SaveN Cane: Developing selection tools for N-efficient sugarcane

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## SRA Research Project Final Report

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<td><strong>Project Title</strong></td>
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<td><strong>Key Focus Area in SRA Strategic Plan</strong></td>
<td>2. Optimally adapted varieties, plant breeding and release (3. Soil health and nutrient management)</td>
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<td><strong>Research Organisation(s)</strong></td>
<td>University of Queensland, SRA</td>
</tr>
<tr>
<td><strong>Chief Investigator(s)</strong></td>
<td>Susanne Schmidt, Prakash Lakshmanan, Mike Cox, Nicole Robinson</td>
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### Project Objectives

1. Quantify the extent of genetic variation for NUE under field conditions
2. Identify the traits that are associated with or confer NUE
3. Develop practically useful screening tools for NUE
4. Establish promising clones for the follow-up project which will
   - test the breeding value of selected clones for cultivar development,
   - implement the developed screening method in the breeding program
5. Establish productive research collaborations with overseas sugar R & D program
6. Raise grower awareness of NUE cultivars

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<td><strong>Date submitted</strong></td>
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<td><strong>Reason for delay (if relevant)</strong></td>
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### Success in achieving the objectives

- ☒ Completely Achieved
- □ Partially Achieved
- □ Not Achieved

### SRA measures of success for Key Focus Area (from SRA Strategic Plan)

- KFA 2: Rate of genetic gain (TCH); Locally adapted cane varieties with reduced inputs
- KFA 2: Varieties identified for increasing nutrient use efficiency within plant and ratoon crops, Reduced impact of farm run off

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Page 1  Sugar Research Australia- Research Funding Unit- Research Project Final Report
PART A
To be completed by the Chief Investigator

Section 1: Executive Summary

This project supports the sugar industry’s intensifying efforts to reduce its nitrogen (N) footprint that is caused by inefficient use of N fertiliser by the crop. The industry aims to minimise N pollution of coastal waters and emission of potent greenhouse gas nitrous oxide from soil without negatively impacting the economic sustainability of sugar production. International research addressing this pervasive problem in grain and other crops indicates that effective approaches combine agronomic innovation of N supply and nitrogen-use efficient (NUE) crop varieties.

This UQ-SRA collaborative project, aimed to advance knowledge of N use efficiency of crop varieties through systematic testing of a considerable number of sugarcane clones with diverse genetic background (commercial varieties from Australia and overseas, identified water-use-efficient clones, crosses with ancestral canes). Additional value was derived from a collaboration with QLD DAFF (Andrew Robson) to advance remote sensing of crop N, and investigations of the effects of N fertiliser on soil biology (Graham Stirling-nematodes, UQ consortium-bacterial and fungal communities). Brazilian researchers (Sao Paulo State) have since established sister experiments based on this project.

Clones were cultivated with low or recommended N rates (20-40 or 160-200 kg N-fertiliser per year) in two field trials (Mackay, Burdekin). The contrasting N rates were based on concepts that (i) NUE traits are only obvious in low-N environments, and (ii) ideal crop varieties will be strongly responsive to N supply and efficiently acquire N from fertiliser and indigenous soil reserves.

NUE traits of 64 clones were characterised over three years (plant crop-1st ratoon crop-2nd ratoon crop) by quantifying the effects of contrasting N supply on growth in early, mid and late season. Clone vigour and ratooning ability were evaluated, as was canopy development and photosynthetic performance, the ability to acquire and store nitrate, N allocation to stalks and leaves, and sugar and biomass yields.

Project deliverables focused on generating knowledge on the genetic variation in N response and NUE traits and ranking of clones across environments with different soils to study the magnitude and the robustness of NUE traits.

The overall deliverables and key findings include:
(i) Establishment of field experimental conditions with limited N availability suitable for screeningsugarcane populations for NUE and N-related crop attributes. The field trial set-up was demonstrably effective in evaluating a considerable number of clones over a 3-year crop cycle;
(ii) Knowledge of genetic variation for NUE in Australian sugarcane germplasm;
(iii) NUE screening for photosynthetic performance, N uptake and accumulation attributes and yield parameters (CCS, sugar and biomass yields) identified benefits/drawback of experimental approaches;
(iv) Generated data on trait variation across clones, crop stages and environments, demonstrating that environmental conditions markedly affected crop performance as evidenced by moderate (22%, Mackay) and strong (45%, Burdekin) reduction in yields with low N supply. Soil characteristics are a likely cause as clones at Mackay acquired on average 3- and 2-fold more N than at the Burdekin site over the plant-1st ratoon cycles at low and recommended N supplies;
(v) Plant vigour appears to be a major determinant of NUE in sugarcane;
Clones with contrasting NUE and N response have been identified for use in next-step NUE trait research;
Remote sensing showed potential for screening sugarcane germplasm, but its application at early stages of crop growth requires further investigation.

Taken together, the project has achieved the stated objective and fulfilled a role in SRA’s focus area of (1) optimally-adapted varieties, plant breeding and release. The project outcomes have been communicated to the industry nationally and internationally, have been evaluated in the context of global efforts in advancing NUE in crop and cropping systems, and are in preparation for peer review and publication in highly ranked international scientific journals. The project is strongly aligned with industry interests as evidenced by interest of growers, national and international collaborators. Logical next steps towards developing N use-efficient sugarcane in the Australian breeding program include advancing understanding the basis of clone sensitivity to N and tools for rapid selection of N-responsive clones.

### Section 2: Background

Maximising nitrogen use efficiency (NUE) is a mutual goal for cropping systems globally due to the economic and environmental implications of wasteful N fertiliser use, especially N leaching to the hydrosphere and emissions of potent greenhouse gas nitrous oxide (Hirel et al. 2011, Tilman et al. 2008). Across global crop systems there is general consensus that a two-tiered approach of crop breeding and management will maximise advances in NUE. With much of the Australian sugarcane industry located in catchments of the Great Barrier Reef (GBR) lagoon and the health of the GBR continuing to deteriorate, there has been heightened pressure to curb N losses to improve water quality (Brodie et al. 2012).

In most recent decades, the Australian sugar industry has intensified efforts to improve NUE (Wood et al. 2010), and governments have enacted regulations that require growers to adopt practices that reduce the risk of loss of sediment and chemicals (Thorburn & Wilkinson 2013). These regulations stipulate that the target for N is a 50% reduction from 2009 to 2013. However, most recent water quality data reveal that only a 10% reduction in N load has been achieved in this time frame although 50% of growers have adopted improved crop management practices (State of Queensland 2014). Further to this, projections have indicated that universal implementation of current best management practices will reduce dissolved inorganic N only by 10 to 15%, and ‘best case’ scenarios based on improved management are expected to achieve a 60% reduction in dissolved inorganic N exported (Thorburn & Wilkinson 2013). Clearly, a comprehensive approach is required in line with global efforts that aim to reduce N losses without yield penalties through selection and breeding of N efficient crop varieties.

