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FINAL REPORT – SRDC PROJECT BSS305

MORE CROP PER DROP

by

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SUMMARY

Water stress is the major constraint to productivity in the Australian sugar industry, with an estimated cost of $260 million/annum (CSE014 Milestone Report 3.5). In 2003 and 2004, the losses exceeded $140 million in Mackay alone. This impact may increase with climate change. About 40% of the Australian sugarcane production is rain-fed with or without supplementary irrigation. With the erratic rainfall and the increasing cost and restrictions on water use, efficient use of available water is an increasingly important priority in irrigated production systems as well.

Varieties that adapt well to drought and use water efficiently are becoming increasingly important for sustainable sugarcane production. Australian sugarcane breeding programs, however, do not explicitly address selection for response to dry conditions. To effectively address this issue there is a need to understand the main physiological mechanisms underlying genetic variation in response to different types of water stress environments in sugarcane. Hence, this project was initiated to determine the potential of Australian sugarcane germplasm for developing water use-efficient and drought tolerant commercial cultivars.

In this project the phenotypic and genetic variations of traits that confer or linked to drought tolerance and water use efficiency (WUE) and their contribution towards productivity were studied in a genetically diverse sugarcane population. Field experiments were conducted for 3 years under rainfed, fully irrigated and managed drought conditions at three locations in the Northern Queensland. The experiment sites were in Home Hill in the Burdekin shire, Crystal Creek in the Herbert Shire and Dalbeg in the upper Burdekin. All trials were conducted in commercial sugarcane farms and followed the best crop management practices. A genetically diverse population (131 genotypes) comprising *S. officinarum* crosses with wild relatives, commercial cultivars including foreign clones, advanced lines in the selection program and some parental clones in the BSES breeding program was used as test clones. Clone performance was evaluated under rainfed, irrigated and managed drought conditions adopting most appropriate statistical field designs for the respective sites. The agronomic and cane yield characteristics, fibre, sugar and sugar quality characteristics and physiological traits related to drought tolerance were collected during the crop growth period and at harvest. Appropriate statistical methodologies were used to analyse and interpret the results at the end of the project.

The water environments of test sites represented the types of drought environments occurring in the respective cane growing region and were able to quantify the responsive cane and sugar yield variation among test lines. The cane yield reduction due to imposed drought conditions ranged from 17 to 52%, depending on the severity of drought, in three crop classes over 3 years. Strong clonal variations and clone-by-environment interaction for biomass yield, cane yield, yield components and sugar yield were recorded under severe water stress condition. The drought response of QCANES was poor for most of the agronomic characters within the cultivars than the clones derived from crosses with wild relatives. Because of the strong clone-by-environment interaction, response of most of clones changed substantially under severe stress condition, suggesting that more than one test environment is needed for efficient screening of population for production regions experiencing different levels of water stress. The crosses with wild relatives showed relatively high yield stability across the range of drought environments than the other clones including breeding lines that are currently being used for cultivar development.

Among the physiological parameters studied, stomatal conductance and relative water content (RWC) of leaf tissue are proving to be good indicators of stress response under field condition. They are showing potential to be used as surrogate traits for identifying clones that are drought tolerance and possibly water-efficient. Both these traits showed strong repeatability across test environments and also across crop classes, and are relatively easy

to measure. However, quantitative genetic analysis revealed that the genetic correlations between physiological traits and yield reduction in the test population under varying test environments are moderate. Considering that, based on the available data, yield reduction in drought condition relative to well-watered condition could be a reliable indicator for drought tolerance.

The analysis of both physiological and yield data showed complex interactions between type of stress, response of traits to stress, trait effects on growth and yield and other environmental parameters. As an example conservative stomatal and high WUE may help in some environments but can be detrimental in others. Similarly deep root system and green leaf retention affect water use over time but that may not be desirable in a production condition with limited water availability unless the cultivar has the ability to control water use through conserved stomatal behaviour. The physiological traits identified in this project are proving useful for characterising clone behaviour under different stress environments to identify desirable trait combinations and genotypes (ideotypes) suitable for different production conditions, which is the focus of the second phase of this research. The data generated also helped develop a theoretical framework to study the mechanistic basis of observed clone responses to varying stress conditions and to develop models to simulate target production environments (both water-limited and fully irrigated) and the traits needed for achieving high productivity in those environments. These aspects research are now continuing in the current project, BSS0334. In brief, all the findings of this study including field observations suggest that a “whole of crop system” view is needed to make any meaningful advancements in this area of research, especially for crop improvement purpose.

1.0 BACKGROUND

Water availability is the most important determinant to crop productivity worldwide (Passioura, 2002). In rain-fed regions productivity can be severely impacted by water stress, and nearly 40% of the Australian sugar industry is in rain-fed condition. With increasing regulations on agricultural use of water, even in irrigated regions innovative solutions are needed to increase or at least maintain crop productivity. There is also increasing pressure to reduce water use for environmental reasons. Significant improvements in drought tolerance and water use efficiency have been made in cereals and legumes and the experience and lessons learned in those crops could be applied to other crops, including sugarcane (Hochman et al. 2009). Large variation in traits conferring or linked to drought tolerance and WUE has been reported in many crops and plant species (Dodig et al. 2010). Plants utilize different traits and strategies to cope with abiotic stresses, but each species evolved its own unique combination of traits to adapt to water-limited environments. Therefore, the challenge for this project is to determine whether there is scope for large gains in drought resistance and WUE in sugarcane, and if so, how to achieve that outcome.

The two essential requirements for crop improvement programs are substantial genetic variation and broad germplasm for the desired traits. The limited literature indicate the existence of genetic variability for WUE and DT in sugarcane (De Silva et al 2007), however it has not been quantified within the Australian sugarcane germplasm. Much of the germplasm characterisation has been done in India, South Africa and Brazil but the morpho-physiological features and genetic elements that confer those traits remain unknown. This project, therefore, aims to break new grounds in this area of research by using a broad-based sugarcane germplasm, including the introgression populations, to unravel the genetic and morpho-physiological determinants of drought tolerance and WUE, which is critical for developing selection systems for cultivar improvement.

This project complements other sugarcane physiology projects such as CSE014, and *Save*N Cane (UQ044) and thus expands the physiological and genetic understanding of traits related to water relations, carbon fixation, biomass partitioning and nitrogen use efficiency.

2.0 OBJECTIVES

The long-term objective of this research is to reduce the impact of recurring drought and the rising cost of water through the development of water use-efficient and drought tolerant sugarcane varieties.

The specific objectives of this project, the first phase of the research, are:

1. Assess the potential of Australian sugarcane germplasm for developing water use-efficient and/or drought tolerant commercially useful varieties
2. Establish a knowledge base and quantitative data of morpho-physiological and genetic traits associated with or conferring drought tolerance and WUE in the Australian germplasm
3. Identify clones with desired WUE and drought tolerance characteristics in the test population for direct commercial deployment and breeding
4. Establish promising populations/clones for the follow up project: a) to develop a rapid screening method to select water use-efficient and drought tolerant cultivars; b) to test the breeding value of selected clones for cultivar development; and c) to implement the developed screening method in the breeding program

For a quick reference, achievements of these Objectives are briefly outlined below.

Objective 1 - Assess the potential of Australian sugarcane germplasm for developing water use-efficient, drought tolerant commercially useful cultivars
A broad genetically diverse germplasm (131 clones) including commercial cultivars, crosses with wild relatives, imported cultivars known to be drought tolerant elsewhere and some promising parental lines were field-tested and ranked them according to their capacity to maintain crop productivity [tonnes of cane per hectare (TCH) and commercial cane sugar (CCS) content] under well-watered and water stress conditions in three different commercial sugarcane production locations. Significant genetic variation for drought tolerance was found in the test population.

Objective 2 - Establish a knowledge base and quantitative data of morpho-physiological and genetic traits associated with or conferring DT and WUE in the Australian germplasm
Important physiological parameters related to WUE and drought tolerance such as stomatal conductance (*gs*), canopy temperature (CT) and relative water content (RWC) were investigated. Substantial genetic variation for *gs*, CT and RWC were identified. Quantitative analyses showed that *gs* and RWC were genetically associated with the reduction in TCH. Clone responses to transpiration efficiency under variable moisture environments (glasshouse) are being investigated as part of the second phase of the project (BSS0334). Mechanistic basis of physiological traits controlling yield in different genotypes under water-limited conditions needs to be understood.

Objective 3 - Identify elite clones with desired WUE and drought tolerance characteristics in the test population for direct commercial deployment and breeding
As noted in the Objective 1 significant variation in crop productivity was observed in the test populations under both well-watered and water stress conditions. Some of the un-selected clones (crosses with wild relatives) performed better than the selected high TCH clones under severe drought environments. Since the drought tolerant un-selected clones are low TCH clones even under non-stress conditions they were not suitable for commercial deployment. However, the breeding values of these selected clones will be assessed in the second phase of the project. Some QCANES and selected advanced clones also had desirable WUE traits but their potential as parental lines needs validation.

Objective 4 - Establish promising populations/clones for the follow up project: a) to develop a rapid screening method to select water use-efficient and drought tolerant cultivars; b) to test the breeding value of selected clones for cultivar development; and c) to implement the developed screening method in the breeding program
Based on the data obtained in this project a theoretical framework to determine possible trait combinations that could assist identifying clones for different production environments has been developed. The test environments used in the clone evaluation represent a range of target production environments in different sugarcane growing regions (Home Hill, Dalbeg and Crystal Creek).

Based on the theoretical framework a set of clones (about 20) with better adaptive traits to different water stress environments was identified. Further testing of these clones in target production environments will be continued in the second phase of this project.

The project achieved all of the objectives as outlined in the proposal.

3.0 METHODOLOGY

3.1 Assessment of genetic variation for WUE and drought tolerance in the Australian sugarcane germplasm

3.1.1 Research methodology

In order to capture maximum genetic diversity in the test population a broad-based germplasm (for details see the section Test Clones) consisting of selected parental clones from the breeding program, F1 and backcross generations of sugarcane introgression clones (include crosses of commercial clones with wild relatives), foreign clones and QCANES was selected for this research. The test population was evaluated in water-limited, rainfed and well-watered field experiments in Burdekin and Herbert regions (for details of test site see the section Field Experiments and experimental designs) designed to maximise expression of genetic variation in drought tolerance and WUE. Various morpho-physiological traits (for details see the section The Measurements) potentially relating to drought tolerance were measured to understand the mechanisms contributing to genetic variation and to identify potential surrogate traits for efficiently selecting cultivars for different production environments. Soil water status in experimental area was monitored using Enviro-scan and Time Domain Reflectometry (TDR) system, and transpiration efficiency (TE) and WUE of selected clones was determined using pot trials in glasshouse conditions.

An outline of the activity of project covering plant crop to ratoon 2 is presented below. Details of experiments and methodology are presented in the subsequent sections.

Year 1 (2007-2008) - Plant Crop 1 (PC1)
The selected 131 genotypes representing commercial clones and clones obtained from crosses between commercial clones and exotic germplasm (*Erianthus* spp. and *S. spontaneum*) were grown in experimental sites in Home Hill (Burdekin) and Crystal Creek (Herbert), commencing from July 2007. There were test clones grown under full field capacity irrigation (1 ML per irrigation and time of irrigation determined by the “water sense” simulation for the area) for control, no irrigation during the early growth period for stress treatment and fully rainfed conditions. Clones were evaluated for stem and leaf growth, canopy temperature, stomatal conductance (gs), relative water content (RWC) and morphological and agronomic parameters. After 12 months the crop was harvested for cane and sugar yield measurements. The first year data was used to identify a sub-set of clones to be planted in 2008 propagation plots for the experiments in Dalbeg in 2009.

Year 2 (2008-2009) - First ratoon crops of PC1
Characters evaluated in PC1 were reassessed in R1 crops.
In addition, 40 clones (Table 3) with contrasting responses in the first year trial were replanted in propagation plots for 2009 experiment. The treatments were repeated as did in plant crop to confirm the performance of clones across a wider range of water environments.

At the end of 12-month crop cycle, the R1 crops were harvested for cane and sugar yield measurements.

The data from PC1 and R1 trials were analysed together to be able to validate the consistency of clones' performance and provide more insight into their response to stress conditions. A sub set of 10 clones was grown in a glasshouse for transpiration efficiency and water use measurements. The most promising 5 clones from these experiments were used for detailed physiological characterisation in a special student project at the Queensland University of Technology (QUT).

Year 3 (2009-2010) - Second ratoon crops of PC1 (R2)
The R2 crops were established after harvesting the respective R1 crops.
In the third year of growth, control clones in the irrigation treatment were continued receiving irrigation and those subjected to water deficit conditions were maintained without any irrigation.

This was taken the trial closer to maximum stress imposition and consequently maximum expression of water stress tolerance. The R2 crops were evaluated with a similar range of measurements made as in year 1 and 2. In addition, 40 clones (Table 3) with contrasting responses to drought in the PC1 and R1 were planted in a trial (PC2) with three levels of water treatments in 2009. No irrigation, 50% irrigation and fully irrigation treatments were applied to generate a series of moisture environments to test the performance of those 40 selected clones. A sub set of 10 clones was identified within this trial (with the information on contrasting pattern of gs, RWC and yield reduction in PC1 and R1) and detailed moisture extraction was investigated with the access tubes and Neutron Moisture Meter (NMM). The PC2 crops were evaluated with a similar range of measurements made as in year 1, 2 and 3.

3.1.1.1 Field experiments and trial designs

Locations and treatments
Two field experiments were established on 17 and 29 June 2007 at two locations with contrasting environments and soil characteristics. One was in Home Hill (HH) in Burdekin Shire (147^{o}23'E, 19^{o}41'S) and the other was in Crystal Creek (CC) in Herbert Shire (146^{o}18'E, 18^{o}59'S).

Average annual rainfall at HH site is 840 mm and irrigation is available throughout the crop season. The soil type is brown dermosol (Australian Soils Classification) with light clay in texture. The organic carbon content is 0.88% and the soil pH ranges from 6.5 to 7.3. The HH experiment was arranged in a randomized split plot design with water as the main treatment and 89 test clones as the sub-treatment. There were two blocks and two water treatments; full irrigation (control) and no irrigation (water stress) from 3 months after planting. The water-limited treatment was irrigated until 17 September 2007 and allowed to grow under rainfed conditions. There were two replications for each treatment. The plant (PC1), ratoon 1 (R1) and ratoon 2 (R2) were maintained for three years from 2007-2010 and each crop was harvested on 17 June in each year. The irrigation system was designed to impose water limitation at any time of the crop growth. Planting in June allowed imposing an early water stress treatment in September-December and late water stress treatment in April - July in each year.

The annual rainfall at CC in Herbert is 1600 mm and there was no irrigation available in that production area. Most of the rain is received in December-March period. The soil type is sandy loam with organic carbon content of 0.71% and the soil pH ranges from 4.7 (1:5, CaCl2-based) to 5.4 (1:5, water-based). The sugarcane is grown under fully rain-fed conditions and growers experience crop productivity loss due to low rainfall. The experimental design at CC is a randomized block design with 2 replications. In total 99 clones were planted in each replication. Plots of both HH and CC experiments were 10 m long with 4 rows (1.6 m row spacing) and the data were collected from the two middle rows.

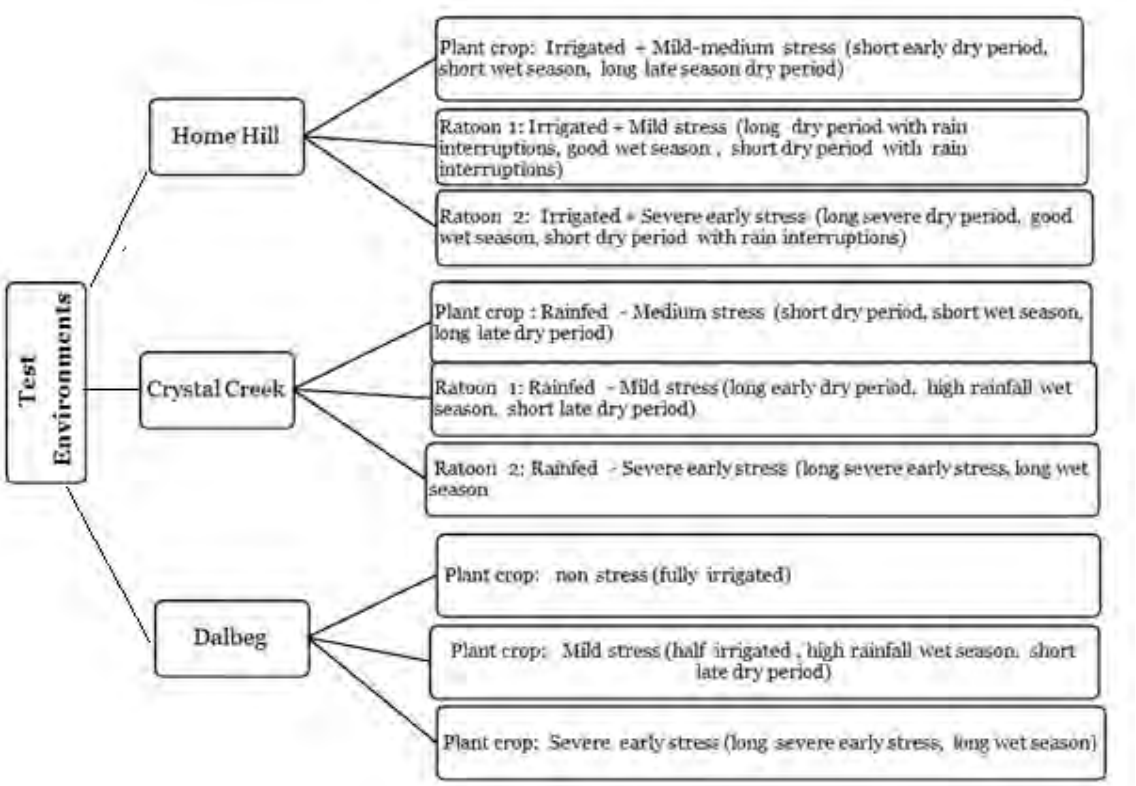

Figure 1 - The twelve test environments at Home Hill, Crystal Creek and Dalbeg with varying levels of water availability during the twelve month crop cycles. Fully irrigated control treatment was included in each crop year at Home Hill and Dalbeg. Crystal Creek is a rainfed production area.

A third field experiment was established on 29 April 2009 at Dalbeg, upper Burdekin Shire (147°17'E, 26°.53'E 58M elevation) with different environments and soil characteristics to the HH and CC sites. Average annual rainfall at Dalbeg is 650 mm and irrigation is available all through the crop season. The soil type is sandy loam (Australian soil classification) with light clay in texture. The organic carbon content is 0.65% and the soil pH ranges from 6.1 to 6.9. The experiment was arranged in a randomized split plot design with water as the main treatment and 40 test clones as the sub-treatment. There were three blocks and three water treatments; full irrigation (control), half-irrigation and no irrigation (water stress) from 3 months after planting.

Between the three field experiments there were 12 test environments (Figure 1). These experiments were managed with timely weed control and adequate fertilizer application based on soil test results. The plots in both experiments were harvested and plot weight was recorded with an electronic balance attached to the weigh bin with an accuracy of ± 0.5kg.

3.1.1.2 Test clones

A total of 131 clones (Table 1) were tested in 12 test environments at 3 locations. There were 42 common clones across HH and CC locations. The plant material was grown in propagation plots in HH and CC location in 2007 and Dalbeg in 2008. These clones were cold-soaked and hot water treated at 50°C for 4 hours and soaked in fungicide to avoid any

contamination of ratoon stunting disease (RSD). The test clones represented a wider genetic background of sugarcane. They were from inter-specific and inter-generic crosses such as *S. spontaneum* × *S. officinarum, Erianthus* × *S. officinarum*, commercial varieties, parental clones and foreign introductions. The details of the genetic diversity of test population are shown in Table 2.

