s was estimated using the following formula:

$$h_b^2 = \frac{\sigma_g^2}{\sigma_g^2 + \frac{\sigma_{gt}^2}{n_t} + \frac{\sigma^2_e}{n_t n_k}}$$

where σ^2_g, σ^2_{gt}, σ^2_e are genetic (G), G×water treatment (GE) interaction and error variance components, respectively. Also, *n, t* and *k* are the numbers of observations, treatments, and blocks, respectively, and *e* is the experimental error.

Genetic correlations between each set of observations were analysed following the method described by Burdon (1977). The genetic and phenotypic correlations between TCH and g_s of

each set of measurements in 17 test environments were estimated using the method described by Kempthorne (1969).

$$\gamma_g = \frac{Cov_{g(xy)}}{\sqrt{\sigma^2_{gx}} \times \sqrt{\sigma^2_{gy}}}$$

where, $Cov_{g(xy)}$ is the genetic covariance of the product of TCH and g_s, and σ^2_{gx} and σ^2_{gy} are the genetic variance of TCH and g_s, respectively. The phenotypic variances and co-variances were used to estimate the phenotypic correlation using the same statistical procedure. The appropriate standard errors for genetic and phenotypic correlations were estimated.

3.2.3.1.2. Calculation of genetic gain by indirect selection for stomatal conductance

The objective of sugarcane breeding programs is to produce varieties that maximise economic profits to the whole industry. Therefore, ideally, the traits used for clone selection in a variety development program should be evaluated under commercial production conditions, or pure stand. Unfortunately, it is not feasible to evaluate thousands of clones in early stages of selection, as each clone would need a large area to avoid issues such as competition between clones planted closely to each other as occurs in selection trials, and GxE effects. Hence, plant breeders have to use indirect traits, or selection criteria, as surrogates for the traits of interest. As mentioned in the 'Background explanation' above, one of the objective traits for sugarcane variety development is cane yield in pure stand (TCH). In general, 2-row plot yield (tch) is used as selection criterion for TCH, and the expected TCH gain from selecting small plot tch can be estimated as described in Falconer and MacKay (1996).

$CR_{TCH} = ih_{TCH}h_{tch}r_{g(TCH,tch)}\sigma_{P_{TCH}}$ ..... *TCH predicted from 2 row plot tch*

where, i is selection intensity, h_{TCH} is heritability for TCH, h_{tch} is heritability for tch, $r_{g(TCH,tch)}$ is genetic correlation between TCH and tch, and $\sigma_{P_{TCH}}$ is the square root of phenotypic variance.

Similarly, expected genetic gains can be estimated if a different selection criterion such as stomatal conductance or canopy conductance is used to select for TCH. The effectiveness of two different selection criteria can be judged by the expected genetic gains. Given the same objective trait and selection intensity, the real difference between the two criteria will be the heritability of selection criteria and genetic correlation between selection criteria (g_s) and objective trait (TCH).

$CR_{TCH} = ih_{TCH}h_{gs}r_{g_{(TCH,gs)}}\sigma_{P_{TCH}}$ .....TCH predicted from g_s at 5-6 months

3.2.3.1.3. Developing an optimal selection index – an example

Sugarcane clones vary for many traits which may be measured in selection trials. In order to select clones in selection trials, breeders need to consider all measured traits and rank clones according to some judgment about their overall worth.

In many programs this ranking and selection of families or clones is done subjectively by breeders, using intuitive judgments on how to weigh different traits and different information. Traits which are both reliable and economically important should be given more weighting than traits that are measured less precisely or are less economically important. However, it is very difficult to do this, and sometimes sub-optimal selection may be used.

Selection index theory (Baker, 1966, Wei *et al.,* 2008) provides a way to rank clones objectively based on all available information. A selection index done properly will rank clones as closely as possible according to their true genetic economic worth, based on all available information.

A selection index is in the form of a linear equation of observed trait values for each genotype with a coefficient for each trait value:

$SI_i = b_1*X_{1i} + b_2*X_{2i} + ... + v_n*X_{ni}$ (equation 1)

where, SI_i = the selection index of genotype i ; b_1, b_2, b_n etc are the index coefficients to be estimated (below) for trait 1, trait 2, trait n; and X_{1i}, $X_{2i,}$ etc are the measurements of trait 1, trait 2, trait n on genotype i.

The mathematical procedure (matrix calculation) in calculating the selection index is described in the results section.

3.3. Results and discussion

3.3.1. Test and validate predictions about the value of key water efficiency and drought tolerance traits in different production environments

3.3.1.1. Production environment characterization

The rainfed environments at Home Hill featured a severe terminal stress in the plant crop, a moderate mid-crop stress followed by a mild terminal stress in the 1R and a very severe mid-

crop stress in the 2R crop. The full irrigation treatments in different crop years were not entirely stress-free. They experienced mild, short-term stress at the end of the plant crop, very mild stress in the 1R crop and moderate but short-duration stress in the 2R crop. Cumulative effect of water stress on biomass accumulation highlights the periods where biomass yields were most affected by water stress but this does not take into account the effects of water stress on canopy development (Figure 1).

All treatments at Dalbeg were subject to some degree of water stress for nearly five months, mid-way through the development of the crop. The rainfed treatment suffered the most and the full irrigation treatment the least. Biomass accumulation dropped by nearly 40% of potential biomass yield in rainfed treatment in Dalbeg compared with just about 20% in the worst environment at Home Hill. Water stress was least prevalent at the Home Hill site where the standard varieties yielded over 100 tha^{-1} for the plant and 1R crop. The 2R crop was stressed to some extent in December 2009 and again towards the end of the crop but soil moisture was never close to being fully extracted even during these relatively dry periods (Figure 1).

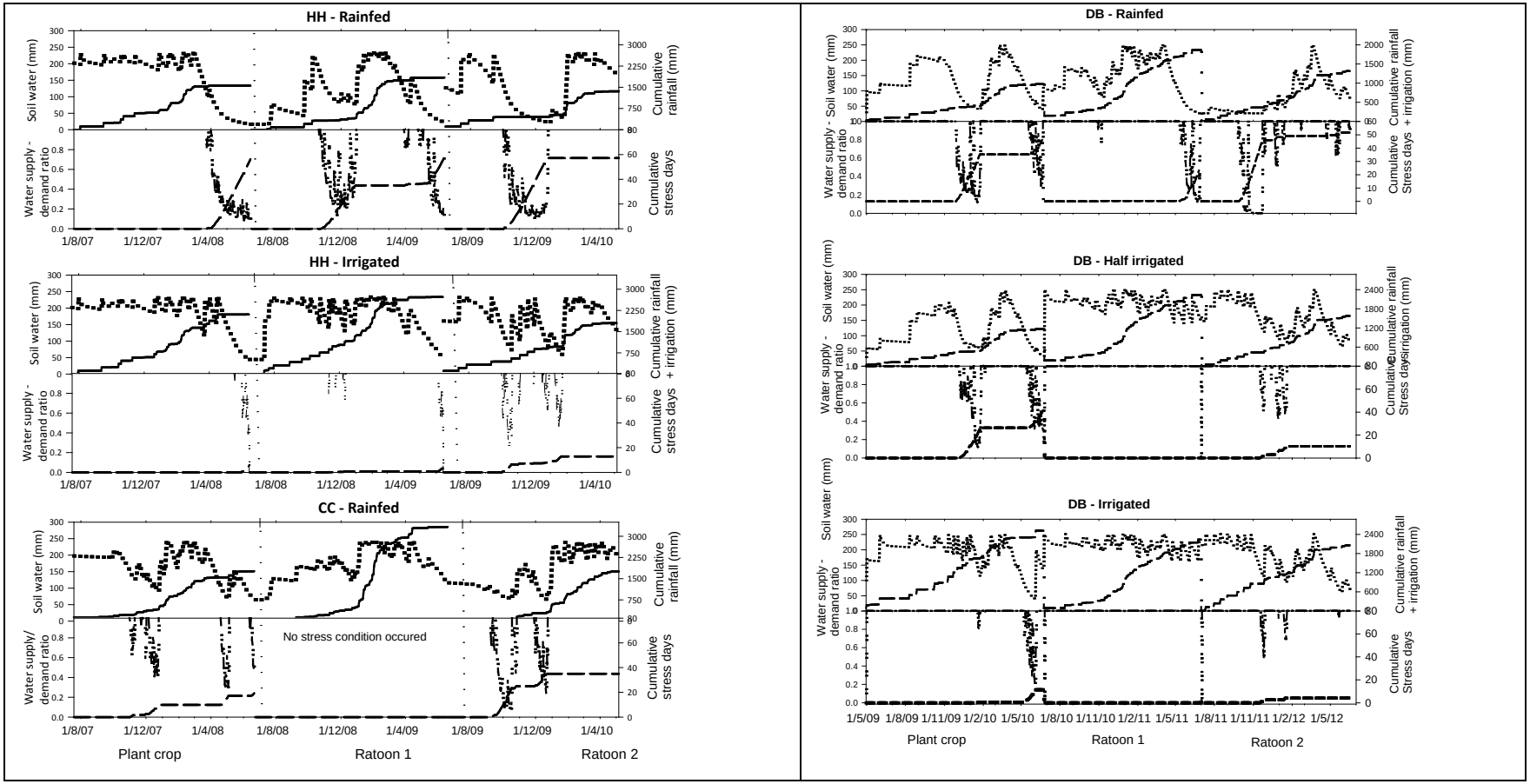


Figure 1. Simulated soil water depth (mm; dotted lines) and cumulative rainfall and irrigation (mm; continuous black line) water supply/demand ratio (dashed & dotted line) and cumulative stress-days (broken line) for the experiments in Home Hill (HH; rainfed and fully irrigated), Crystal Creek (CC; rainfed) and Dalbeg (DB; fully irrigated, half irrigated and rainfed).

3.3.1.2 Modification of APSIM sugar model to improve the yield prediction based on variable TE and hourly temperature

A recent theoretical study based on the APSIM Sugar indicated that increased transpiration efficiency (*TE*) at the leaf level would almost always help to improve sugarcane biomass yields in water-limited environments if the increased *TE* arose from improved intrinsic water use efficiency. However, if increased *TE* was increased through reduced conductance, yields could be reduced in high rainfall climates and shallow soils, and they could increase in moderate rainfall climates and deeper soils. Increased rooting depth, increased intrinsic water use efficiency and to a lesser extent, reduced conductance leading to increased *TE*, were suggested as the best traits to consider for selection of sugarcane clones in water-limited environments in the tropics and sub-tropics (Inman-Bamber et al., 2012).

Recent glasshouse studies have shown significant variation in *TE* between clones and also an increase in *TE* with water stress (Basnayake *et al.*, 2012b; Jackson *et al.*, 2014). The APSIM sugar model however was unable to simulate the observed *TE* variation in response to water stress. Diurnal interactions between transpiration, photosynthesis, vapour pressure deficit (VPD) and water stress had not been studied in sugarcane before the start of the MCPD project, and our simulation study (Inman-Bamber *et al.*, 2012) highlighted the need to do this in order to better understand and model the proposed benefits of increased *TE*. This led to a significant APSIM upgrade in order to improve the accuracy of complex trait modelling.

The APSIM sugar model has been upgraded and successfully tested for trait and crop modelling. The requested changes were completed by Shaun Verrall (CSIRO, CES) largely by December 2013. The changes involve three areas of programming.

1. The first was ‘deep’ level Fortran code which when compiled produces a ‘sugar.dll’ file which the user has no ability to change
2. The second was the ‘sugar.xml’ file which contains nearly all of the physiological parameters that define sugarcane and distinguishes sugarcane clones from each other
3. The third is the ‘Manager module’ which is where ‘casual’ users of APSIM can introduce their own code, some of which can influence the way the model runs through feedback to state variables. The new Manager2 module has more ‘power’ than Manager1 to introduce changes to the way the model runs. Manager2 can work with more elaborate programming languages such as C# than Manager1. The requested changes incrementally

added and each change has its own APSIM setup, Manager code, sugar.dll and sugar.xml files.

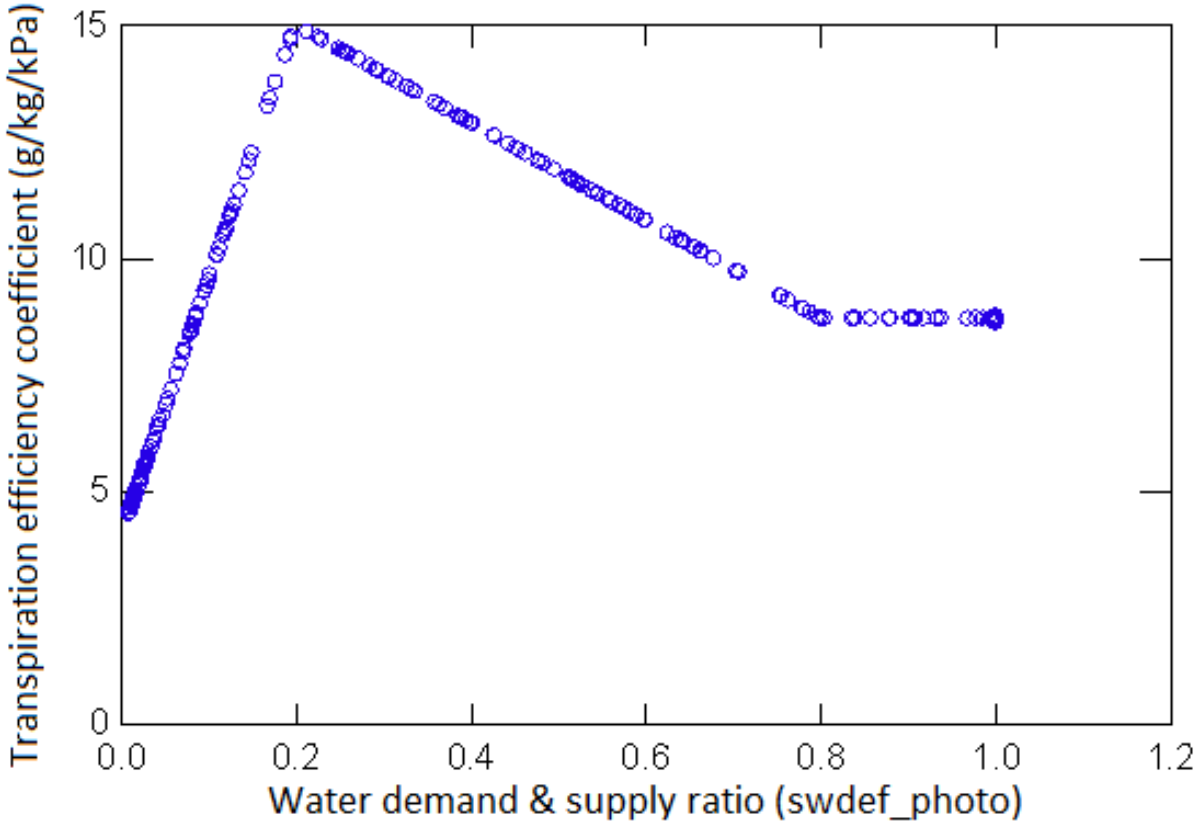
The project consultant (Dr Geoff Inman-Bamber) checked each change as it was made in its own folder and then again using common and most recent *.dll and *.xml files with all the traits included but where changes could be disabled or enabled and re-parameterized. The numbering of changed steps are the same as in the change request document (SRA FU). As with any software development, it will take a long time to determine if all combinations of the changes are bug-free, so users are asked to report any inconsistencies they may have observed. Dr Inman-Bamber has tested a fair number of combinations using the changes and confirmed that they worked mostly (>90%) as designed.