Until recently, efforts to improve NUE of sugarcane cropping of the Australian industry have focussed on changed management practices. However, minimising N losses by optimising N rates and timing of N application remains a difficult task due to the limited capacity to predict weather, accompanying soil processes and crop growth. Thus, there is a need to maximise crop N uptake and utilisation through management in combination with improvement of varieties. Attracted by possible gains, breeding programs of crops of grain, feed and biofuel industries are making significant investments to improve NUE via traditional breeding (Hirel et al. 2007) and transgenic approaches (Xu et al. 2012).

Improving NUE through breeding is a challenge due to its complexity; genetically and physiologically. NUE is composed of multiple efficiencies that are the result of integrated N pathways ranging from N uptake from the soil solution, metabolism, internal use, storage, remobilisation and various loss pathways. Because N is an essential building block of structural and functional molecules and quantitatively the most important nutrient
that plants acquire from soil, acquisition and use of N are regulated across all levels of organisation, from molecule, cell, organ, plant, to whole crop. The greatest efforts to understanding and improving NUE have targeted grain crops (maize, wheat, rice) due to their global significance as food crops and the need to maximise grain N (protein) content (Hirel et al. 2007). Research into NUE of biomass crops and forage grasses is now gaining momentum to maximise production with low N input (Brégard et al. 2000, Yang et al. 2009, Yu et al. 2013).

In sugarcane, there has been limited characterisation of genotypic N response and N physiology traits and fragmented efforts across global industries so far have not focussed on variety improvement through breeding. The majority of NUE studies are reported from Brazil, South Africa, US and Australia, and most are limited to a small number of genotypes. The South African sugar industry practises variety-specific N fertiliser recommendations and has recently invested in NUE improvement through transgenes (Arcadia Biosciences; www.arcadiabio.com) that has shown promising results in canola and rice (Good et al. 2007).

The evaluation of genetic variation for NUE in sugarcane has been limited by the small number and narrow range of genotypes tested and the difficulties of controlling N supply and other contributing factors in the field. To evaluate a substantial number of genotypes with diverse genetic background our UQ-BSES team initially assessed genetic variation for NUE in a bi-parental mapping population at limiting and replete N supply in the glasshouse (Robinson et al. 2007, Whan et al. 2010). The male parent was Australian variety Q165, bred for high sugar yield with high N fertiliser rates, while the female parent was S. officinarum accession IJ76–514 (not selected for yield or performance in high N environments). Screening 61 progeny over a 3 month period showed that biomass production of genotypes varied 9-fold with low N supply and 4-fold with high N supply (Robinson et al. 2007). Motivated by the genetic variation in internal NUE observed in young plants, and successful field screening for genetic variation in water stress response (Basnayake et al. 2012) the current project was initiated to evaluate the genetic variation in N response in the field and to identify the traits that contribute to NUE in sugarcane.

Section 3: Outputs and Achievement of Project Objectives
Project objectives, methodology, results and discussion

Project Objective 1: Quantify the extent of genetic variation for NUE under field conditions

To investigate the scope for gains in NUE through variety improvement it is necessary to establish the variation for NUE that exists in the germplasm pool. NUE is a complex trait that can be defined in many ways. To assess the relative performance of genetically diverse sugarcane germplasm we have compared yield tonnes cane per hectare (TCH), commercial cane sugar (CCS) and N accumulation (kg N ha⁻¹ yr⁻¹) of 64 genotypes at two sites with two levels of N supply. Plants were grown over three crop cycles at Mackay and Burdekin with low (20-40 kg N ha⁻¹ yr⁻¹) and industry-recommended (160-200 kg N ha⁻¹ yr⁻¹) fertiliser rates. Prior to trial establishment sorghum or maize crops were grown without added fertiliser and all biomass removed from the site with the aim to deplete available soil N reserves. Fifty-three genotypes were common across both sites and comprised of Australian commercial varieties (30%), foreign varieties (23%), parental lines from the Australian breeding program (36%), introgression clones including backcrosses of species cane S. spontaneum and Erianthus spp. with commercial varieties (11%). The experimental trials were split plot design with three replicate blocks and 10/12m long and 4-row wide plots. Data were analysed for variance (ANOVA, ASREML) testing genotype, N supply and their interactions.
supply, and genotype x N supply interaction. For variance component analysis, genotypes and N supply environments were treated as random factors.

At Mackay and Burdekin, yield reduction with low N supply averaged 22% and 45%, respectively, over three year crop cycles (plant to 2nd ratoon, Table 1), highlighting that environmental effects are considerable drivers for performance at low N fertiliser supply.

Table 1 Range of tonnes of cane per hectare (TCH) across 64 genotypes at low and recommended (rec) N supply and percentage (%) yield reduction for each crop class (Plant- plant crop, 1R-1st ratoon, 2R-2nd ratoon) at sites in Mackay and Burdekin.

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<tr>
<th>Parameter</th>
<th>Site</th>
<th>N supply</th>
<th>Crop</th>
<th>Mean</th>
<th>Variance</th>
<th>Genetic variance</th>
<th>Heritability H²</th>
<th>Coefficient of variation</th>
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<td>Mk</td>
<td>Low</td>
<td>P</td>
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<td>284</td>
<td>204</td>
<td>0.68</td>
<td>0.21</td>
</tr>
<tr>
<td></td>
<td>Mk</td>
<td>Low</td>
<td>1R</td>
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<td>235</td>
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</tr>
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<td>Low</td>
<td>2R</td>
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<td>90</td>
<td>50</td>
<td>0.62</td>
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At the Burdekin site, broad-sense heritabilities for yield (TCH) were moderate to high (>0.6, Table 2) in plant and 1st ratoon crops indicating that the observed genetic variability at both N supply rates was high relative to experimental error. The low heritability observed in the 2nd ratoon at the Burdekin site is likely attributable to declining soil N availability, the severity of the N stress decreasing observable genetic variance and increasing environmental variance. Low N plots received only 100 kg N in total over three years with annual rates of 20-40 kg N fertiliser. Contrasting results to those identified at the Burdekin site were observed at the Mackay site. Moderate broad-sense heritabilities were observed for plant and ratoon crops, but did not decrease with increasing N limitation across crop classes.
Analysis of the Burdekin field trial results indicates that the treatment response is largely explained by N supply. However, ‘genotype x treatment (N supply)’ interaction was small in the plant crop but proportionally increasing with crop class to be closer to genotype variance in the 2nd ratoon. This indicates that the soil N reserve at the trial site was still large enough to mask the treatment effect in the plant crop. This initial soil N reserve declined gradually with N treatment effects becoming more apparent in ratoon crops.