Table 1 - The genetic diversity of sugarcane test population used for the evaluation of drought tolerance and the identification of traits that confer drought adaptation in sugarcane

Genetic background of the test lines	No. of lines tested
F1 *Erianthus arundinaceus* × Commercial varieties	5
BC1 *Saccharum spontaneum* × Commercial varieties	1
Saccharum officinarum × *Erianthus rockii*	1
S. officinarum × *E. arundinaceus*	1
S. officinarum × *S. spontaneum*	4
Commercial varieties × *E. arundinaceus*	6
Commercial varieties × *S. spontaneum*	3
Commercial varieties × F1 *S. spontaneum*	24
S. spontaneum BC1	3
Commercial varieties	25
Parental lines (breeding program)	17

Table 2 - The test population of 131 clones used for the field experiments in three locations and their genetic backgrounds

CLONE	TYPE	CLONE	TYPE	CLONE	TYPE	CLONE	TYPE
	ERIANTHUS ARUNDINACEUS CROSSES	**CT04-250**	COMMERCIAL X F1 SPONTANEUM		**NEAR COMMERCIAL**		**COMMERCIAL**
CT04-33	COMMERCIAL X ERIANTHUS ARUNDINACEUS	**CT04-258**	COMMERCIAL X F1 SPONTANEUM	**QN91-3028**	MQ75-932 X QN66-2008	**Q200**	COMMERCIAL
CT04-40	COMMERCIAL X ERIANTHUS ARUNDINACEUS	**CT04-269**	COMMERCIAL X F1 SPONTANEUM	**QB01-18**	POJ2878XMANDALAY	**Q207**	COMMERCIAL
CT04-50	COMMERCIAL X ERIANTHUS ARUNDINACEUS	**CT04-290**	COMMERCIAL X F1 SPONTANEUM	**QB01-21**	POJ2878XMANDALAY	**Q208**	COMMERCIAL
CT04-58	COMMERCIAL X ERIANTHUS ARUNDINACEUS	**CT05-563**	COMMERCIAL X F1 SPONTANEUM	**QB01-3**	POJ2878XMANDALAY	**Q209**	COMMERCIAL
CT04-61	COMMERCIAL X ERIANTHUS ARUNDINACEUS	**CT05-570**	COMMERCIAL X F1 SPONTANEUM	**QB01-38**	POJ2878XMANDALAY	**Q219**	COMMERCIAL
CT04-69	COMMERCIAL X ERIANTHUS ARUNDINACEUS	**CT05-582**	COMMERCIAL X F1 SPONTANEUM	**QB01-5**	POJ2878XMANDALAY	**Q220**	COMMERCIAL
CT04-116	F1 ERIANTHUS ARUNDINACEUS X COMMERCI	**CT05-583**	COMMERCIAL X F1 SPONTANEUM	**KQ99-3265**	Q117 x CP74-2005	**Q229**	COMMERCIAL
CT04-119	F1 ERIANTHUS ARUNDINACEUS X COMMERCI	**CT05-594**	COMMERCIAL X F1 SPONTANEUM	**QC93-1647**	Q117 X Q135	**Q231**	COMMERCIAL
CT04-120	F1 ERIANTHUS ARUNDINACEUS X COMMERCI	**CT05-605**	COMMERCIAL X F1 SPONTANEUM	**MQ93-538**	Q96 X MQ77-340	**N29**	COMMERCIAL
CT04-28	F1 ERIANTHUS ARUNDINACEUS X COMMERCI	**CT05-608**	COMMERCIAL X F1 SPONTANEUM	**QS95-6004**	QC90-6006 QC80-203	**H56-752**	COMMERCIAL
CT04-30	F1 ERIANTHUS ARUNDINACEUS X COMMERCI	**CT05-626**	COMMERCIAL X F1 SPONTANEUM	**QN83-1072**	QN68-1797 X QN77-374	**KQ228**	COMMERCIAL
CT04-83	S.OFFICINARUM X ERIANTHUS ARUNDINACEU	**CT05-632**	COMMERCIAL X F1 SPONTANEUM	**QC93-1863**	QN80-3425 X Q162	**MQ77-340**	COMMERCIAL
		CT05-645	COMMERCIAL X F1 SPONTANEUM	**QN92-157**	QN82-1241 X QN77-409	**NCO-310**	COMMERCIAL
	OFFICINARUM CROSSES	**CT05-646**	COMMERCIAL X F1 SPONTANEUM	**QC91-580**	QN83-636 X Q142	**QS85-7325**	COMMERCIAL
CT04-559	S.OFFICINARUM X SPONTANEUM	**CT05-681**	COMMERCIAL X F1 SPONTANEUM			**QN66-2008**	COMMERCIAL
CT04-577	S.OFFICINARUM X SPONTANEUM	**CT05-703**	COMMERCIAL X F1 SPONTANEUM		**COMMERCIAL**	**QN80-3425**	COMMERCIAL
CT04-845	S.OFFICINARUM X SPONTANEUM	**CT05-706**	COMMERCIAL X F1 SPONTANEUM	**Q117**	COMMERCIAL	**R570**	COMMERCIAL
CT04-951	S.OFFICINARUM X SPONTANEUM	**CT05-735**	COMMERCIAL X F1 SPONTANEUM	**Q119**	COMMERCIAL	**SP85-3877**	COMMERCIAL
CT04-99	S.OFFICINARUM X E.ROCKII	**CT05-753**	COMMERCIAL X F1 SPONTANEUM	**Q124**	COMMERCIAL	**TELLUS**	COMMERCIAL
	SPONTANEUM CROSSES	**CT05-827**	COMMERCIAL X F1 SPONTANEUM	**Q138**	COMMERCIAL		
CT04-303	SPONTANEUM BC1	**CT05-830**	COMMERCIAL X F1 SPONTANEUM	**Q142**	COMMERCIAL		
CT04-305	SPONTANEUM BC1	**CT05-851**	COMMERCIAL X F1 SPONTANEUM	**Q157**	COMMERCIAL		
CT04-309	SPONTANEUM BC1	**CT05-853**	COMMERCIAL X F1 SPONTANEUM	**Q158**	COMMERCIAL		
		CT04-450	COMMERCIAL X S. SPONTANEUM	**Q171**	COMMERCIAL		
CT04-166	BC1 SPONTANEUM X COMMERCIAL	**CT04-495**	COMMERCIAL X S. SPONTANEUM	**Q183**	COMMERCIAL		
		CT05-199	COMMERCIAL X S. SPONTANEUM	**Q190**	COMMERCIAL		

3.1.1.3 The measurements

Morphological and agronomic measurements
The crop establishment (% germination and gaps in the rows), leaf development, tillering, stalk length, stalk diameter, millable stalk number, plot yield (weight of millable stalk per plot; cane harvested from 2 x 10 m long inner rows from each plot), total green leaf number at harvest, Brix, pol and fibre % and days to maturation were measured as described previously (Robertson et al. 1996). Three months after planting or ratooning, the total shoot count was taken from the middle two rows and the gaps (space >60 cm without shoots) were recorded for the final yield adjustments. The cane was harvested at physiological maturity when the total soluble solid content (Brix %) of the standard clone Q208$^{\phi}$ (a popular commercial variety) reached 20-24 %, which is usually about 11-12 months after planting. A sub-sample of 8 stalks were collected randomly from the middle two rows of each plot before harvesting and used to measure the agronomic and sugar attributes. Middle two rows were then machine-harvested and plot yields were recorded. The plot yields of each crop class were adjusted to the respective gaps and used in the statistical analysis.

Sugar and fibre analyses
Brix, pol or sucrose percentage, fibre content and purity were assessed from the sub-sample of 8 stalks at harvest (Laboratory Manual, BSES, Vol 4. 1984). Formulae developed for commercial cane sugar (CCS) calculation was used to measure sugar yield (Laboratory Manual, BSES, Vol 4. 1984). Sugar yield (tonnes of sugar per hectare; TSH) was calculated using the product of cane yield and CCS. Juice Brix refers to the total soluble solid content present in juice and expressed as a percentage. Brix includes sugars as well as non-sugars. The juice sucrose per cent is the actual cane sugar present in juice measured using polarimeter. The sucrose per cent is also referred to as pol per cent. For all practical purposes pol % and sucrose % are synonyms. The juice purity coefficient refers to the percentage of sucrose present in the total solids content in juice. A higher purity indicates the presence of higher sucrose content out of the total solids present in juice. The purity percentage along with sucrose percentage aids in determining maturity time. The CCS refers to the total recoverable sugar precent in cane.

Soil moisture status
The probes of automated soil moisture monitoring system (EnviroSCAN®) were installed in each plot of Q208$^{\phi}$ in each replicate of the water treatments at HH experiments. The sensors were placed at 10, 20, 50, 80, 100, 150, 180 and 200 cm depths and connected to a Campbell CRX10 data logger via an SDI 12 interface. The software program was uploaded to the interface to enable automated data collection from the sensors every 15 minutes and transmit the hourly average to the main data logger. The soil moisture data was downloaded from the logger using a computer interface. The sensors were not calibrated for each soil type and depth, but normalized to the air and water as a standard practice. The soil moisture profiles were used to characterize the soil water environments across water treatments in the three crop classes.

Physiological measurements
A series of physiological measurements on stomatal conductance (Grantz and Moore, 1987), canopy temperature (Blum et al, 1989), leaf relative water content (Barrs and Weatherley, 1962), and leaf elongation were measured. The temporal soil moisture variation was monitored using four EnviroSCANS® installed in each treatment within each block where Q208$^{\phi}$ is planted. These moisture measurements were used to characterize the water environments at HH.

Stomatal conductance
Abaxial stomatal conductance (gs) was measured between 10:00 and 14:00 h on three youngest fully expanded leaves per plot using a leaf porometer (SC-1 Decagon Devives Inc., USA) according to manufacturer's instructions. The PC1 measurements were recorded approximately fortnightly during the drought treatments from mid-September to mid-December. More intensive measurements from PC from Dalbeg and RI, and R2 in all locations were collected from irrigated, semi-irrigated, water-limited and rainfed treatments at weekly intervals.

Canopy temperature
Canopy temperature (CT) easured just above the leaf canopy of every plot in each treatment using the infra-red thermometer (Mikron-Mi-N15, Mikron Infrared Inc. USA) according to manufacturer's instructions. Measurements were made by keeping the Thermometer 2 m away from the canopy. A total of four measurements were recorded from each plot with two from either side of the plot orientation. However, CT was not considered as a useful observation for DT based on the PC1 results and the consultative group recommendation (please refer Milestone report 5, Achievement Criterian 2).

Leaf relative water content (RWC)
The leaf relative water content was measured at the end of the drought period in PC1 simultaneously with *gs* measurements during the early growth of in all crop classes. The method described by Barrs and Weatherley (1962) was used to estimate RWC under drought and irrigated conditions.

$$RWC = \left[\frac{(Fresh\,weight - Dry\,weight)}{(Turgid\,weight - Dry\,weight)} \right] \times 100 \qquad (Equ1)$$

Leaf elongation
The leaf elongation was measured from 40 clones in two treatments as described by Inman-Bamber (Inman-Bamber, 1995). The youngest fully expanded leaf (YFEL) was used to measure leaf growth. The spindle and the YFEL were held together and the length of the spindle tip was marked on the YFEL. Three samples were taken from each plot at the time of measurements. The expansion of the spindles were measured daily from day 1. The rate of development was estimated by using the total expansion and the days taken to complete the total expansion. Comparison was made between treatments and clones.

3.1.1.1 Transpiration efficiency (TE) in sugarcane under glasshouse conditions

Background
The field experiments results indicated that there is a wide variation among the test clones for drought adaptation. A considerable G×E interaction for cane yield, total biomass and physiological measurements (*gs* and RWC) was observed in these experiments. This indicates a varying potential in the test clones to respond to water-limited conditions under field environment. The total biomass production is highly correlated with the transpiration efficiency (TE) of the crops and it is being used in crop modelling to predict the productivity of many crops under varying soil moisture environments. Thus, the important factor for contrasting drought responses among the test clones could potentially be the variation in TE under well-watered and water-limited conditions. It needs quite extensive experimentation protocol to quantify TE under field conditions as there are uncontrollable environmental variables affecting crop TE under field conditions. Thus, quantifying TE in the number of clones under controlled environment condition would be more practical and less affected by environmental variables. A pot experiment with 10 clones was designed to determine the variation in biomass production under variable moisture conditions and quantify the GxE for water use and TE under glasshouse conditions.

Objectives

The objectives of this study are to test the genetic variation for TE among selected set of sugarcane clones and quantify the GxE for TE under glasshouse conditions.

Materials and methods

Glasshouse conditions

In order to provide accurate irrigation and controlled rainfall, this experiment was conducted under glasshouse conditions. The glasshouse temperature was maintained at 27 ± 2^0C. Automated ventilation was effective to remove accumulated hot air through ceiling shutters during mid-day. As there were no measures to control humidity, there was a possibility of experiencing mid-day depression of humidity with temperature increases during experimentation (January – May, 2009). Irrigation water was available throughout the experiment. North-south pot orientation provided uniformity of lighting during the day.

Test clones

Based on the previous field data, ten clones were selected for the experiment. They were Q183$^{\Phi}$, QC91-580, Q190$^{\Phi}$, KQ228$^{\Phi}$, CT04-69, QB01-5, CT04-951, CT05-753, CT05-645 and Q208$^{\Phi}$. These clones were included in the field trial at Dalbeg. Moisture extraction of these clones in the field was measured using the neutron moisture meter (NMM) through access tubes installed in the plots. The *gs*, RWC and drought responses in terms of biomass production were quite contrasting among these clones under experimental conditions in Dalbeg.

Planting and pot preparation.

The hot water treated planting materials were collected from the propagation plots in BSES, Brandon and single-eye setts were planted in trays containing a commercial potting mixture and incubated in the germinator at 38^{0}C. Nine pots of 45 - 35 cm in diameter (top and the base, respectively) and 35 cm in depth were filled with 25 kg potting mixture containing peat moss (50%) and sand (50%). Uniform soil compaction was obtained by applying weight to each pot. The top 5 cm of pots were not filled. The pots had a number of holes at the base to drain excess water freely after first irrigation. For uniform initial establishment, adequate water and nutrients were provided to all the pots. During water treatments, the pots used for dry treatment were watered below the field capacity. Five soil samples (500g) were collected to determine the initial gravimetric moisture content at the time of potting.

Out of the 9 pots (replicates) maintained for clone, three pots were used for the early biomass harvest at the time of water treatment. The other pots were maintained for the two water treatments in the glasshouse. A sub-set of 3 pots without plants were used to monitor moisture variation through-out the experiments. To avoid evaporation, the soil surface was covered with 5 cm thick layer of white PVC plastic beads after commencement of water treatments (Figure 2). The pots were weighed several times until it reached a constant weight. Once the drainage was completed, the holes around the base were sealed with plastic rivets to prevent losing any water from the base. The pot weight was recorded at the saturation/upper limit and the quantity of water available at the full field capacity was determined. At this point, the moisture content of those 3 pots was measured using the TDR and average moisture content was determined. The moisture content at saturation was considered as the approximate upper limit, the full field capacity (FC). Based on the dry soil weight in each plot, the amount of water required to reach the 100% and 50% field capacity for the soil was estimated.

After 3 weeks the pots were arranged in the split plot design for water treatments. Clones were randomly allocated within each water treatment. There were three replicates (3 pots/clone), completely randomized, for each treatment. The moisture treatment was applied when the plants were approximately at 7-8 leaf stage. The moisture content of the soil was measured in the dry treatment until it reached to 50% moisture content.

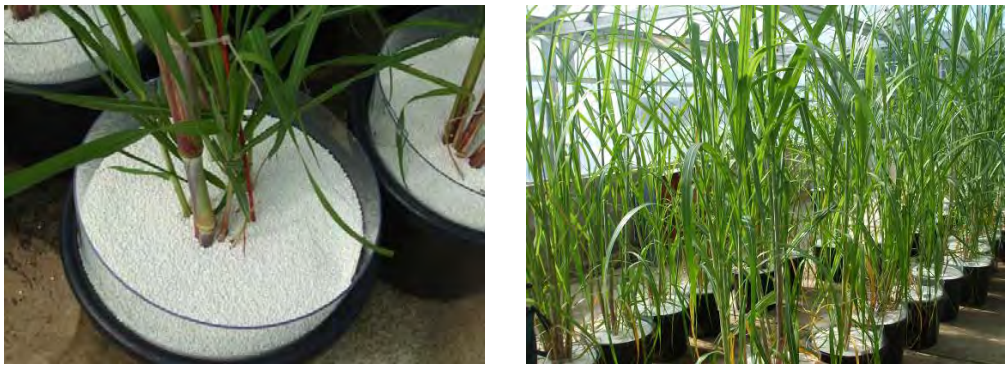

Figure 2 - Pot experiment to measure the transpiration efficiency in selected sugarcane clones under glasshouse conditions. A thick layer of PVC beads stopped evaporation losses from the pots.

The treatments were commenced when the last pot of the dry treatment reached the deficit below or equal to 50% FC. The required amount of water (as per calculation for FC) was added to the wet and 50% FC treatments. The moisture content of each pot was measured manually (using TDR) daily between 9-10am. As watering was done once a day, in each pot, there was approximately 5- 10% water deficit (based on FC) in the wet treatment and 50-60% deficit (based on FC) in the dry treatment. Water was injected into the soil with a dispenser attached to a funnel to avoid and surface evaporation following watering. At the end of the experiment the total water used for each pot was calculated. This was used to estimate the TE of each clone.

Other measurements.
Photosynthesis (using Licor 6400), stomatal conductance (using porometer) and RWC were measured on the youngest fully expanded leaf. Most of the measurements were taken during 9 am-12 noon. Hourly photosynthesis, *gs* and RWC were recorded during cloudless sunny days.

Biomass harvest
There were nine pots for each clone for biomass assay. Six were used for the two (wet and dry) treatments and the other three pots were harvested at the commencement of water stress treatment. Plants in these three pots were used to estimate the initial biomass of each clone at the commencement of the water stress treatments. All leaf, stem and root biomass were taken into account. At the end of the treatment, the biomass was partitioned into leaf, stem and root and the weights were recorded separately. The total biomass was used to calculate the TE of each clone. The quantity of water added to each pot was calculated at end of the treatment. The TE during 5-month period of growth was estimated as;
TE = Total biomass (g)/total water used = gram BM produced/ Kg water used.

Statistical analysis
Appropriate statistical and genetic analyses were conducted for the observations with the data adjusted to special variations. Treatment, clone and treatment-by-clone interactions were statistically tested for each crop class. More description on statistical analyses are given in respective sections.

4.0 RESULTS

4.1 Environment characterization

4.1.1 Rainfall and soil moisture profiles at Home Hill experiment

The pattern of rainfall and the soil moisture availability at different soil layers in three crops at Home Hill are shown in Figure 3. Rainfall distribution was highly variable during the three years of experimentation. The PC1 had a short dry period at the early vegetative phase and a long dry period during the late maturation period. In contrast, both R1 and R2 crops had long wet period during the late maturation period. There were rain interruptions during the early dry period of R1 crop whereas R2 crop had little or no rain during the early vegetative period.

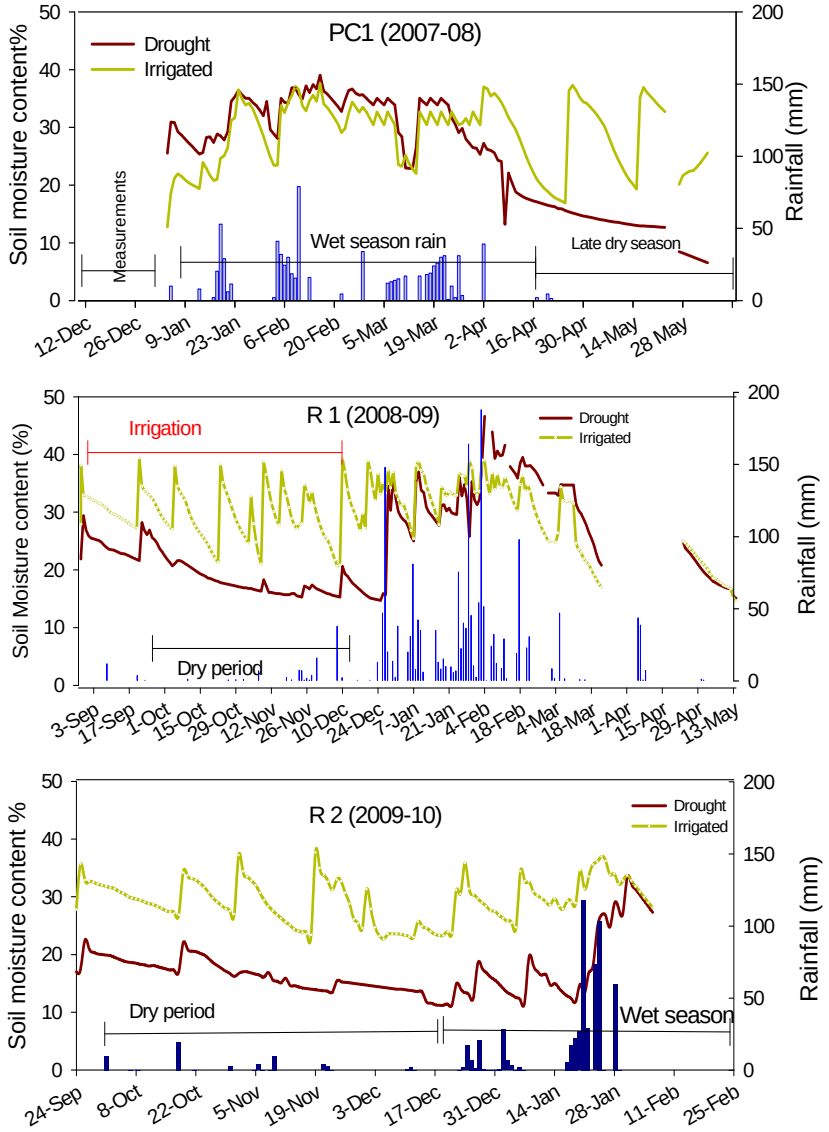

Figure 3 - Rainfall (mm) and average soil moisture profile at 80 cm depth in irrigated and drought treatments of three crop classes at Home Hill

The soil moisture status during the crop growth period showed that there were differences in soil moisture between the irrigated and drought treatments in 3 crop classes at HH (Figure 3). Similarly, there was a distinct variation in moisture deficit during the imposed stress periods in the drought treatments of the three crop classes. The water stress during early vegetative phase was severe in R2 (2009) than PC1 (2007) and R1 (2008) crops. The soil moisture variation across water treatments and crop classes suggested that a range of moisture environments and consequently severity of stress were experienced during drought period.

The moisture content at different depths were measured in early October to late December in each year when the stress conditions in the water stress treatment were mild to severe (Figure 4). Measurements indicated a significant water extraction at 50 and 80 cm depths in both well-watered and WS treatments with more pronounced effect on the latter. Some extraction also occurred at 200 cm depth in the water stress treatment. Monsoonal rains commenced in the middle of December and continued till March to April.