For testing, it used the 'Basic' code in SYSTAT, essentially to replicate the requested changes to ensure that the new changes gave the expected and required results. However, APSIM does not report all the relevant intermediate variables making it possible only to get approximate answers in the case of some traits. The 'Sugar.xml' file can be renamed by the user and these files came in many forms and names in refined APSIM settings. All these settings are stored in an '.apsim' file which the user can change. It simply refers to all the forms of the 'sugar.xml' file as such, or just as '.xml'. The .xml file replaces the older 'sugar.ini' file but the node for this file can still be called 'ini' or any name the user chooses.

3.3.1.2.1. Transpiration efficiency (TE)

Step 1. Intrinsic water use efficiency (k) = transpiration efficiency (TE) = 'transp_eff_cf'.
The results clearly shows that the transpiration efficiency coefficient (TEC) changes with the changes in water demand and supply ratio. The SYSTAT codes were used to test the changes.

Earlier, the user must be able to set the plant response to water stress [example *TE* = 8.7 g kPa/kg when demand is fully met by root water supply and *k* increases proportionally to 10.0 g kPa/kg when water supply is diminished (closer to 0)]. Now the APSIM extrapolation system can accommodate a more complex relation to "*S*" if required. Data given below, obtained from glasshouse experiments (Basnayake *et al.*, 2013 & Jackson *et al.*, 2014), suggest a response to stress similar to predictions of *k* (Figure 2).



Sugar.xml code				
< x_s wd ef_photo>	0.0	0.2	0.8	1
Y_transp_eff_cf unit = "g Kpa/Kg" >	4	15	8.7	8.7

Figure 2. 'Transpiration efficiency coefficient (*k* or TEC) in relation to Water Demand/Supply (S). TEC is not an output variable in APSIM and was derived as in the SYSTAT code. The lower box has the sugar.xml code for the relationship shown in the graph.

Step 2. Develop hourly temperature, radiation and VPD values from daily data

This initial check was done with a dataset from Kalamia (Burdekin), and for validation, datasets from Kalamia and the Ord covering a wide range of climatic conditions were used. In the Figures 3 and 4, automatic weather station (AWS) data was compared to hourly data generated in the new version of APSIM. In the case of Kalamia, temperature was estimated very precisely with R^2>0.90 as required in the change. In the Ord, R^2 was 0.87, close to the required level of agreement.

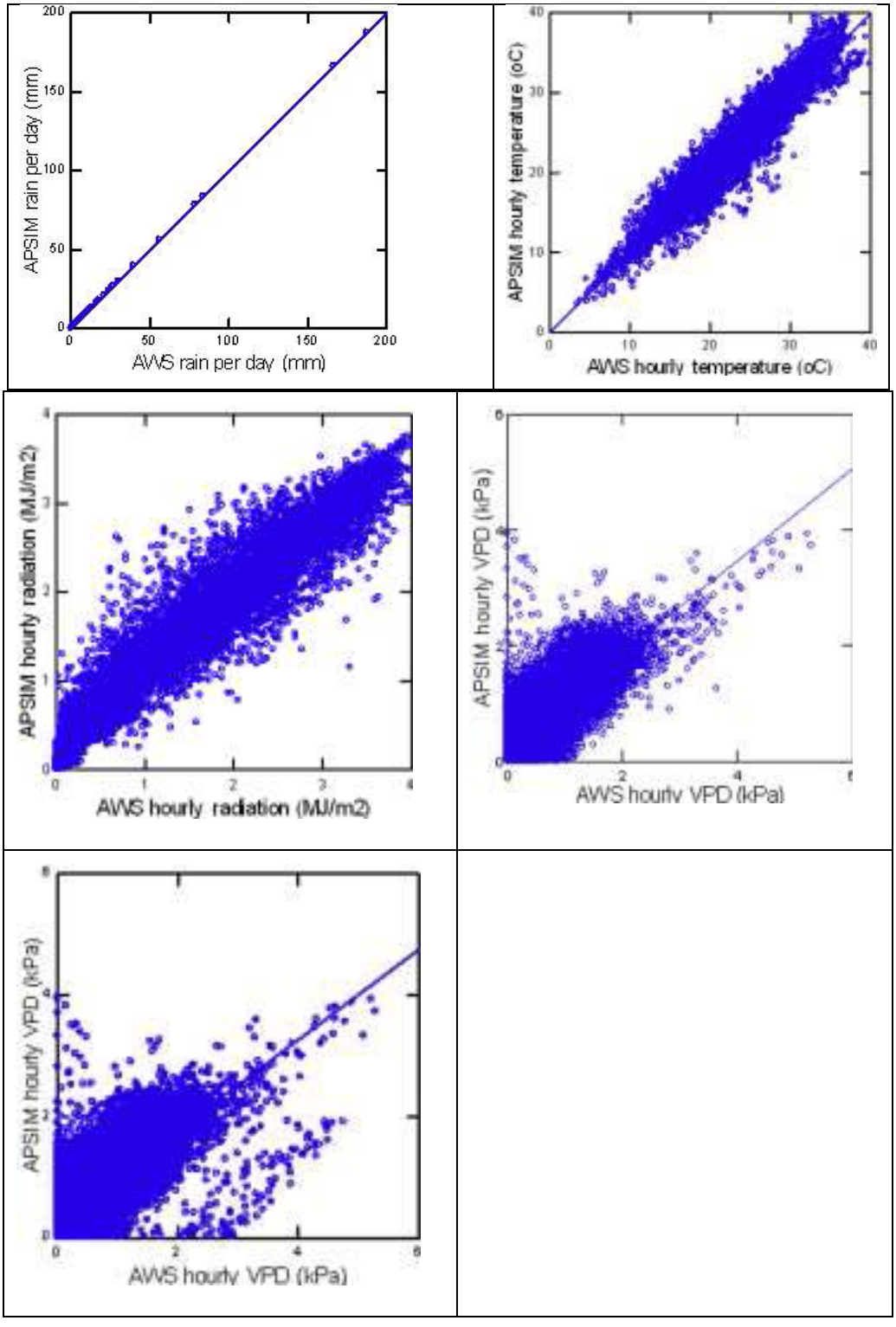
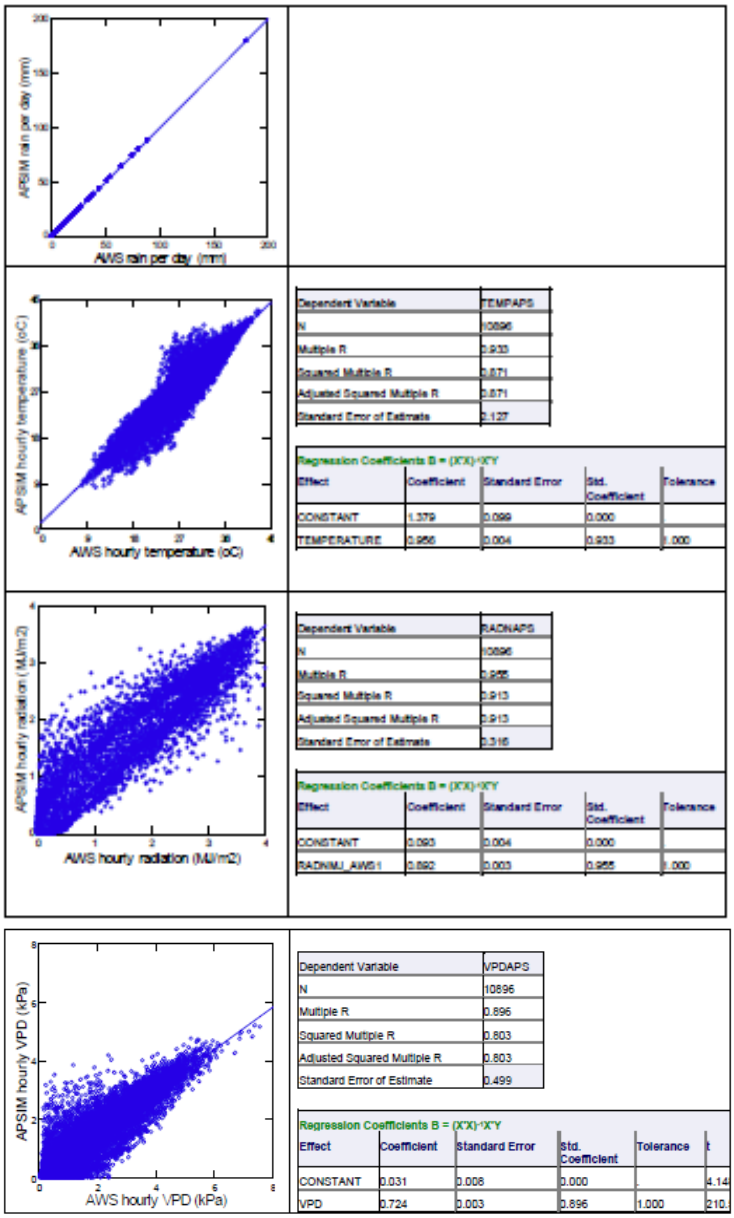


Figure 3. Daily rainfall, hourly temperature, radiation and VPD derived from new APSIM version compared to values measured hourly at the automatic weather station in Kalamia (2003-2005). The second VPD plot was done after correcting errors in the C# script.



Dependent Variable	TEMPAPS
N	10896
Multiple R	0.933
Squared Multiple R	0.871
Adjusted Squared Multiple R	0.871
Standard Error of Estimate	2.127

Regression Coefficients B = (X'X)-1X'Y

Effect	Coefficient	Standard Error	Std. Coefficient	Tolerance
CONSTANT	1.379	0.099	0.000	
TEMPERATURE	0.956	0.004	0.933	1.000

Dependent Variable	RADNAPS
N	10896
Multiple R	0.956
Squared Multiple R	0.913
Adjusted Squared Multiple R	0.913
Standard Error of Estimate	0.316

Regression Coefficients B = (X'X)-1X'Y

Effect	Coefficient	Standard Error	Std. Coefficient	Tolerance
CONSTANT	0.093	0.004	0.000	
RADNMJ_AWS1	0.892	0.003	0.956	1.000

Dependent Variable	VPDAPS
N	10896
Multiple R	0.896
Squared Multiple R	0.803
Adjusted Squared Multiple R	0.803
Standard Error of Estimate	0.499

Regression Coefficients B = (X'X)-1X'Y

Effect	Coefficient	Standard Error	Std. Coefficient	Tolerance	t
CONSTANT	0.031	0.008	0.000	.	4.14
VPD	0.724	0.003	0.896	1.000	210.

Figure 4. Daily rainfall, hourly temperature, radiation and VPD derived from new APSIM version compared to values measured hourly at the automatic weather station in the Ord (2005-2007).

Radiation was estimated with the required degree of precision at both sites (R^2>0.9, with regression coefficients of 0.917 and 0.892 for Kalamia and the Ord, respectively. Hourly VPD was not estimated very precisely with R^2=0.7 and 0.80 respectively.

A procedure was developed for determining hourly from daily values in a folder labelled Script(C#). The user can modify the way VPD is determined, for example by changing the estimate of dew point temperature. The user can also supply vapour pressure data (VP) from the SILO database.

Step 3. Derive maximum hourly transpiration rate from hourly radiation, and VPD estimates, APSIM derives potential biomass gain (dlt_dm) first and then potential transpiration (demand) from the equation 2. However, potential dlt_dm (hence RUE) is limited by temperature, N supply, oxygen supply and lodging before this calculation is done. In the new version, RUE is also affected if the user invokes leaf number and CO_2 responses. Thus, when demand is calculated on an hourly basis, all these limitations and effects have to be accounted for in the hourly biomass gain (*dWi*) value.

In the new version, the introduction of constrictions on hourly demand and transpiration after all these effects have been accounted for, hence hourly biomass gain will need to be recalculated to achieve the aim of having the diurnal pattern of transpiration to influence transpiration efficiency, otherwise the exercise is a bit pointless.

The hourly time step will have no effect unless the shape of the diurnal transpiration pattern is altered passively by water stress or actively by the user. Both active and passive effects could be deployed in the same run but transpiration will be limited by one or the other on any given day depending on which is more limiting.

Simulation 1. When root water supply is limiting

When water supply is limiting, hourly biomass gain in the new version will be greater than in the old version because transpiration will meet demand both early and late in the day when VPD is low thus effectively reducing daily VPD and increasing the transpiration efficiency for that day.

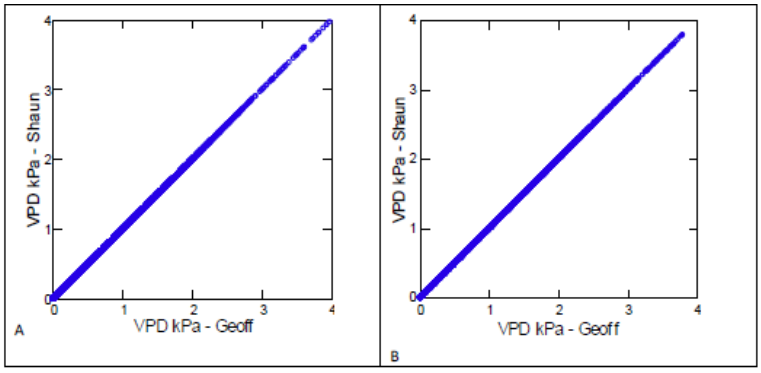


Figure 5. Hourly VPD derived from new APSIM version compared with the derivation of hourly VPD as requested in the change request document, using daily values of maximum and minimum temperature from A) the automatic weather station at Kalamia (2003-2005) and B) daily values of maximum and minimum temperature for Ingham from the SILO data base (1998-2000).

Vapour pressure is derived in units of hPa in C# script. This led to estimates of crop water demand (sw_demand) that were 10 x greater than were possible. By changing the units to kPa the estimate of hourly VPD and Modified APSIM estimates now agree (Figure 5) when both using the daily maximum and minimum temperature data rather than actual hourly data from the AWS as in step 2 above.

It was expecting hourly transpiration to be derived as:

Potential hourly transpiration (T_{Oi}) for the i^{th} hour

$$T_{Oi} = R_i(1\text{-}exp(\text{-}E.LAI)) \ * RUE * \ VPD_i \ / k_a \qquad (4)$$

where, R_i = hourly radiation (MJ/m^2) = 1.8 for plant and 1.65 for ratoon crops, VPD_i = hourly vapour pressure deficit, k_a = as derived in step 1, set at a constant = 8.7 g kPa/kg in this example.

APSIM new model hourly VPD values are now close to those expected (91% in this example, when there is no water stress, Figure 5A) but because APSIM does not report RUE after it is modified by the range of factors mentioned previously (temperature, N supply, oxygen supply and lodging), it is not possible to get exactly the same results as new simulations.

Values were obtained as $T_{ai} = R_i(1\text{-}exp(\text{-}E.LAI)) \ * RUE * \ VPD_i \ * S / k_a$, from eqns 1 and 4 while modification has worked out the maximum supply for each hour and capped *Ta* (actual transpiration) at that value as was requested. Without this capping in the checking process, the hourly values will deviate when there is some stress (Figure 6A).