The results from the Mackay trial were different to those observed in the Burdekin trial. At Mackay, ‘genotype x treatment (N supply)’ interaction was minimal. This may be attributed in part to the lack of a large N supply effect on yield until the 2nd ratoon and the severity of the N stress. The estimated yield reduction in Mackay for the plant, 1st ratoon and 2nd ratoon crops were 11, 22 and 35%, respectively compared with 39, 46
and 49% for the three crop classes at the Burdekin site. At the Mackay site, the variance of ‘genotype x treatment (N supply)’ interaction compared to the variance of genotype was relatively low in the plant crop but it showed an upward trend in the 2nd ratoon crop, suggesting the increasing intensity of ‘genotype x treatment (N supply)’ interaction with increasing N deficit (Table 3). However, by contrast to the Burdekin the result indicates that the overall ranking of clones at Mackay will not be different in moderately N-limited and non-limited environments and the key driver therefore is the vigour of the clone and those with high growth rate will remain at the top of the ranking list (Table 3). The severity of the N stress influences the expression of the genotype by N treatment interaction and there was only moderate N stress at the Mackay site, particularly in the plant and 1st ratoon, due to the high N mineralisation.

Commercial cane sugar (CCS) showed high broad-sense heritabilities at both N supply rates at both sites (Table 2). Variance for genotype was much greater than that of N supply and the interaction between the two factors indicating that this is a genotype effect not influenced by reduced N supply (Table 3). N accumulation (kg N ha\(^{-1}\)) had lower heritabilities compared to biomass accumulation due to the greater variability of the data, reflected by the higher coefficients of variance observed for stem N concentration and total N accumulation (Table 2). However, heritability of N accumulation did not decrease with increasing N limitation.

### Table 3
Variance components for genotype and N supply main effects and ‘genotype × N supply’ interaction for each crop class at Burdekin and Mackay for cane yield TCH, total dry matter, commercial cane sugar and N accumulation. N supply rates were: Burdekin low supply (20, 40, 40 kg N ha\(^{-1}\)) and recommended N supply (180, 200, 200 kg N ha\(^{-1}\)) Mackay low supply (40, 40, 40 kg N ha\(^{-1}\)) and recommended N supply (160, 160, 160 kg N ha\(^{-1}\)).

<table>
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<th>Parameter</th>
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<tr>
<td></td>
<td>G*N</td>
<td>39</td>
<td>34</td>
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<td>MV</td>
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<tr>
<td></td>
<td>G*N</td>
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<td>75</td>
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<td></td>
<td>Error</td>
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While the combined analysis indicated low genotype x N supply interaction for TCH, some clones had a relatively low yield loss under reduced N supply, suggesting large variation in N responsiveness exists in the test population. This is best explained by the cumulative yield variation of a selection of representative clones for the three crop cycles at the Burdekin site shown in Figure 1. At recommended N supply, yield of Q186 was
only 1.4-fold of that at low N supply, despite an additional 480kg N applied. In contrast, Q208 was more responsive to N application as yield at recommended N rate was 2.4-fold of that produced at low N supply. The greatest difference in genotype N responses were observed in the plant crop and the broadest range in genotype yields was observed at low N supply in the Burdekin plant crop with yields varying 4-fold across genotypes reducing to 2-3 fold differences in the subsequent ratoon crops. It is likely that different factors will drive genotype response in these crop types with differences in establishment, investment in root systems and tillering capacity. As genotypic variance has been established, the next step needed is further testing of a subset of genotypes in additional sites with varied N supply environments including reduced and recommended N rates as well as moderate N supply or controlled release fertiliser to characterise genotype N response.

**Figure 1** Cumulative yield (TCH) of representative genotypes Q208, Q186, KQ228 and SP80-1816 grown at low and high N supply for the three crops (plant to 2nd ratoon) at the Burdekin trial site. Different letters indicate statistically significant differences (ANOVA Tukey’s post hoc p<0.05) between genotypes. Note that ‘total N’ refers to the cumulative fertiliser application over the three year crop cycle.

**Project Objective 2: Identify the traits that are associated with or confer NUE**

Understanding the effect of N supply level or N stress on traits, and their relationship to yield, is necessary to understand plant function to develop varieties with improved NUE. Additionally, understanding physiological traits at the crop level may help to identify indirect selection criteria that could be applied to accelerate breeding for NUE improvement. However, there is very limited knowledge on the effect of N supply on physiological processes in sugarcane generally, and even less on the extent of variation that exists between genotypes. Response of sugarcane to N availability manifests across plant structure and function. Nitrogen availability influences root growth, tillering, canopy development, leaf pigment characteristics, stalk...
development, water relations, N uptake and use, photosynthesis and radiation use efficiency among others. These and related attributes determine crop performance and yield. Hence, maximising the impact of the most N responsive and yield-determining traits is critical for NUE improvement.

Based on N responses observed in the Burdekin and Mackay plant crops, a subset of genotypes (Q232, KQ228 Q208, Q186, Q138, Q183, Q218, QN01-1075, QN01-1735, QS95-6004, QC91-580, SP80-1816, SP79-2313 N14, R570, QBYC04-10559) were selected for more intensive study. Comparisons of genotype performance across sites and years combined with assessments of biomass and N accumulation and allocation, leaf area development, and photosynthetic parameters in controlled conditions have identified potential traits for further assessment. Rather than one single trait, a number of N-linked traits influence genotype performance and the importance of these traits is likely to change in different N supply environments, such as the Burdekin compared to Mackay trial site where access to soil N sources (other than N fertiliser) differ.

*(i) Nitrogen dilution throughout crop season*

Nitrogen dilution in aboveground tissues is due to the relationship between metabolic and structural plant growth and as plants or crops develop the amount of structural tissue increases. Changes in N concentrations of aboveground biomass with time have been compared between genotype to explore if luxury uptake capacity and pattern of N dilution differs between genotypes (Figure 2). Nitrogen concentrations were lower in the low N supply treatment early and mid-season, but did not differ significantly between the selected genotypes at the times sampled. However, a broader range of genotypes needs to be evaluated and final yield based comparisons of N response and genotype N interactions indicate examination of dynamics in plant crops is required.

![Figure 2](Image)

**Figure 2** Nitrogen concentration in the aboveground biomass for selected genotypes (KQ228, Q186, SP80-1816, Q208) grown with limiting N (40 kg N ha⁻¹, A) and recommended N supply (160 kg N ha⁻¹ B) in 1st ratoon Mackay site. Values are averages n=3, vertical bars represent 95% confidence interval.
(ii) Nitrate accumulation

Nitrate is a major N form in sugarcane soils and is the most mobile and vulnerable to loss from the plant-soil system. In contrast to ammonium-N acquired from soil that results in toxicity if it remains in cells in the same form, nitrate can be stored in tissues and assimilated at a later stage when carbon is available to generate amino acids and demand for N occurs. Analysing N source preferences, commercial varieties, accessions of *S. spontaneum*, and *S. officinarum* supplied with replete and equimolar concentrations of nitrate and ammonium, we demonstrated that sugarcane discriminates against nitrate acquiring half or less of the amount of ammonium in the form of nitrate (Robinson et al. 2011). In contrast, sorghum and maize acquired similar amounts of nitrate and ammonium, and *Erianthus* took up to 80% of N as nitrate relative to ammonium (Robinson et al. 2011). Higher ammonium-relative-to-nitrate-uptake has also been demonstrated in several South African sugarcane varieties using an in vitro experimental system (Hajari et al. 2014). Thus, sugarcane varieties and progenitor species appear to have a lower capacity to acquire, transport and store nitrate than related grain and grass species. The ability of genotypes to acquire, transport and store nitrate is of interest as a NUE trait. To evaluate the extent of genotypic variation in nitrate accumulation in young field-grown plants, tissue nitrate concentrations were compared approximately six weeks after fertiliser additions in the Burdekin 2nd ratoon and Mackay 1st ratoon (approximately 7 green leaves growth stage).