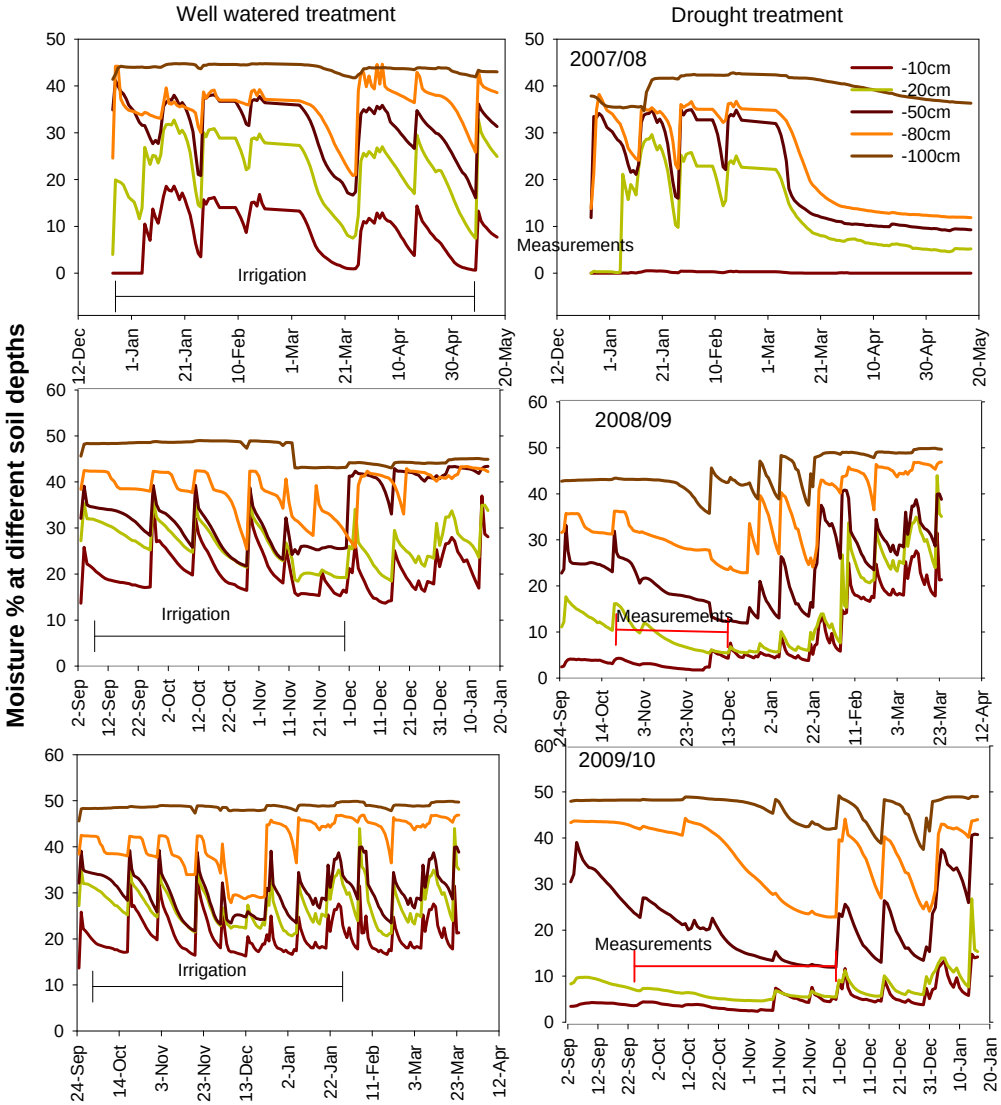

Figure 4 - The soil moisture variation in different soil profiles of root depths in irrigated and drought treatments in 3 crop classes at Home Hill

The rainfall pattern and soil moisture variations in R2 crop were significantly different from the plant (PC) and R1 crop. There was a prolonged drought period from early September 2009 to early January 2010. The wet season started from January and had 871 mm rainfall from January to June 2010. During the early dry season (Sept 2009 to Dec 2009) most of the clones suffered a severe water stress (Figure 4) and started to recover during the wet season in Jan-May 2010. The stress environment in R2 can be defined as a prolonged un-interrupted early drought period followed by highly saturated wet season.

4.1.2 Rainfall and soil moisture profiles at Crystal Creek

The rainfed environment at CC received relatively high rainfall than HH in high rainfall years. The monthly and the cumulative rain fall for the three crop years are presented in the Figure 5. As the soil profile was less than 0.9 m, the soil moisture characterization using EnviroSCAN was not carried out in the CC trial. However, in order to establish the pattern of moisture variation during the stress period, regular moisture measurements were collected using the TDR minitraser.

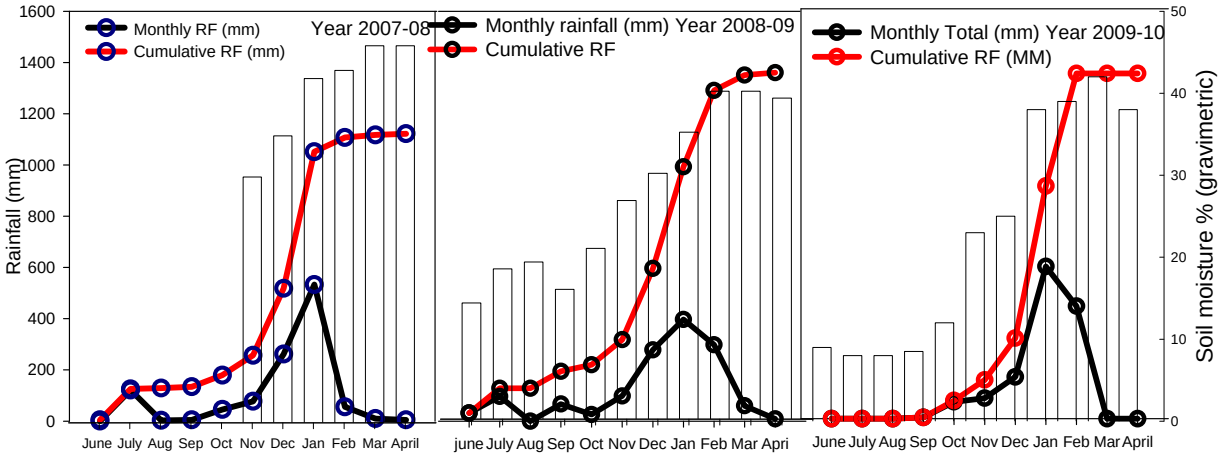

Figure 5 - Monthly rainfall distributions and the cumulative rainfall in 3 years at Crystal Creek. Vertical bars indicated the average gravimetric soil moisture content at 60cm depth measured from TDR.

The PC1 and R1 crops received some rainfall during the early phase (June- December) of growth and had fairly good crop establishment. The drought had been frequently interrupted by the occasional rain during this period. The R2 crop received no rainfall during July to September and resulted in severe setback on growth in most of the test clones. The drought in R2 was considered as a severe stress environment for growth.

4.1.3 Rainfall and soil moisture profiles at Dalbeg.

The pattern of rainfall and the soil moisture variation at different soil layers in drought and irrigated treatments are shown in Figure 6. Rainfall during the early period of growth was extremely low (May-November, 108 mm) and the crop was badly suffered from water stress. The wet season started in early December and most of the rainfall was during December to April 2010. The drought treatment in PC had an uninterrupted long dry period at the early vegetative phase and a long wet period during the late maturation period. In the same experimental area, both semi-irrigated and irrigated treatments had irrigation during the early vegetative phase and rainfall during the late maturation period.

The soil moisture contents during the crop growth period showed that there were different levels of moisture status between the irrigated and drought treatments in the PC (Figure 6). The water stress during early vegetative phase was severe in the drought treatment of PC (Dalbeg) than PC and R1 crop at HH.

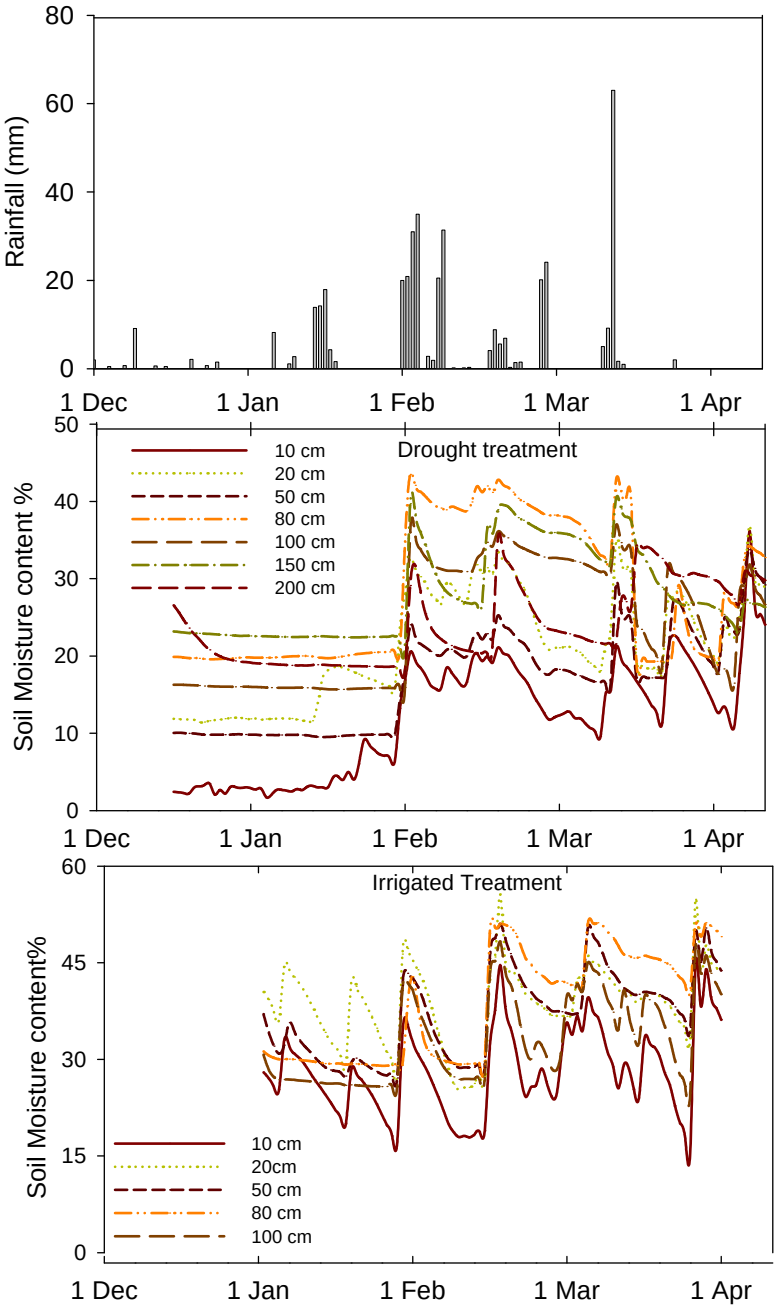

Figure 6 - Monthly rainfall distribution and the soil moisture variation in different depths in irrigated and drought treatments at Dalbeg trial

4.2 Variation in agronomic characters and sugar attributes

Trails at Home Hill and Crystal Creek were harvested in June every year and the growth and yield data were analysed separately for each crop class and collectively for 3 crops. In order to reduce the spatial variation within the experiment, each trial was analysed separately. Appropriate statistical analyses for variance tests were conducted with the spatially adjusted data from each experiment.

4.2.1 Agronomic characters in three crops at Home Hill

The results of the 3 experiments in 3 crop years at Home Hill were summarised in Table 3. There was significant variation (combined analyses) among clones for all the agronomic traits measured in three crops. Analysis of trial data showed that crop growth was substantially affected by water stress (Table 3). It also revealed some distinct and interesting growth effects. Water stress did not affect stalk population and stalk diameter despite significant growth inhibition, however, it substantially reduced stalk height and cane yield. From the data collected, the stalk height reduction was found to be the main contributor to the large reduction in cane yield (TCH) at Home Hill.

Table 3 - Population means of cane yield (TCH), total dry matter (TDM), harvest index-sugar (HI) and agronomic characters under well watered and drought conditions at Home Hill. Percentage reduction and the Lsd 5% to compare crop classes and treatments are presented.

Trait	Crop class	Drought	Crop class effects	Irrigated	Treatment effects	Reduction (%)
		Mean	Lsd 5%	Mean	Lsd 5%	
TCH	Plant	66.43	16.08	93.4	8.78	28.9
	R1	92.59		109.81		15.7
	R2	45.28		96.83		53.2
TDM	Plant	23.32	5.07	32.47	6.46	28.2
	R1	32.65		39.27		16.9
	R2	17.01		33.55		49.3
HI (Sugar)	Plant	0.22	0.1	0.26	0.12	15.3
	R1	0.24		0.25		4.4
	R2	0.2		0.25		18.6
Stem diameter (mm)	Plant	24.59	ns	24.79	ns	0.8
	R1	24.4		24.41		0.1
	R2	21.59		24.11		10.5
Stalk length (mm)	Plant	288.26	35.15	348.79	41.37	17.4
	R1	318.51		356.39		10.6
	R2	234.59		346.37		32.3
Green leaf / stalk	Plant	5.74	1.91	9.81	1.55	41.5
	R1	6.21		9.07		31.5
	R2	7.66		8.31		7.8
Stalk number / m	Plant	16.34	4.35	16.52	ns	1.1
	R1	13.89		13.98		0.7
	R2	10.12		13.45		24.7

Under water stress conditions, TCH reduction varied from 15 and 53% in R1 and R2, respectively (Table 3). The strong association between stalk height and TCH suggests that stem elongation is highly sensitive to water stress and it could be a useful indicator of clone selection for drought tolerance.

Despite the rain interruptions during the early drought between November and February, the late season drought from March to June caused pronounced water stress in the plant crop (PC1) at Home Hill. As a consequence, a 29% TCH reduction was observed at harvest. The stress was not pronounced in R1 crop and hence the TCH reduction was only about 15%. The treatment mean difference for TCH was not significant (P=0.08). The prolonged

drought in R2 severely affected the TCH by reducing stalk number as well as stalk length. Though late maturation period of R2 received fairly high rainfall, most of the clones were unable to recover and produce similar yield as in the irrigated treatment. The R2 crop had a significant treatment effect on TCH and the yield loss was 53%.

4.2.2 Variation in sugar, fibre and related characters in three crops at HH

The combined analyses showed that there were no significant treatment effects on Brix, fibre and pol % in 3 crops. The estimated CCS of test population was reduced by 23 % under late drought in PC1, which is indeed small compared to TSH reduction (Table 4). As expected, an increase in CCS was observed in many clones in the drought treatment as compared to those in well-watered condition. From the data it is clear that the observed substantial TSH reduction was mainly caused by the large reduction in TCH due to severe and prolonged stress during the late crop growth in PC1. However, Brix and pol were recovered well in R1 and R2 as the crops received rainfall during maturation period.

Table 4 - Effects of drought on sugar, fibre and juice quality under well watered and drought conditions at Home Hill in 3 crop classes

Trait	Crop Class	Drought		Irrigated		Reduction (%)
		Mean	Lsd 5% Crops	Mean	Lsd 5% Treatments	
TSH	Plant	5.39	3.09	8.73	4.29	38.3
	R1	8.14		10.31		21
	R2	3.74		8.61		56.5
CCS	Plant	7.22	ns	9.4	1.4	23.2
	R1	8.58		8.7		1.4
	R2	8.15		8.48		3.8
Brix %	Plant	20.04	ns	18.62	ns	-7.6
	R1	20.03		19.76		-1.4
	R2	19.09		19.55		2.3
Fibre %	Plant	15.53	ns	16.37	ns	5.1
	R1	15.42		16.09		4.1
	R2	15.16		15.47		2
Pol %	Plant	59.8	ns	65.32	ns	8.5
	R1	65.74		65.45		-0.4
	R2	61.58		63.07		2.4

The clone variations for TCH, TDM, TSH and CCS were highly significant. Some of the commercial cultivars were badly affected by severe drought in R2. On average >50% TCH reduction was recorded in commercial clones (Table 5) in R2 crop. In contrary, few improved commercial cultivars showed less reduction in TCH than the population average. The crosses of commercial breeding lines with wild relatives had low TDM and TCH under irrigated conditions but they also showed more yield stability than commercial clones under severe drought conditions. However, one of the breeding line, QC91-580 and few other commercial cultivars including NCo310, had relatively high TCH than all other commercial canes in the drought treatments at Home Hill.

Table 5 - Average performance of selected ten high-yielding clones (QCANES) in 3 crop classes and their TCH reduction in R2 at Home Hill

Clone	Crop avaerage yield		Reduction % under Stress conditions		
High reduction	Drought TCH (t/ha)	Irrigated TCH (t/ha)	Medium stress (Plant)	Mild stress (R1)	Severe sterss (R2)
QN66-2008	77.67	114.59	19.91	22.40	75.28
R570	66.05	133.10	44.53	39.50	75.10
Q124	58.13	94.93	30.56	31.05	73.74
Q138	57.42	108.94	32.52	38.18	71.80
Q209	63.73	100.53	14.25	28.84	67.53
Low reduction					
Q190	72.75	114.94	39.72	26.02	45.87
NCO310	89.43	113.43	14.32	8.42	43.09
Q171	71.58	93.18	19.67	13.85	41.23
QC91-580	86.77	128.78	22.00	11.63	39.23
QC93-1863	84.47	127.20	33.99	18.95	39.08
Population average	68.25	102.30	24.41	18.43	53.13

Table 6 - Average performance of ten selected clones (crosses with wild relatives) in 3 crop classes and their TCH reduction in R2 at Home Hill

Clone	Treatments		Reduction % under stress condition		
High reduction	Drought TCH (t/ha)	Irrigated TCH (t/ha)	Medium (Plant)	Mild (R1)	Severe (R2)-
CT04-50	63.48	69.71	6.05	0.00	15.56
CT05-583	93.94	95.88	2.72	3.00	18.01
CT04-30	67.12	76.64	16.76	0.06	31.15
CT04-33	57.70	72.29	1.08	18.27	31.67
CT04-450	63.91	79.90	10.99	6.57	32.19
CT04-495	74.51	99.31	19.11	22.11	34.35
CT05-608	83.25	101.88	15.23	6.01	35.36
CT05-626	80.73	98.87	21.12	1.47	36.32
CT05-605	75.36	89.39	1.77	7.83	38.27
Population Average	68.25	102.30	24.41	18.43	53.13

The lowest TCH reduction was observed in clones obtained from crosses with wild relatives (Table 6). Among these clones, CT04-50, CT04-30, CT04-33 and CT04-450 were inherently low in sugar and TCH under non-stressed condition.

There was no significant variation among crops for CCS. However, both late season medium stress and early season severe drought conditions affected CCS of the plant and R2 crops. In general, the mild water stress conditions during late maturation enhanced the CCS in most of the commercial cultivars. The CCS in R1 crop with mild stress highly correlated with the CCS in R1 irrigated crop. This association was strong for both commercial and the untested clones in R1. However, there were re-ranking of clones for TCH and TSH across treatments in PC1 and R2 showing significant clone × treatment interaction for TCH and TSH with increased severity of stress (Figure 7).

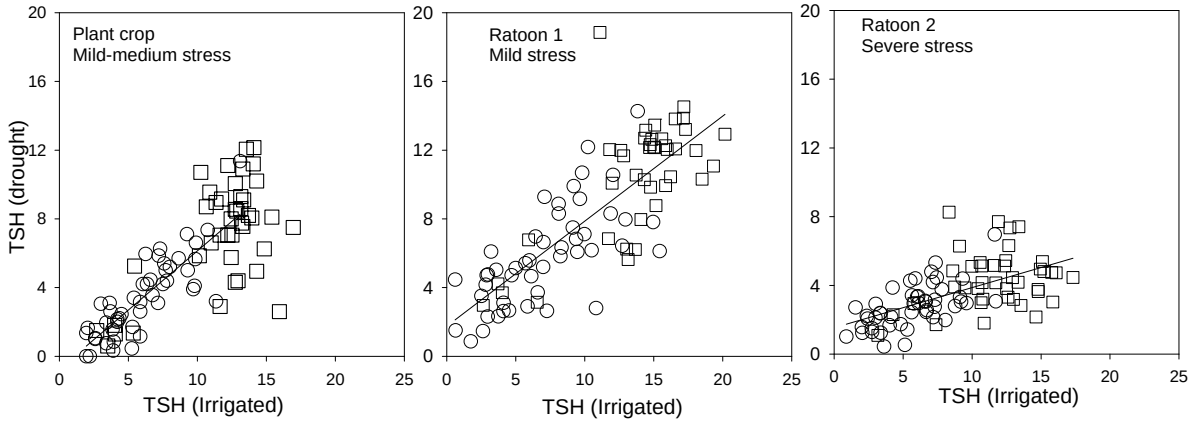

Figure 7 - Comparison of TSH between irrigated and drought treatments in 3 crop cycles in 3 years at Home Hill. (□) commercial cultivars and (○) clones derived from the crosses with wild relatives.

4.2.3 Variation in cane yield, agronomic characters and sugar yield under rainfed conditions at Crystal Creek

The TCH in three crop classes in CC trial were generally higher than that obtained in HH trial. There was a significant TCH reduction across plant and ratoon crops. The severe drought conditions in R2 had contributed more for the TCH reduction. About 33% TCH was dropped in R2 in comparison to plant crop. The agronomic traits such as stalk length, stalk diameter, green leaf number /stalk and nodes/stalk were greatly reduced in R2 crop due to severe stress (Table 7).

As observed in Home Hill experiment, the green leaf number per stalk, stalk diameter and stalk length had high correlation with TCH in all three crop classes (Table 7). Though there was a variation in stalk number per meter, it had negative effects on the final TCH in 3 crops. The negative correlation was mainly because of the low TCH and high stalk number/m in many wild relatives in the test population. The association between TCH and the stalk number among QCanes in the test population was also negative. There was a re-ranking of clones between R2 and other 2 crop classes (PC1 and R1) causing significant clone × crop class interaction.

Table 7 - TCH and other agronomic characters of each crop class and averages of the 3 crops (a) and phenotypic correlations between agronomic traits and TCH for each crop and averages of the 3 crops (b) at Crystal Creek

(a) Crop	Green leaf No per stalk	No of nodes per stalk	Stalks number per meter	Stalk diameter (mm)	Stalk length (cm)	TCH t/ha)
Plant crop	7.60	28.48	14.26	25.26	394.41	109.84
Ratoon 1	6.03	27.59	12.35	23.21	354.33	102.84
Ratoon 2	4.42	20.27	13.46	21.98	281.04	73.60
Averag of 3 crops	6.02	25.45	13.36	23.48	343.26	95.42
(b) Phenotypic correlations with TCH						
Plant crop	0.41	0.25	-0.48	0.58	0.29	
Ratoon 1	0.38	0.29	-0.14	0.57	0.40	
Ratoon 2	0.34	0.23	0.10	0.47	0.49	
Averag (3 crops)	0.53	0.32	-0.31	0.65	0.39	

4.2.4 Variation in sugar and other quality characters in rainfed conditions at Crystal Creek

The clone variation was highly significant for sugar, fibre and related characters studied. The average sugar and other quality characters of 99 clones in 3 crop classes at Crystal Creek are presented in Table 8. The early severe drought conditions in R2 at Crystal Creek caused severe TCH losses in all clones. However, there was a strong correlation between 3 crops for TCH and TSH in Crystal Creek.

