

UTILISATION OF WILD CANES FROM CHINA

By

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Abstract

IN 2002 a collaborative program of breeding and research was commenced, aiming to utilise wild germplasm from China for sugarcane improvement in both China and Australia. Some results and recommendations arising from this program to date are reported here. The program aimed to: 1. Characterise genetic diversity in Chinese *S. spontaneum* and *Erianthus arundinaceus*; 2. Conduct crossing between Chinese *S. spontaneum* and *Erianthus* spp. and sugarcane, and initial evaluation of the resulting progeny; 3. Assess if and how DNA markers can be used to identify genome regions of positive or negative value from wild clones, and to assist in programs aiming to introduce new genes from wild canes into commercial cultivars; 4. To quantify genotype × environment interactions between Australia and China. Results from the genetic diversity studies indicated a high level of genetic diversity in both *S. spontaneum* and *Erianthus arundinaceus* in China, and provide a basis for targeted sampling and use of this material in future breeding efforts or for core collections. Two hundred and two crosses from a range of *S. spontaneum* and *Erianthus* clones generated viable seeds, and 100 of these crosses have been verified (to date) using DNA markers as producing true hybrids. These results were significant in relation to *Erianthus* spp. in providing (to our knowledge) the first report of verified fertile *Saccharum* × *Erianthus* hybrids in the world, despite many past efforts. Several case study populations derived from *S. spontaneum* and *Erianthus* were used for Quantitative Trait loci (QTL) mapping. Several apparently important QTL for cane yield were identified from *S. spontaneum*. An approach to apply DNA markers in future introgression breeding in sugarcane is recommended, based on the results and experience obtained. Another significant result was the first (to our knowledge) documentation of genotype × country interactions. Somewhat surprisingly, moderate to high (>0.6) genetic correlations in performance of families and clones between trials in China and Australia were observed, despite some contrasting environmental and management conditions. This result supports ongoing collaboration between China and Australia via exchange of selection trial results and selected germplasm.

Introduction

Genetic improvement in most crop species has been characterised by incremental gains over time, with occasional larger ‘jumps’ in progress when some favourable new germplasm or genes are

found and used. In sugarcane, a major 'jump' arose with interspecific hybridisation in the early part of the 20th century when noble cane cultivars (*Saccharum officinarum*) were crossed with the wild species *S. spontaneum* and with hybrids derived from this wild species, by Indonesian and Indian breeders (Roach, 1989). Genotypes arising from these early programs (such as POJ2878, Co205) quickly became utilised not just as cultivars but also as parental material in sugarcane breeding programs world-wide. Following the success of these early interspecific hybridisations, sugarcane breeding programs have since largely concentrated on utilising clones derived from these early programs in further cycles of crossing and selection. However, this has contributed to a situation where most parent clones within sugarcane breeding programs around the world trace back to a relatively small number of key ancestors (Arceneaux, 1967; Roach, 1971). This small sampling of clones for breeding, combined with a perception among some sugarcane breeders of desirable traits in wild canes such as adaptation to environmental stresses, has led to ongoing interest from breeders in the introgression of new sources of germplasm (Roach, 1989).

Some serious attempts since the initial interspecific hybridisations to introgress new sources of germplasm have been made in several countries and are still ongoing. The focus in most has been on use of *S. spontaneum* but effort on other species including *S. robustum* in Hawaii (Heinz, 1967) and *Miscanthus spp.* in Taiwan (Lo et al., 1986) has also occurred. More recently, activity targeting *Erianthus arundinaceus* in several countries (eg. Miller and Tai, 1992; Legendre, 1989; Piperidis et al., 2000) has occurred, motivated by apparently desirable characters in this species such as vigour, drought tolerance, water logging tolerance, and disease resistance (personal communication with various breeders).

Generally, the procedure used in introgression breeding involves initial crossing between *S. officinarum* or commercial type parents and the wild species followed by several cycles of backcrossing to commercial sugarcane parents. However, the process of introgression breeding is generally very long, with each cycle of crossing and subsequent selection of progeny usually taking between six and eight years. Given that two or more backcrossing cycles are usually necessary to regain high levels of sucrose, it is clear that introgression breeding represents a long-term commitment. Further, commercial success has not always been achieved, with some major efforts (mostly not reported in the literature) not yet leading to released cultivars. The time and risk factors have acted to reduce the level of resources devoted in most sugarcane breeding programs to introgression breeding despite universal awareness of its potential value. Roach (1984, 1989), based partly on direct experience with the CSR program in Australia, listed several reasons for the failure of some efforts to provide more productive varieties. These reasons were largely related to inferior traits in the wild donor clones, and difficulties in selecting and combining the desirable portions of both the wild type and the recurrent parents during subsequent selection cycles. Another problem noted was the lack of cytological or genetic backup to confirm the hybrid nature of initial clones derived from interspecific hybridisation and selected for further crossing.