Nitrate concentrations of shoot tissue varied across genotypes but consistent patterns emerged when comparing concentrations across sites and seasons (Figure 3). Australian commercial varieties, Qcanes, tended to have lower nitrate concentrations than *Saccharum spontaneum* introgression hybrid QBYC04-10559 and Brazilian variety SP79-2313. Analysis of nitrate concentrations of plant crops at both sites (= six weeks after fertiliser application) showed a similar relatively high nitrate concentration in QBYN04-10559 of (20 μmol nitrate g dw⁻¹) and SP79-2313 (18 μmol nitrate g dw⁻¹). In comparison, Q canes had < 2 μmol nitrate g dw⁻¹, with the exception of KQ228 which had 4-10 μmol nitrate g dw⁻¹. While these values represent comparatively low nitrate concentrations (approximately 1.5% of the total shoot N pool) the consistent differences in nitrate content between genotypes point to differences in N storage and transport between genotypes that could be exploited as a NUE trait.
Figure 3 Nitrate concentration (μmol nitrate g⁻¹ dw) in shoot tissue of genotypes measured 6 weeks after fertiliser application at the (A) Burdekin site 2nd ratoon crop and (B) Mackay site 1st ratoon crop. Data are averages (n=3) and standard deviations. Crops received low or recommended (high) N rates.

(iii) N utilisation efficiency

To effectively improve NUE of varieties it is necessary to dissect the processes controlling NUE. The first process, “N-uptake efficiency” denotes the amount of N acquired by the crop from fertiliser and/or soil (kg N in the crop per available in soil). The second process, “N utilisation efficiency” or internal NUE denotes the capacity of the crop to use the acquired N for biomass (yield) production (tonnes crop biomass per kg N in the crop). Internal NUE is determined by the N concentration of the tissues and biomass allocation to these tissues. At the end of the crop season and when considering only above ground tissues there is a strong relationship between internal utilisation efficiency and stem N concentration (Figure 4). Evaluation of the extent of genetic variation in stem concentration at harvest and sensitivity to N supply is an interest as NUE trait even though internal NUE is not the only driver of yield at low N supply.
Figure 4 Relationship between stem N concentration (%) and internal NUE (kg dry weight kg\(^{-1}\) N) of aboveground tissues at final harvest of the plant crop Mackay plots supplied with recommended and low N supply (n=380).

In the Mackay trial plant crop, average stem N concentration varied 3-fold between genotypes, ranging from 0.1% N in QN80-4367 to 0.32% N in CP88-1762 and significant (P<0.05) genotype and treatment (N supply) effects but no ‘genotype x treatment (N supply)’ interaction was observed (Figure 5). The greatest response of stem N concentration to N supply was observed in Q231 and Q251 which contained 0.1% N at low N supply and 0.24% N at high N supply. In contrast, N supply rate had little effect on stem N concentration in Q237 (0.25 to 0.29% N). In contrast to the plant crop, no significant differences in stem N concentrations were determined between genotypes in the ratoon crops at Mackay.
Figure 5 Stem N concentration (%N) of genotypes grown with recommended N (red bars) and low N (blue bars) supply in plant crop of the Mackay trial. Nitrogen content was quantified in pooled samples of 4 stalks from each plot. Values are averages (n=3) with bars representing standard error.

Figure 6 Stem N concentration (%N) of genotypes grown with recommended N (red bars) and low N (blue bars) supply in 1st ratoon crop of the Burdekin trial. Nitrogen content was quantified in pooled samples of 4 stalks from each plot. Values are averages (n=3) with bars representing standard error.

Overall, stem N concentrations in the Burdekin trial were consistently lower than in the Mackay trial. At low N supply, stem N concentrations ranged from 0.05 to 0.11 % and at high N supply from 0.07 to 0.22% and significant genotype, treatment (N supply) and ‘genotype x treatment (N supply)’ interaction effects observed. Comparing the plant and 1st ratoon crop in Burdekin, S. spontaneum introgressions (QBYN04-20119, QBYC04-10559) and Q240 were consistently ranked highly for stem % N at high N supply in the 1st ratoon (3,2,1) and plant crop (6, 7, 13). However, there was no consistent genotype ranking across N supply rates and trial sites. Similarly, a study on juice quality showed that juice amino acid concentrations varied 2 -fold across genotypes but with considerable re-ranking of genotypes between years (Jackson et al. 2006). This was possibly associated with the large differences in N status of the crop between years. This demonstrates the difficulties in comparing varieties across seasons and fields due to considerable interactions with environment and season. Therefore genotype or variety comparasions need to be in replicated blocked trials with detailed analysis of soil and weather.

(iv) Leaf area development

Links between leaf traits, photosynthesis and NUE are well established in many crops. However, evaluation of a broader range of sugarcane genotypes and the response of leaf area development to N supply is necessary to establish the relationship between NUE and canopy development. Destructive sampling of green leaf area of a subset of genotype was undertaken at 2 time points, early and mid-season, as well as at harvest. Leaf area peaked mid-season at both N supply rates and this was the time of greatest difference between genotypes. Leaf area differed between genotypes over the course of the season at both trial sites. The cumulative impact of traits in addition to leaf area is exemplified with the similar yields achieved by variety Q208 and SP80-1816.
although the Q208 displayed lower leaf areas. This result indicates the need to incorporate additional measures including canopy architecture, time to canopy closure when assessing genotype development.

**Figure 7** Representative leaf area (m² m⁻²) at three time points (weeks since harvest) of KQ228, Q186, Q208 and SP80-1816 grown with low (A, C) and recommended (B, D) N fertiliser applied (week 9) for the 2nd ratoon Burdekin (A, B) and 1st ratoon at Mackay (C, D). Values are averages (n=3) and standard deviation.

*(v) Specific leaf N*

Sugarcane cultivated with recommended fertiliser rates displayed a decline in specific leaf nitrogen (SLN, amount of N per unit leaf area) from upper to lower canopy leaves and throughout the crop season (Ludlow et al. 1991, Allison et al. 1997). In maize and sorghum, maximum net assimilation rates (45-50 μmol CO₂ m⁻² s⁻¹) were achieved at SLN of ~1.0-1.5 g N m⁻² and declined when SLN was below this threshold level (Muchow & Sinclair 1994). In sugarcane, the SLN threshold for maximal assimilation rates has been shown to be higher at 1.7-2.0 g N m⁻² (Ludlow et al. 1991). In over half of the sugarcane crops with high N fertiliser rate (>160 kg N ha⁻¹) evaluated in Australia, SLN was <1.2 g N m⁻² at the 4000 g m⁻² biomass growth stage (Park et al. 2005). This finding indicates that photosynthesis is limited by low SLN during much of the crop cycle, which could not be reversed by external N supply. It is suggested that SLN decline can be attributed in part to a physiological constraint rather than N availability alone as all crops showing declining SLN received >200 kg N ha⁻¹ (Park et al.
2005). This suggests the potential to contribute to NUE and therefore SLN was measured in a subset of genotypes at Mackay and Burdekin to determine genotypic variation in this trait.