Table 8 - Quality characteristics and TSH of 99 clones in 3 crops classes in Crystal Creek

Crop Class	CCS	Brix	Pol	Fibre	TSH
CC-Rainfed-PC1	9.5	21.2	71.1	16.0	10.9
CC-Rainfed-R1	9.4	20.1	69.2	16.9	10.1
CC-Rainfed-R2	9.7	21.3	72.0	16.7	7.7
Mean	9.6	20.9	70.8	16.5	9.6

4.2.5 Variation in agronomic characters and cane yield at Dalbeg trial

Dalbeg trial was planted in April 2009 to investigate the performance of 40 clones selected based on HH and CC trial results. They were tested at three different water treatments (Full, 50% and no irrigation) and to assess the magnitude of genotype × environment interaction for agronomic and physiological characters.

4.2.5.1 Early dry matter harvest at Dalbeg

The purpose of this early harvest of 7-month-old crop was to test whether an early biomass measurement will have any strong correlation to final yield of fully-grown crop under drought and well-watered condition. In total 16 shoots were randomly harvested from the middle 2 rows of each plot and total fresh weight, leaf weight and stem weight were recorded. Sub samples (about 500g) of leaf and stem were taken to determine dry/fresh weight ration. The dry weights of leaf and stem were determined after 7 days drying under 60^0C in the oven. The total leaf and stem biomass were calculated using the ratio of each sample.

The shoot dry weight of clones was determined in all three water treatments; well watered (WW), half irrigation (HI) and water stress (WS) (Figure 8). Approximately at 7-month stage, there was a clear variation in biomass accumulation among three treatments. The average shoot biomass (dry weight) of WW, HI and WS treatments were 11.27, 6.06 and 4.28 t/ha, respectively. The biomass between WW and WS treatments was strongly correlated (r^2 = 0.51) while the correlation was relatively low (r^2 = 0.28) between HI and WS treatments (Figure 8a, 8b). The correlation between WW and HI however was strong (r^2 =0.57) (Figure 8c). Some of the high biomass clones in irrigated treatment were severely affected by water stress. The average shoot biomass reduction caused by water stress in HI and WS treatments were approximately 45% and 62%, respectively.

The statistical analysis showed significant clone, treatment and clone × treatment interaction for the biomass accumulation in the test clones under different water deficits at Dalbeg (Figure 8).

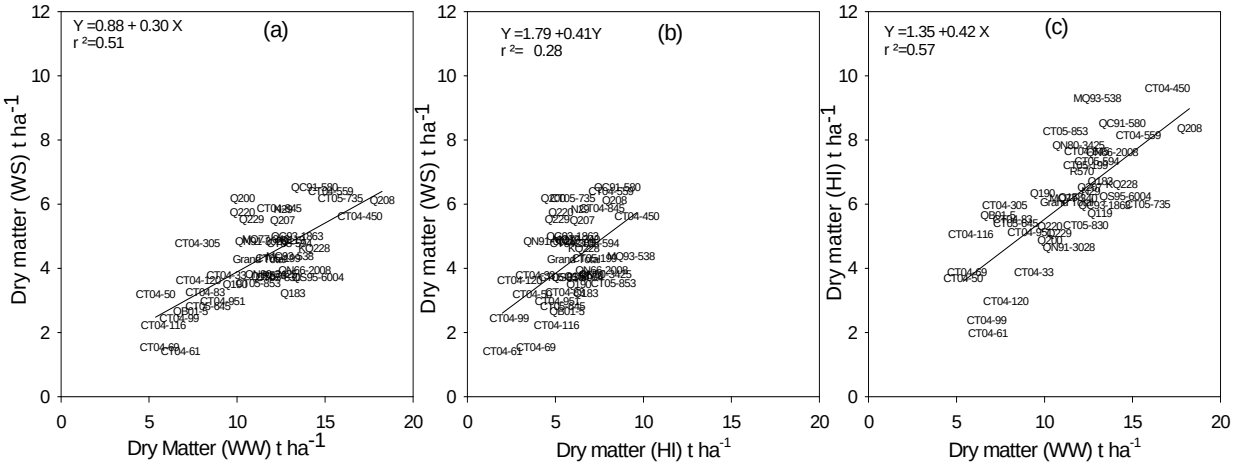

Figure 8 - The association between three water treatments for shoot biomass accumulation in 40 clones at 7 months stage in Dalbeg trial. WW = well watered, WS = water stressed, HI = half-irrigated

The biomass production under different water status in two groups of clones with varying capacity for biomass accumulation is presented in Table 9. The results suggest that substantial biomass reduction could occur both in high and low biomass clones (e.g. KQ228$^{(b)}$ and CT04-99).

Table 9 - Shoot biomass (t/ha) accumulation of 7-month-old plants and the % biomass losses due to water limitation in high and low biomass clones (PC) under well-watered, half-irrigated and water stressed conditions at Dalbeg

Clones	Dry matter (t/ha)			Reduction%	
High biomass	Drought	Half -irrigated	Full-irrigated	Half -irrigated	Drought
Q208	6.11	8.35	18.24	54.26	66.49
CT04-450	5.61	9.62	16.97	43.28	66.92
CT05-735	6.20	5.98	15.86	62.30	60.93
CT04-559	6.40	8.16	15.32	46.73	58.20
QS95-6004	3.73	6.25	14.59	57.18	74.44
QC91-580	6.52	8.52	14.40	40.81	54.73
KQ228	4.61	6.61	14.38	54.06	67.97
Low biomass					
QB01-5	2.66	5.65	7.35	23.16	63.76
CT04-61	1.43	1.99	6.78	70.59	78.84
CT04-99	2.46	2.37	6.71	64.64	63.31
CT04-116	2.24	5.06	5.80	12.86	61.42
CT04-69	1.55	3.89	5.59	30.39	72.29
CT04-50	3.20	3.68	5.37	31.52	40.42
Lsd5% clone =1.89, treatments= 1.79 and interaction =3.5					

4.2.5.2 Variation in cane yield and agronomic characters at Dalbeg

The imposition of water stress conditions for the drought treatments was successful in the plant crop. In drought and semi-irrigated treatments TCH and TDM were reduced by 40% and 43% respectively (Table 10). The treatment effects on agronomic characters are shown in Table 10.

Table 10 - The effects of water treatments on agronomic and morphological characters of 40 clones tested at Dalbeg trial (PC) in 2009/2010 crop season

Characters	Drought	Reduction %	Semi-irrigated	Reduction %	Irrigated	Average
TCH (t/ha)	71.4	39.9	95.6	19.5	118.8	95.3
TDM (t/ha)	22.4	42.6	30.7	21.2	39.0	30.7
Stalk length (cm)	266.8	25.1	326.5	8.4	356.4	316.5
Stalk diameter (mm)	24.8	1.7	24.8	2.0	25.3	24.9
Green leaf/stalk	7.1	-1.3	7.1	0.8	7.0	7.0
Nodes/stalk	21.1	12.7	23.6	2.1	24.1	22.9
Stalks/m	8.6	26.0	10.0	14.1	11.6	10.1

The most affected morphological characters in the water stress treatments were stalk number per meter (26%) and stalk height (25%). As the intensity of rainfall increases, some clones in the drought treatment recovered steadily. The key yield parameters, TCH and TSH, in semi-irrigated treatment were reduced by 20 and 35 % respectively (Table 10). This reduction was nearly 50% of what was recorded in the dry treatment. There was a slight increase in the number of green leaves (1.3%, $p>0.05$) at the time of harvest in the drought-affected crop. This increase in green leaf number could be due to the rapid and continued growth of drought-affected crop during the following the wet season. Fibre content and the stalk diameter did not change significantly in drought treatments.

Statistical analysis showed that treatment $\times$ clone interactions were significant for TCH, TSH, TDM and stalk length. Water stress had no significant effect on green leaf number, fibre content and stalk diameter. The lack of stress treatment for green leaf content at harvest could be due to the recovery of growth following the late wet season towards the end of crop cycle.

4.2.6 Variation in sugar, fibre and related quality characters at Dalbeg

In the drought treatment, the average CCS had declined by 34% and this reduction was highly significant (Table 11). Consequently, the TSH was reduced by 60% when compared with fully irrigated treatment (Table 11). CCS, Brix and pol % were changed with water stress but there were no clone x treatment interactions.

Two groups of test clones, high and low reduction clones, were identified by comparing the variation in TSH reduction due to water stressed conditions (Table 12). There was an overall reduction of 60.5% in TSH due to drought (Table 12). The average reductions in these two groups (high and low reduction) were 76% and 48%, respectively. Similarly, a drastic reduction, as high as 75%, in CCS was also observed in many clones, including commercial selections. This may have caused by the delay in physiological maturation of drought-affected crop and the slow recovery (in some clones) during the wet season.

Table 11 - The effects of water treatments on sugar, fibre and related quality characters of 40 clones tested at Dalbeg trial in 2009/2010 (Trial was harvested in June, which was the lowest CCS period in the season)

Characters	Drought	Reduction %	Semi-irrigated	Reduction %	Irrigated	Average
CCS	6.1	33.6	7.8	15.2	9.2	7.7
TSH	4.8	60.5	7.9	34.7	12.0	8.2
Harvest Index	0.2	31.1	0.2	18.2	0.3	0.3
Brix	18.3	5.9	18.7	3.8	19.4	18.8
Fibre %	13.4	2.7	13.9	-1.1	13.8	13.7
Pol %	52.9	19.3	60.0	8.5	65.6	59.5

Table 12 - Clones with high and low reduction of TSH in the PC2 at Dalbeg

Clone	TSH_Drought	TSH -Semi	TSH- Irrigated	% Reduction	TCH_Drought	TCH -Semi	TCH- Irrigated	% Reduction	CCS_Drought	CCS -Semi	CCS- Irrigated	% Reduction
Low TSH reduction												
CT05-830	4.99	6.79	8.21	39.14	64.60	86.70	106.20	39.18	8.86	9.05	10.03	11.59
Q200	10.40	10.86	17.98	42.14	98.90	133.60	142.40	30.53	10.57	10.78	12.63	16.25
CT04-305	5.12	6.67	9.25	44.61	74.20	94.40	115.60	35.84	4.95	5.46	6.02	17.67
Q190	9.00	6.94	16.86	46.63	84.30	126.90	137.70	38.81	10.80	11.63	12.77	15.46
Q119	7.57	10.55	15.46	51.06	96.80	136.10	162.40	40.40	8.01	9.09	10.44	23.29
Q207	7.34	12.35	15.30	52.03	77.70	108.30	122.70	36.68	9.66	11.50	12.47	22.57
QN80-3425	7.20	11.79	15.02	52.05	83.50	111.50	128.70	35.15	8.56	10.56	11.58	26.05
Q208	9.63	16.16	20.29	52.53	96.10	148.40	168.90	43.09	9.99	10.96	12.03	16.96
MQ93-538	7.91	13.07	16.69	52.63	88.30	131.40	144.50	38.93	8.93	9.95	11.59	22.97
CT04-50	2.53	1.86	5.49	53.81	69.40	80.80	101.00	31.32	0.97	2.18	4.78	79.63
High TSH reduction												
CT04-33	2.49	3.76	8.66	71.23	62.40	72.10	86.60	28.01	1.37	3.38	5.70	75.89
MQ77-340	3.07	4.76	10.78	71.52	86.30	118.10	141.40	38.97	4.50	5.52	6.79	33.68
CT04-120	2.09	6.31	7.82	73.35	64.10	90.80	106.30	39.69	3.07	7.40	8.63	64.46
QB01-5	1.87	2.87	7.08	73.64	47.50	56.60	86.40	45.07	3.41	4.33	6.64	48.70
QS95-6004	4.17	11.96	16.15	74.18	49.20	95.20	114.50	57.01	8.50	12.57	14.05	39.52
CT05-645	0.59	2.97	2.33	74.63	40.10	43.20	52.40	23.57	1.92	4.02	4.39	56.20
CT05-594	1.18	2.88	5.20	77.41	61.70	73.80	95.40	35.38	2.00	3.83	5.34	62.60
CT04-559	0.87	2.54	4.10	78.67	30.50	47.60	61.40	50.38	2.84	5.45	6.64	57.19
CT04-116	1.18	1.97	5.79	79.68	58.00	67.60	106.30	45.42	2.03	2.95	5.21	61.00
CT04-61	1.30	1.92	8.12	83.94	47.20	61.90	88.00	46.34	2.18	3.14	5.62	61.26
Average all	4.76	7.86	12.04	60.47	71.40	95.60	118.80	39.90	6.06	7.81	9.24	34.38
Stand. error	2.70	4.28	5.48		20.10	31.40	33.20		3.24	3.28	3.09	

The cane yield (TCH) of 40 clones in the PC at Dalbeg were compared with that of R2 crop at Home Hill. There was strong association between 2 crops for TCH under irrigated and drought conditions (Figure 9a). However, the differences in slope and the interception indicated that the pattern of clone response to irrigated and drought environments across 2 locations were different (Figure 9b). This variation could be attributed to the clone × location × treatment interaction for TCH. As a result there was no association between estimated yield reduction % between two locations. This G x E interaction could potentially complicate the process of clone selection for drought tolerance based on observations in one location.

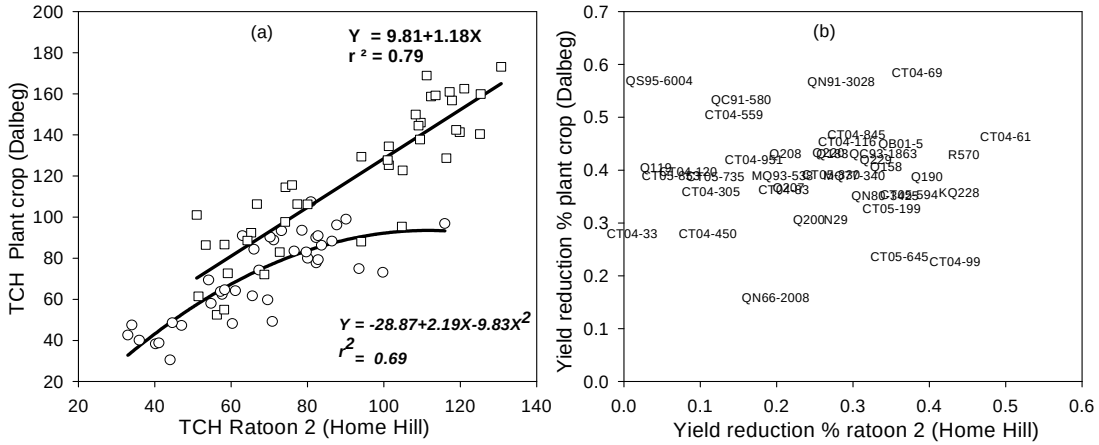

Figure 9 - (a) Yield comparison of 40 clones under irrigated (□) and drought (○) conditions at Home Hill (R2) and Dalbeg (PC2) in 2009-2010 and (b) comparison of yield reduction % of those 40 clones at 2 experiments

4.2.7 Clone × water treatment interaction for cane yield and sugar traits

Combined analyses were conducted for the common 40 clones across 12 water environments (Table 13). The result indicated a significant clone × environment interaction for TCH, CCS and TSH which was mainly caused by the variation in clone responses to different drought conditions at Home Hill and Dalbeg. Particularly, the R2 at HH and PC at Dalbeg were severely affected by drought and some clones had responded differently to water stress conditions across these 2 locations. Beside the variation in soil and climate factors, the patterns of drought development at the early growth stages across these two locations were comparable. The PC at Dalbeg was planted in April 2009 and had a very slow growth during the early drought period. In contrast, R2 crop at HH had a rapid establishment after June 2009 harvest and subjected to a long dry period during the early vegetative period. The intensity of moisture stress for these 2 crops was comparable.

Table 13 - Genetic variances estimated from the individual analysis of variances agronomic characters across the 12 test environments

Crop	TCH (t/ha)	TSH (t/ha)	TDM (T/ha)	Salk length (cm)	Stalk diameter (mm)	Green leaf No	Stalk Number /m
CC-Rainfed-Plant	508.1	30.2	52.3	650.0	18.1	18.1	27.1
CC-Rainfed-Ratoon 1	340.8	20.1	33.6	1068.0	14.5	14.5	14.9
CC-Rainfed-Ratton 2	312.3	16.0	26.3	1243.0	14.9	14.9	23.3
HH-Drought-Plant	205.4	8.6	13.8	844.7	17.2	17.2	19.1
HH-Drought-Ratton 1	240.2	11.6	25.3	738.8	26.0	26.0	19.5
HH-Drought-Ratoon 2	44.5	1.4	7.4	846.3	15.1	15.1	15.1
HH-Water-Plant	258.5	16.1	26.6	1418.0	18.1	18.1	20.3
HH-Water-Ratoon 1	306.0	22.5	37.5	426.4	10.9	10.9	19.4
HH-Water-Ratoon 2	245.9	12.6	17.2	1337.0	17.7	17.7	20.9
DB-Water-Plant	380.3	20.1	32.6	412.5	13.2	12.4	17.6
DB-Semi water-Plant	3321.5	14.3	23.6	652.3	12.1	9.9	16.4
DB-Drought-Plant	42.3	1.6	9.6	652.2	19.2	13.2	14.7

Table 14 - Genetic variances of sugar characters across 12 environments in 3 locations

Crop	CCS	Brix	Fibre %
CC-Rainfed-Plant	12.23	27.06	3.87
CC-Rainfed-Ratoon 1	8.95	14.94	6.76
CC-Rainfed-Ratoon 2	13.43	23.34	11.60
HH-Drought-Plant	11.58	19.06	13.64
HH-Drought-Ratoon 1	8.28	19.45	10.21
HH-Drought-Ratoon 2	5.09	15.07	6.97
HH-Water-Plant	10.81	20.33	14.95
HH-Water-Ratoon 1	11.79	19.41	11.01
HH-Water-Ratoon 2	6.71	20.85	9.01
DB-Water-Plant	9.36	13.23	5.36
DB- Semi water-Plant	11.23	13.25	5.63
DB-Drought-Plant	3.65	5.36	5.11

Among all 12 environments, R2 crop (drought) in Home Hill and PC (drought) in Dalbeg showed the lowest genetic variation for CCS, Brix and fibre% (Table 14). The genetic correlations of these two environments with other 10 were also comparatively low.

Combined analyses were conducted for each crop in each location to estimate the treatment × clone interaction for agronomic and sugar traits. The results for TCH, CCS, TSH and TDM are presented in Table 15. The clone variation was highly significant for all the traits. Among 3 HH crops only R2 (early severe stress) showed significant clone × treatment interaction for TCH. The interaction for TCH was significant with 3 levels of water treatments at Dalbeg (Table 15). The clone × treatment interaction for CCS was significant in PC and R1 at Home Hill where some stress occurred at maturity stage. However, TSH estimated from TCH and CCS showed clone × treatment interaction in all 4 crops.

Table 15 - The probability and significance of the treatment, clone and treatment × clone interactions of total biomass, cane yield, CCS and sugar yield in HH and Dalbeg trials

	Probability	Home Hill			Dalbeg
Characters	Variance	Plant	Ratoon 1	Ratoon 2	Plant
	Treatment (T)	0.178	0.096	0.05	<0.001
TCH	Clone (C)	<0.001	<0.001	<0.001	<0.001
	T x C	0.387	0.063	0.002	<0.001
	Treatment (T)	0.091	0.6	0.67	<0.001
CCS	Clone (C)	<0.001	<0.001	<0.001	<0.001
	T x C	0.004	0.017	0.53	0.644
	Treatment (T)	0.155	0.216	0.141	<0.001
TSH	Clone (C)	<0.001	<0.001	<0.001	<0.001
	T x C	<0.001	0.002	<0.001	0.04
	Treatment (T)	0.116	0.104	0.094	<0.001
TDM	Clone (C)	<0.001	<0.001	<0.001	<0.001
	T x C	0.72	0.161	0.019	0.178

4.3 Variation in physiological characters

4.3.1 Stomatal conductance (*gs*) variation in multiple crops

4.3.1.1 Clone variation for *gs* at Home Hill and Crystal Creek

Repeated *gs* measurements were made at HH and CC in 3 crop classes. During each data collection, 3 measurements from each plot were recorded. The mean *gs* in R1 and R2 under non-stressed conditions in Home Hill were 311.5 and 279.9 mmol m^{-2} s^{-1}, respectively. The *gs* for the 3 crop classes under moderate and severe stress conditions in Home Hill were 156.5, 249.5 and 126.4, respectively (Table 16). The *gs* under non-stress conditions are comparable while the stress conditions showed some variations. This indicated that the imposition of water stress treatment was successful at Home Hill trial enabling quantify the genetic variation for *gs* response to varying water stress condition.

In 2009, the R2 crop at HH experienced the longest stress period and recorded the lowest *gs*, 72.7 mmol m^{-2} s^{-1} was recorded during this study. The dry period in R1 crop was interrupted by rain and consequently the minimum *gs* observed in water stress treatment in R1 was much higher (198.2 mmol m-2s-1) than that of the R2 crop. As expected, the well-watered treatment recorded the highest *gs* (Table 16). In general, *gs* measured across crop classes were consistent for each drought treatment (Figure 10). The variation in *gs* among clones was significant with strong repeatability between measurements within the same location.

Table 16 - The statistics for stomatal conductance of test clones at 95% confidence limit in wet and dry treatments in the plant and ratoon crops in Home Hill during 3 consecutive years (crop classes)

Statistics	PC1-drought	R1 -drought	R1-Irrigated	R2 -drought	R2-Irrigated
Mean	156.6	249.5	311.5	126.4	279.8
Std Deveation	29.2	25.2	31.7	25.3	23.4
Minimum	99.6	198.2	248.7	72.7	224.2
Maximum	224.3	301.2	376.1	193.3	325.2
95% Confidence Limit	6.1	5.3	6.7	5.3	4.9
Number od clones	89.0	89.0	89.0	89.0	89.0

Analysis of *gs* data across 3 crop classes at Home Hill showed that there was no significant clone × crop interaction (Table 17). This means the ranking of clones for *gs* does not change with crop class. The treatment × clone interaction across the means of three crops was significant ($P<0.05$). This indicated at there was no re-ranking of clone expressions for *gs* across varying moisture stress environments. Thus, *gs* is showing potential for using as a surrogate trait for water stress response.