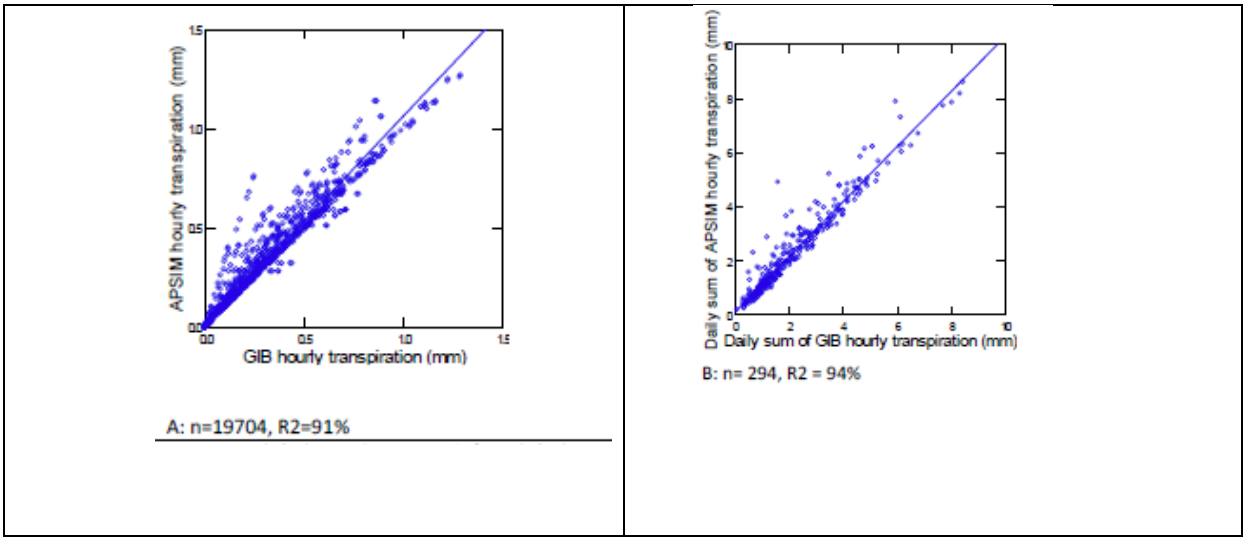


Figure 6. A) Hourly potential (no stress) transpiration derived from APSIM and from eqn 4 and B) daily totals of hourly actual transpiration (with stress) from APSIM vs values from eqn 4.

Water stress effect on transpiration use efficiency was disabled and there was no user limitation on hourly transpiration.

However the daily totals for hourly transpiration are close (94%, Figure 6B) even with water stress (but no user limitation on transpiration) showing that the capping of hourly transpiration due to water stress did not change the total daily transpiration much, only the diurnal distribution (Figure 6B), which is what we wanted. The .xml settings are shown in the box in Figure 6B, were such that transpiration efficiency coefficient (TEC) was set not to change with water stress.

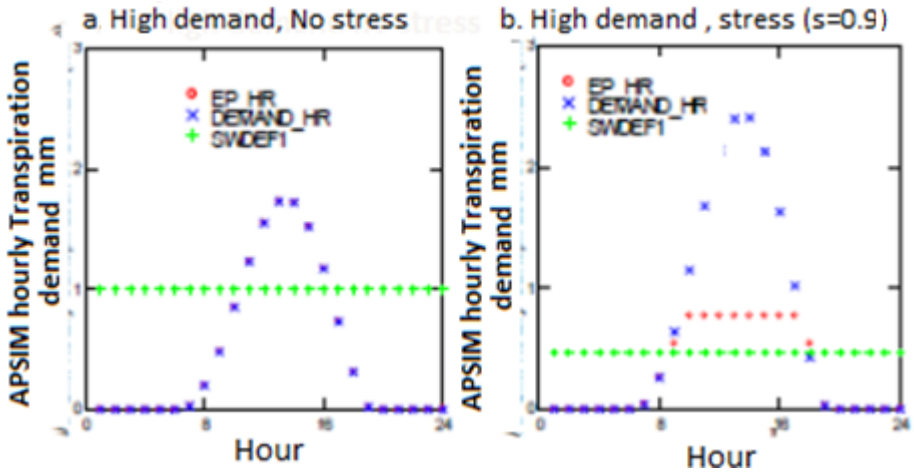


Figure 7. Examples of three days in December of actual hourly transpiration (o), hourly transpiration demand (X) and the daily supply/demand ratio (*S*,+). Note: the apparent numerical equality for o and + in Fig 8E is coincidental.

Simulation 2. Where stomatal conductance (g_s) is limiting

For this, the user can set a maximum hourly transpiration rate and in so doing allow the crop to avoid some effects of water stress.

The example in Figure 8A shows a setting of 1.6 mm/h transpiration when there is sufficient water supply (no stress) and a setting of 0.6 mm/h when there is no water supply (full stress) but water limitations would no doubt prevent such a limit being applied because of the passive limitations on transpiration. The lowest hourly demand in this example was about 1.1 mm/h with S~0.35.

In Figure 8E, the capped transpiration was not quite as expected. Transpiration was actually greater than demand at 7, 8 and 9 AM which is impossible in nature.

For simulations over 821 days using the Ayr AWS data, this error (expressed as transpiration minus demand, ignoring zero or negative values) was very small when water stress was the cause of flattening (capping) of the diurnal transpiration pattern (Figure 9A) and was only slightly more serious when the user was responsible for a marked reduction in hourly respiration (Figure 9B).

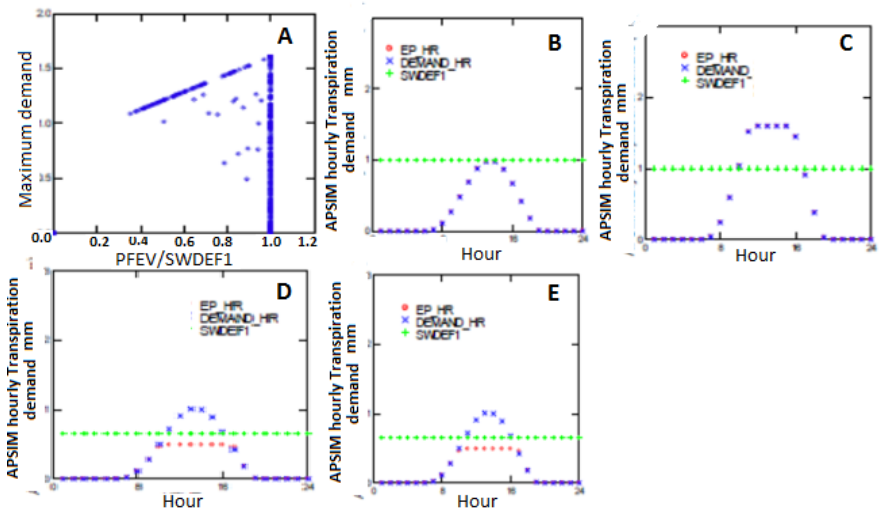


Figure 8. A) Maximum hourly demand constrained by the user to 1.6 mm/h with no stress and 0.8 mm/h for total stress. B-E) Examples of 4 days of APSIM-derived hourly transpiration (o), transpiration demand (X) and the supply/demand ratio (S,+). B) A day when there was no stress and demand was not limited by the user C) A day when there was no stress and demand was limited by the user D) A day when there was stress and demand was not limited by the user and E) A day when there was stress and demand was limited by the user.

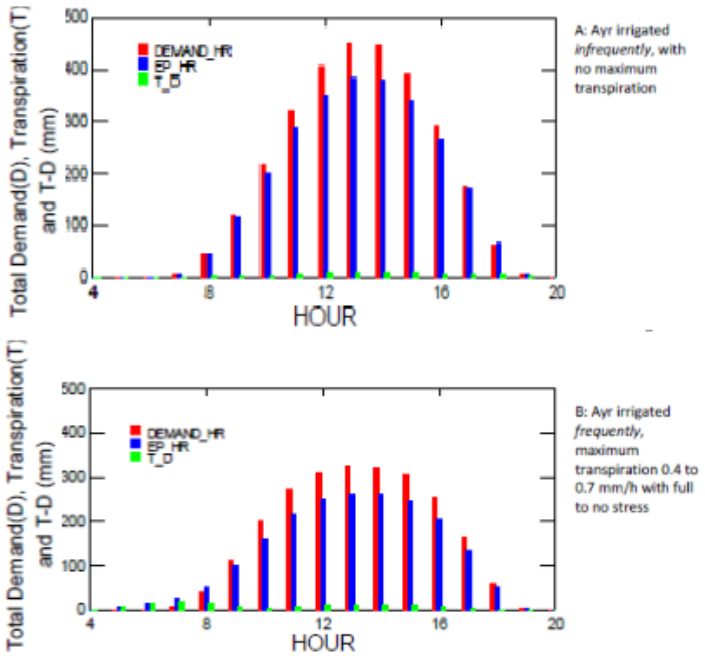


Figure 9. Examples of the effect of capping transpiration in the Ayr climate (A) with infrequent irrigation and in the Ayr climate (B) with frequent irrigation.

Figure 10 provides examples of the impact on biomass yield, transpiration and transpiration efficiency (*TE*) by the modifications to the sugar model in regard to derivation of hourly transpiration demand and hourly actual transpiration with passive and active capping. In a high yield potential and high water demand environment like the Ord, active capping of transpiration can have a large impact on yield and *TE* (Figure 10 A,B,C) because of high VPD and consequently high demand values in the middle of the day. If the user limits transpiration to 1.6 mm/h in this environment, the effect of VPD is reduced considerably. In a less demanding environment (Ayr) the benefit of capping transpiration can also be large when water stress is prevalent but not when the stress is minimal. If water supply is sufficient to meet the demand there is no advantage of having a trait which constrains transpiration and photosynthesis through reduced stomatal conductance. Such a trait could lead to yield losses while conserving water which may not be necessary.

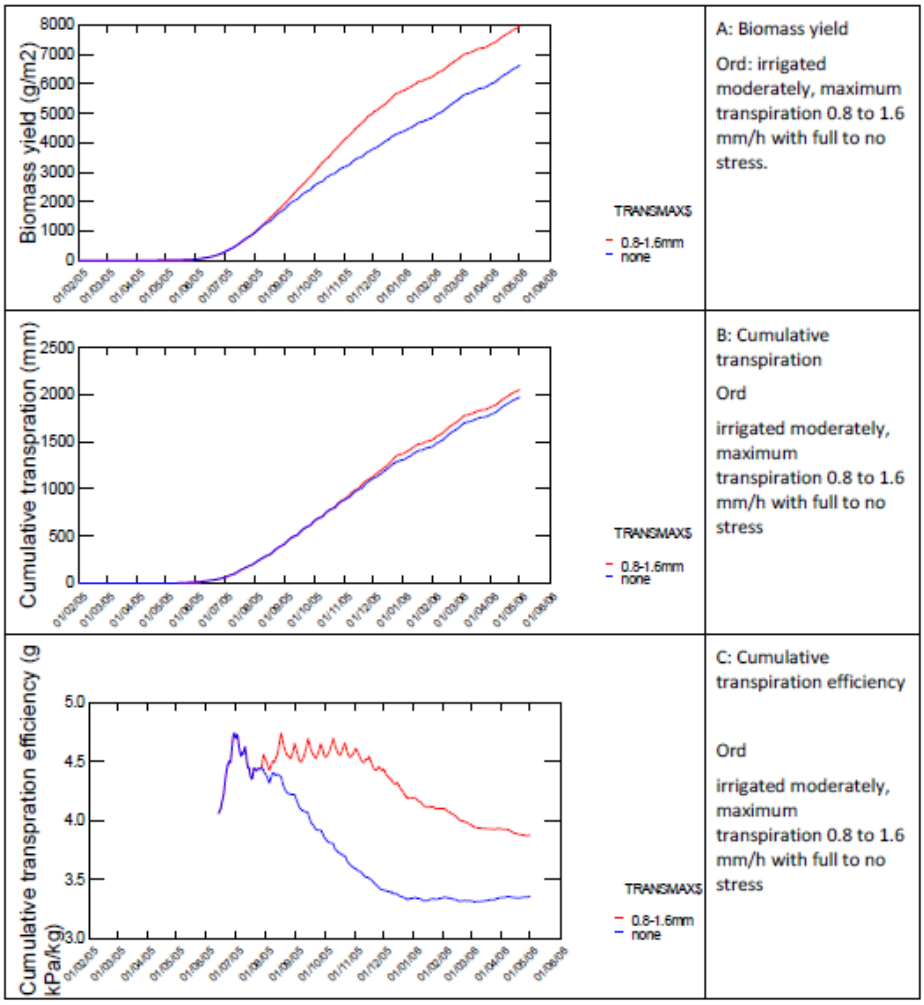


Figure 10. Examples of the effect of capping transpiration in the Ord climate (A-C) with moderate irrigation (note biomass yields).

Simulation 3. When root water supply derived from root length density

In the standard version of APSIM-Sugar, traits for vigour (leaf area and RUE) do not link with root water uptake (RWU) capability even though this link appears to be described in the Keating et al (1999). After the January 2014 consultative committee review, it was decided to investigate the possibility of getting this link to work in APSIM as an alternative to the current approach based on the concept that soil unsaturated hydraulic conductivity (*k*), and root length volume (*RLV or l*) can be combined to obtain an easily measurable index of root water uptake capacity (*kl*). Note that *k* here and in the *TE* equations is used by convention but the meaning is entirely different. The new sugar module now separates *k* and *l* to determine root water uptake (RWU) for each soil layer (*n*):

RWU(n) = RLV(n) * 100 * KL(n) * ESW(n) mm/d (5)

where, *l*=RLV and *k*=KL.

It is important to note that the user interface for APSIM has not changed in regard to the KL term and users of the new version must remember that entries for KL are actually for *k*.

The RLV is reported in mm/mm^3 in APSIM so the 100 multiplier in eqn 5 converts RLV to cm/cm^3. Extractable soil water (ESW) is in mm per layer rather than cm^3/cm^3, so RWU is also in mm/layer/d which is what we want to determine potential or actual water uptake per day.

Data in Figure 11A confirms that APSIM is now determining root water supply from root length volume (*RLV*). Root water supply or use depends on water available in each layer and *RLV* which increases with time at depth but can also decrease as roots die back (Figure 11). The plant crop is 'hard wired' to have *RLV* of 40, 30, 10 and 5 mm/mm^3 initially in the top 4 layers of soil. This is a generous offer by the early modellers to allow the crop access to water to get things going but this should be reviewed in the light of the changes we have made. The user can make the top 4 layers very shallow to make initial rooting depth more realistic. Note that RWU is the supply side of the supply/demand relationship. If the demand is less than supply, then the amount of water used will be less than what the roots can offer. The rapid fluctuation in RWU in the upper layers (1 to 3) in this example (Figure 11) is due to the frequent irrigation allowed in the management options.

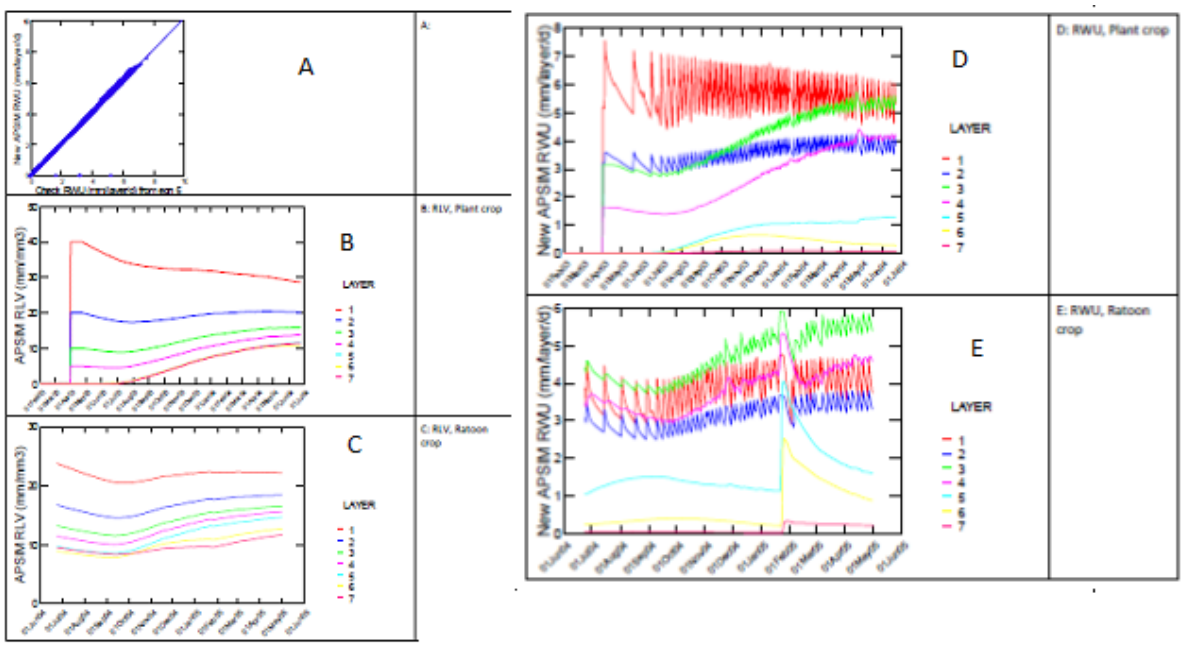


Figure 11. A) Potential root water use (RWU) from APSIM and from eqn 5, B) Root length volume (RLV) time series for a plant crop, C) and ratoon, D) Potential root water use (RWU) time series for a plant crop and E) ratoon.