Table 4 Specific leaf N (g N m\(^{-2}\)) measured early and mid-season in representative crops 1\(^{st}\) ratoon Mackay and 2\(^{nd}\) ratoon Burdekin for 14 genotypes. Values are averages (n=3), with 8 stalks sampled per plot.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Mackay 1(^{st}) ratoon</th>
<th>Burdekin 2(^{nd}) ratoon</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Early season</td>
<td>Mid-season</td>
</tr>
<tr>
<td></td>
<td>Low N</td>
<td>Rec N</td>
</tr>
<tr>
<td>KQ228</td>
<td>1.7</td>
<td>1.9</td>
</tr>
<tr>
<td>N14</td>
<td>1.6</td>
<td>1.9</td>
</tr>
<tr>
<td>Q138</td>
<td>1.7</td>
<td>1.8</td>
</tr>
<tr>
<td>Q186</td>
<td>1.8</td>
<td>1.9</td>
</tr>
<tr>
<td>Q208</td>
<td>1.7</td>
<td>2.1</td>
</tr>
<tr>
<td>QBY04-10559</td>
<td>1.6</td>
<td>1.6</td>
</tr>
<tr>
<td>QCS1-980</td>
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<td>1.9</td>
</tr>
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<td>QN01-1075</td>
<td>1.5</td>
<td>1.7</td>
</tr>
<tr>
<td>QN01-173S</td>
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<td>1.9</td>
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<td>SP79-2313</td>
<td>1.5</td>
<td>2.1</td>
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<td>SP80-1816</td>
<td>1.5</td>
<td>1.7</td>
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<tr>
<td>average</td>
<td>1.7</td>
<td>1.8</td>
</tr>
<tr>
<td>95% confidence interval</td>
<td>0.3</td>
<td>0.1</td>
</tr>
</tbody>
</table>

Source of variance

<table>
<thead>
<tr>
<th>Source of variance</th>
<th>Genotype (G)</th>
<th>N supply (N)</th>
<th>G*N</th>
</tr>
</thead>
<tbody>
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<td>ns</td>
<td>*</td>
<td>ns</td>
</tr>
<tr>
<td>Genotype (G)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N supply (N)</td>
<td>*</td>
<td></td>
<td>*</td>
</tr>
<tr>
<td>G*N</td>
<td>ns</td>
<td>ns</td>
<td>Ns</td>
</tr>
</tbody>
</table>

\(P<0.05\)

Similar to observations in previous studies SLN declined with crop age at both sites (Table 4). In the Burdekin 2\(^{nd}\) ratoon, SLN of all genotypes except the S. spontaneum introgression hybrid were below reported ‘SLN threshold’ of 1.2 at mid-season at both N supply rates. Despite significant genotype differences there was only a narrow range of SLN observed with the exception of early season sampling in Burdekin. The narrow range of SLN observed indicates that an additional focus should be to determine the relationship between photosynthesis and SLN and if this relationship differs between genotypes.

(vi) Photosynthesis and Photosynthetic NUE

Photosynthetic proteins (RuBisCo, light harvesting complexes) and chlorophyll, contain a large proportion of leaf N. There are consistent and strong relationships between maximal photosynthetic rates and leaf N concentration, which is described as ‘photosynthetic NUE’ (Evans 1989). In addition to maintaining high leaf N status in low N environments, adapting leaf anatomy and maximising N allocation to RuBisCo and light-harvesting complexes can improve photosynthetic NUE (Ranjith & Meinzer 1997). Insight into physiological
traits that affect NUE came from comparative analysis of CO₂ fixation in two Hawaiian sugarcane varieties (H65-8235, H65-7052) (Ranjith & Meinzer 1997). Photosynthetic NUE (PNUE) differed between varieties at all N supply rates compared to dry weight accumulation which was significantly higher in H65-8235 only at high N supply. While photosynthetic NUE varies, this trait has only been linked to higher biomass as high N supply, and we therefore evaluated this trait in five genotypes in the glasshouse, based on different performance in the field trials.

Figure 8 Photosynthetic rate, SLN and photosynthetic NUE (PNUE) of the youngest fully expanded leaf for five genotypes grown with limiting N (grey) and replete (black) supply for 4 months in the glasshouse. Values are mean (n=4) and bars standard error. Different letters indicate significant difference (p<0.05) ANOVA, Tukey’s HSD test.
Genotypes differed significantly in photosynthetic response to N supply (Figure 8). The two Brazilian genotypes, SP80-1816 and SP79-2313, maintained higher photosynthetic rates relative to those measured at high N supply. In contrast, photosynthesis in youngest fully expanded leaves of genotypes Q208, Q186 and QN01-1075 was significantly lower at low N supply compared to that at high N supply (Figure 8A). Specific leaf N declined with decreased N availability and did not differ between genotype at low or high N supply (Figure 8b). PNUE of Q208 was significantly lower than SP80-1816 and SP79-2313 at low N supply (Figure 8c).

![Figure 9](image)

**Figure 9** Relationship between photosynthetic rate and stomatal conductance of youngest fully expanded leaf of five genotypes at limiting N and replete N supply grown in the glasshouse for 4 months.

There is a strong relationship between photosynthesis and stomatal conductance and therefore conductance of SP80-1816 and SP79-2313 was less sensitive to N stress compared to the other genotypes (Figure 9). Similarly, genotypic differences in response of photosynthesis and conductance were observed in a comparison of genotypes, Q183 and QC91-580, to water and N stress in the glasshouse (Figure 10). Water and N stress individually and combined reduced photosynthesis of QC91-580 approximately 30%. Photosynthetic response of Q183 to individual water and N stresses was similar to QC91-80. However, Q183 was more sensitive to combined water and N stress and photosynthetic rate and conductance were one third that of the well supplied plants. While the link between PNUE and NUE is still to be established, these results highlight the potential of stomatal conductance as a tool to screen genotype sensitivity to N stress.
Figure 10 Photosynthesis and stomatal conductance of youngest fully expanded leaf of Q183 and QC91-580 after 3 months growth with replete water and N (+W+N), replete water and limiting N (+W-N), limiting water and replete N (-W+N), and limiting water and N (-W-N).

Project Objective 3: Develop practically useful screening tools for NUE

As a first step in developing practically useful screening tools satellite image parameters such as normalised difference vegetation index (NDVI) and other remote sensing parameters as well as on-ground leaf area index sensors were evaluated as indicators of biomass and N accumulation throughout the crop season and potential screening tools.