The estimated correlation efficiencies between *gs* (drought) measured in PC, R1 and R2 crops in HH trial are presented in Figure 10. Though these clones were experiencing different levels of water stress conditions in 3 different years, there was significant correlation between plant and ratoon crops for *gs*. This further attests the robustness of the *gs* in quantifying genetic variation among clones for responses to water stress. The correlations between dry treatments across crops were stronger than the dry & wet and wet & wet treatments. The lowest correlation between dry and wet conditions was recorded in R1 (r^2=0.36).

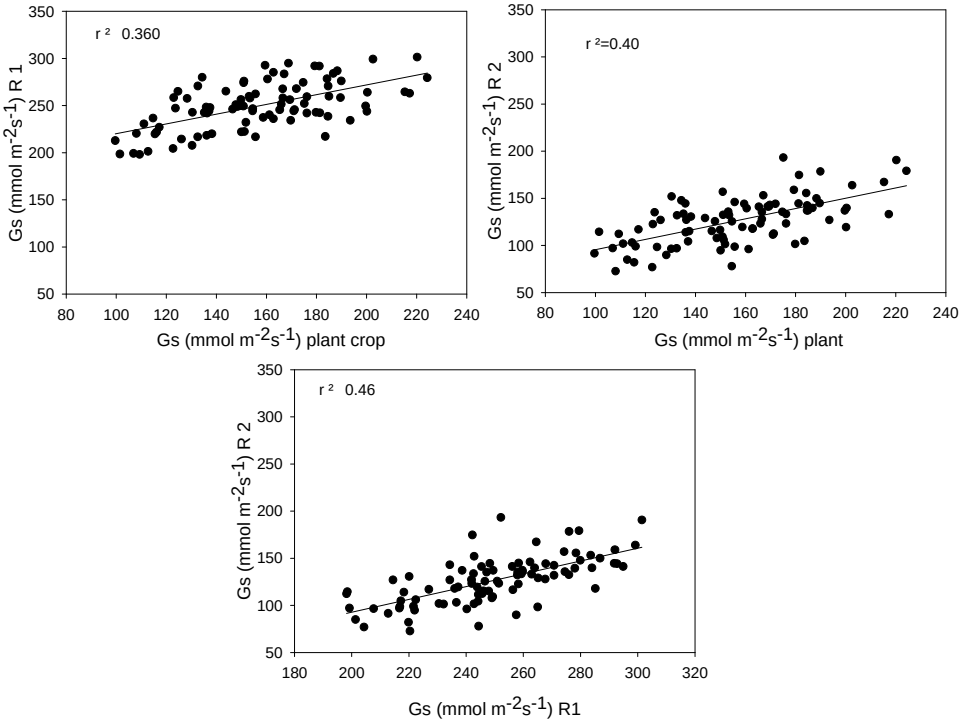

Figure 10 - Comparison of *gs* between crops (PC, R1 and R2) with 89 clones tested under water stress conditions at Home Hill

In R1 crop, when the stress conditions were modest, some clones maintained stomatal activity similar to that of non-stress condition while others maintained a significantly reduced *gs* level. This clonal variation in *gs* caused low correlation in R1. However, it is important to note that a highly significant treatment × crop interaction was observed in all trials (Table 17).

Table 17 - Analysis of variance for combined gs data across three crop classes* in 3 years

Source of variation	Df	Mean sq.	Variance Ratio	Probability
Treatment	1	4104591.0	6840985.0	<.001
Error 1	1	0.6		
Clone	88	6588.9	6.2	<.001
Treat x Clone	88	1557.5	1.5	0.050
Error 2	88	1057.2	2.6	<.001
Crop	2	76017.8	170.4	<.001
Treat x Crop	2	7671.2	13.6	<.001
Clone x Crop	176	694.0	1.2	0.060
Treat x Clone x Crop	176	564.2	1.3	0.040
Error 3	356	446.0		
Total	1067			

* Only the observations with significant treatment effects were pooled for the combined analysis.

4.3.1.2 Genetic correlation between test environments for *gs*

Genetic correlations between plant and ratoon crops (9 test environments) were estimated from 3 years of crop data in 2 locations. The assumption was that there was a strong environmental correlation between crops as they were grown in the same field over time but with varying environmental conditions because of the year-to-year variation. The genetic and environment covariance components between two crops were assessed using the method described by Burdon, 1977, and genetic correlations between crops in the same and between locations were estimated (Table 18). In brief, the genetic correlations were higher than the total phenotypic correlation between test environments at Home Hill.

Table 18 - Genetic correlations between test environments for average *gs* at different times in irrigated, rainfed and drought conditions at Home Hill (HH) and Crystal Creek (CC)

	CC-R1	CC-R2	HH-PC1_Dr	HH-PC1_Irri	HH-R1-Dr	HH-R1-Irri	HH-R2-Dr	HH-R2-Irri
CC-PC1	0.44	0.87	0.81	0.93	0.75	0.67	0.90	0.99
CC-R1		0.45	0.42	0.48	0.39	0.35	0.47	0.51
CC-R2			0.37	0.42	0.34	0.30	0.40	0.45
HH-PC1_Dr				0.83	0.67	0.59	0.80	0.88
HH-PC1_Irri					0.63	0.56	0.75	0.82
HH-R1-Dr						0.64	0.85	0.94
HH-R1-Irri							0.69	0.76
HH-R2-Dr								0.68
HH-R2-Irri								1.00

CC & HH are 2 locations, Dr =drought treatment, Irri = Irrigated treatments

4.3.1.3 Genetic variance components and broad-sense heritability estimation for *gs*

Variance component analyses were conducted to estimate the genetic, phenotypic and environmental variance components for *gs*. The genetic (σ^2g) and phenotypic (σ^2p) variance components were estimated from the combined analysis and the heritability was estimated on the experiment mean. High heritability for *gs* was observed at Home Hill trial (0.72) across 3 crops (Table 19).

The broad sense heritability for *gs* was estimated using the following formula.

$$h^2 = \frac{\sigma_g^2}{\sigma_g^2 + \sigma_{gc/(n_t n_b)}^2 + \sigma_{gt/(n_c n_b)}^2 + \sigma_{gtc/(n_b)}^2 + \sigma_{e/(n_r n_b n_c)}^2}$$

where σ2g, σ2gc, σ2gt and σ2gtc are genetic (g), clone×crop (gc), clone×treatment (gt) and clone×crop×treatment (gtc). σ2e is the error variance component. n = number of observation, b = block, g = clones, t = treatment, c = crop, e = error.

The proportion of genetic component is almost 3 times greater than that of G×E interaction variance component (Table 19). This indicated that the genetic component for the variation for *gs* in the test population is stronger than that of the non-genetic, and environment components. This further confirms the value of this trait as a potential tool for genetic improvement.

Table 19 - Estimation of variance components for clone, clone × crop, clone × treatment and clone × crop × treatment interactions and the heritability (broad sense) for gs

Source of Variance	Variace Components	Standard error for vc.
Clone (g)	336.00	80.50
Clone x Crop	47.60	40.40
Clone x Treatment	72.60	33.60
Clone x Crop x Treat.	0.12	0.05
Error	816.60	
Heritability	0.72	

The multiple crop data from the HH experiment demonstrated high genetic variation, high heritability and substantially high genetic correlation (than phenotypic) in *gs* between test environments of plant and ratoon crops.

4.3.2 Variation in relative water content (RWC) in leaf tissues in multiple crops

4.3.2.1 Clone variation

There was no clonal variation in RWC under non-stress conditions in well-watered treatment (data not presented). This observation is consistent with the findings reported by a Brazilian team earlier (De Silva et al, 2007). Therefore, the combined analysis was conducted only for the water stress treatments across plant and ratoon crops (Table 20). There was significant clone variation for RWC under stress. However, there was no significant variation in RWC

between plant and ratoon crops under stress condition (Table 20). This non-significant crop × clone interaction indicates that the ranking of clones for RWC remains consistent across crop years as observed in *gs*.

The variation in mean leaf RWC across three crops is presented in Table 21. As expected the non-stress treatments recorded the highest RWC with low standard deviations and low confidence limits in all three crops (Table 21).

Table 20 - Analysis of variance of combined data for RWC across crop classes during 3 year period and the mean RWC of each crop

Source of variation	DF	Mean sq.	Variance ratio	Probability
Block	1	114.62		
Crop	2	122.47	1.35	0.426
Error 1	2	90.94		
Clone	88	76.68	3.34	<0.001
Clone x Crop	176	24.73	1.08	0.294
Error 2	264	22.99		
Total	533			

Table 21 - The statistics for leaf RWC for test clones at 95% confidence limits for wet and dry treatments in plant and ratoon crops in 3 years at Home Hill

Statistics	PC1-Drought	PC1-Irrigated	R1 - Drought	R1-Irrigated	R2 - Drought	R2-Irrigated
Mean	88.2	97.2	88.7	95.0	89.8	94.9
Std Deviation	6.0	1.6	4.9	1.6	3.3	1.4
Minimum	72.4	92.4	76.2	91.2	82.0	92.5
Maximum	97.0	99.6	97.4	99.5	96.8	98.0
95% CL	1.3	0.7	1.0	0.3	0.7	0.3
Number of clones	89.0	89.0	89.0	89.0	89.0	89.0

4.3.2.2 Genetic correlation between test environments for RWC at Home Hill

The estimated genetic correlation between test environments for RWC in Home Hill and Crystal Creek are presented in Table 22. There were no correlations between wet treatments across crops as the clones in irrigated crop did not show any significant genetic variation for RWC. Unlike gs genetic variances of RWC were not significant for well water environments and hence not included in the estimation of genetic correlations between test environments. There were relatively high genetic correlations between plant and ratoon crops for RWC measured under water stress and rainfed conditions. This indicated the robustness of RWC measurements in quantifying genetic variation among clones for response to water stress.

There was a considerable consistency in ranking of clones across crops for RWC. This is desirable as similar ranking of clones across crop years would increase chances of selecting clones with desirable levels of RWC from the plant crop itself. Practically, achieving repeatable water stress environments across crop years for field level drought screening trials is difficult.

Table 22 - Genetic correlation between test environments (drought) for RWC among 3 crops with 89 clones tested under three water treatments at HHl and R1 crop at CC

Test environment	HH-PC1-Drought	HH-R1-Drought	HH-R2-Drought
CC-R1-Rainfed	0.69	0.76	0.40
HH-PC1-Drought		0.67	0.78
HH-R1-Drought			0.55

However, the results so far indicate that both *gs* and RWC have the potential to develop as surrogate traits for discriminating clones for water stress response as expression of both traits were proven to be consistent across successive crop cycles and multiple regions.

4.3.2.3 Variance components and broad sense heritability estimation for RWC

As for *gs*, combined analyses were conducted to estimate the variance components for clones, crops (PC, R1 and R2) and clone × crop interactions. The treatment and treatment × clone interaction effects were not included as there was no genetic variation in the irrigated treatments. The genetic (σ^2g) and phenotypic (σ^2p) variance components were estimated from the combined analysis and the heritability was estimated on the experiment mean. High heritability for RWC was observed in both Home Hill (0.67) and Crystal Creek (0.78).
The broad sense heritability for RWC was estimated using the following formula.

$$h^2 = \frac{\sigma_g^2}{\sigma_g^2 + \sigma_{gc/(_t n_b)}^2 + \sigma_{e/(_r n_b n_c)}^2}$$

where, σ^2g and σ^2gc are for clone (g) and clone×crop (gc). The error variance component is σ^2e. n = number of observation, b = block, g = genetic (clone).

As observed with *gs*, because of high genetic variation, high heritability and positive and high genetic correlations between plant and ratoon crops, leaf RWC can be considered as a potential trait for selecting clones for water-limited environments.

4.3.3 Clone variation for *gs* in plant crop in Dalbeg

Dalbeg experiment had 3 test environments; well-watered (WW) half-irrigated (HI) and water stress (WS). The soil type and environment were different from Home Hill and Crystal Creek. The rainfall was lower than HH and CC and the supplementary irrigation water was available for the dry period.

A series of *gs* measurements were taken during the long dry period in the PC. These measurements were collected during 10 am and 2 pm on clear sunny days. Data from each observation was analysed to identify the significance of clone, treatment and clone × treatment interactions. A significant clone variation was observed for *gs* at different time of measurements. September and November measurements were taken 2-3 days after irrigation of semi-irrigated and irrigated treatments. The October and December measurements were taken 2-3 days before the next irrigation of the irrigated treatments (Figure 11).

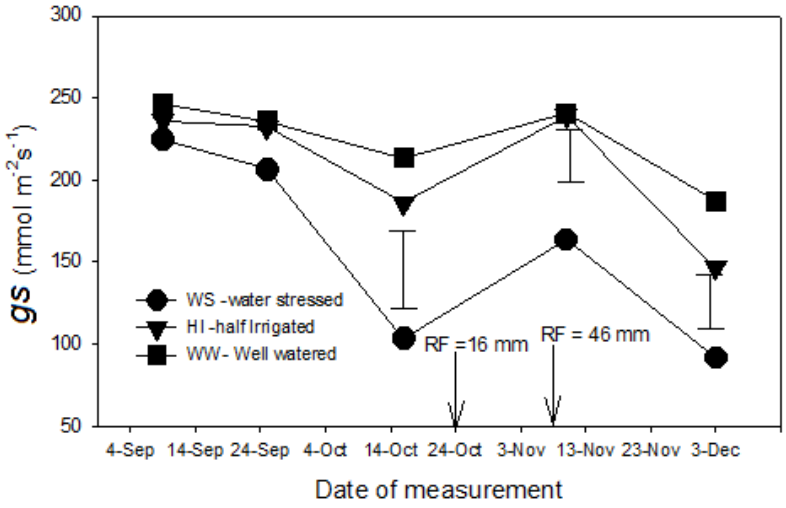

Figure 11 - Mean stomatal conductance in 40 clones at five different occasions in Dalbeg. Vertical bars indicate Lsd 5% for comparison of treatment means.

The *gs* was maximum in all three treatments in early September when the first irrigation was completed. The *gs* decreased rapidly with the progression of water stress in the WS treatment. After 84 days the mean *gs* in the water stress treatment reached 91.8 mmol m^{-2} s^{-1} which was about 50% of the full irrigation treatment (187 mmol $m^{-2}s^{-1}$) (Table 23).

Interestingly, the interaction between clones and water treatments was highly significant (10 Nov) when the treatments were affected by rainfall in early November (Table 23). Some clones in the semi-irrigated treatment recovered rapidly after the rain. This suggested that the stomatal responses of different clones to water stress vary with the severity of water stress.

Table 23 - Descriptive statistics for *gs* from 40 clones in PC grown under well-watered (WW), half- irrigated (HI) and water stress (WS) treatments at Dalbeg.

Date	Statistics	Conductance (mmol $m^{-2}s^{-1)}$			Statistical varaition - P-values		
		WS	HI	WW	Clone	Treatment	Interaction
9-Sep	Mean	224.0	232.2	246	<0.001	<0.087	<0.118
	Minimum	193.0	176.8	219			
	Maximum	256.0	289.5	272			
	St. Dev.	69.9	30.2	56.6			
23-Sep	Mean	206.7	232.2	236.0	<0.001	<0.099	<0.099
	Minimum	186.8	186.8	186.8			
	Maximum	270.7	299.5	316.5			
	St. Dev.	32.4	30.2	36.9			
16-Oct	Mean	103.3	185.96	213.6	<0.001	<0.001	<0.112
	Minimum	81.8	162.1	182.1			
	Maximum	143.0	285.4	303.1			
	St. Dev.	19.3	30.9	30.5			
10-Nov	Mean	163.8	239.2	240.7	<0.001	<0.001	<0.015
	Minimum	129.8	149.8	189.8			
	Maximum	261.7	238.7	342.7			
	St. Dev.	31.3	24.6	41.5			
3-Dec	Mean	91.8	146.8	187.0	<0.001	<0.011	<0.087
	Minimum	193.1	129.4	134.0			
	Maximum	62.3	231.7	302.2			
	St. Dev.	31.3	31.3	30.2			

** significant at P< 0.01, ns = not significant.

The HH-R1 results suggested that non-stressed and stressed clones could be discriminated for *gs* when the average *gs* in WS treatment reach below 200 mmol m^{-2} s^{-1}. This observation was consistent with the results of R2 crop at Home Hill and the PC in Dalbeg trial where a significant treatment effect was observed when the average *gs* was < 200 mmol m^{-2} s^{-1}.

The significance of clone × treatment and clone × time interactions were explored in a combined analysis with measurements from 5 different occasions. Both treatment × clone and time × clone interactions were significant (Table 24). This was consistent with the observations made earlier in Home Hill trial where a significant clone × treatment interaction was observed both in R1 and R2 crops. Different clones had different stomatal responses when grown under contrasting moisture conditions at Dalbeg. During September – December period, some clones had maintained higher *gs* in three water regimes, while others had reduced their stomatal responses when cell water status declined under increasing level of water stress.

Table 24 - Combined analysis of variance for the gs measurements taken at five occasions during water stress period in Dalbeg

Source Var.	DF	SS	MS	VR	Prob
Block	2	8540	4270	0.02	
Treatment	2	3164789	1582394	9.2	0.032
Residual	4	687833	171958	24.32	
Clone	39	1887192	48390	6.84	<.001
Treat×Clone	78	731176	9374	1.42	0.023
Residual	234	1541553	6588	1.12	
Time	4	7011548	1752887	299.03	<.001
Clone×Time	156	1386883	173360	29.57	<.001
Treatment ×Time	8	1664590	10670	1.82	<.001
Treat.Clone.Time	312	2142535	6867	1.17	0.04
Residual	960	5627396	5862	2.16	
Total	5399	36593163			
Total	5399	36593163			

4.3.3.1 Genetic variance components and broad-sense heritability estimation for gs

The variance components of clone, clone × treatment, clone × time, clone × treatment × time and error variance were estimated from the combined analysis. The total interaction component was approximately 22% of the clone variance and the estimated heritability (plot mean) for *gs* was 0.78. This indicates that the genetic component for the total variation is stronger for *gs*, which is a desirable characteristic when exploiting traits for variety improvement.

The patterns of *gs* variation with time among five contrasting clones are shown in Figure 12. Those five clones had different levels of *gs* under non-stress condition in WW treatment. Clone CT05-735 showed rapid recovery in HI (Figure 12) and WS treatments after November rain, while CT05-645 had fast response to increased soil moisture conditions only in WS treatment. The clones were severely stressed in WS treatment on 16 October and showed very low conductance. Interestingly, conductance in CT05-951 was consistently low in WW and HI treatments and showed a slow recovery in WS treatments. These clones recovered from stress at different rates after November rainfall. There were clone × time and clone × treatments interactions partly because of the differences in the rate of recovery after a prolonged dry period.

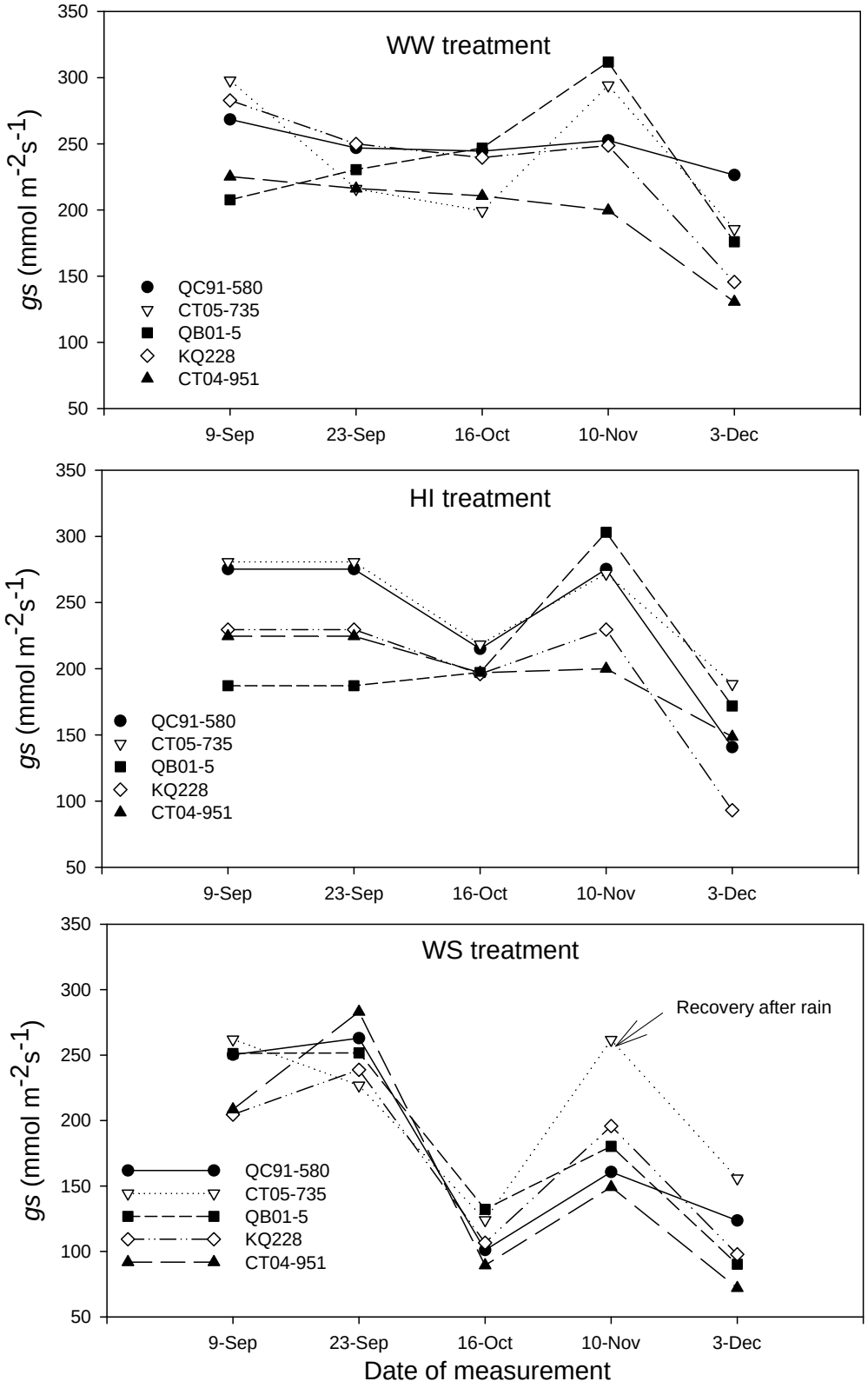

Figure 12 - Stomatal conductance variation in 5 clones at each time of measurements under different soil moisture conditions

4.3.3.2 Effect of drought on the pattern of stomatal conductance variation within a day

The hourly variation in *gs* during the day was investigated in six clones with differing *gs* and biomass production. Data collected at 5 time points between 9 am to 3 pm indicated a significant time and clone variation. The pattern of *gs* varied among clones. The *gs* was higher in all clones under irrigated treatment (Figure 13). However, *gs* declined in the afternoon and this same trend was observed at the last measurement at 2.30 pm. In contrast, same clones in the drought treatment showed an increase in gs after mid-day and by 2 pm it was equal to or higher than that recorded in irrigated treatment. These responses indicated the differential influence of the moisture status in plant and soil on stomatal behaviour in different clones during the day, which may have an implication for water conservation and crop adaptation to water-limited conditions.