Simulation 4. Transpiration efficiency and internal CO_2

CO_2 has a dominant effect on *k* (transp_eff_cf) or *TE* (please note that this *k* is not *k* from KL) so the new changes allow for the kind of response below and they also allow CO_2 levels to be set in the manager file or to be read in from the climate (met) file.

y_transp_eff_cf is a multiplier for modifying *k* as in step 1

transp_eff_cf_fact = 1.0 1.25

x_CO_2 = 375 720 ppm

There were some errors in the way CO_2 levels are chosen by the user which were fixed in the script for yearly changes in climatic factors. The user is prompted to select a change coefficient which is then multiplied by the difference between the selected base year and the year in the met record. The equation for CO_2 was the same as for the other variables so the default change coefficient (~390) was added for each year from the selected base year, ending up with some very large CO_2 levels. The code now simply uses the CO_2 entry as the selected CO_2 level for the run.

The transpiration efficiency coefficient (TEC) is not an output variable in APSIM and it cannot be derived precisely from other output variables but the TEC values derived and presented in Figure 12 indicate that the response of TEC to CO_2 and to water stress are working as required. In this example, TEC was expected to increase 40% by a rise in CO_2 from 360 to 720

ppm. At 360 ppm, TEC was set to increase from 8.7 g kPa/kg to 16.0 g kPa/kg as the demand ratio decreased to 0.2 and the response would be 40% greater at 720 ppm. The model is thus working correctly.

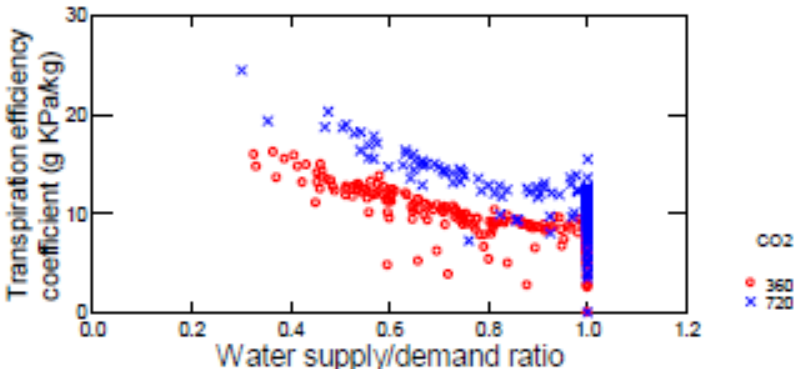


Figure12. Transpiration efficiency coefficient (TEC) for two CO_2 levels in relation to water stress expressed as the water supply/demand ratio (S).

Radiation use efficiency and CO_2

Radiation use efficiency (RUE) is not an output variable and is affected by many factors but the data presented in Figure 13 indicate that the response of RUE to CO_2, in this case a 10% increase with twice normal CO_2, is working as expected. The maximum RUE for a plant and ratoon crop was set at 1.8 and 1.65 g/MJ respectively.

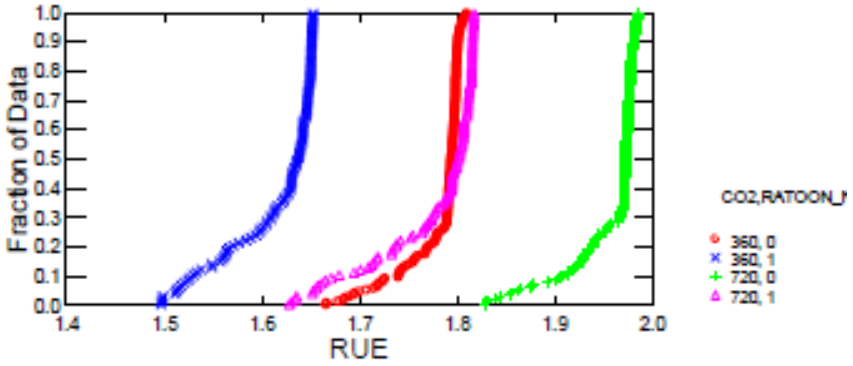


Figure 13. RUE derived as the increment in biomass divided by intercepted radiation for days when there was no water stress and intercepted radiation exceeded 2 MJ/m^2. The data are for two CO_2 levels and a plant (0) and ratoon crop (1).

Sugar respiration and temperature

The fraction of sucrose lost to respiration each day was related to daily mean temperature as expected (Figure 14A), and biomass and CCS were reduced by respiration as anticipated (Figure 14B and 14C). The inclusion of respiration increased the impact of seasonal temperatures on ripening which is interesting.

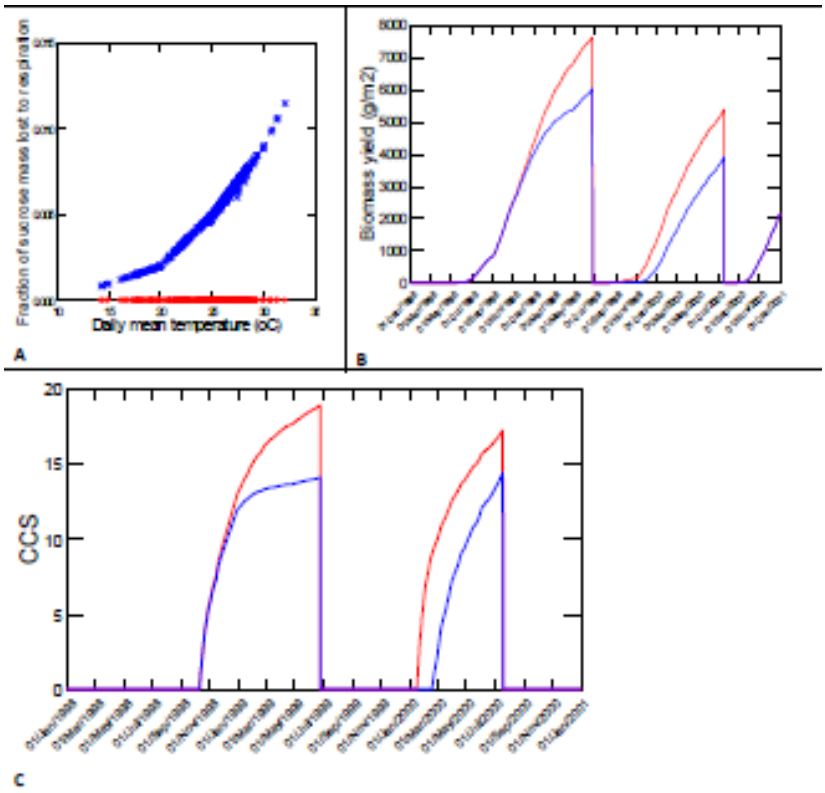


Figure 14. A) Sucrose respiration fraction in relation to daily mean temperature and B) biomass accumulation with time, with (blue) and without respiration (red).

Radiation use efficiency and leaf number

In this example RUE was set to decrease to 80% of the starting value (1.8 g/MJ for plant crops and 1.65 g/MJ for ratoon crops) when leaf number increased from 30 to 50. The model performed as expected apart from reduced values of RUE in the early stages, probably because of leaf senescence after tillering (Figure 15).

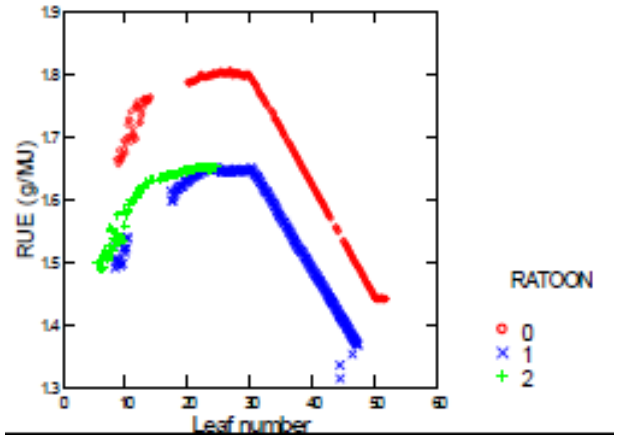


Figure 15. RUE derived as dlt_dm/radn_int for SELECT SWDEF_PHOTO =1 AND RADINT >2 and nfact_photo = 1, for a plant and ratoon crop in relation crop phenology (leaf number).

Conclusion

The modification of APSIM sugar module in 3 main areas, which had some limitations, were successfully completed. The hourly temperature and humidity variation that improves the predictive capacity of APSIM can now be accommodated for TCH simulation.

3.3.1.2.2. APSIM sugar model to improve the yield prediction of test clones based on variable TE and hourly temperature (Assessment of improved sugar module using actual data from the current project)

1. Transpiration efficiency (*TE*) (g biomass/kg water transpired) and biomass prediction

Results on transpiration efficiency (*TE)* of Brandon 2012 glasshouse experiment was used for the *TE*-based yield prediction on above ground dry matter.

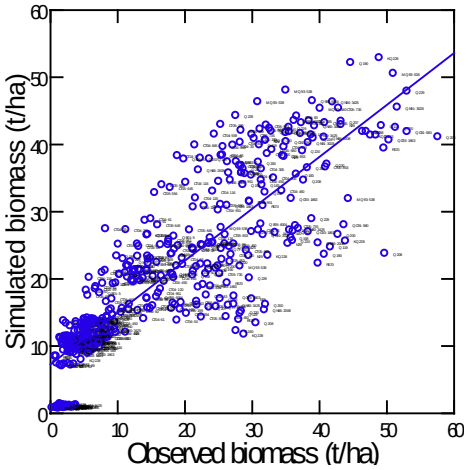
Table 3. Mean *TE* (g/kg) based on above ground biomass from the glasshouse experiment at Brandon in 2012 and Transpiration efficiency coefficients (TEC, kg kPa/kg) supplied to APSIM for four stress levels (swdef_photo)

Clone	TE dry treatment	TE wet treatment	TE relative to Q183 dry %	TE relative to Q183 wet %	TEC (0)	TEC (0.25)	TEC (0.5)	TEC (1.0)
CT04-50	8.3	5.55	86.5	97.4	0.0055	0.0159	0.0120	0.0077
CT04-559	7.55	5.4	78.6	94.7	0.0049	0.0141	0.0106	0.0082
CT04-951	8.5	5.4	88.5	94.7	0.0051	0.0148	0.0112	0.0077
CT05-583	7.7	5.05	80.2	88.6	0.0054	0.0157	0.0118	0.0082
CT05-735	7	5.55	72.9	97.4	0.0051	0.0147	0.0110	0.0084
CT05-853	8.65	5.5	90.1	96.5	0.0058	0.0168	0.0127	0.0086
KQ228	7.35	5.1	76.6	89.5	0.0050	0.0144	0.0109	0.0080
MQ239	8.7	6.15	90.6	107.9	0.0054	0.0157	0.0118	0.0088
N29	7.4	5.25	77.1	92.1	0.0049	0.0142	0.0107	0.0075
Q119	6.55	5.15	68.2	90.4	0.0048	0.0138	0.0104	0.0081
Q183	9.6	5.7	100.0	100.0	0.0060	0.0174	0.0131	0.0087
Q190	7.8	5.6	81.3	98.2	0.0052	0.0149	0.0113	0.0085
Q200	7.45	5.9	77.6	103.5	0.0050	0.0146	0.0110	0.0087
Q208	7.9	5.05	82.3	88.6	0.0052	0.0151	0.0113	0.0079
Q229	8	5.35	83.3	93.9	0.0055	0.0159	0.0119	0.0082
QB01-5	6.75	5.2	70.3	91.2	0.0047	0.0137	0.0103	0.0076
QC91-580	7.3	6.05	76.0	106.1	0.0049	0.0143	0.0107	0.0089
QN66-2008	9.4	5.4	97.9	94.7	0.0066	0.0193	0.0145	0.0078
QS95-6004	7.7	5.65	80.2	99.1	0.0054	0.0155	0.0117	0.0085
R570	6.3	5.15	65.6	90.4	0.0043	0.0124	0.0094	0.0082

The *TE* values in Table 3 were used to define the differences between clones in regard to the response of *TE* to water stress in the new version of the APSIM model for the simulations reported here. All other traits (area per leaf, stalk population, green leaf number, RUE, kl and extractible soil water (ESW)) were unchanged from previous simulations.

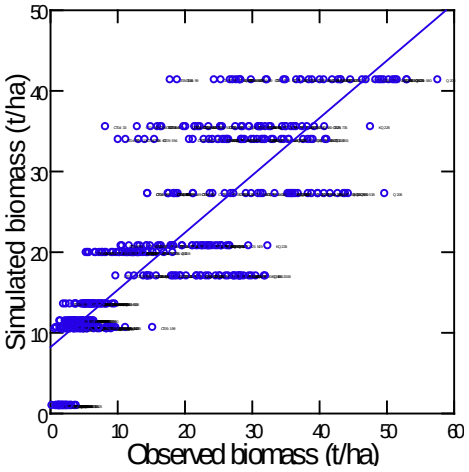
2. Yield simulation for Dalbeg experiment based on *TE* estimated for 20 clones in 2012 glasshouse experiment at Brandon

A) All data (plant and 2R) with all measured traits



Dependent Variable	BIOMASS
N	480
Multiple R	0.855
Squared Multiple R	0.731
Adjusted Squared Multiple R	0.730
Standard Error of Estimate	6.397
Regression Coefficients B = $(X'X)^{-1}X'Y$	
Effect	Coefficient Standard Error
CONSTANT	7.283 0.465
TDM	0.772 0.021

B) All data with no measured traits



Dependent Variable	BIOMASS
N	480
Multiple R	0.836
Squared Multiple R	0.699
Adjusted Squared Multiple R	0.699
Standard Error of Estimate	6.364
Regression Coefficients B = $(X'X)^{-1}X'Y$	
Effect	Coefficient Standard Error
CONSTANT	8.234 0.463
TDM	0.711 0.021

Figure 16. Simulated and measured biomass for 40 clones at Dalbeg where biomass was measured both at harvest and pre-wet season (December) in a plant and 2R crop both receiving three irrigation treatments (fully irrigated, half-irrigated and rainfed), where clonal variation was simulated (A) and where no clonal variation was simulated (B).

The model accounted for 70% of the variation in biomass yield when considering only variation in the environment resulting from two crops (plant and 2R), three treatments and two ages for which biomass (12 environments) was determined (Figure 16). When data for traits was included, only 3% more variation was 'explained'. It is therefore necessary to consider each environment independently or to normalize the data with respect to mean yield of the environment. The rainfed environments are those of particular interest in this study.