(i) Remote sensing

A satellite image (Ikonos) was taken of the Burdekin site in June 2011, one month before the final plant crop harvest and a strong relationship between normalised difference vegetation index (NDVI) and yield (TCH was observed (NDVI=0.0021TCH+0.3686, $R^2=0.55$) indicating the potential of satellite imagery for estimating biomass and N accumulation. Based on this result, we evaluated the potential to develop remote sensing as a tool for biomass estimation earlier in season. When screening large numbers of genotypes plot size is constrained by practical issues and cost and hence accurate biomass measurement throughout season is not possible. A major constraint is the large quantity of material needed to be destructively harvested at regular intervals, making the experimental trials excessively large. This work was in collaboration with another project evaluating remote sensing for foliar nitrogen mapping (Dr Andrew Robson ‘Developing remote sensing as an industry wide yield forecasting, nitrogen mapping and research aide’). Satellite images were obtained of the Burdekin 2nd ratoon (5, 9 months) and 1st ratoon Mackay (3, 7 months) and the image of the 2nd ratoon in Burdekin is shown as an example of the treatment block and genotype plot resolution at 5-month growth (Figure 11).
Figure 11  A. False colour ‘Geoeye’ image of Burdekin site 2\textsuperscript{nd} ratoon crop including three replicate blocks consisting of 64 genotypes grown with low N supply (40 kg N ha\textsuperscript{-1}) and high N supply (180 kg N ha\textsuperscript{-1}) taken on 14 January 2013.

Satellite images of the Burdekin site provided resolution between treatment (N supply) throughout season (Figure 11) and a significant positive relationship explaining 31\% of the data was observed between biomass of 14 genotypes sampled in February 2013 and NDVI of January image (Figure 12A). A comparison of yield (TCH) and NDVI of all plots at final harvest showed a stronger correlation ($r^2=0.47$, Figure 12B). In contrast to that observed in Burdekin, early season (3-month) and mid-season measures in Mackay showed no relationship between NDVI and sampled biomass.
Figure 12 Relationship between NDVI and biomass for (A) image captured in February 2013 and biomass (t DW ha\(^{-1}\)) sampled in December 2012 for 14 genotypes (84 plots) and (B) image captured in May 2013 and biomass (TCH) sampled for 384 plots in July 2013 at the Burdekin site.

The weaker relationship between biomass and NDVI observed when sampling younger crops is likely a result of smaller number of plots sampled and the size of the destructive sample possible in small plots as well as genotypic variation in canopy architecture. Further assessment with more time points and robust destructive biomass sampling at multiple sites is necessary to further dissect these factors.

(ii) Hand-held sensors

Leaf area index (LAI) measured with a plant canopy analyser, Li-COR LAI-2200 was also evaluated as a screening tool to compare genotype performance. There was a moderate association between LAI2200 measurements
and destructively sampled leaf area index and biomass at the time of LAI-2200 reading (Figure 13A).
Statistically significant (p<0.05) differences were observed between genotypes and N supply early (5-month stage) in season, but no significant differences were detected at 8-months growth. The S. spontaneum introgression clone, QBYC04-10559, which visually has a very dense canopy, gave the highest canopy cover, 1.9 and 2.7 at low and high N supply, respectively. This highlights the integration of canopy architecture traits (leaf orientation) in the canopy analyser indicating its potential role in early season measures. This can be used in conjunction with destructive sampling to establish the relationship between early growth and NUE.

**Figure 13** Relationship between LAI measured with hand held LICOR2200 and (A) LAI measured by destructive sampling and (B) biomass (T dw ha\(^{-1}\)) measured in the 2\(^{nd}\) ratoon Mackay in December (5-month since harvest).

**Project Objective 4:** Establish promising clones for the follow-up project which will (a) test the breeding value of selected clones for cultivar development, and (b) implement the developed screening methods in the breeding program.
Principal component analysis (PCA) was conducted for the Burdekin trial based on the internal NUE, total nitrogen accumulation and cane (TCH) and sugar yield (tonnes of sugar per hectare- TSH) to develop two components (PC1, PC2) to be used as predictors in subsequent analysis grouping genotypes with similar characteristics (Figure 14). PCA showed that PC1 and PC2 explained 49% of the variation within the population with PC1 scores (36.8%) mostly related to the TCH and TSH and a lesser extent to total N and internal NUE. The correlations of PC1 with TCH were higher particularly for low N treatment. PC2 score explained only 12 % of the variation and had a strong negative correlation (-0.5 and -0.6, high and low N, respectively) with NUE and positive correlation with total N content of high and low N treatments in the 1st ratoon. Zone A-D define contrasting quadrants with positive and negative relationships with the principle components, PC1 and PC2. Genotypes with common characteristics TCH, TSH, Total N and NUE to N supply responsiveness defined by the circled regions. Genotype grouping are further explored by cluster analysis (Figure 15). The clones in the A and B zones are potentially desirable ones, with more cane and sugar yield under the experimental conditions. They are also likely to be high vigour clones.

TCH =cane yield/ha
TSH = sugar yield /ha
TN = Total N t/ha
NUE = N use efficiency (kg TDM/Kg N
REC =Recommended N supply 180, 200 kg N ha
Low =20/40 kg N/ha
PC, 1R and 2R  are crop classes

Figure 14 The vectors (traits) and their association with individual clone as shown in the Principal Component Analysis.

Therefore, the cluster analysis (Figure 15) was conducted using TCH and TSH of all three crop classes, and total N and NUE for plant and 1st ratoon crops to identify groups of clones with common characteristics to select a
sub-set of test materials for further investigations. The sub-set of clones (20) were selected for further studies based on the yield performance in low N and high N conditions and their responses to tissue N content and NUE at Burdekin trial (Table 5).

![Figure 15](image)

**Figure 15** The genotype grouping based on the cluster analysis of the clone x trait matrix, including TCH and TSH of all three crop classes, and total N and NUE for plant and 1\textsuperscript{st} ratoon crops

Most of the clones in Zone C and D in Figure 14 grouped together and formed 2 distinct groups at 3 group level truncation (Figure 15). However, clones in Zone A and B (Figure 14), mostly closer to TCH and TSH vectors in positive direction of PC1, grouped together and formed one distinct group. Generally clones in Zone D had low ranking among all others (QBYN clones). Based on the TCH and the ratio of TCH low/TCH rec N (Fraction-TCH) data from all three crop classes, and NUE and ratio of NUE low N/NUE rec N (Fraction-NUE) from plant and 1\textsuperscript{st} ratoon, test clones were ranked and 20 clones with high average ranking and representing selections from each group are presented in Table 5.
Due to the contrasting results obtained in the Mackay trial and the small treatment effect observed in the plant and 1st ratoon, genotypes were ranked based on TCH and NUE measured in the 2nd ratoon where the greatest treatment effect was observed. Twenty clones were selected based on a high ranking for TCH at low N supply rank of <10 in the TCH and to a lesser extent at high N supply. Additionally clones were included with poor yield but high ranking (1-10) for internal NUE. Nine of the genotypes selected based on these parameters in the Mackay trial were also selected in the Burdekin subset, based on criteria over the 3 year crop cycle. Five of the genotypes selected based on these parameters were only present at the Mackay trial.

Table 5 The 20 selected clones and their ranking for TCH, NUE, fraction TCH and NUE and average ranking across 3 crop cycles in Burdekin. (NUE ranking based on plant and 1R crops only).