A large fluctuation in day time *gs* was observed in high biomass producing CT05-735 and QC91-580 clones under irrigated condition. In these 2 clones, maximum *gs* were recorded after mid-day and dropped steadily after 1pm. An entirely opposite pattern of *gs* was observed when they were grown under drought condition (Figure 13). In contrast, low biomass producing CT04-645 and a high biomass commercial cultivar Q119 did not show any pronounced variation in *gs* under both stressed and non-stressed condition. From this data, it appears that different high biomass clones employ different gas and moisture exchange strategies to achieve the same biomass production. Grantz and Meinzer (1991) reported a similar behavior of stomata in response to humidity variation during the day.

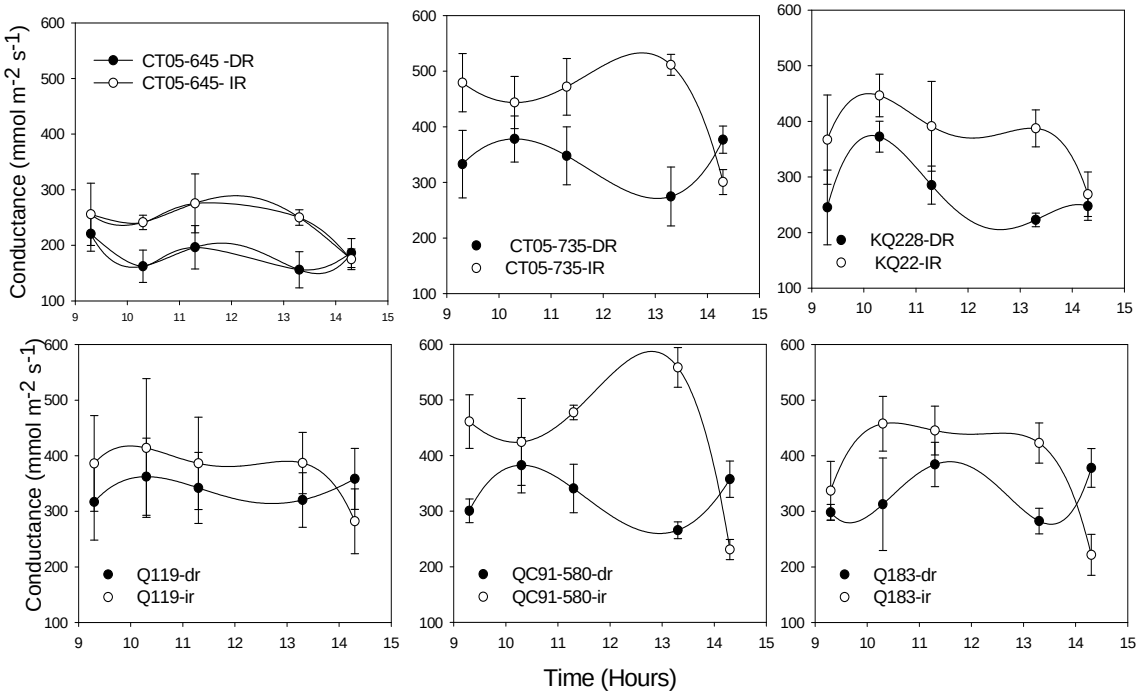

Figure 13 - Day time variation in stomatal conductance (gs) in 6 clones at five different time points at Dalbeg trial in November 2010. Vertical bars indicate the SE of measurements.

A combined analysis was conducted for the last 2 time points of measurements (10 Nov and 3 Dec). The time, time × clone and treatment × clone interactions were significant, which suggested variable responses by clone to moisture deficit across water treatments. The most contributing factor for clone × time interaction was the clone responses on moisture variation in HI and WS treatments. Clones in the HI and WS treatments had recovered at different rates just after the mid-November rainfall.

The variance components of clone, clone × treatment, clone × time, clone × treatment × time and error variance were estimated from the last 2 measurements in November and December 2009. The total interaction component (0.61) was approximately 33% of the clone variance (1.95), and the estimated broad sense heritability for RWC was 0.69.

Table 25 - Descriptive statistics for RWC at four occasions in 40 clones grown under well-watered (WW), half- irrigated (HI) and water stress (WS) treatments at Dalbeg

Date	Statistics	Drought	HI	Irrigated	Average	Variation
23-Sep	Mean			95.5	95.5	ns
	Minimun	Similar moisture levels		87.4	87.4	
	maximum			99.5	99.5	
	Std			2.0	2.0	
1-Nov	Mean	91.8			91.8	clone
	Minimun	85.6	Slow strees and no		85.6	
	maximum	96.4	measurements were taken		96.4	
	Std	2.3			2.3	
10-Nov	Mean	89.8	92.3		91.0	clone
	Minimun	73.0	87.7	No stress	73.0	Treatment
	maximum	98.6	96.8		98.6	Interaction
	Std	5.4	2.1		4.3	
3-Dec	Mean	89.3	90.9	94.1	91.4	clone
	Minimun	82.0	83.2	89.1	82.0	Treatment
	maximum	98.3	97.7	98.5	98.5	Interaction
	Std	3.7	3.2	2.2	3.7	

4.3.4 Effect of drought on the pattern of RWC

The hourly variation in RWC during the day was investigated in six clones with the gs measurements. The pattern of RWC varied among clones as in gs. All clones in irrigated treatment had higher RWC (Figure 14). The RWC variation during the day was narrower than gs and was not significantly different for most measurements. As observed with gs, RWC also showed a trend towards the opposite direction after mid-day. Q119 and CT05-645 showed very little response to the hourly variation in soil and environment while a noticeable variation in RWC was evident during mid-day in CT05-735, KQ220, Q183$^{(\dagger)}$ and QC91-580. The pattern of RWC variation thus showed possible association with the variation in gs.

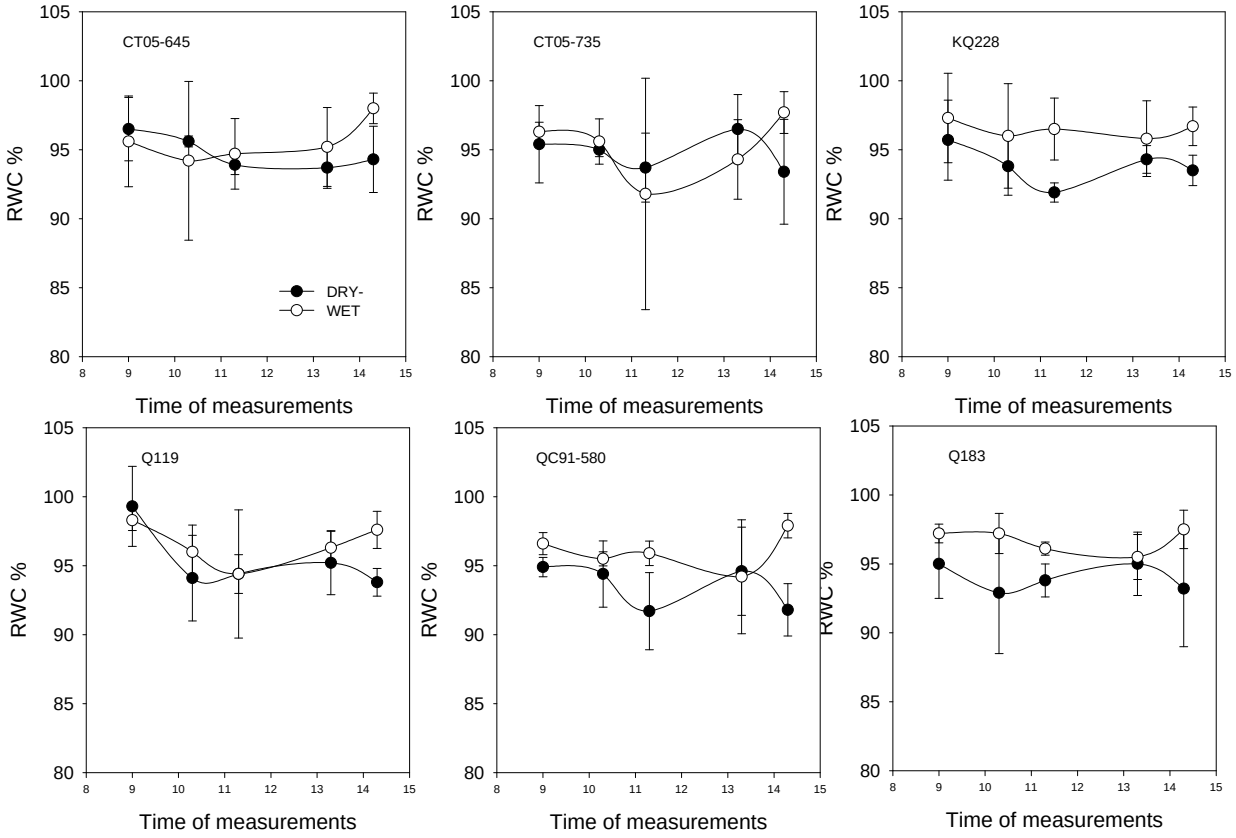

Figure 14 - Day time variation in RWC in 6 clones at five different time points at Dalbeg trial in November 2010. Vertical bars indicate the SE of measurements.

4.4 Glasshouse experiment to assess the transpiration efficiency (TE) in sugarcane

A glasshouse experiment investigating the variation of transpiration efficiency was conducted during January-March 2010. Ten selected clones were planted in 20 kg pots and grown in glasshouse facility at CSIRO Davies Laboratory, Townsville. The humidity in the glass house reached to 100% in the predawn (1:00-2:00 am) and remained unchanged for 5-6 hours. The minimum humidity was recorded just after noon when the temperature was at maximum. The maximum temperature varied from 34-42^{o}C while the minimum humidity varied from 35-65% in the mid-day. The vapour pressure deficit (VPD) fluctuated from 1.7 to 4.1 during this period (Figure 15).

Results of the total biomass (total dry weight) accumulation, total water use and transpiration efficiency (TE) are presented in Table 26. There was a significant difference between treatments for biomass accumulation and TE. The average biomass reduction was 39.6% whereas the reduction in water use was 58.5%. The TE was higher in 50% FC (18.2) than in the treatment with full FC (12.5). It is worth noting that commercial clones or commercial selections (all QCANES) generally have much higher TE than most of the other clones under well-watered treatment, but not under stressed condition. And, most of them recorded a higher reduction in biomass than the untested clones under 50% FC.

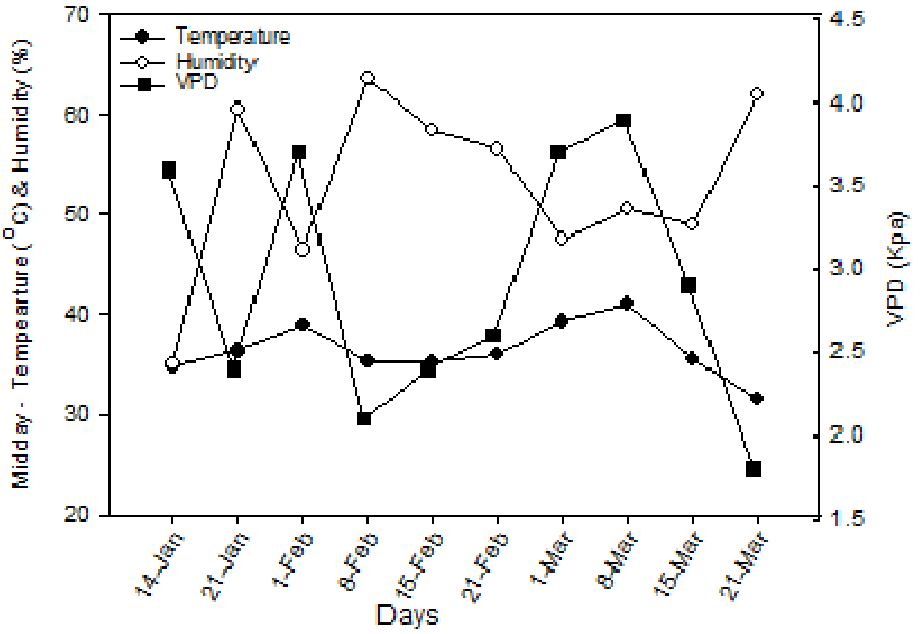

Figure 15 - Climate conditions in the glasshouse showing weekly mid-day temperature (^{0}C), humidity and VPD (Kpa) during the experiment at CSIRO, Townsville

Some QCANES showed lowest TE than the unselected introgression clones under water limited (50% FC) conditions. Interestingly, broadly adapted Q208$^{\phi}$ substantially increased TE (33.6 %) under water-limited conditions while other commercial cultivars such as KQ228$^{\phi}$, Q190$^{\phi}$ and Q183$^{\phi}$ had the lowest TE under 50% irrigated conditions (Figure 16). This data suggests the existence of considerable genetic variability for TE in sugarcane germplasm and the necessity for further understanding in order to exploit this variation for variety improvement program.

Table 26 - Clone variation for total biomass accumulation, total water use and transpiration efficiency (TE) under controlled moisture conditions at 100% and 50% field capacity (FC) in the pot experiment

	Total biomass (g)			Total water used (kg)			water use efficiency (g DM/kg H_2O)		
Clones	50% Fc	100% FC	Reduction%	50% Fc	100% FC	Reduction%	50% Fc	100% FC	Increased%
CT04-69	431.1	537.1	19.7	23.4	54.9	57.3	18.4	9.8	46.7
CT04-951	510.5	679.5	24.9	23.0	54.9	58.2	22.3	12.4	44.3
CT05-645	330.0	488.0	32.4	20.1	49.6	59.5	16.4	9.8	40.1
CT05-830	293.8	339.5	13.5	19.7	41.6	52.5	14.8	8.2	45.0
KQ228	336.9	765.2	56.0	21.7	53.1	59.2	15.5	14.3	7.7
Q183	360.7	854.7	57.8	22.1	56.3	60.6	16.3	15.2	6.9
Q190	345.9	768.9	55.0	22.2	53.3	58.4	15.6	14.4	7.9
Q208	473.9	767.6	38.3	21.7	53.0	59.1	21.8	14.5	33.6
QB01-5	537.6	687.9	21.9	21.5	51.9	58.5	24.8	13.3	46.4
QC91-580	350.8	689.1	49.1	21.2	52.9	60.0	16.4	13.0	20.6
Mean	397.1	657.7	39.6	21.7	52.1	58.5	18.2	12.5	31.5

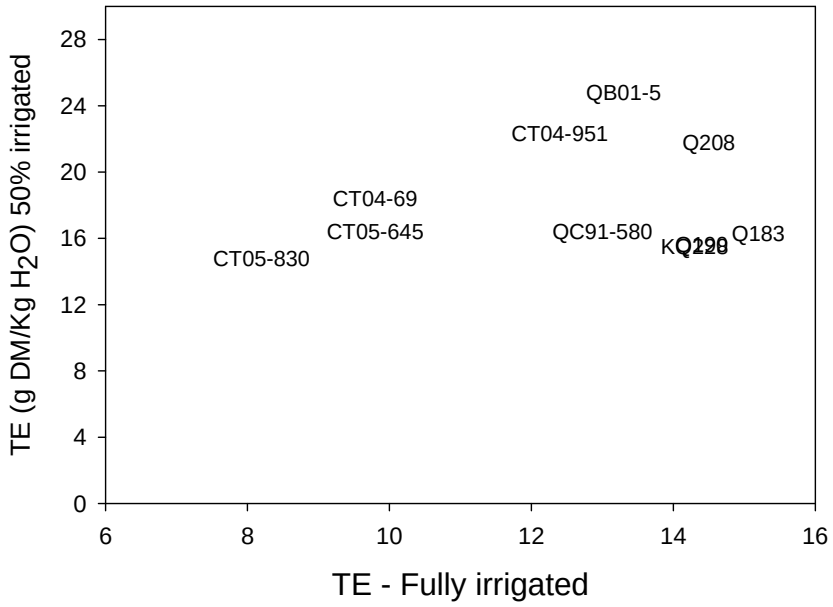

Figure 16 - Comparison of transpiration efficiency of 10 clones during 5-month growth period under 100% and 50% field capacity in the pot experiment

4.5 Variation in soil moisture extraction under drought in 10 clones in plant crop at Dalbeg

In addition to the pot trial to determine the variation in TE, the patterns of root water extraction of those 10 selected clones were investigated at Dalbeg. Neutron moisture meter readings were recorded for a depth up to 3.3 m on four occasions in the drought treatment in PC at Dalbeg (2009-2010), and the means of three replicate plots were calculated for each occasion and for each depth. Maximum and minimum values were obtained from these means over all sampling occasions, and these values were considered as the drained upper limit (DUL) and the lower limit (LL). Some vigorous clones such as KQ228$^{\phi}$, Q190$^{\phi}$ and Q183$^{\phi}$ (Figure 17) showed contrasting differences in DUL and LL given the extensive dry period before 30 November (please refer Figure 7). These readings were the lowest while readings after 30 November were generally relatively higher. The high capacity of these 3 clones in rapid water extraction under dry soil condition in the field partly explains the variation in low TE in the pot experiment (rapid development of water deficit).

4.6 Variation in leaf elongation in relation to drought effects

As an indicator of response to drought, leaf elongation (Inman-Bamber, 1995) was measured on 7, 8 and 9th leaf in 40 clones selected for the PC at Dalbeg. The daily elongation of the first emerging spindle was manually recorded. The maximum and minimum temperatures were also recorded during this period to compare the temperature responses for leaf elongation rates in different clones. The average elongation of six clones during different temperature periods are presented in Figure 18. The pattern of leaf elongation was highly related to temperature variation during leaf growth period. There was a variation among clones for these responses. Generally, clones with high elongation rates were more sensitive to elevated temperature and showed rapid increment in elongation.

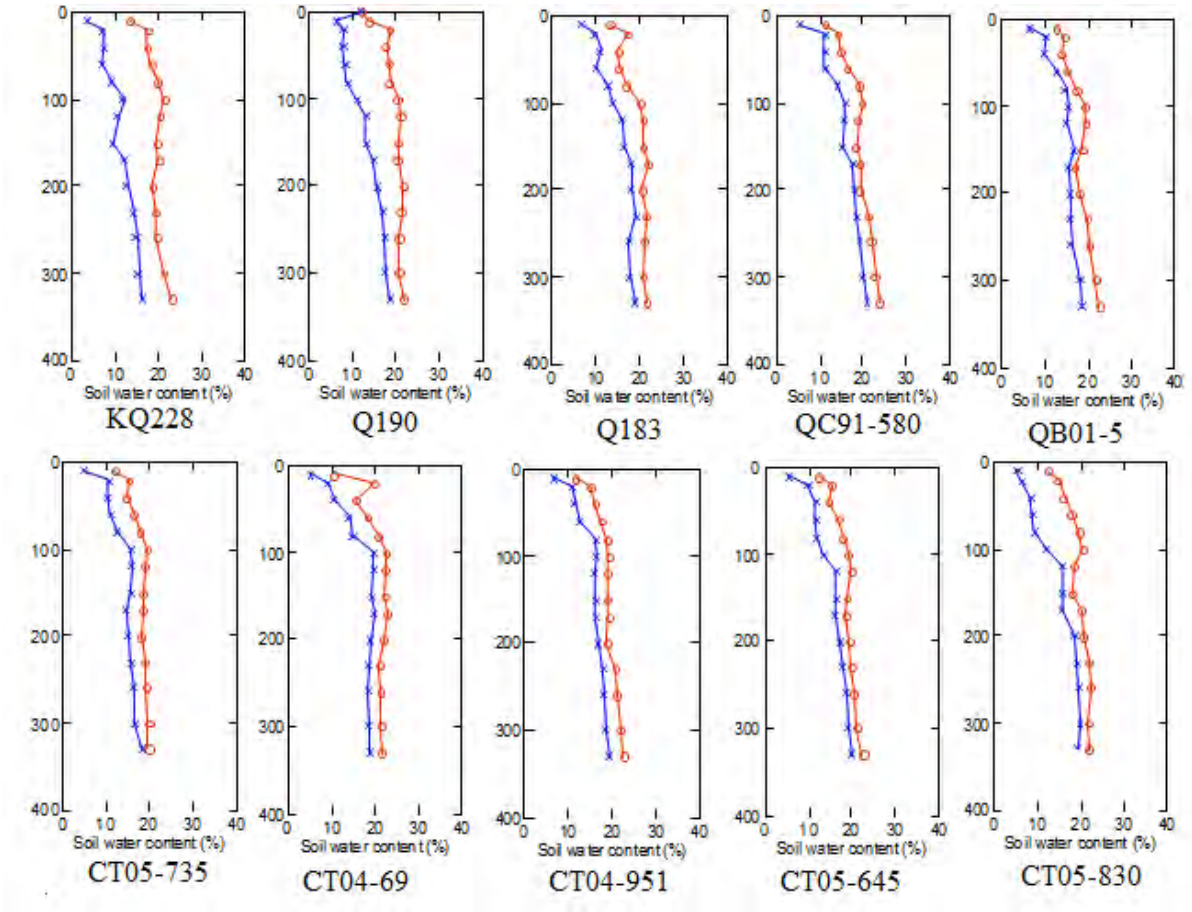

Figure 17 - The drain upper limit (DUL - in red) and the lower limit (LL - blue) of moisture levels in 10 clones in PC2 grown under severe drought conditions (May to November) at Dalbeg. The space between red and blue lines indicates moisture extraction.