Table 4. The coefficient of determination (R^2) for the plant (0) and 2R crop (2) at Dalbeg, for simulated versus biomass measured at harvest and prior to the wet season (pre-wet) for three irrigation treatments; (fully irrigated, half-irrigated and rainfed). Leaf area traits, radiation use efficiency (RUE) and hydraulic conductance traits (kl) were estimated for all clones, but extractible soil water (ESW) for only 10 clones and *TE* for 20 clones

Number of clones			All clones (n=40)			n=10	n=20
Traits included in model			All traits	- ESW	- ESW - TE	+ ESW+ TE	+TE
Treat	Crop	Stage					
Rainfed	0	Harvest	0.064-	0.005	0.024	0.446-*	0.213-*
Rainfed	0	Pre-wet	-0.060	-0.118*	0.204-**	0.072-	0.044-
Rainfed	2	Harvest	0.434 **	0.349 **	0.348 **	0.607 **	0.352 **
Rainfed	2	Pre-wet	0.078	0.069	0.113 *	0.034	0.069
Half irri.	0	Harvest	0.117 *	0.159 *	0.225 **	0.013	0.005
Half irri.	0	Pre-wet	0.003	0.005	0.107 *	0.011	0.029
Half irri.	2	Harvest	0.474 **	0.405 **	0.388 **	0.797 **	0.369 **
Half irri.	2	Pre-wet	0.465 **	0.464 **	0.465 **	0.295	0.413 **
Fully irri.	0	Harvest	0.352 **	0.32 0**	0.309 **	0.574 *	0.343 **
Fully irri.	0	Pre-wet	0.223 **	0.19 0**	0.196 **	0.512 *	0.258 *
Fully irri.	2	Harvest	0.418 **	0.336 **	0.328 **	0.674 **	0.416 **
Fully irri.	2	Pre-wet	0.567 **	0.571 **	0.567 **	0.400	0.350 **

Pre-wet = end of the early stress period (5-6 month age); -Negative slope; * = significant with *P*=0.05; ** = significant with *P*=0.01 or less

a) All 40 clones

i. Harvested biomass yield

The model accounted for significant variation for the half-irrigated and fully irrigated treatments of both plant and ratoon crops and for the rainfed treatment of the 2R crop (R^2 ranged 0.12 to 0.43). Clonal variation in yield of the rainfed treatment of the plant crop was not explained at all (Table 4).

ii. Sampled biomass yield (pre-wet season)

The model accounted for significant variation in the yield of the irrigated treatment of both crops and of the half-irrigated treatment of the 2R crop (R^2 range 0.22 to 0.57).

iii. Removing the extractable soil water (ESW) trait

When ESW removed, the coefficient of determination (R^2) declined in all cases apart from the rainfed treatment, (pre-wet season yields of the plant crop) where there was a weak negative correlation (R^2=- 0.118). There was a slight improvement (0.12 to 0.16) of harvest yields of the half irrigated treatment of the plant crop where R^2 improved slightly. Thus ESW is considered to have added capability to account for yield variation in the 40 clones even though only 10 clones were characterized for this trait (Table 4).

iv. Removing the ESW and TE traits

The ability to account for yield variation when removing *TE* as well as ESW declined in 3 of the cases in Table 4 and improved in 4 cases, the others remaining practically unchanged. Measuring the *TE* trait did not help in accounting for yield variation in all 40 clones bearing in mind that 20 clones out of 40 clones were not characterized for *TE* in the 2012 glasshouse experiment.

b) 10 clones for which all traits (Leaf area, RUE, kl, ESW and TE) were measured

Use of the knowledge of all traits in the model helped to explain a large amount of variation (up to 80%) in six situations (Table 4) but not in the others. The most interesting cases were the harvest results of the rainfed treatment of both plant and ratoon crops where in the plant crop, the correlation was negative (R^2=0.45) and in the ratoon crop it was positive (R^2=0.61). A more detailed study of how this could occur is warranted. One explanation was the cyclone Yasi severely damaged the half irrigated experiments and to a lesser extent the fully irrigated.

c) 20 clones for which leaf area, RUE, kl and *TE* were measured

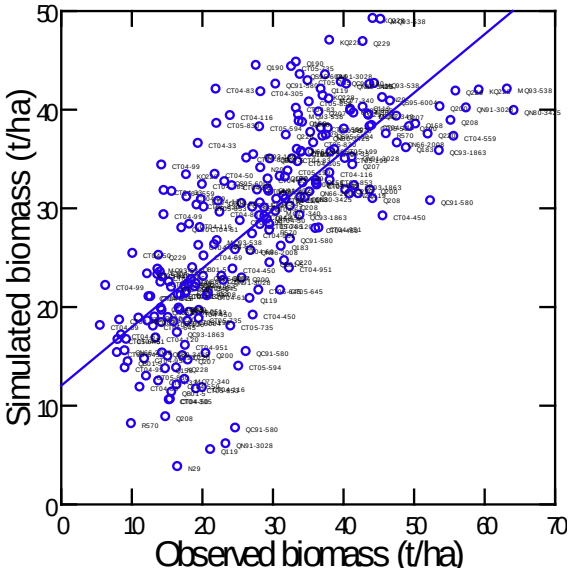
R^2 was generally not improved by excluding the 20 clones for which *TE* was not measured, indicating that measured *TE* has not greatly helped in the explanation of clonal yield variation.

Conclusion

The yield prediction (TCH) by the modified model was increased in 6 crop environments at Dalbeg when the absolute values of traits were incorporated for 10 clones. However, the physiological traits derived from conductance and absolute values of *TE* improved the biomass prediction with the hourly estimation of VPD as modified in the APSIM sugar model.

3. Yield simulation for Home Hill experiment based on *TE* estimated for 20 clones in 2012 glasshouse experiment in Brandon

C) All data (plant and 2R) with all measured traits



Dependent Variable	BIOMASS
N	240
Multiple R	0.747
Squared Multiple R	0.559
Adjusted Squared Multiple R	0.557
Standard Error of Estimate	6.548

Regression Coefficients B = $(X'X)^{-1}X'Y$

Effect	Coefficient	Standard Error
CONSTANT	12.030	1.046
TDM	0.594	0.034

Figure 17. Simulated and measured biomass for 40 clones at Home Hill where biomass was measured at harvest in a plant and two ratoon crops both receiving two irrigation treatments (irrigated and rainfed).

a) All 40 clones

i. Harvested biomass yield

Significant variation in biomass was accounted for by the model for all irrigated crops as well as the 1R crop of the rainfed treatment. In the 1R crop of the irrigated treatment, 46% of the variation in biomass was explained by the traits included in the model.

ii. Removing the ESW trait

Including information for ESW helped to improve the R^2 marginally only for the plant and 2R of the irrigated treatment. Removing ESW increased the R^2 substantially for the rainfed treatment of the plant crop. The clones are therefore not benefiting from higher root vigour or suffering from low root vigour in the rainfed treatment, which is unexpected.

iii. Removing the ESW and TE traits

R^2 did not change much when *TE* was removed in addition to the removal of ESW. Knowledge of the variation in *TE* between clones therefore did not help to explain the variation in yield in either irrigated or rainfed conditions.

Table 5. R^2 for a plant (0) and two ratoon crops (1 and 2) at Home Hill, for simulated versus biomass measured at harvest from two irrigation treatments; irrigated or rainfed. Leaf area traits, radiation use efficiency (RUE) and hydraulic conductance traits (kl) were estimated for all clones, but extractible sol water (ESW) for only 7 clones and *TE* for 18 clones at Home Hill

Number of clones			All clones (n=40)			n=7	n=18
Traits included in model			All traits	-ESW	-ESW-TE	+ESW+TE	+TE
Treat	Crop	Stage					
Rainfed	0	Harvest	0.063	0.281**	0.268**	0.001-	0.004
Rainfed	1	Harvest	0.254**	0.355**	0.358**	0.518**	0.116
Rainfed	2	Harvest	0.057-	0.049-	0.032-	0.406**-	0.077-
Irrigated	0	Harvest	0.272**	0.237**	0.224**	0.545**	0.266*
Irrigated	1	Harvest	0.459**	0.457**	0.452**	0.159	0.269*
Irrigated	2	Harvest	0.345**	0.294**	0.310**	0.519**	0.383**

-Negative slope; * = significant with *P*=0.05; ** = significant with *P*=0.01 or less

b) 7 clones for which all traits (Leaf area, RUE, kl, ESW and TE) were measured

Observed biomass was significantly associated with simulated biomass in many of the cases in Table 5.

c) 18 clones for which leaf area, RUE, kl and *TE* were measured

R^2 was generally not improved by excluding the 22 clones for which *TE* was not measured indicating that measured *TE* has not greatly helped in the explanation of clonal yield variation.

Conclusion

The yield prediction by the modified model was improved in 4 crop environments at Home Hill when the absolute values of traits were incorporated for 7 clones. However, the physiological traits derived from conductance and absolute values of *TE* improved the biomass prediction with the hourly estimation of VPD as modified in the APSIM sugar model.

3.3.2. Evaluate the results of trait validation experiments in breeding program

A decision was made to conduct a field experiment in a relatively dry (rainfed) environment in Burdekin and Bowen to validate the main hypotheses developed in the project. Twenty cultivars with variation of different physiological parameters were selected based on the results from the previous experiments and tested under three variable moisture environments in Bowen. The underlying physiological mechanisms were investigated, and the genetic association between traits and crop productivity in varying moisture environments was quantified.

In addition to Bowen trial, another trial (similar to FAT) with 20 cultivars (eleven clones from the current project and nine clones from the SRA sugarcane breeding program) was established with three moisture environments at Brandon in the Burdekin in 2013. A series of stomatal conductance (g_s) and canopy temperature measurements (as indicator of transpiration efficiency - *TE*) were made from this trial, and early prediction was made based on these trait variation.

In both trials, the genetic correlation between g_s and the TCH was estimated. Results of Bowen and Dalbeg experiments are presented in two separate sub-sections below.

3.3.2.1. Trait validation experiments with selected (20) and unselected introgression lines (20) at Dalbeg

Genetic correlation between conductance (g_s) and TCH in PC, 1R and 2R crops in Dalbeg

The hypothesis on the genetic association (γ_g) between canopy conductance (g_c) and biomass production was tested with the 3 crops data of Dalbeg trial. The genetic correlations between TCH and stomatal conductance as well as TCH and g_c were estimated (Figure 18).

The impact of leaf area on stomatal conductance (g_s) was investigated for the environment in Dalbeg, where leaf area was estimated in all clones at approximately 6 months after planting (plant crop) or harvesting (ratoon crops). Analyses of leaf area data indicated a significant genotype main effect but also of note is a genotype x environment interaction effect (particularly interactions involving crop class). An estimate of canopy conductance (g_c) for each time of g_s was made from the product between g_s and *LAI* (g_s weighted for canopy size). The genetic correlation between g_c and TCH was stronger than that of stomatal conductance (g_s)-TCH relationship. It should be noted that the leaf area values used for this exercise was

estimated from sampling on one date, and therefore did not represent the actual leaf area at each time the stomatal conductance was measured.

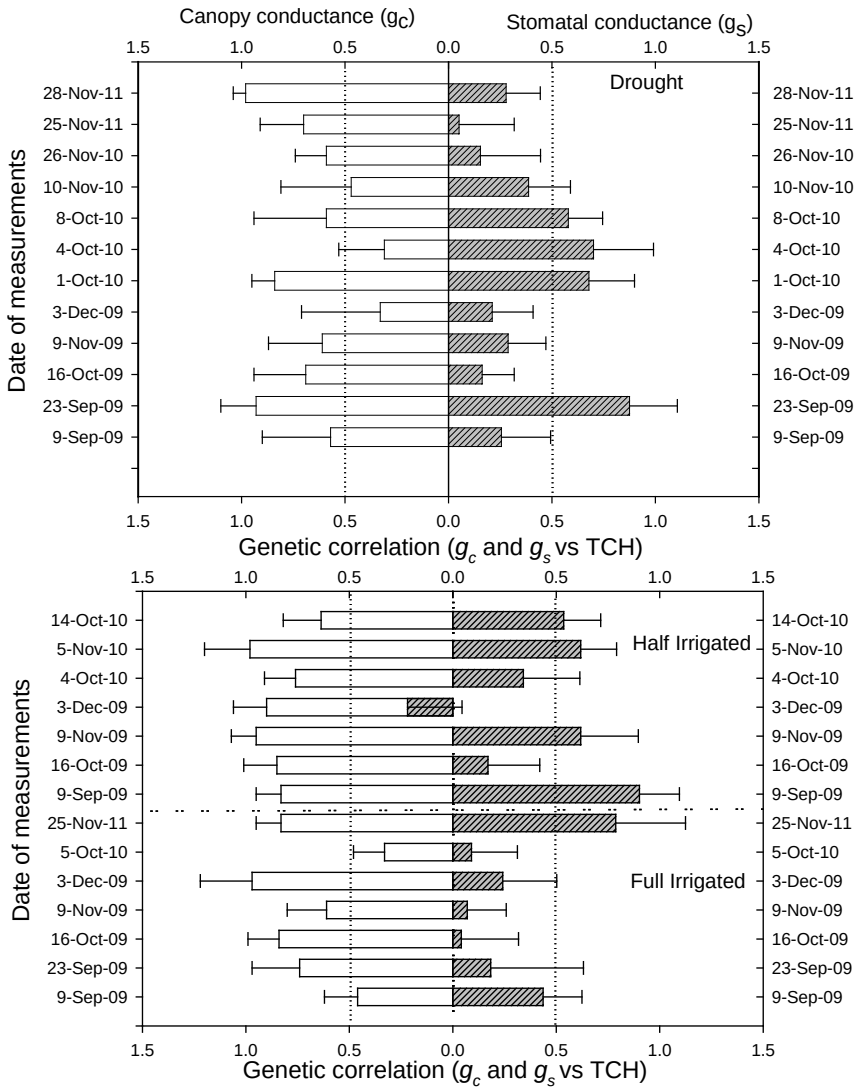


Figure 18. Genetic correlation (bars) and SE estimates (whiskers) between canopy conductance (g_c) and cane yield (TCH) (open), and stomatal conductance (g_s) and TCH (shaded) in 26 observations within 8 test environments (rainfed, half-irrigation and full irrigation) at Dalbeg. TCH data represent site mean for each treatment in each crop class (plant crop, 1R and 2R crops) of Dalbeg trial.

3.3.2.2. Trait validation experiments with selected (10) and unselected introgression lines (10) at Bowen

The plant crop and 2 ratoon crops at Bowen were harvested at 12 months in June (2011-2013). Physiological and yield data were analysed and the results were presented in the Milestones 6, 7 and 8. The magnitude of genetic variances for genotype (G) and G x water

treatment interaction and the ratio of G : GxW were estimated for TCH, total dry matter (TDM), commercial cane sugar (CCS) and tons of sugar per ha (TSH) for the 3 crop years. The genetic variance component was larger than the GxE variance components in all 3 years. This is comparable with the results reported earlier (Basnayake *et al*., 2012a).

Leaf stomatal conductance

Stomatal conductance was measured in three treatments in all three crops from September - November. Data analysis showed a significant clone variation in most occasions. However, treatment and treatment x clone interactions were not significant when measurements were taken at the early stages of the stress development in the field. In November, the average g_s in rainfed and irrigated treatments were 180 mmol m^{-2} s^{-1} and 274 mmolm^{-2} s^{-1} respectively.