<table>
<thead>
<tr>
<th>Clone</th>
<th>TCH Rec</th>
<th>TCH Low</th>
<th>Fraction-TCH</th>
<th>NUE Rec N</th>
<th>NUE Low N</th>
<th>Fraction-NUE</th>
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</thead>
<tbody>
<tr>
<td>CP70-1133</td>
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<td>2</td>
<td>47</td>
<td>5</td>
<td>2</td>
<td>14</td>
</tr>
<tr>
<td>KQ228</td>
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<td>11</td>
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<td>12</td>
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<td>58</td>
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</tbody>
</table>

Table 6 The 20 selected clones and their ranking for TCH, NUE, fraction TCH and NUE in the 2nd ratoon in Mackay. Fraction NUE=low N supply NUE/rec N supply NUE.
Based on the performance across both sites, with slightly greater weighting given to the Burdekin trial ranking due to the inclusion of multiple crop classes, the following genotypes are suggested as the best candidates to test at additional sites to further dissect traits contributing to NUE (Table 7). These genotypes, which include widely grown cultivars such as Q208, together with four to five cultivars released at least five decades ago such as, A28, Q57, Q63 and Q86 should be further evaluated for performance at reduced and recommended N supply and trait characterisation. Establishment of these trials would also allow further evaluation of the used of remote sensing as a screening tool and development of relationships with biomass and N content as discussed in objective 4. This is necessary before it is possible to implement this as a screening tool in the breeding programme.

Table 7 Genotypes with proposed characteristics selected for further evaluation characterisation of NUE traits in controlled conditions and low N input field trials

<table>
<thead>
<tr>
<th>Clone</th>
<th>Origin</th>
<th>Proposed characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>CP70-1133</td>
<td>US cultivar</td>
<td>High TCH at low N input, high internal NUE</td>
</tr>
<tr>
<td>KQ228</td>
<td>Australian cultivar</td>
<td>High TCH, less responsive to applied N</td>
</tr>
<tr>
<td>Q186</td>
<td>Australian cultivar</td>
<td>High relative yield at low N, less responsive to applied N</td>
</tr>
<tr>
<td>Q232</td>
<td>Australian cultivar</td>
<td>High TCH at low N input</td>
</tr>
<tr>
<td>Q138</td>
<td>Australian cultivar</td>
<td>High internal NUE at low N input</td>
</tr>
<tr>
<td>Q208</td>
<td>Australian cultivar</td>
<td>High TCH at high N, Responsive to N applied</td>
</tr>
<tr>
<td>Q240</td>
<td>Australian cultivar</td>
<td>High TCH at high N, Responsive to N applied</td>
</tr>
<tr>
<td>QN01-1301</td>
<td>Parental line</td>
<td>High TCH low N input, less responsive to applied N</td>
</tr>
<tr>
<td>QN01-1075</td>
<td>Parental line</td>
<td>Low TCH, Low internal NUE</td>
</tr>
<tr>
<td>QN80-3425</td>
<td>Parental line</td>
<td>High internal NUE</td>
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<tr>
<td>QN80-4367</td>
<td>Parental line</td>
<td>Moderate TCH, high internal NUE</td>
</tr>
<tr>
<td>SP80-1816</td>
<td>Brazilian cultivar</td>
<td>High TCH at low N input</td>
</tr>
<tr>
<td>N14</td>
<td>South African cultivar</td>
<td>Less responsive to applied N, high internal NUE</td>
</tr>
</tbody>
</table>
Collaborative research links have been established with several research institutions, scientists and industry people both within Australia and internationally. They are listed below.

1. Research on nitrogen and water interactions and nitrogen uptake and re-mobilisation in sugarcane
   (a) Dr. Kuldeep Singh
       Agronomist (Sugarcane)
       Punjab Agricultural University
       Regional Research Station, Faridkot, India
       1 February 2011- 30 April 2011

   (b) Dr. Henrique C. Junqueira Franco
       Agricultural Researcher
       Brazilian Bioethanol Science and Technology Laboratory, CTBE, Campinas/SP, Brazil.
       June 2011- 30 March 2012

   (c) Dr. Sandra Jämtgård
       Research Fellow
       Swedish University of Agricultural Sciences, Umeå, Sweden
       March 2012-August 2012

   (d) Dr. Yang Liu
       Research Fellow
       Sugarcane Research Institute of Guangxi Academy of Agricultural Sciences, Nanning China
       Nov 2013-May 2014

As part of the research collaboration with Sugarcane Research Institute of Guangxi Academy of Agricultural Sciences (GXAAS), Nanning China, Professor Hong-Wei Tan, Director, Sugarcane Research Institute, GXAAS, Dr. Dong-Liang Huang, Deputy Director, Sugarcane Research Institute, GXAAS and Prof Yang Rui Li (Director, Sugarcane Research Center, Chinese Academy of Agricultural Sciences, Nanning) visited UQ Schmidt laboratories and SRA Indooroopilly on October 16th, 2014 and discussed possible collaborations between Australian and Chinese sugarcane scientists involved in NUE research. As an initial step exchange of doctoral students and post-doctoral researchers between UQ and GXAAS is planned and a post-doctoral fellow from GXAAS will be visiting the UQ Schmidt/Robinson group for a year starting from April or May 2015.

Research on NUE also attracted many local sugar industry stakeholders to get involved in research exploring different aspects of NUE in relation to different N fertilizer forms. These local links include Wilmar, Herbert Cane Protection and Productivity Board, and Brian Granshaw- a Burdekin cane grower.
The relevance of genetic improvement of NUE, focusing on project aims and findings have been communicated at international conferences including:

i) Presentation title: Implications of inefficient use of nitrate by sugarcane  
   Attendees: Susanne Schmidt, Prakash Lakshmanan (note: Nicole Robinson could not attend due to pregnancy)  
   5th International N conference N2010, New Delhi, India, 3-7 December 2010

ii) Presentation title: Improving N use efficiency of sugarcane  
    Attendee: Susanne Schmidt  
    1st conference on resource use efficient tropical agriculture, Haikou, Hainan Island, China, November 2011

iii) Presentation title: Nitrogen efficient sugarcane  
    Attendees: Susanne Schmidt, Prakash Lakshmanan  
    Sugarcane physiology for agronomic application, Campinas, Brazil, November 2012

iv) Presentation title: Addressing the nitrogen problem in sugarcane production  
    Attendees: Susanne Schmidt, Prakash Lakshmanan  
    Green technologies for sustainable growth of sugar and integrated industries in developing countries. Nanning, China, November 2014

**Project Objective 6: Raise grower awareness of NUE cultivars**

Grower awareness of the need for efficient and environmentally responsible use of N is at a peak as there have been numerous industry reviews and forums, and regulatory policy responses in the recent past. In this context, communication of project aims and outcomes have heightened awareness of the potential of variety improvement to address NUE. Also, in all communications the research team have highlighted the medium- to long-term nature of genetic solutions as the research outcome ultimately will be realised through breeding N use efficient varieties.