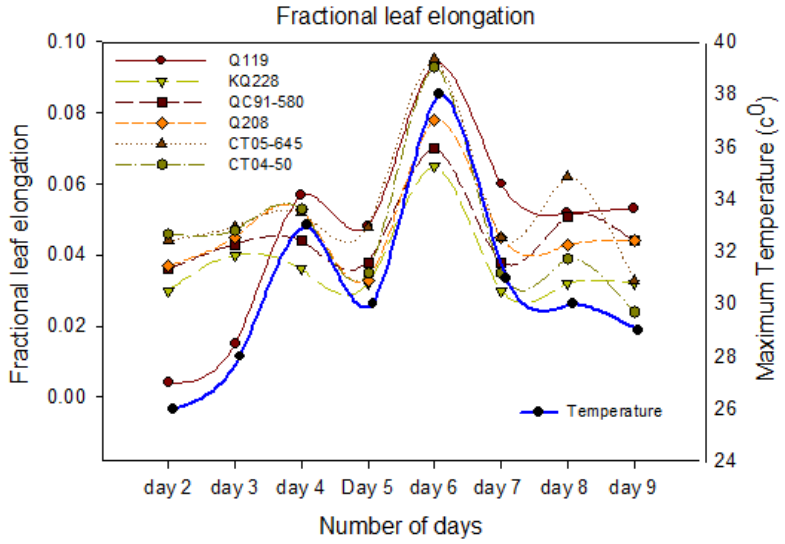

Figure 18 - Daily temperature (maximum) variation and fractional leaf elongation of 6 clones grown under severe drought conditions in R2 at Home Hill

The variation in leaf extension rate (LER) between irrigated and drought treatments was quantified. The rate of elongation varied from 3.6-6.5 cm/day under irrigated condition while it was 2.9-5.3 cm/day under severe stress conditions (Figure 19). Under severe stress conditions R570 and Q208$^{\phi}$ maintained the highest leaf elongation (Table 27).

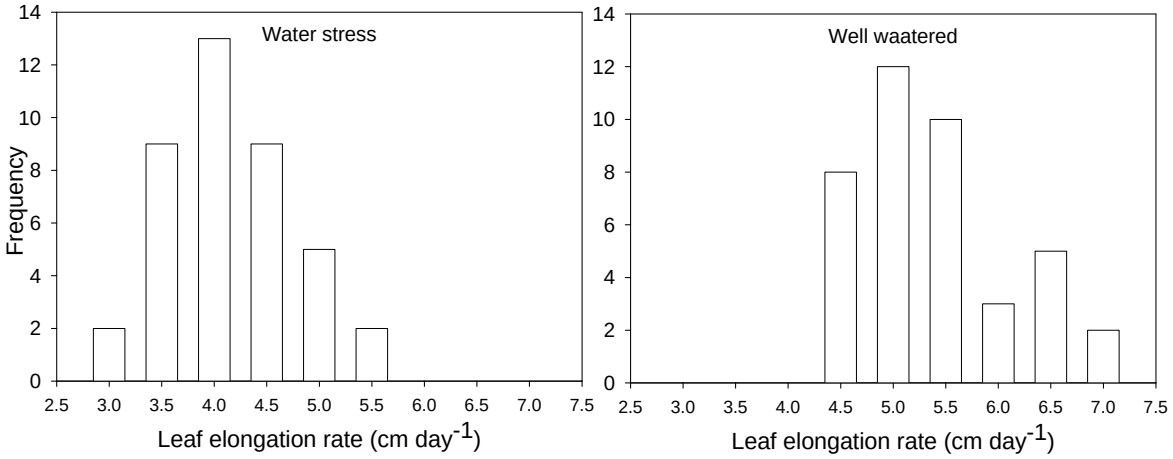

Figure 19 - Frequency distributions of average rate of leaf elongation in 7, 8 and 9th leaf in 40 clones under fully irrigated and drought conditions in March 2009

Table 27 - Observations on growth measurements in 3 leaves each in 10 clones grown under well-watered and drought conditions in R2 crop at Home Hill

Clone	Elongation (cm/day) Irrigated	Elongation (cm/day) Drought	% Elongation under drought relative to irrigated clone	Leaf length fully grown (cm)
Q208	6.28	5.16	82	107.97
QC93-1863	5.78	4.42	76	104.60
Q119	5.56	4.16	75	85.90
CT04-845	6.03	4.35	72	84.27
Q229	6.02	4.59	76	83.30
R570	6.01	5.3	88	79.77
Q183	5.79	3.93	68	78.57
CT04-99	5.5	3.8	69	73.70
CT05-199	6.58	3.84	58	65.30
Q158	5.69	3.55	62	61.87

The fractional elongation (dry/wet treatment) varied widely (34 to 88%) among 40 clones in this experiment. The well adapted commercial variety Q208$^{\phi}$ and R570 had the highest fractional elongation. It was also noted that the variation in rate of elongation and the fractional elongation were not related to the variation in actual leaf length. Although the variations in leaf elongation of those 40 clones across two water treatments were correlated (r^2 = 0.3), there was no strong correlation between leaf elongation in early growth phase and the variation in final biomass production of those 40 clones.

4.7 The genetic correlation between physiological traits and crop productivity

Genetic, phenotypic and environmental correlations between TCH reduction (as a percentage of TCH reduction due to drought over the realized TCH), *gs* and RWC were estimated for Home Hill (Table 28). The data were collected from the six water environments (plant crop and 2 ratoon crops). Genetic variances and covariances components were estimated using the combined analysis of 3 crop classes and two water treatments. Genetic correlations usually had moderate and negative values for RWC and *gs*. Cane yield reduction generally was best correlated with RWC measured during water stress period in stressed plants. The phenotypic and environmental correlations were relatively low for RWC. Stomatal conductance and cane yield reduction were also negatively correlated indicating that maintenance of high *gs* under water-limited conditions could be advantageous in minimizing yield losses in this population.

Table 28 - Genetic, phenotypic and environmental correlations between physiological traits and mean yield reduction (%) of 3 crops of Home Hill

Correlations	RWC%	Stomatal conductance (gs)
Genetic	-0. 42	-0.36
Phenotypic	- 0. 28	-0.35
Environmental	- 0.14	-0.28

4.8 Identification of clones for further experimentation

We investigated scientifically relevant and practically useful physiological traits for clone evaluation for drought adaptation and efficient use of water. This study revealed some of the technical challenges/complexities when exploring physiological traits in large genetically diverse populations of sugarcane under field conditions. It is evident that more mechanistic understanding of the regulation of traits under varying moisture environments is needed for exploiting physiological traits for crop improvement.

Table 29 - Clusters of genotypes with different levels of RWC, gs and yield reductions identified from the multivariate analysis

Groups No	RWC %	*gs* Drought (mmol $m^{-2}s^{-1}$)	*gs* irrigated (mmol $m^{-2}s^{-1}$)	Yield Irrigated	Yield Reduction%	Remarks
Group 81	90.2	158.0	346.0	106.6	38.3	High *gs*, low RWC
Group 82	93.0	161.8	335.6	87.9	20.5	High *gs*, high RWC
Group 84	91.8	146.7	313.3	109.1	29.3	Average *gs*, high RWC
Group 85	88.0	120.3	298.6	93.1	31.8	Low *gs*, low RWC
Average	90.4	142.6	318.8	99.8	30.7	

Principal component analysis (PCA) was conducted with the complete set of data from 3 crop cycles to gain more insight into trait and clone association. Score of PC 1 (Principal Component one) had explained 36% association with the population and was highly correlated with RWC. PC 2 (Principal Component two) score had 29% association with the population and was correlated with gs. Four groups of clones were identified with different gs and RWC expression patterns (Table 29). These results are consistent with the previous PCA with plant crop data in 2008. Most clones in group 82 (Table 29) had high *gs* and RWC in all 3 crop classes. The average TCH reduction in this group was lower than the other 3 groups.

Framework for identification of possible trait combinations for target production environments (TPEs)

A theoretical framework based on the results of PCA and cluster analysis has been developed to determine possible trait combinations which could assist in identifying clones for different production environments (Figure 20).

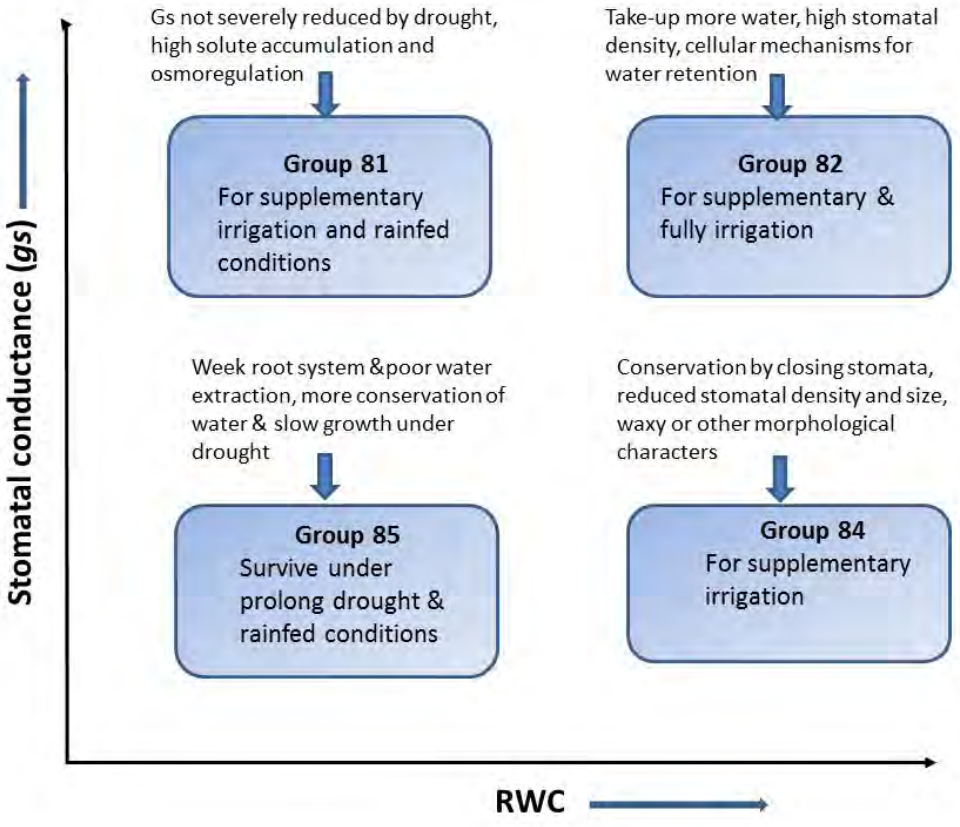

Figure 20 - Theoretical framework based on stomatal conductance, relative water content of tissue and yield parameters for understanding and validating trait-clone-production environment relationships in target regions for the next phase of research

The productivity (TCH) of clones under well-watered and drought conditions were used as the primary selection criterion for identifying clones from those groups (Table 30, 31 & 32). Those clones with significant interaction in stress environments were considered for further investigation. The assumption was that those clones have specific adaptation to particular combination of environment stress factors which could overcome the production barriers in those conditions. Further systemic studies are needed to understand the contribution of those physiological traits to continue productivity under different stages of growth in those

clones. There are some clones among those selections with broad adaptation across varying stress environments (Table 32). Those different types of adaptive mechanisms in different groups within a range of stress environments need to be future investigated. The test environments used in the clone evaluation represented the most target production environments (TPEs) in the Northern Queensland. Further testing of these clones in TPEs will be continued in the second phase of this project in Central and North Queensland.

Population differences between QCANES and non-selected clones

The productivity (TCH) of improved cultivars and the clones derived from the crosses with wild relatives across 12 test environments were investigated (Figure 21). Both groups showed a similar TCH under severe stress environments. The group difference increased with the relief of stress under highly favourable environments. However, there were some clones within each group with specific adaptability to high stress environments (Figure 22). The low yield in improved clone could be attributed to poor response to water-limited conditions or their inherent drought susceptibility.

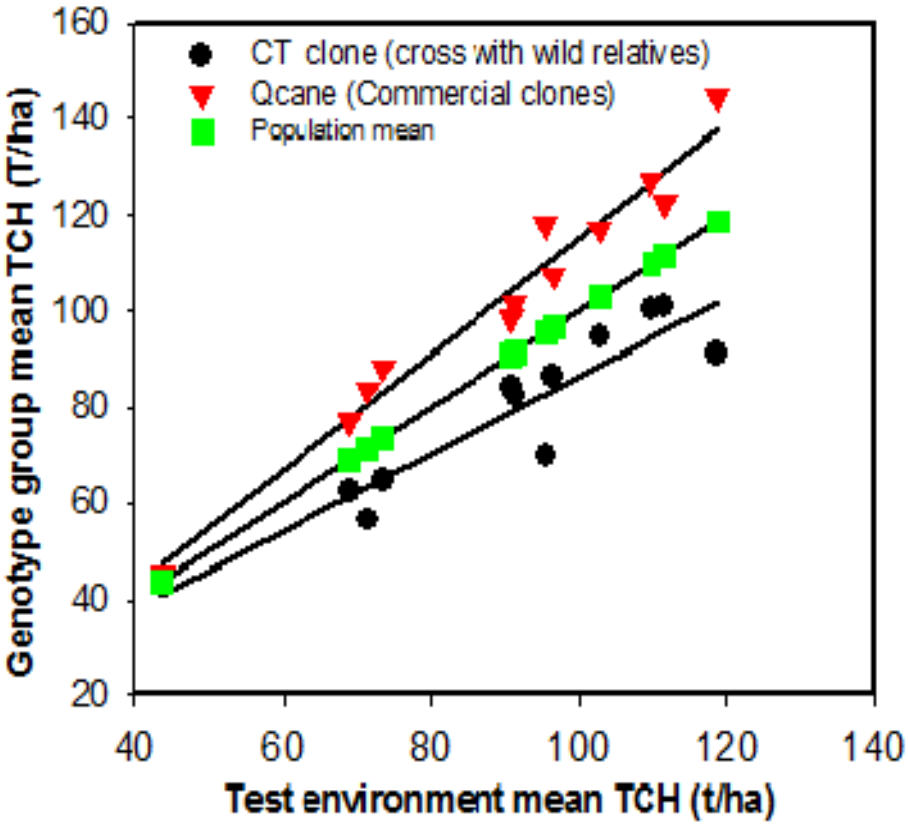

Figure 21 - Group mean TCH variation against the stress environments and non stress environments within the 12 environment mean TCH

Table 30 - Performance of 21 clones at Crystal Creek those were selected for further experimentation on trait and productivity relationship under different target production environments

Crystal Creek	TCH			*gs*	RWC
clone	Plant_CC	R1-CC	R2-CC	CC-CD	CC-RWC
CT04-50	113.25	70.5	29.4	214.7	97.4
CT05-594	90.9	82.3	54.85	174.4	94.5
CT04-116	118	114.4	71.6	180.4	94.7
CT04-33	90.75	67.7	41.75	260.3	98.0
CT04-450	92.65	82.95	53.6	172.6	97.0
CT05-853	82.15	95.95	87	193.0	98.0
QB01-5	89.35	74.2	64.7	225.8	96.8
QC93-1863					
QC91-580					
CT04-120	90.6	93	33.35	208.8	95.2
CT04-99	85.4	56.15	36.1	199.6	94.8
CT04-559	64.65	75.8	45.5	166.5	97.5
Q190	131.8	139.5	95.75	232.4	97.7
Q158	172.45	106.3	77.4	179.0	94.7
CT04-951	83.05	98.6	57.5	182.4	97.0
Q183	133.95	130.9	107.7	207.9	96.2
MQ93-538					
CT05-645	80.3	70	52.8	198.0	97.0
R570					
QN66-2008	157	129.1	76.6	170.5	96.0
CT05-570	120.15	81.05	65.8	204.6	92.1
Avearge	**106.9**	**93.8**	**62.8**	**124.9**	**95.9**
Maximum	**172.5**	**142.8**	**109.2**	**301.9**	**99.7**
Minimum	**64.7**	**56.2**	**25.2**	**92.1**	**92.1**

Table 31 - Performance of 21 clones (at Home Hill) selected for further experimentation on trait and productivity relationship under different target production environments

	TCH						gs mmol m-2s-1		RWC%	yield reduction%		
Clone	PC1-dr	R1-Dr	R2-Dr	PC1 -Irri	R1-Irri	R2-Irri	Drought	Irrigate	Drought	R2	PC1+R2	Rank
CT04-50	54.1	79.0	51.5	51.0	65.8	61.0	150.7	285.7	94.0	**15.6**	**0.7**	1.0
CT05-594	65.6	83.8	58.8	104.7	102.6	71.4	153.8	310.2	88.6	**17.7**	**0.7**	2.0
CT04-116	54.7	90.5	53.6	77.3	101.8	75.5	128.3	263.2	91.8	**29.0**	**0.7**	5.0
CT04-33	57.6	74.6	36.2	58.3	91.2	53.0	150.8	294.1	92.1	**31.7**	**0.8**	7.0
CT04-450	57.2	79.0	55.7	64.3	84.5	82.2	111.0	285.4	91.3	**32.2**	**0.8**	8.0
CT05-853	69.6	95.9	53.0	74.2	97.7	86.0	150.8	334.2	93.4	**38.4**	**0.8**	13.0
QB01-5	34.0	62.1	40.4	53.4	76.6	66.1	159.0	347.0	85.3	**38.8**	**0.6**	14.0
QC93-1863	82.8	112.6	51.6	125.4	138.9	84.7	119.4	307.3	89.5	**39.1**	**0.6**	15.0
QC91-580	99.7	141.5	65.0	117.8	111.4	106.9	205.8	345.5	93.0	**39.2**	**0.7**	16.0
CT04-120	61.1	75.4	39.3	66.7	83.6	65.9	119.8	275.5	89.2	**40.3**	**0.8**	17.0
CT04-99	33.0	45.9	23.6	58.2	74.3	42.9	140.6	309.8	81.9	**44.9**	**0.6**	21.0
CT04-559	44.0	62.6	36.5	51.4	106.7	67.4	128.0	281.8	84.4	**45.8**	**0.7**	22.0
Q190	66.0	95.6	45.1	109.5	129.2	83.3	146.5	309.8	91.6	**45.9**	**0.6**	23.0
Q158	71.1	94.4	51.0	108.4	127.8	98.1	142.6	310.4	89.0	**48.0**	**0.6**	26.0
CT04-951	60.3	80.4	41.3	72.7	100.9	81.4	123.4	286.7	89.1	**49.3**	**0.7**	28.0
Q183	79.7	94.2	36.1	109.7	120.2	105.6	118.1	299.3	87.2	**65.8**	**0.5**	77.0
MQ93-538	86.4	111.4	38.5	109.1	155.0	118.4	161.9	374.7	91.0	**67.5**	**0.5**	81.0
CT05-645	35.9	48.5	19.1	56.3	74.0	76.2	97.5	275.7	83.6	**74.9**	**0.4**	87.0
R570	63.0	77.5	32.8	113.5	128.1	131.6	159.7	309.6	86.5	**75.1**	**0.4**	88.0
QN66-2008	80.9	96.2	26.1	101.0	124.0	105.7	126.8	280.1	87.1	**75.3**	**0.5**	89.0
Average	**68.3**	**89.4**	**41.8**	**92.3**	**112.7**	**98.0**	**143.3**	**307.2**	**88.9**			
Maximum	**115.9**	**141.5**	**79.3**	**138.5**	**163.2**	**141.2**	**205.8**	**374.7**	**95.1**			
Minimum	**33.0**	**45.9**	**19.1**	**51.0**	**65.8**	**42.9**	**97.5**	**230.3**	**80.3**			

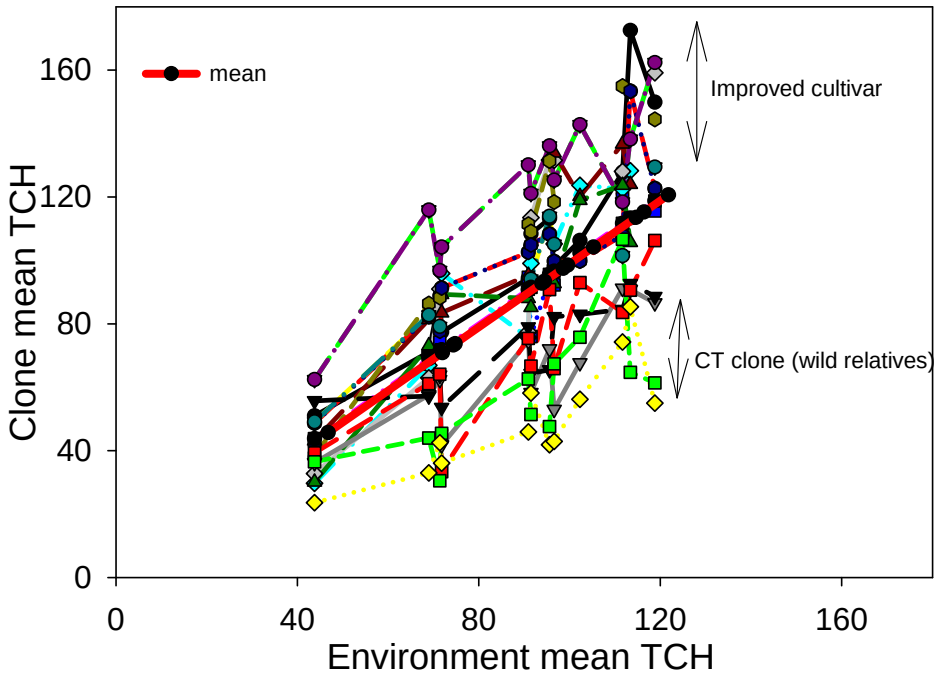

Figure 22 - Variation in clone stability across 12 test environments. The red line indicates the mean of the whole population.