Genetic correlation between stomatal conductance (g_s) and TCH in plant, 1R and 2R crops in Bowen

The hypothesis on the genetic association (γ_g) between canopy conductance and biomass production was tested. Generally low to moderate genetic correlations between stomatal conductance (g_s) and TCH across 3 water environments were observed in all the three crops (Table 6)

Table 6. Genetic correlation between stomatal conductance (g_s) and the TCH at harvest in 3 crop classes in 3 years (2011-2013) at Bowen experiment

Crop	Treatments	Site observation	Genetic correlation	Standard error
Plant	Rainfed	BW-1	0.534	0.155
	Half irrigated	BW-1	0.066	0.205
	Fully irrigated	BW-1	0.476	0.088
Ratoon 1	Rainfed	BW-1	0.674	0.116
		BW-2	-0.193	0.199
	Half irrigated	BW-1	0.084	0.100
	Fully irrigated	BW-1	0.377	0.148
Ratoon 2	Rainfed	BW-1	0.443	0.104
		BW-2	0.499	0.156
		BW-3	0.542	0.156
	Half irrigated	BW-1	0.509	0.141
		BW-2	0.624	0.339
	Fully irrigated	BW-1	0.419	0.162
		BW-2	0.922	0.308

As indicated earlier, the leaf area values used for 6 month crop stage were estimates of one sampling date, and therefore may not represent the true leaf area at each stomatal conductance measurement time. There was considerable variation in canopy size during the stress period in rainfed treatment as well as in irrigated treatments. Normalising g_s across canopy size (*LAI*) would provide a better assessment of g_c in different growth stages.

3.3.2.3. Trait validation experiments with breeding lines at Brandon

Stomatal conductance varies greatly under water stress and showed strong clone x treatment interaction in the clone evaluation trial. The variation among clones in all 3 treatments was significant. The differences among water treatments were significant when the stress level in the rainfed treatment was severe. The clones were grouped into selected breeding lines and advanced clones, and the data were analyzed to test whether they are different for g_s. The results did not show any significant difference between group means at mild to moderate stages of stress (Figure 19). However, individuals within groups showed significant variation for g_s when the stress was moderate.

Genetic correlation between g_c and TCH was estimated for 12 month old crop. The genetic correlation between TCH and g_c estimated using 12 month *LAI* was stronger than that of with 6 month *LAI* (Table 7). Therefore, stomatal conductance may provide a useful predictor of yielding ability of clones under appropriate weather conditions.

Table 7. Genetic correlations between g_s, g_c (with 6 month *LAI*), g_c (with 12 month *LAI*) and TCH of rainfed, half-irrigated and fully irrigated treatments and their standard error estimates in 2R crop at Bowen

Treatments	Site observations	Genetic corr. (LAI 6m & TCH)	Standard error	Genetic corr. (LAI 12m & TCH)	Standard error
Rainfed	BW-1	0.443	0.104	0.833	0.204
	BW-2	0.498	0.156	0.784	0.194
	BW-3	0.542	0.155	0.813	0.200
Half irri.	BW-1	0.509	0.141	0.344	0.174
	BW-2	0.627	0.338	0.349	0.154
Fully irri.	BW-1	0.415	0.161	0.546	0.195
	BW-2	0.922	0.307	0.589	0.212
	BW-3	-0.170	0.156	0.271	0.192

Plate 1. Irrigated and rainfed treatment effects on growth of 20 clones in the trait validation experiments at Brandon in 2013.

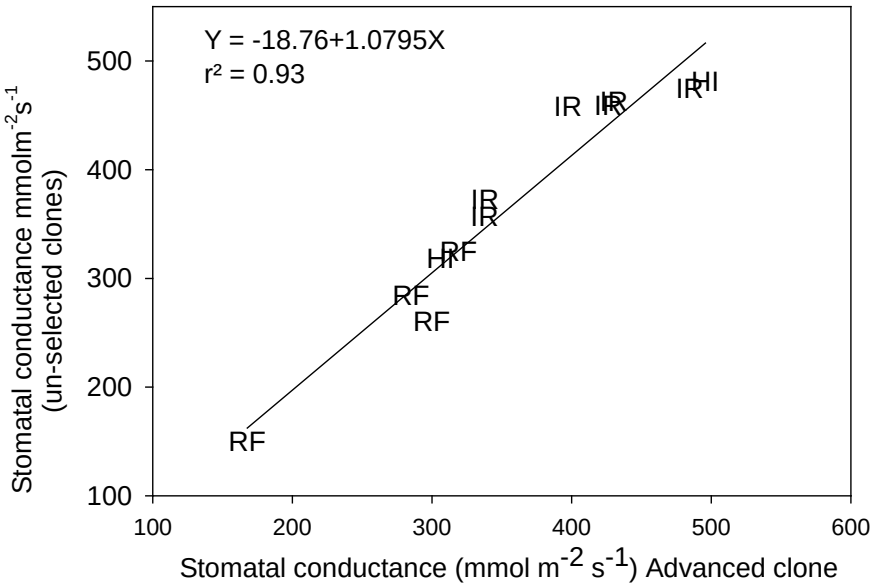


Figure 19. Comparison of mean g_s (4th Nov 2013) in nine advanced lines (clones from FAT trials of the breeding program) and selected clones (from the current MCPD experimental populations) in 3 water treatments (RF - rainfed, HI – half irrigation and IR – full Irrigation). Lsd 5% for treatment differences = 39.5.

Leaf area and canopy development

Most clones in the rainfed treatment produced only 12-14 leaves during the dry period. Leaf area development varied significantly under irrigated and rainfed conditions (Figure 20). Canopy development (leaf area index, *LAI*) was measured in rainfed, half-irrigation and full

irrigation treatments consecutively on 6, 7 and 8 Nov 2013 using the LiCOR 2200 canopy analyser. The measurements were taken just after dawn when the zenith angle of the sun was below 28^0. A significant variation among treatments and clones (P<0.05) was observed. However, clone-by-water stress interaction was not significant. The objectives of the canopy measurement is to develop an index for the canopy level photosynthesis (A) and conductance (g_s), and to predict the crop base intrinsic transpiration efficiency of the test clones under varying moisture environments.

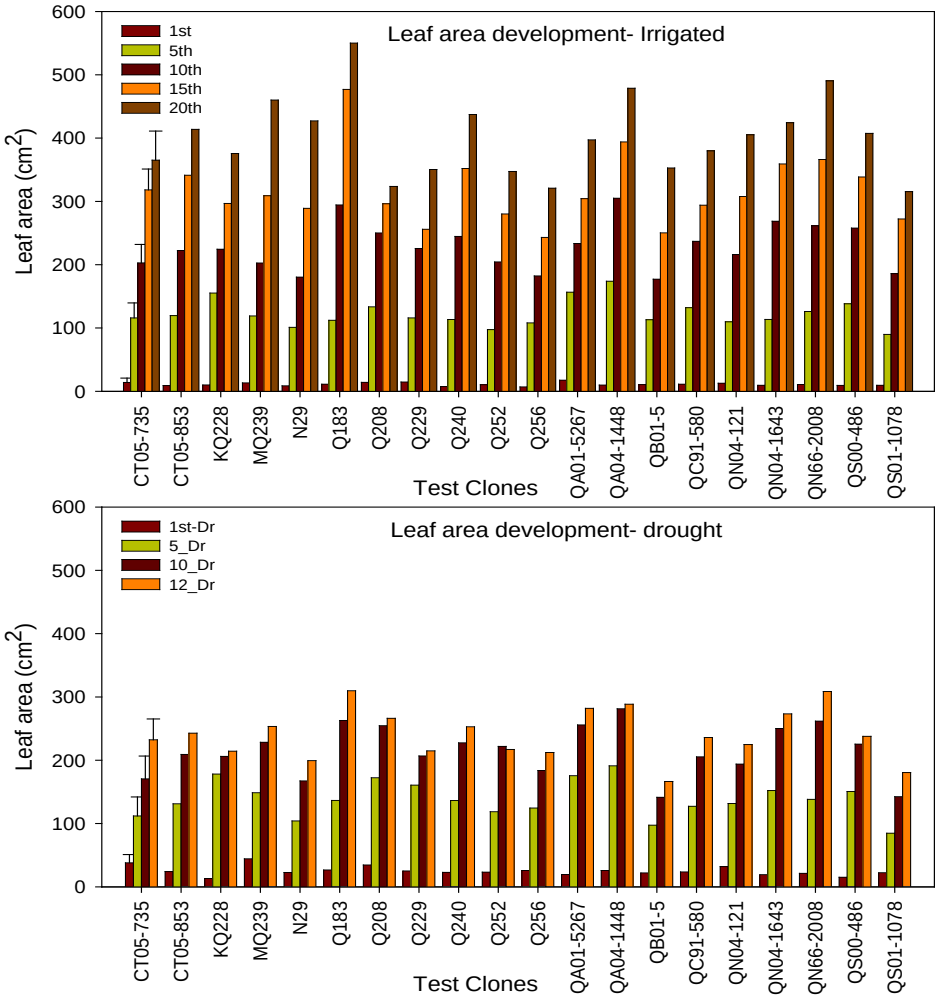


Figure 20. Genotypic variation in leaf area development (every 5th leaf) in 20 clones under irrigated (top) and rainfed (drought) conditions (bottom). At 21 week stage, clones in irrigated treatment had 20 leaves whereas only about 12 in the rainfed treatment. The vertical lines on the 1st set of bars indicate the 5% Lsd values for the mean comparison of leaf area at the same leaf age.

Soil moisture extraction

Soil moisture extraction of 20 clones was measured up to 3 m depth in the rainfed experiment. The first measurement was taken on 12 September 2013 when the average g_s was approximately 230 mmol m^{-2} s^{-1}. It was reduced to 150 mmol m^{-2} s^{-1} by 15th October indicating the reduction in water availability, and soil moisture extraction, with time. Total water extraction of 20 clones in 3 replications was measured, and the clone variation for moisture extraction was statistically analyzed. In this observation, clones QN66-2008 and MQ239 extracted more water than the other clones in the rainfed treatment and had largest canopy cover under stress condition. Clones QS00-486, QB01-5, Q183, Q229 and Q252 were severely affected by drought and had significantly lower water extraction than Q208, KQ228, MQ239 and QN66-2008. Two popular commercial varieties, Q208 and KQ228, had relatively larger canopies and higher water extraction than the others. Measurements were continued during the progression of stress period. A positive correlation between soil moisture extraction and *LAI* and g_s (R^2=0.53, 0.48) was observed under rainfed conditions (Figure 21). The clones with larger canopies were able to extract more water and maintain high conductance during the early stage of stress.

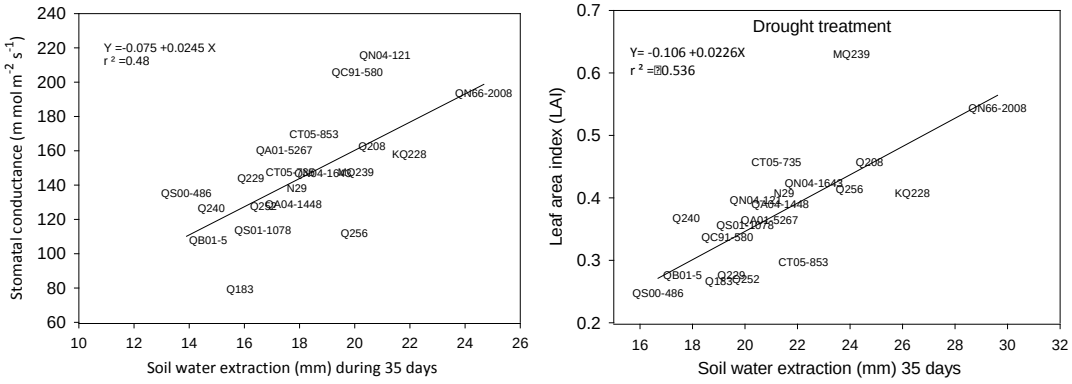


Figure 21. Relationship between root water extraction (2 m depth) during 35 days and stomatal conductance (g_s) and leaf area index (*LAI*) of 20 clones tested under rainfed conditions. Lsd 5% for mean comparisons of root water extraction, g_s and LAI are 4.5 42.2 and 0.15, respectively.

Relationship between Photosynthesis (A) and stomatal conductance (g_s)

The relationship between *A* and g_s as a predictor of intrinsic transpiration efficiency (TE_i) was investigated in both selected and advanced clones grown different water treatments. However, it should be noted that the stress conditions experienced was mild to moderate in rainfed treatment at the time of measurements. There was a strong positive correlation

between *A* and g_s under water stress condition in both groups of clones but not so when water was not limiting (Figure 22). The selected breeding lines and unselected MCPD clones showed similar relationship between *A* and g_s under 3 water treatments.

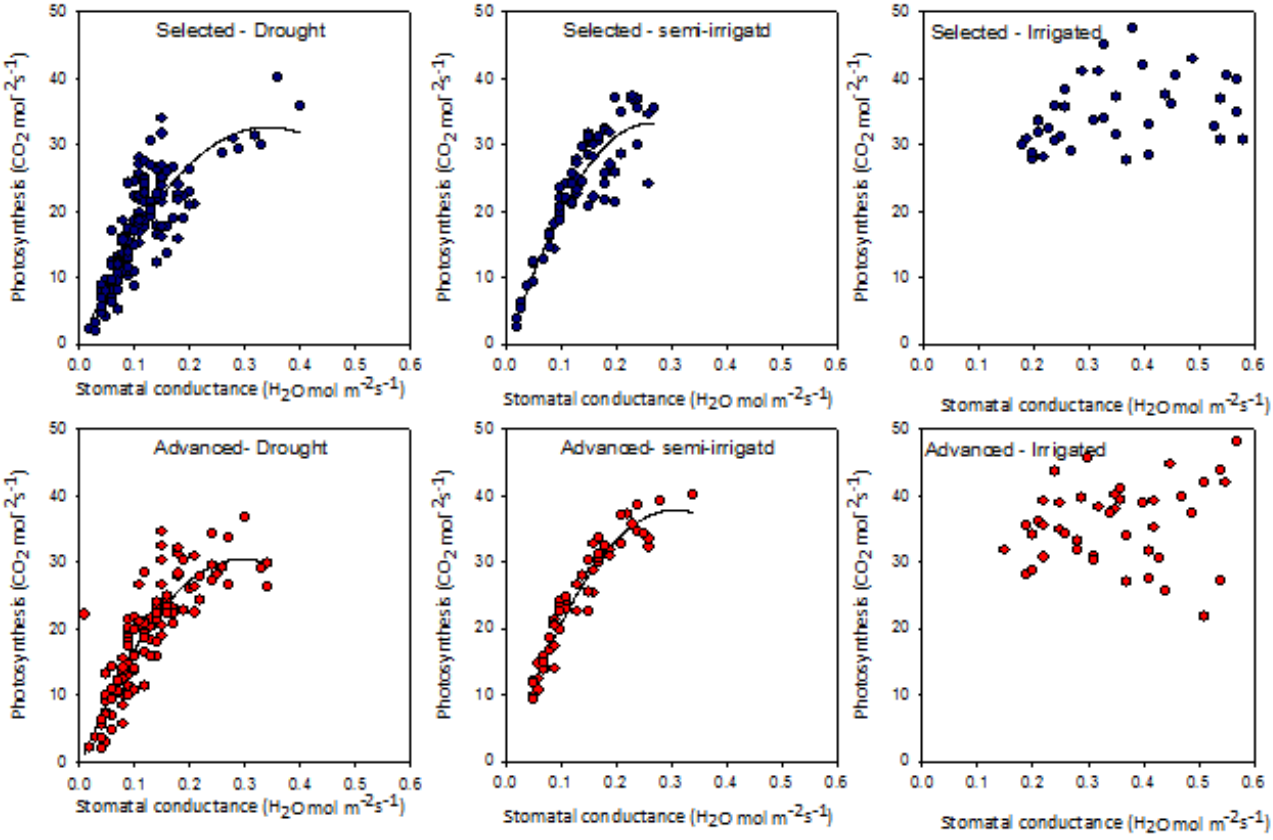


Figure 22. Association between stomatal conductance (g_s) and photosynthesis (*A*) in selected (from previous experiments) or advanced (from breeding program) clones under rainfed (drought), half (semi) irrigated and fully irrigated conditions in 2013 plant crop.