The highlights of the current project outcomes and the potential for these areas of research have been conveyed through engagement with SRA PEC units and productivity boards including Burdekin Productivity Services and Herbert Cane Protection and Productivity Board. The following grower orientated presentations were delivered:

(a) Caneclip 2014- Developing nitrogen efficient cane varieties  

(b) Burdekin Productivity- PEC grower presentation March 2014 attended by growers and extension personnel

(c) SRDC Regional expos  
Project aims and initial results were presented by NUE team members (N Robinson, P Lakshmanan, S Schmidt, J Basnayake) at the following SRDC regional expos  
9 May 2012 Maryborough  
10 May 2012 Ingham  
11 May 2012 Ayr
These sugar industry and grower-centered communication efforts were found to be effective as Herbert Cane Protection and Productivity Board (evaluation of regional varieties and selections coming from the project in Herbert), Wilmar (varietal response to mill mud from N use perspective) and a sugarcane grower Brian Granshaw (variety and legume interaction and N uptake) were keen to initiate research in exploring variety to improve crop NUE.

References


Section 4: Outputs and Outcomes

Outputs-
1) Scientific knowledge and quantitative data on genetic variability of NUE in sugarcane germplasm. The results suggest considerable genetic variation for NUE exists in sugarcane;
2) Increased understanding of the relative contribution of above-ground plant attributes to NUE;
3) Assessment of remote-sensing as a tool for screening sugarcane clones for NUE. This technique showed some potential as screening tool for NUE and should be evaluated at more growth stages with large genetic populations;
4) Effective communication of project results to sugar industry stakeholders;
5) Research training of university undergraduates through short-term projects. Increased exposure of sugarcane N fertiliser issue and the importance of NUE to plant science and agriculture undergraduates at UQ.

Outcomes-
1) Increased awareness of NUE and potential for genetic improvement of NUE in Australian sugar industry;
2) Initiation of research interaction with growers (Brian Granshaw- Burdekin and Troy Apps-Sunshine Coast), milling sector (Wilmar) and Herbert Cane Protection and Productivity Board;
3) Contribution to SRA PEC unit communication program and SRDC regional workshops and roadshows;
4) Contribution to SRA and federal DAFF N research review.

Section 5: Intellectual Property (IP) and Confidentiality
All commercial cultivars and other clones used in this project form part of the SRA breeding program and hence are protected by appropriate germplasm ownership rights by SRA. Results of N use efficiency of test clones needs to be tested widely and in different regions to determine its IP value. Please note that fundamental concepts on NUE are widely discussed in the scientific literature. This includes the application of remote and proximal sensing technologies for germplasm characterisation for N use and productivity. The results of this project are not commercially sensitive.

Section 6: Industry Communication and Adoption of Outputs
a) What key messages have come from the project to date, when and how they have been communicated and to whom? Has there been any communication with the relevant SRA Professional Extension and Communication (PEC) officer or unit?
The key messages of this project are (i) there is genetic variation for NUE in sugarcane; (ii) leads have been identified to direct further investigation to identify traits conferring NUE; (iii) remote sensing technologies have potential for screening sugarcane germplasm for N response, but the accuracy needs to be validated in large genetic populations in different production scenarios; (iv) there is value in advancing variety-based genetic solutions to complement and strengthen N management strategies.

b) What new information, if any, is available on the adoption of project outputs?
The test population was ranked according to N response and internal NUE (iNUE), a measure of how well the N acquired by crops of different germplasm is used to produce biomass. The best performing or high iNUE clones, subject to quarantine approval, may be tested in different growing regions for N efficiency. Similarly, considering the large genetic variation for N uptake and retention observed in the test population, it is worth testing the N accumulative capacity of regional varieties to further improve N use in commercial crop production.

c) List any newsletters, fact sheets or any other media coverage.
d) **Identify any further opportunities to disseminate and promote project outputs at seminars, field days etc.**

1. Publication of research results in scientific journals are planned;
2. Willing to provide seminars on project results and the need to have genetic solutions to complement N management at appropriate sugar industry events.

### Section 7: Environmental Impact

*Outline any new information on adverse/beneficial environmental impacts of conducting the project and/or implementing its findings.*

There were no adverse impacts of conducting this project. This project has demonstrated that there is genetic variation in cultivar response to applied fertilizer N and use of indigenous soil N. However, this results needs to be validated these require further testing with reduced N input across a greater number of sites, particularly in the targeted production regions. After further confirmation of yield and N use response with reduced N input, including plant crops, genotype-specific fertilizer recommendations may be possible for those production areas identified as high priority in the Australian Government 2014 Reef Water Quality Protection Plan 2013-prioritisation report. A reduction in fertilizer input would have direct environmental benefits with reduction in N loads entering GBR in addition to reduced nitrous oxide emissions. Quantitative information on the positive impact in applied N savings has not been addressed in this study as it requires a complete tracking of applied and indigenous N in the cropping system and related environment.

### Section 8: Recommendations and Future Industry Needs

*Include activities or other steps to further develop, disseminate, commercialise or exploit the Project Outputs and realise the industry benefits.*

1. Better understanding of the extent of genetic variation for NUE in the local germplasm and crop-specific N physiology is fundamental for developing successful strategies for genetic improvement of NUE. Screening of Australian sugarcane breeding lines and current varieties, and a detailed physiological investigation, including belowground attributes, focusing on the regulation of N uptake, use, storage and remobilisation in the context of sugarcane commercial production is a priority.

2. Initiate multidisciplinary research to identify large effect NUE traits to be used as a selection trait for NUE improvement in breeding program. This should be conducted in parallel with the evaluation of rapid screening tools such as remote and proximal high-throughput sensing technologies for selecting highly productive, N use-efficient clones.

3. Based on the developmental features of sugarcane and commercial sugarcane cropping systems the key targets for NUE improvement are increased N uptake and radiation use efficiency. Any improvement to increase N uptake at the most N demanding phase of crop development- the stalk-filling phase, and retaining more N in leaf would improve radiation use efficiency, yield and N relations of the whole cropping system.

4. The experience in grain crops suggest that more gain (improved varieties for reduced N fertiliser use) can be realised by selecting for yield under low N input, or alternating high and low N conditions. This
information is not available for sugarcane. Evaluating various selection strategies relevant to sugarcane should be undertaken at least in those regions where N leakage is a pressing issue. The aims of the project were to determine the scope of genetic variation and begin to dissect NUE traits, for long term development in the breeding programme. However, results suggest that N response curves should be developed for selected current commercial cultivars in those production areas identified as high priority for reduced N input.

5. A well-defined, coordinated, long-term targeted research program involving a multi-disciplinary team of breeders, physiologists, soil scientists, agronomists and modellers will be a productive approach for improving NUE in sugarcane.

Section 9: Publications

Robinson et al. Genetic variation of sugarcane yield and N accumulation in response to N supply, in preparation


Paungfoo-Lohienne et al. Nitrogen fertilizer dose alters fungal communities in sugarcane soil and rhizosphere. Scientific Reports, accepted Jan 2014