Table 32 - Performance 21 clones (at Home Hill) selected for further experimentation on trait and productivity relationship under different target population of environments

	TCH			RWC%			Yield	gs mmolm-2s-1		
Clone	**Drought**	**Semi-Irri**	**Irrigated**	**Drought**	**Semi-Irri**	**Irrigated**	**reduction**	**Drought**	**Semi-Irri**	**Irrigated**
CT04-50	69.4	80.8	101.0	94.2	96.1	93.6	**31.3**	174.1	230.7	234.5
CT05-594	61.7	73.8	95.4	89.7	94.0	89.7	**35.4**	167.7	227.5	179.4
CT04-116	58.0	67.6	106.3	91.1	95.1	94.0	**45.4**	151.2	202.4	181.5
CT04-33	62.4	72.1	86.6	92.4	94.2	91.5	**28.0**	136.6	249.1	195.0
CT04-450	63.8	65.5	88.6	90.7	94.4	92.4	**28.0**	125.0	192.0	197.6
CT05-853	59.7	79.1	97.6	90.3	94.2	90.9	**38.9**	140.7	236.2	198.1
QB01-5	47.5	56.6	86.4	87.9	94.3	89.6	**45.1**	180.8	233.7	209.4
QC93-1863	91.0	123.5	159.9	90.4	93.9	90.2	**43.1**	132.0	190.8	180.1
QC91-580	73.1	143.0	156.7	91.5	94.0	89.5	**53.3**	176.5	250.9	236.3
CT04-120	64.1	90.8	106.3	89.6	93.6	87.1	**39.7**	145.0	233.2	195.6
CT04-99	42.5	41.9	55.0	87.9	95.3	91.2	**22.6**	174.2	241.2	195.6
CT04-559	30.5	47.6	61.4	88.0	93.2	91.7	**50.4**	121.0	201.8	178.8
Q190	84.3	126.9	137.7	91.9	94.4	91.8	**38.8**	172.9	195.6	212.2
Q158	88.8	113.2	149.9	89.1	93.9	92.1	**40.7**	173.0	252.4	249.9
CT04-951	48.2	52.4	83.0	88.5	92.5	90.4	**41.9**	147.0	209.9	199.0
Q183	83.0	109.0	146.0	88.6	93.1	92.0	**43.2**	128.1	195.4	193.0
MQ93-538	88.3	131.4	144.5	90.3	95.6	90.9	**38.9**	185.5	242.5	205.1
CT05-645	40.1	43.2	52.4	88.3	94.6	94.0	**23.6**	157.1	228.5	205.8
R570	91.0	131.7	159.2	88.9	95.5	91.0	**42.9**	131.2	254.4	190.5
QN66-2008	107.5	124.9	127.8	91.4	95.6	92.8	**15.9**	154.6	190.6	190.9
Average	**71.9**	**96.7**	**119.7**	**90.2**	**94.7**	**91.5**	**15.2**	**158.2**	**223.8**	**208.4**
Maximum	**107.5**	**148.4**	**173.1**	**94.2**	**96.4**	**94.0**	**53.3**	**203.9**	**261.0**	**251.1**
Minimum	**30.5**	**41.9**	**52.4**	**85.3**	**92.5**	**87.1**	**0.4**	**121.0**	**190.4**	**171.9**

5.0 DISCUSSION AND CONCLUSIONS

Numerous putative traits have been identified and promoted as potentially useful selection criteria for breeding cultivars for water-limited agriculture in arid and semi-arid zones worldwide (Ludlow and Muchow, 1990). For most of them, their real value for crop improvement has never been proven at the whole plant or crop level. These traits are mostly the components of stress survival mechanisms with little or no major value in contributing to crop yield. Sugarcane germplasm has never been systematically screened for their genetic value for improving WUE and crop productivity under water deficit conditions. Conducting field level sugarcane water stress research involving a large number of germplasm is a huge, time-consuming, resource-intense and expensive exercise. To manage such a situation, limiting the selection process to a small number of major physiological processes that are directly contributing to growth, development, water relations and ultimately to yield would be more useful and practical. By this approach all the proposed unproven putative traits in other plants have been excluded in our research. This would maximise the opportunity for success as key traits controlling main physiological processes are likely to direct towards large genetic gains in breeding programs.

The key physiological traits that are selected for investigation in this project are directly related to carbon fixation, water relations, growth and sugar and cane yield. Stomatal conductance is a major component of photosynthesis and transpiration and plays a regulatory role in plant water use. Tissue, water status, though a simple physiological parameter, indicates cell functional status and adaptive capacity of plants to water stress. These traits, in addition to their impact in major physiological processes, are also easy to measure and thus could be exploited as indirect selection tools for germplasm screening. Sugarcane, being a large plant, with stem as the harvestable part of the crop, spanning across different climatic zones and requiring 12 months to complete a crop cycle, poses its own challenges to develop a screening system and identify suitable traits for genetic improvement for water-limited environments.

Taken into consideration of all these matters, we have selected three locations within the prime sugarcane production environments in North Queensland. Those locations were within the high and low rainfall areas and reasonably mapped for deep ground water levels. We have selected the most practical way of field screening of a manageable size of a germplasm with 133 clones representing a broad genetic background in Saccharum spp. We developed 12 contrasting moisture environments within three locations with the aid of “water sense” (CSIRO) by manipulating irrigation cycles during the crop growth.

The soil moisture status across drought and irrigated treatments in plant and ratoon crops indicated that there were contrasting moisture profiles across different root depths. Drought treatment created a considerable moisture deficit either early or later stages of growth. The variation in cane yield and other agronomic characters between well watered and water stress treatments in experimental locations clearly indicated that the imposition of drought treatment was successful to achieve our objectives. Though the intensity of drought and water status across the 12 test environments were not unique, it provided a considerable random variation of the occurrence of water stress environments those similar to the target population of water limited environments. Fortunately, those experimental years were recorded as the driest and lowest rainfall years within the last ten year period. The dynamic moisture extraction during drought treatments observed by the Enviro-scans structured moisture measurements from the neutron moisture meters *via* access tubes installed in deep soil layers. Observations on the variation in rate of leaf expansion between drought and irrigated treatments suggested that the drought imposition was successful in relation to growth and development of test clones.

Based on first year plant crop results and the recommendation of the consultative committee, the project decided to concentrate on two aspects of drought tolerance and water use efficiency in the sugarcane population. Two main physiological traits known to be promising for drought adaptation for other crops (Ludlow and Muchow, 1990), namely stomatal conductance (*gs*) and relative water content (RWC), were measured in 133 sugarcane clones in response to irrigated and imposed drought conditions in 12 test environments. The variations in RWC were more distinct under imposed drought conditions than the fully irrigated conditions. The effects of these changes among genotypes were investigated in relation to the changes in agronomic and quality characteristics of the test clones.

Imposition of drought in both plant and ratoon crops was successful at Home Hill and Dalbeg. Drought on plant crop resulted in a significant reduction in TCH and CCS which was about 25-75% for TCH. Genotypic variations in both traits were significant when the stress condition was severe. Genotypic variations in stalk height, stalk number and leaf senescence (%) were also significant and affected by drought. These traits showed approximately 17%, 14% and 40% reductions respectively, in the drought treatment.

Clone variations of *gs* and RWC in plant and ratoon crops were significant. Stomatal conductance was more consistent across measurements and between plant and ratoon crops.

The treatment and treatment × clone interactions were significant for *gs* and RWC only in few occasions where the stress levels were considerably high. Changes in micro environment [radiation and low vapor pressure deficit (VPD)] could influence mostly on the expression of these traits during measurements. Significant phenotypic and genetic correlations between yield reduction and physiological traits (*gs* and RWC) in the drought treatment indicated that the yield variation due to water stress can be partly explained by the expression of these traits. Both traits had positive genetic effects on maintaining low yield production under drought. The genetic correlations of these traits with productivity explain that there are no negative pleotropic effects. However, it is important to mention that these genetic correlations were moderate and there is no simple and straight forward approach for selecting clones based on phenotypic associations for commercial adaptation. The large G×E interaction for TCH in this population indicated the plasticity of clones in adopting to different stress environments. One exciting example is that the productivity of QN66-2008 and QC91-580 across HH and Dalbeg environments. The TCH reduction was completely opposite in these two clones across 2 locations. When the drought intensity was high at HH, the productivity had decreased by 75% and 39% in QN66-2008 and QC91-580, respectively. There was a contrasting difference in RWC and *gs* between these 2 clones (Table 31,32). In another stress conditions at Dalbeg, QN66-2008 showed the lowest reduction of 15%. Interestingly, QN66-2008 maintained slightly higher *gs* (190 mmol m^{-1} s^{-1}) than QC91-580 (176 mmol m^{-1} s^{-1}) while both clones had similar RWC (91%) under stress conditions (Table 29, 30). Therefore, it is important to investigate the behaviour of individual clones across varying environments more closely to understand the mechanistic relationship of the physiological traits with the productivity components of those individual clones.

The positive results of genetic correlation with TCH reduction showed that *gs* and RWC could be valuable indicators of drought tolerance and can be used in characterizing test populations. Principle component analysis assisted in identifying four groups of genotypes with contrasting levels of RWC and *gs*. The PC score in vector 1 mostly associated with the RWC whereas PC score in vector 2 was with *gs* in plant crop. The statistical information provided the base to develop a theoretical framework to understand the adaptive mechanisms of those clones using these two traits in different target moisture environments. A number of test clones were identified for further experimentation and to conduct more

research to understand the mechanistic and functional relationship with productivity (TCH) in sugarcane.

- Putative traits related to stress survival mechanisms identified as potentially useful tools in selecting breeding cultivars for water-limited conditions
- *RWC* and *gs* identified as indicators of drought tolerance in characterizing test populations. These traits may have contrasting contributions for the drought adaptation in different target environment.
- A total of 133 clones representing a broad genetic background in Saccharum spp. tested for their genetic value for improving WUE and crop productivity under 12 contrasting moisture environments within three locations during the crop growth. Significant genotypic variations in *gs*, RWC, TCH reduction%, stalk height, stalk number and leaf senescence observed during severe stress conditions
- Significant genetic variation which could be exploited in a breeding program exists for transpiration efficiency (TE) among the test clones
- Large G×E interaction for TCH indicated the plasticity of clones in adopting to different stress environments, e.g. productivity of QN66-2008 and QC91-580 across Home Hill and Dalbeg environments.
- Physiological information gathered to develop a theoretical framework to understand the adaptive mechanisms of those clones using these two traits in different target moisture environments.

6.0 OUTPUTS

1. Development of a quantities database on DT and WUE of the Australian germplasm

   The project developed quantitative and qualitative data on genetic variability of several important agronomic, yield and sugar characters for the 133 clones. These data were from 12 different moisture environments across 3 locations. Similarly, physiological observations on stomatal conductance, relative water content, leaf elongation (LER), transpiration efficiency and root water extraction were collected. Spatial variations of some data were adjusted using appropriate statistical analyses. This database has been used to understand trait-environment association and their interaction.

2. Knowledge and understanding about DT and WUE traits

   Project developed the basic knowledge on role of a key of morpho-physiological traits related to drought adaptation and WUE using a large, genetically diverse sugarcane germplasm. We acknowledged the complexity of drought tolerance and water use efficiency mechanisms in sugarcane and adopted a best and efficient research approach to enable us to establish fundamental understanding of trait productivity relationship across varying target populations of environments.

3. Selected clones for detailed investigations on drought tolerance and WUE traits

   The research findings helped us to identify some elite clones with desired drought adaptation and WUE characteristics for further experimentation. The information generated so far would assist us to make a fair comparison among clones for their productivity across the test environments. One of the exciting information is that the patterns of TCH relationship under irrigated and drought conditions across two test locations (Home Hill and Dalbeg) (please refer Figure 9). It is too early for cultivar

recommendation for direct commercial deployment and further breeding as we collected limited information on mechanistic relationship with productivity and the causes for large G×E interactions.

7.0 EXPECTED OUTCOMES

1. Recommendations of clones for further breeding for DT and WUE and direct commercial deployment
2. Developing rapid screening methods for sugarcane for DT and WUE
3. Improved simulation capability of APSIM-sugarcane for better understanding of traits×environment interactions

Genetic variation across test environments (GxE) is incorporated into the crop growth simulation model APSIM-Sugar to predict responses of particular genotypes across a wider range of environments. These predictions will be tested in further field experiments in the coming years. Ways in which potential application of the results to undertake effective breeding for water stress will be discussed.

8.0 FUTURE NEEDS AND RECOMMENDATIONS

Translate the progress made into pre-commercial stage for breeding water use efficient and drought tolerant cultivars through following activities/steps

1. Test/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)
2. Evaluate the results of trait validation experiments in breeding program
3. Identify elite clones with desired water use efficient and drought tolerant capacity
4. Make specific recommendations to breeding programs to breed cultivar for different target environments affected by water stress

9.0 PUBLICATIONS ARISING FROM THE PROJECT

Oral presentation

1. "Genotypic Variation for Drought Tolerance in Sugarcane"
   The results were presented at the 9th ISSCT Plant Breeding and Germplasm Workshop held in Cairns, Australia in August 2009. The abstract and the presentation have been published in the World Wide Web:
   http://www.bses.org.au/bses_01.asp?page_id=8212214

2. "Breeding sugarcane for water stress"
   The abstract submitted for the 10th ISSCT Plant Breeding and Germplasm Workshop to be held in Brazil has been accepted.

Journal papers

Two manuscripts for journal publication are in preparation.

Poster presentation
A poster paper was presented in the “International conference on Abiotic Stress” in Vienna, Austria.

A poster paper has been accepted for the “Plant Biology Conference” to be held in Melbourne, Australia in July, 2011.

10.0 ACKNOWLEDGEMENTS

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APPENDIX 1

Appendix 1. Genetic variances, genetic co-variances and genetic correlations between test environments (TE) for CCS, TDM, TSH, Gs and RWC

Genetic variance for CCS

Genetic Variance								
CC-Rainfed-P	12.23							
CC-Rainfed-R1	8.951							
CC-Rainfed-R2	13.43							
HH-Drought-P	11.58							
HH-Drought-R1	8.275							
HH-Drought-R2	5.088							
HH-water-P	10.81							
HH-water-R1	11.79							
HH-water-R2	6.712							
Genetic Correlation	CC-Rainfed-1	CC-Rainfed-2	HH-Drought-0	HH-Drought-1	HH-Drought-2	HH-water-0	HH-water-1	HH-water-2
CC-Rainfed-P	0.9633	0.9587	0.9086	0.9795	0.9799	0.9799	0.9769	0.9799
CC-Rainfed-R1		0.9619	0.9116	0.9827	0.9831	0.9831	0.9801	0.9831
CC-Rainfed-R2			0.9073	0.9781	0.9784	0.9784	0.9755	0.9784
HH-Drought-P				0.927	0.9273	0.9273	0.9245	0.9273
HH-Drought-R1					0.9997	0.9997	0.9967	0.9997
HH-Drought-R2						1	0.997	1
HH-water-P							0.997	1
HH-water-R1								0.997
HH-water-R2								
Genetic Co-variance	CC-Rainfed-0	CC-Rainfed-1	CC-Rainfed-2	HH-Drought-0	HH-Drought-1	HH-Drought-2	HH-water-0	HH-water-1
CC-Rainfed-P								
CC-Rainfed-R1	10.08							
CC-Rainfed-R2	12.29	10.55						
HH-Drought-P	10.81	9.279	11.31					
HH-Drought-R1	9.853	8.457	10.31	9.072				
HH-Drought-R2	7.729	6.634	8.088	7.116	6.486			
HH-water-P	11.26	9.668	11.79	10.37	9.453	7.415		
HH-water-R1	11.73	10.07	12.28	10.8	9.845	7.723	11.25	
HH-water-R2	8.878	7.62	9.29	8.174	7.45	5.844	8.517	8.87

Genetic variances for TDM

	Genetic Variance							
CC-Rainfed-P	52.33							
CC-Rainfed-R1	33.58							
CC-Rainfed-R2	26.25							
HH-Drought-P	13.78							
HH-Drought-R1	25.32							
HH-Drought-R2	7.447							
HH-water-P	26.63							
HH-water-R1	37.51							
HH-water-R2	17.15							
Genetic Correlation	CC-Rainfed-R	CC-Rainfed-R2	HH-Drought-P	HH-Drought-R1	HH-Drought-R	HH-water-P	HH-water-R1	HH-water-R2
CC-Rainfed-P	0.7881	0.7473	0.6106	0.7092	0.3736	0.7389	0.7882	0.8
CC-Rainfed-R1		0.9201	0.7518	0.8732	0.46	0.9097	0.9704	0.985
CC-Rainfed-R2			0.7129	0.828	0.4362	0.8626	0.9202	0.9341
HH-Drought-P				0.6765	0.3564	0.7048	0.7518	0.7632
HH-Drought-R1					0.4139	0.8187	0.8733	0.8865
HH-Drought-R2						0.4312	0.46	0.467
HH-water-P							0.9098	0.9235
HH-water-R1								0.9851
Genetic Co-variance	CC-Rainfed-0	CC-Rainfed-1	CC-Rainfed-2	HH-Drought-0	HH-Drought-1	HH-Drought-2	HH-water-0	HH-water-1
CC-Rainfed-P								
CC-Rainfed-R1	33.03							
CC-Rainfed-R2	27.69	27.31						
HH-Drought-P	16.4	16.17	13.56					
HH-Drought-R1	25.81	25.46	21.34	12.64				
HH-Drought-R2	7.374	7.273	6.098	3.61	5.684			
HH-water-P	27.58	27.2	22.8	13.5	21.26	6.072		
HH-water-R1	34.92	34.44	28.87	17.09	26.91	7.689	28.75	
HH-water-R2	23.97	23.64	19.82	11.73	18.47	5.277	19.74	24.99

Genetic variances for TSH.

Genetic Variance								
Genetic Variance								
CC-Rainfed-P	30.17							
CC-Rainfed-R1	20.09							
CC-Rainfed-R2	16.02							
HH-Drought-P	8.616							
HH-Drought-R1	11.58							
HH-Drought-R2	1.39							
HH-water-P	16.11							
HH-water-R1	22.47							
HH-water-R2	12.55							
Genetic Correlation	CC-Rainfed-1	CC-Rainfed-2	HH-Drought-0	HH-Drought-1	HH-Drought-2	HH-water-0	HH-water-1	HH-water-2
CC-Rainfed-P	0.9216	0.9348	0.8741	0.91	0.8932	0.9348	0.9343	0.9348
CC-Rainfed-R1		0.9859	0.9219	0.9598	0.942	0.9859	0.9854	0.9859
CC-Rainfed-R2			0.9351	0.9735	0.9555	1	0.9995	1
HH-Drought-P				0.9104	0.8935	0.9351	0.9347	0.9351
HH-Drought-R1					0.9302	0.9735	0.9731	0.9735
HH-Drought-R2						0.9555	0.955	0.9555
HH-water-P							0.9995	1
HH-water-R1								0.9995
HH-water-R2								
Genetic Co-variance	CC-Rainfed-0	CC-Rainfed-1	CC-Rainfed-2	HH-Drought-0	HH-Drought-1	HH-Drought-2	HH-water-0	HH-water-1
CC-Rainfed-P								
CC-Rainfed-R1	22.69							
CC-Rainfed-R2	20.55	17.68						
HH-Drought-P	14.09	12.13	10.99					
HH-Drought-R1	17.01	14.64	13.26	9.095				
HH-Drought-R2	5.785	4.978	4.509	3.092	3.733			
HH-water-P	20.61	17.73	16.06	11.02	13.3	4.522		
HH-water-R1	24.33	20.94	18.96	13.01	15.7	5.338	19.02	
HH-water-R2	18.19	15.65	14.17	9.722	11.74	3.99	14.22	16.78

Genetic variances for Conductance

Genetic Variance								
CC-Rainfed-P	613.6							
CC-Rainfed-R1	827							
CC-Rainfed-R2	520.8							
HH-Drought-P	626.1							
HH-Drought-R1	450.8							
HH-Drought-R2	348.4							
HH-water-P	546.3							
HH-water-R1	714.3							
HH-water-R2	530.7							
Genetic Correlation	CC-Rainfed-1	CC-Rainfed-2	HH-Drought-0	HH-Drought-1	HH-Drought-2	HH-water-0	HH-water-1	HH-water-2
CC-Rainfed-P	0.5075	0.4389	0.8656	0.8128	0.9273	0.7477	0.6681	0.8961
CC-Rainfed-R1		0.2291	0.4519	0.4243	0.4841	0.3903	0.3488	0.4678
CC-Rainfed-R2			0.3908	0.3669	0.4187	0.3376	0.3016	0.4046
HH-Drought-P				0.7236	0.8256	0.6657	0.5949	0.7978
HH-Drought-R1					0.7752	0.6251	0.5586	0.7492
HH-Drought-R2						0.7132	0.6373	0.8547
HH-water-P							0.5139	0.6892
HH-water-R1								0.6159
Genetic Co-variance	CC-Rainfed-0	CC-Rainfed-1	CC-Rainfed-2	HH-Drought-0	HH-Drought-1	HH-Drought-2	HH-water-0	HH-water-1
CC-Rainfed-R1	361.5							
CC-Rainfed-R2	248.1	150.4						
HH-Drought-P	536.5	325.2	223.1					
HH-Drought-R1	427.4	259.1	177.8	384.4				
HH-Drought-R2	428.7	259.9	178.3	385.6	307.2			
HH-water-P	432.9	262.4	180.1	389.3	310.2	311.1		
HH-water-R1	442.3	268.1	184	397.8	317	317.9	321	
HH-water-R2	511.4	309.9	212.7	459.9	366.4	367.5	371.1	379.2