Genetic correlation between photosynthesis (A) and TCH in plant crops in Brandon

The mean gas exchange measurements of 20 clones in rainfed and irrigated treatments were presented in Table 8. Significant treatment and clone variations were observed for photosynthesis (*A*) and stomatal conductance (g_s). The variations among clones were significant for C_i/C_a ratio and instantaneous transpiration efficiency *(TE_i)* only under stress conditions. All genotypes in the non-stress treatment maintained significantly higher *A* and g_s than the stress treatment. However, significant clone × treatment interactions were observed for photosynthesis and stomatal conductance (Table 8).

Table 8. Genotypic variation in instantaneous carbon assimilation rate (A) (µmol [CO_2] m^{-2} s^{-1}), stomatal conductance to water vapour (g_s) (mol [H_2O] m^{-2} s^{-1}), internal CO_2 to ambient CO_2 concentration ratio (C_i/C_a) and instantaneous transpiration efficiency (TE_i) (µmol/mol)

Clone	A (µmol [CO_2] m^{-2} s^{-1})		g_s (mol [H_2O] m^{-2} s^{-1})		C_i/C_a		TE_i (µmol/mol)	
	Rainfed	Irrigated	Rainfed	Irrigated	Rainfed	Irrigated	Rainfed	Irrigated
KQ228	13.20	41.18	0.11	0.66	0.45	0.57	122.32	76.85
MQ239	11.69	38.21	0.11	0.51	0.50	0.51	112.33	91.71
N29	18.01	39.58	0.13	0.37	0.37	0.37	139.49	120.92
Q183	15.82	36.36	0.12	0.38	0.42	0.41	128.24	115.75
Q208	11.96	30.17	0.08	0.36	0.40	0.50	135.30	100.19
Q229	13.92	43.56	0.12	0.65	0.47	0.56	116.97	76.18
Q240	8.45	38.32	0.07	0.53	0.50	0.54	112.76	83.46
Q252	15.53	36.35	0.12	0.30	0.42	0.35	127.58	129.25
Q256	12.83	33.99	0.10	0.27	0.43	0.35	126.99	128.93
QA01-5267	22.27	35.90	0.21	0.34	0.47	0.40	112.75	117.70
QA04-1448	12.57	35.02	0.09	0.43	0.40	0.49	134.54	98.31
QB01-10005	12.64	40.74	0.10	0.52	0.47	0.51	117.27	89.06
QBYC05-20735	14.77	37.83	0.12	0.40	0.40	0.43	134.74	110.48
QBYC05-20853	21.48	37.61	0.20	0.32	0.44	0.36	118.75	125.46
QC91-580	19.38	33.04	0.16	0.50	0.37	0.53	137.74	90.67
QN04-121	22.09	35.33	0.20	0.66	0.46	0.64	115.32	65.31
QN04-1643	17.83	30.70	0.13	0.28	0.36	0.37	141.56	129.41
QN66-2008	15.06	35.16	0.13	0.41	0.46	0.43	118.82	111.92
QS00-486	14.44	38.99	0.11	0.32	0.40	0.35	133.08	126.78
QS01-1078	26.31	36.47	0.20	0.33	0.37	0.41	132.67	113.66
Mean	16.01	36.73	0.13	0.43	0.43	0.45	125.96	105.10
F-value	3.62	2.11	2.62	4.06	0.66	3.12	0.59	3.15
Probability	0.0003	0.0233	0.0053	<.0001	0.8359	0.0012	0.892	0.001
Significance of effect								
Treat	0.0039		0.0025		0.2354		0.0502	
Clone	0.0009		<.0001		0.0009		0.0014	
Treat × Clone	<.0001		<.0001		0.6002		0.7076	

Some clones in the stress treatment continued to maintain high C_i/C_a ratio similar to non-stress treatment. Increase in intrinsic transpiration efficiency (TE_i) in stress treatment was associated more with higher intercellular CO_2 concentration when conductance was low due to water limitation. KQ228^A, Q229^A and QN04-121 showed high conductance in the non-stress treatment whereas in the stress treatment, KQ228^A and Q229^A showed substantially

reduced conductance but QN04-121 continued to show relatively higher conductance (Table 8). Clones QA01-5267 and QS01-1078 also showed higher conductance under stress which was also reflected by the higher photosynthesis (*A*) values. Under non-stress conditions, g_s and *A* increased simultaneously and reached to a maximum of 45 µmol [CO_2] m^{-2} s^{-1} beyond which *A* increased more slowly than g_s.

Genetic correlation between gas exchange traits and TCH

Table 9. Genetic, phenotypic and error correlations for TCH and the gas exchange traits measured in rainfed and irrigated treatments, and the heritability of the traits

Treatment	Trait	Genetic correlation	Phenotypic correlation	Error correlation	Heritability Broad sense
Rainfed	Photosynthesis	-0.596	-0.273	0.002	0.72
	Conductance	-0.880	-0.160	-0.025	0.18
	Internal C_i/C_a	0.808	0.254	-1.564	0.58
	TE intrinsic	-0.395	-0.016	0.050	0.13
Irrigated	Photosynthesis	-0.259	-0.269	-0.281	0.64
	Conductance	0.561	0.474	-1.561	0.97
	Internal C_i/C_a	0.493	0.001	0.001	0.29
	TE intrinsic	0.057	-0.086	-0.210	0.71

Severity of drought (165 days of water stress) caused the stomatal closure during the driest conditions and most of the clones survived by conserving moisture during this period. The severe dry conditions particularly affected the rainfed treatment and it was reflected in low heritability of g_s in rainfed treatment. Consequently, leaf level stomatal conductance showed a strong negative genetic correlation between early biomass and TCH (Table 9). Clones in the irrigated treatment had a positive genetic correlation between g_s and TCH, though photosynthesis had a small negative genetic correlation with TCH. Conductance was not a limiting factor for photosynthesis, as the irrigated treatment did not suffer any stress. The intrinsic TE and TCH had a negative genetic correlation under water stress in rainfed treatment while C_i/C_a ratio showed a strong positive genetic correlation with TCH under rainfed than irrigated treatments.

Genetic correlation between canopy conductance (g_c) and TCH in plant crop in Brandon

The analyses were done to investigate the genetic association between g_s and canopy conductance (g_c) with the yield in the test population. The objectives of this analysis was to quantify the genetic correlation (γ_g) between g_s, g_c and TCH among mixed population of commercial clones and unselected introgression lines. Each g_s measurement in water treatments was multiplied by the respective *LAI* value to obtain the canopy conductance (g_c) at physiological maturity (Table 10).

Table 10. Genetic correlations between TCH, stomatal conductance (g_s) and the canopy conductance (estimated using *LAI* at physiological maturation) for a series of g_s measurements from 3 water treatments at Brandon experiment (BK)

Treatment	Site observations	Stomatal conductance	Standard error	Canopy conductance	Standard error
Rainfed	BK-1	0.481	0.302	0.714	0.263
	BK-2	-0.048	0.393	0.324	0.461
	BK-3	-0.084	0.345	0.173	0.017
	BK-4	-0.064	0.472	0.130	0.443
Half irri.	BK-1	0.895	0.504	0.935	0.271
	BK-2	-0.108	0.604	0.634	0.424
Fully irri.	BK-1	-0.413	0.377	0.671	0.298
	BK-2	-0.659	0.241	0.835	0.301
	BK-3	0.544	0.261	0.843	0.125
	BK-4	-0.375	0.377	0.569	0.441
	BK-5	-0.778	0.391	0.521	0.725

The impact of leaf area on g_s was investigated for the environments in Bowen and Burdekin, where leaf area was estimated in all clones approximately at 6-month and 12-month stages. Analysis of leaf area data indicated that there was a significant genotype main effect, which is important for assessing canopy level conductance. There was variation among clones for leaf area at physiological maturity and hence the canopy level conductance prediction at 12

month *LAI* would also provide an important evidence for the genetic correlations between TCH.

Conclusion: The key results from these 2 experiments were 1) the moderate genetic correlations between TCH and both stomatal conductance and canopy conductance, estimated early and late in crop growth 2) under appropriate conditions, canopy conductance may be a useful predictor of general yielding ability of clones for incorporation into selection indices in early stages of sugarcane breeding programs.

3.3.3 Identify elite clones with desired water use efficiency and drought tolerance traits (stomatal and canopy conductance)

Having found a strong genetic correlation between canopy conductance and cane yield (TCH) at field level in most occasions, it is necessary to assess the value of this relationship in developing a selection index for breeding more productive clones and to identify a sub-set of elite clones for further selection index optimisation studies.

3.3.3.1. Calculation of genetic gain by indirect selection for canopy conductance

Theory of correlated response was applied to compare the genetic gain between current selection method based on tch in 2 row plots (CAT trials) and the proposed early selection for canopy conductance as a predictor for TCH. However, genetic parameters for canopy conductance estimated at 3-5 month stage prior to inter-plot competition in 4 row plots was assumed to be similar to that of 2 row plots.

The genetic correlation between tch in CAT trials and TCH in FAT trials was estimated to be 0.49 with an average heritability of 0.59 (Jackson and McRae, 2001). In recent MCPD analyses (BSS334), genetic correlation between canopy conductance and TCH of 4 row plots was 0.72 with an average heritability of 0.68.

Assuming selection differential (i) = 2.67 (1% selection rate), $\sigma_{P_{TCH}}$ = 14 (square root of phenotypic variance based on plant and 1st ratoon crops of FATs harvested in Burdekin), the expected genetic gain for TCH:

by selecting tch in 2 row plots,

Expected genetic gain = 2.67 * 14 * $0.59^{1/2}$ * 0.49 = 14.1

and by selecting for conductance,

Expected genetic gain = 2.67 * 14* $0.68^{1/2}$* 0.72 = 22.2

This increase of 8.1 t/ha (22.2-14.1) in expected genetic gain with conductance could result in an approximate annual industry benefit of 3 million tonnes of cane.

3.3.3.2. Developing an optimal selection index – background information and an example

It is important to determine the b coefficients in equation (1) mentioned in the methods. The actual genetic parameters estimated from the experiments were used for this purpose.

$\mathbf{b} = [V]^{-1}.[C].\mathbf{a}$ (eqn 1)

where, $\mathbf{b}$ = m x 1 vector of traits to be measured (i.e. there are m traits measured),

V = m x m matrix of the phenotypic variances and co-variances among the measured traits

C = an m x q matrix of co-variances between the measured traits and genetic values of traits of economic value (i.e. there are q traits affecting economic value)

$\mathbf{a}$ = q x 1 vector of the economic weightings of traits affecting economic value

As explained below, this calculation required two key groups of information.

(i) The statistics needed to determine the V and C matrices.

As much of this information should come directly from the trials in which the selection is being done. However, some information, such as covariance with performance measured in small plots with that in pure stands, and size of GxE interactions, will need o be assumed (based on knowledge from past research).

(ii) The economic weightings.

This needs to be determined based on information from industry people – based on a combination of experts' best judgments and modelling. Determining economic weightings is a major task, but is important.

Example of a selection index

The following example uses some assumptions based on data obtained from Australian sugarcane selection trials and economic weightings.

Consider an early stage sugarcane clone trial, in which CCS, cane yield (TCH) and stomatal conductance (g_s) are measured in small single-row plots.

3.3.3.2.1. Determination of phenotypic variances and co-variances for the three traits, for determining the 3 x 3 V matrix

The linear model assumed for each trait in doing an analysis of variance of any trait which is commonly assumed is as follows:

$y_{ijk} = \mu + g + c + gxe + error$

where, y_{ijk} = phenotypic value of genotype i in environment j and replicate k (i.e. the observed performance of the genotype, and which we use in selection), μ = mean of all observations, g = true genetic effect (i.e. the effect we would really like to estimate and use for selection but can never know precisely because of the other effects), c = competition effect of genotype i (i.e. deviation of the performance of genotype i of expected value in small plots from its performance in pure stand; pure stand means if it is grown in a commercial field by itself), gxe = genotype x environment interaction of genotype i in environment j, error = random experimental error effect of genotype i in environment j in replicate k

Based on this model,

$\sigma_p^2 = \sigma_g^2 + \sigma_{comp}^2 + \sigma_{gxe}^2/(n_e) + \sigma_{error}^2/(n_e.n_r)$

where, σ_p^2 = phenotypic variance of the estimates of means of genotypes, σ_g^2 = (true) genetic variance of genotypes under test, σ_{comp}^2 = variance due to competition effects, σ_{gxe}^2 = variance due to g x e interaction, σ_{error}^2 = variance due to experimental error variance, n_e = number of environments used for testing, n_r = number of replicates per environment.

(Note that this model assumes no co-variances between genotype and competition effects, which may or may not be valid. We have obtained results from research experiments which shows situations where there is zero covariance, but also some situations where there is a positive covariance (i.e. better genotypes are more competitive. If there is a covariance, then a covariance component should be added to the above model. But for purpose of simplicity this is assumed to be equal to zero in this example).

Based on results obtained from past analyses of variance in some trials in Australia, the following fairly realistic values for these statistics are assumed for stage 2 of the clonal selection trials each of the three traits being measured:

CCS (in single row):	TCH (in single row):	Stomatal conductance (based on a series of measurements under a range of optimal conditions)
$\sigma_g^2 = 1.0$ $\sigma_{comp}^2 = 0$ (No effect) $\sigma_{gxe}^2 = 0.4$ $\sigma_{error}^2 = 1.0$	$\sigma_g^2 = 350$ $\sigma_{comp}^2 = 1050$ $\sigma_{gxe}^2 = 52$ $\sigma_{error}^2 = 900$	$\sigma_g^2 = 500$ $\sigma_{comp}^2 = 0$ (no competition effect 3-6 month) $\sigma_{gxe}^2 = 0$ $\sigma_{error}^2 = 200$

It is further assumed there is only one environment (n_e = 1) used for selection and two replicates (n_r = 2). Therefore based on the above assumptions, the following phenotypic variances (σ_p^2) were calculated:

CCS	TCH	Stomatal conductance (gs)
for = 1.9	σ_p^2 for = 1902	σ_p^2 for = 700

It is further assumed that there is zero genetic correlation and error correlation between CCS and cane yield, and a genetic correlation of stomatal conductance and cane yield of 0.8. Therefore phenotypic covariance between CCS and cane yield is zero.

For stomatal conductance and cane yield:

$r_{g(gs.TCH)} = cov_g/(\sigma_{g,gs} . \sigma_{g.TCH}) = 0.8$

Therefore based on above assumptions, $cov_{g(gs.TCH)} = 0.8 * (500*350)^{0.5} = 334$

For the 3x3 V matrix (i.e. the variances and co-variances between the phenotypes of the measurements in the single row plots), the following values are determined based on the above assumptions:

	CCS	TCH	gs
CCS	1.9	0	0
TCH	0	1902	334
gs	0	334	700

For considering co-variances between phenotypes measurements made in a single row plot and true genetic values in multi-row plots in determining, the only variance in common is the true genetic variance, and hence this is the covariance. Therefore the 3 x 2 C matrix is as follows:

	CCS (pure stand)	TCH (pure stand)
CCS (single row)	1.0	0
TCH (single row)	0	350
Gs (single row)	0	334

3.3.3.2.2. Economic weightings

Based on prior work, it is assumed for this example that a 1% unit increase in CCS (e.g. going from 12% to 13%) results in a total industry benefit of $400/ha, and 1 ton per hectare increase in cane yield (in a pure stand) results in a total industry benefit of $20/ha.

Therefore the (2 x 1) **a** vector is:

CCS (pure stand)	400
TCH (pure stand)	20

3.3.3.2.3. Calculating the b coefficients

Given equation 2,

b = $[V]^{-1}$. [C] .**a**

then

$$b = \begin{pmatrix} 1.9 & 0 & 0 \\ 0 & 1902 & 334 \\ 0 & 334 & 700 \end{pmatrix}\text{-1} \cdot \begin{pmatrix} 1.0 & 0 \\ 0 & 350 \\ 0 & 334 \end{pmatrix} \cdot \begin{pmatrix} 400 \\ 20 \end{pmatrix}$$

The inverse of a matrix can be determined in Excel or other software (e.g. R):

$$b = \begin{pmatrix} 0.526316 & 0 & 0 \\ 0 & 0.000574 & -0.00027 \\ 0 & -0.00027 & 0.00155 \end{pmatrix} \cdot \begin{pmatrix} 1.0 & 0 \\ 0 & 350 \\ 0 & 334 \end{pmatrix} \cdot \begin{pmatrix} 400 \\ 20 \end{pmatrix}$$

and the matrices multiplied together (using standard matrix algebra rules) to give:

$$b = \begin{pmatrix} 210.52 \\ 2.187 \\ 8.498 \end{pmatrix}$$

Therefore, the selection index to use for the above assumptions is:

Selection index = 210.52 x CCS + 2.187 x TCH + 8.498 x g_s

3.3.3.3. Clones with improved water productivity under well-watered and water deficit conditions

The selection indices were calculated for each genotype in Brandon experiment where commercial and unselected clones were tested together (Table 11), and the genotypes are ranked according to the selection index in three water environments. The FAT clones QA01-5267 and QN04-121 ranked 1st and 2nd, respectively, in rainfed and half-irrigated environments, while QN04-121 became the top clone for fully irrigated condition. This preliminary estimation should be considered only as an example as to how canopy conductance could be integrated in the selection index to select clones with high productivity and wide adaptation. The ranking of clones indicated that the best commercial clones selected for high CCS and TCH were not necessarily the best performing clones for contrasting water environments. For instance N29 the second best under full irrigation was dropped to 7th position in half-irrgation treatment and the 2nd last in the rainfed areas. So, this indicate that addition of water relations trait like canopy conductance could greatly refine target

production environment-specific variety selection/recommendation. Clearly the full extent of the value of this approach will be evident only when we test the system in large selection trials in different regions.

Table 11. An example of selection index calculation incorporating canopy conductance for ranking clones with wide adaptability and productivity. Test clones grown under 3 different commercially-relevant water environments (full irrigation, half-irrigation and rainfed) in Brandon, Ayr were used for this analysis. Plant crop yield (TCH), sugar content (CCS) at maturity and canopy conductance of different water treatments were used for this analysis. In each treatment measurements with TCH and g_c genetic correlation and their heritability more than 0.7 were used for this calculation.

		Rainfed					Half irrigated						Irrigated				
Clone	CCS	TCH	gs	Selection Index	Rank	Clone	CCS	TCH	gs	Selection Index	Rank	Clone	CCS	TCH	gs	Selection Index	Rank
QA01-5267	2887.3	101.3	3246.3	6234.9	1	QA01-5267	3372.4	255.6	4938.6	8566.6	1	QN04-121	2786.8	300.8	5097.7	8185.3	1
QN04-121	2147.5	127.1	3593.6	5868.2	2	QN04-121	2640.2	271.3	4955.3	7866.7	2	N29	2968.4	299.7	4722.4	7990.5	2
QS00-486	3099.1	151.8	2367.1	5618.0	3	Q252	3130.2	234.8	4411.1	7776.2	3	Q229	3014.8	347.0	4378.3	7740.1	3
Q252	3128.0	136.1	2298.0	5562.1	4	CT05-735	2995.8	282.4	4485.0	7763.1	4	QA01-5267	3187.1	306.6	4073.8	7567.4	4
QC91-580	2497.5	113.0	2816.0	5426.6	5	QC91-580	2725.8	281.0	4749.9	7756.7	5	KQ228	3492.5	337.7	3687.3	7517.5	5
MQ239	2482.2	157.3	2627.8	5267.3	6	QS00-486	3024.7	302.2	4197.4	7524.3	6	QC91-580	2737.9	341.7	4226.7	7306.2	6
Q208	2655.8	149.0	2445.6	5250.5	7	N29	2654.6	252.2	4547.0	7453.8	7	Q252	3377.9	353.7	3499.0	7230.6	7
KQ228	2906.4	152.3	2188.0	5246.6	8	QN04-1643	2602.4	244.8	4546.5	7393.7	8	Q240	3270.5	361.0	3532.9	7164.4	8
CT05-735	3015.5	135.0	2090.5	5240.9	9	Q183	3234.2	264.6	3754.0	7252.8	9	QS00-486	2917.3	330.8	3894.8	7143.0	9
QN04-1643	2887.8	120.4	2213.6	5221.8	10	MQ239	2439.3	277.8	4385.5	7102.6	10	CT05-735	2850.5	313.5	3955.2	7119.2	10
CT05-853	2736.9	110.7	2030.0	4877.6	11	Q229	2819.8	299.0	3945.1	7063.9	11	Q183	3300.7	292.1	3499.4	7092.1	11
QN66-2008	1989.8	141.4	2745.3	4876.5	12	KQ228	3303.3	258.8	3485.8	7047.9	12	CT05-853	2507.2	240.4	4093.9	6841.4	12
Q256	2632.4	161.0	2074.3	4867.7	13	Q240	3232.3	252.2	3477.0	6961.5	13	QA04-1448	2856.5	421.3	3375.3	6653.0	13
Q229	2746.0	141.9	1961.4	4849.3	14	QA04-1448	2705.5	300.4	3926.9	6932.8	14	MQ239	2618.4	323.4	3576.7	6518.6	14
QS01-1078	2536.8	126.0	2126.8	4789.5	15	QS01-1078	2771.4	246.6	3858.9	6877.0	15	QB01-5	1802.9	200.7	4307.1	6310.7	15
Q183	2538.1	115.6	2119.7	4773.3	16	CT05-853	2213.2	202.1	4440.9	6856.2	16	Q208	2755.3	308.4	3232.6	6296.4	16
Q240	2674.7	127.6	1901.9	4704.3	17	Q256	2934.7	281.4	3448.3	6664.5	17	QS01-1078	2437.2	270.8	3515.3	6223.4	17
QA04-1448	2355.5	165.2	1778.1	4298.7	18	Q208	2850.0	316.5	3375.4	6541.9	18	Q256	2788.1	356.4	3014.0	6158.5	18
N29	2174.6	119.7	1755.3	4049.6	19	QN66-2008	2497.0	257.0	3736.8	6490.8	19	QN04-1643	2975.8	366.1	2610.0	5951.9	19
QB01-5	2087.2	65.7	1471.4	3624.3	20	QB01-5	1619.6	180.4	4115.4	5915.4	20	QN66-2008	2336.1	327.8	3283.3	5947.2	20

Values given for CCS, TCH and g_s were derived by multiplying CCS, TCH and g_s with the respective coefficients of b1, b2 and b3

4. Conclusions

The More Crop Per Drop stage II project (BSS334) focused mainly on a) validation of the genetic basis of trait (stomatal and canopy conductance)-yield (TCH) relationship,b) quantifying the predictability of canopy conductance (g_c) as a selection trait for cane yield (TCH) in the early-stage selection trials, and c) development of trait and crop simulation capability using APSIM sugar module.

APSIM sugar module refinement for improved trait and crop simulations: In the trait modelling component of this project transpiration efficiency (*TE*) in APSIM sugar module was re-defined as a dynamic cultivar-specific variable rather than a crop constant. This has greatly improved trait and crop simulation, and showed how physiological knowledge can be used to improve sugarcane crop simulation for different production environments. Identifying important trait parameters and understanding their regulation are critical for improving the accuracy of simulation. For example, in the research reported here hourly modulation of stomatal conductance (g_s) and root water extraction, and how it is influenced by different environmental parameters considerably improved our understanding of sugarcane water relations and crop productivity in different production scenarios. This will expand trait modelling capability and help strengthen crop model as a decision support system for environment-specific management recommendations.

Stomatal and canopy conductance and its genetic relationships with cane yield

1. Genetic variation of stomatal conductance (g_s)

Highly significant genotypic variation was found for g_s for most times of measurements. Analyses of variance across treatments showed that genetic variance was significant in all cases and for most cases greater than genotype x treatment interaction variance, indicating relative consistency in genotype performance across different water availability conditions. Further, genetic correlations between g_s across all site x treatment combinations were relatively high and positively correlated. This is indicative of a generally consistent pattern of ranking of genotypes for g_s across different crop production environments. Commercial clones consistently showed high g_s than that of unselected introgression and species clones across all test environments.

2. *Genetic correlations between stomatal conductance and cane yield*

Genetic correlations between g_s and cane yield were measured for all environments studied. Results indicate an overall positive association between g_s and average cane yield among the genotypes, but with varying strength of the relationship. Studies on the factors influencing g_s –TCH relationship showed that the maximum temperature during the time of measurement was negatively related with the magnitude of these correlation values. Mean temperature, vapour pressure and water stress at the time of measurement had much lower impact on g_s –TCH relationship.

In addition to the environmental parameters the impact of leaf area on stomatal conductance was also investigated. A significant genotype main effect and genotype x environment interaction effects, particularly interactions involving crop class was evident. Hence, g_s was estimated at canopy level, called "canopy conductance (g_c)", (g_s weighted for canopy level), was determined. This greatly improved trait-yield correlation with g_c showing much larger genetic correlation (γ_g= 0.7-0.9) with TCH that between g_s –TCH.

3. Potential of canopy conductance as a selection trait for cane yield potential and WUE in sugarcane.

The genetic correlation between mature cane yield in single row plots, typically used in early-stage breeding programs, and multi-row plots at the same site with a set of unselected clones is about 0.5 (Jackson and McRae, 1998). Correlations between yields estimated in individual seedlings would be expected to be even smaller (<0.5). If measurements of canopy conductance could be made to provide moderate to high (e.g. greater than 0.5) genetic correlations with cane yield consistently, then with similar levels of broad-sense heritability, these could add considerable value to selection indices and variety development.

Use of stomatal or canopy conductance as a selection trait in breeding programs will require rapid, high-throughput and cost-effective screening methods suitable for large selection trials. Semi-automated methods for continuous aerial imaging of leaf area or canopy size and measurement of canopy conductance are being developed for a range of crops (Rebetzke *et al.* 2013; Chapman *et al.* 2014) and this approach would seem to provide a system for practical and low-cost data collection. Understanding how conductance changes under different conditions (in response to various internal and external cues) may improve additional predictive power to g_s and g_c for selecting sugarcane clones with increased water

use efficiency, drought tolerance and overall yield potential. Further research is required to determine the conditions under which the genetic correlation between canopy conductance and yield is maximized.

5. Outputs and Outcomes

5.1. Outputs

1. Identification of traits related to water use efficiency (WUE) and drought adaptation in sugarcane. The key, potentially practically useful traits are stomatal conductance, canopy conductance and leaf area index,
2. Quantitative data on genetic variability of sugarcane water use efficiency (WUE)-related traits (stomatal conductance, canopy conductance and leaf area index) and their genetic relationships with cane yield and crop adaptation
3. Successful experimental validation of canopy conductance as a potential trait for selecting sugarcane clones with high yield and broader adaptation
4. Elite clones with desired WUE characteristics for further trait-yield relationship studies and breeding experiments
5. Improved APSIM sugar module for sugarcane crop modelling
6. Publications and conference presentations (please see the list in the Publication sub-section below)

5.2. Outcomes

1. The long-term impact of this research will be increased industry productivity by minimizing the risk of recurring water stress and yield fluctuation through high-yielding, water-efficient, drought tolerant cultivars. The key output of this project, establishment of a water relations-related trait, canopy conductance, as a potential selection criteria for selecting high-yielding clones early in the selection stage, if applicable in large selection trials will be a significant boost to variety development in general. Based on the data from experimental trials, it is estimated that if the same result can be translated to actual selection trials that will lead to better more resilient varieties with an additional yield advantage of 8.1 tons of cane/ha.

2. More close and active interactions with Chinese sugarcane researchers in the water stress area.

3. Fostering multi-disciplinary (physiologists, breeders, crop modelling experts, agronomists), multi-organisational (SRA, CSIRO, overseas institutions) research collaboration in the sugar industry

4. Capacity building in the area of crop physiology and breeding. Improved trait development research and modelling capacity in the area of sugarcane drought, WUE and crop adaptation. This research work also led to a PhD project by Sijesh Natarajan based at SRA Brandon.

6. Intellectual Property (IP) and Confidentiality

No protectable IP at this stage.

7. Industry Communication and Adoption of Outputs

Update on this work have been made in SRDC industry roadshows, presentations in SRA breeders' meetings, SRA project development meetings and Cane Clip presentation through PEC unit.

8. Environmental Impact

None at this stage

9. Recommendations and Future Industry Needs

The next logical steps would be to road-test the research findings of this project in the SRA regional selection trials. If they are yielding positive results, then develop automated large-scale, cost-effective screening systems for practical application. A project to this effect has been submitted to the SRA Funding Unit for consideration.

10. Publications

List of Publications:

Article: J. Basnayake, P.A. Jackson, N.G. Inman_Bamber, P. Lakshmanan. 2014. Sugarcane for water- limited environments. Canopy conductance is a potential selection trait for high-yielding clones for diverse production conditions. Will be submitted to Journal of Experimental Botany soon (a copy attached- Appendix 2).

Conference Paper: Phillip Jackson, Jaya Basnayake, Geoff Inman-Bamber, Prakash Lakshmanan. 2014. Selecting sugarcane varieties with higher transpiration efficiency. 36th Annual ASSCT Conference, , 28 April- 1 May, Gold Coast, Queensland, Australia.

Conference Paper: J Sexton, Ng Inman-Bamber, Y Everingham, J Basnayake. 2014. Investigating simulations of cultivar response to water stress. ASSCT 36th Conference, 28 April- 1 May, Gold Coast;, Australia.

Conference presentation (invited talk): Prakash Lakshmanan, Jayampathi Basnayake, Philip Jackson, Sijesh Natarajan, Geoff Inman-Bomber and Chris Stokes. 2014. Sugarcane for water-limited environments: challenges and opportunities. International Conference "Green Technologies for Sustainable Growth of Sugar & Integrated Industries in Developing Countries" (IS-2014) November 25-28, Nanning, China.

Conference Paper: Jayampathi Basnayake, Philip Jackson, P, Inman-Bamber, NG, Prakash Lakshmanan. 2013. Substantial genetic variation for response to water stress exists in the Australian sugarcane germplasm. IV Inter-Drought international conference, September 2-6 Crown Metrapol, Perth, Australia,.

Conference presentation: Jayampathi Basnayake, Philip Jackson, P, Inman-Bamber, NG, Prakash Lakshmanan. 2013. Stomatal conductance is a major determinant of sugarcane productivity in water-limited environments. Substantial genetic variation for response to water stress exists in the Australian sugarcane germplasm. IV Inter-Drought international conference, September 2-6 Crown Metrapol, Perth, Australia,.

Article: Basnayake, J, Jackson, P.A, N. G. Inman-Bamber, and Prakash Lakshmanan. 2012a. Sugarcane for water-limited environments. Genetic variation in cane yield and sugar content in response to water stress. Journal of Experimental Botany 63(16): 6023-6033. Impact Factor 5.79.

Conference Paper: Jayampathi Basnayake, Philip Jackson, Geoff Inman-Bamber and Prakash Lakshmanan. 2012b. Sugarcane for water-limited environments: 3.Transpiration efficiency of commercial and wild relatives of sugarcane. Australian Agronomy Conference, 10/2012.

Conference presentation. (invited talk): Prakash Lakshmanan, Jayampathi Basnayake, Philip Jackson and Geoff Inman-Bamber. 2012. An integrated research framework to develop sugarcane for water-limited environments. 6th International Crop Science Congress, Aug 6-10, Bento Goncalves, Brazil.

A PhD student, Prapat Punpee, is administratively linked to this project. He conducted transgenic research on improving drought tolerance sugarcane and he is in the final stage of thesis writing.

11. Appendix 1

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