The surge in development and application of DNA markers in plant improvement programs starting in the 1980s potentially opens new horizons in crop improvement, and particularly introgression breeding. Two broad applications of DNA markers in relation to better utilisation of exotic germplasm can be readily identified. First, DNA markers may help determine genetic relationships among materials in collections, helping identify core sets of clones sampling major genetic diversity for breeding. Second, markers may be used to map quantitative trait loci (QTL) and facilitate identification and introgression of favourable genetic components from exotic germplasm.

Tanksley and Nelson (1996) and Tanksley and McCouch (1997) argue that, for most quantitative traits of commercial value, the phenotype of exotic genotypes will nearly always be dominated by the presence of unfavourable alleles, making identification of sources of potentially valuable genes using phenotype impossible for most quantitative traits. They suggested that the old

paradigm of utilising exotic germplasm involving 'looking for the phenotype' is being replaced by a new one involving 'looking for the genes' because of availability of QTL mapping.

In China, large scale collection of sugarcane related germplasm from the wild occurred during the 1980s and 90s. Many clones arising from these collections are maintained in the National sugarcane germplasm collection in Yunnan province (maintained by Yunnan Sugar Research Institute, YSRI) and in another collection in Hainan province (maintained by Guangzhou Sugar Industry Research Institute, GSIRI). Sugarcane breeders expect many of these clones to contain individual traits and genes of commercial value if they could be identified and recombined in other agronomically suitable genetic backgrounds. In 2002, breeders and scientists from China and Australia began a collaborative program designed to study and utilise wild germplasm from China, targeting eventual development of cultivars in both countries as the end goal. Of particular interest was to explore the role of DNA markers in assisting in introgression breeding in sugarcane.

This project had four main objectives as follows:

- I. To characterise the genetic diversity available in the Chinese collections.
- II. To generate progeny derived from Chinese *S. spontaneum* and *Erianthus* spp, and to commence field evaluation of these in China and Australia.
- III. To determine if and how DNA markers could be used to help introgression breeding.
- IV. To assess if selection in China could be used for Australia, and vice versa, through GE studies.

The aims of this paper are to summarise some progress with this collaborative program, some results to date, and possible future directions.

Genetic diversity studies

S. spontaneum

A diversity study was carried out on a collection of 443 clones of *S. spontaneum* sampled from across its geographic range and maintained in germplasm collections at BSES Ltd (Australia), Copersucar (Brazil), United States Department of Agriculture (USA) and the collections in China. 676 polymorphic AFLP markers were scored across all clones and the data subjected to principal component analysis (PCA). Two main clusters were identified with PCA which corresponded to clones collected from the tropics (mostly from southern India and SE Asia) and the sub-tropical (mostly northern India and China) regions. As seen in other *S. spontaneum* diversity studies, within each of these two clusters there was a tendency for clustering to be based on local geographic origin. Overall, an extremely high level of polymorphism was detected, with a large number of markers appearing in fewer than 10% of the clones, supporting the view that, of all the *Saccharum* species, *S. spontaneum* is the most diverse. While new sources of diversity were identified within the Chinese *S. spontaneum* collections, there was no obvious grouping of this material overall from other *S. spontaneum* clones collected in other parts of Asia.

Erianthus

A diversity study was also carried out with 220 clones of *Erianthus arundinaceus*, of which 123 clones were sourced from the National Nursery of Sugarcane Germplasm Resources (NNSGR) at YSRI in China, 74 clones from the BSES Ltd. Collection in Australia, and 23 clones taken from the collection of Copersucar in Brazil. Similar methods as used for the *S. spontaneum* study were applied. We found that *E. arundinaceus* from China was quite dissimilar and much more diverse than that collected from Indonesia (the latter comprised the other source of most clones studied). Within China, there was a major differentiation among material collected from eastern versus western China (ie. in contrast to differentiation on latitude). The results highlighted the uniqueness and importance of the genetic diversity within China for this species.

We also conducted research to help resolve the position of the species *Erianthus rockii*, a species unique to China and which is of interest in developing drought resistance, and which was used to produce hybrids with sugarcane during the collaborative program. The results suggested this species was distinct from other *Erianthus* and *Saccharum* species and was more similar to *Miscanthus* species. Details of this study were reported by Cai *et al.* (2005).

Generation of progeny from *S. spontaneum* and *Erianthus*

Within the collaborative breeding program, 202 crosses were made by YSRI and GSRI. Microsatellite markers that were highly polymorphic in sugarcane were used to check samples of progeny from crosses, following methods described by Cai *et al.* (2005). Samples of progeny from one hundred and forty-six crosses have been checked to date with DNA markers (example shown in Figure 1) with 100 showing at least some true hybrid progeny (Table 1). As expected, highest proportions of crosses delivering viable seed but ‘non-true progeny’ (arising from selfing of the female or pollen contamination) were those involving *Erianthus*, with crosses between sugarcane (*S. officinarum* or commercial cultivars) and *S. spontaneum* delivering true hybrids. Of particular significance and importance in this project was the verification of fertile hybrids derived from (*Saccharum* × *Erianthus*) × *Saccharum*. This result was the first validated backcross material from *Erianthus* in our experience, despite considerable efforts by other breeding programs in the past. This important result means that it should be possible to utilise the *Erianthus* genome in future sugarcane improvement.

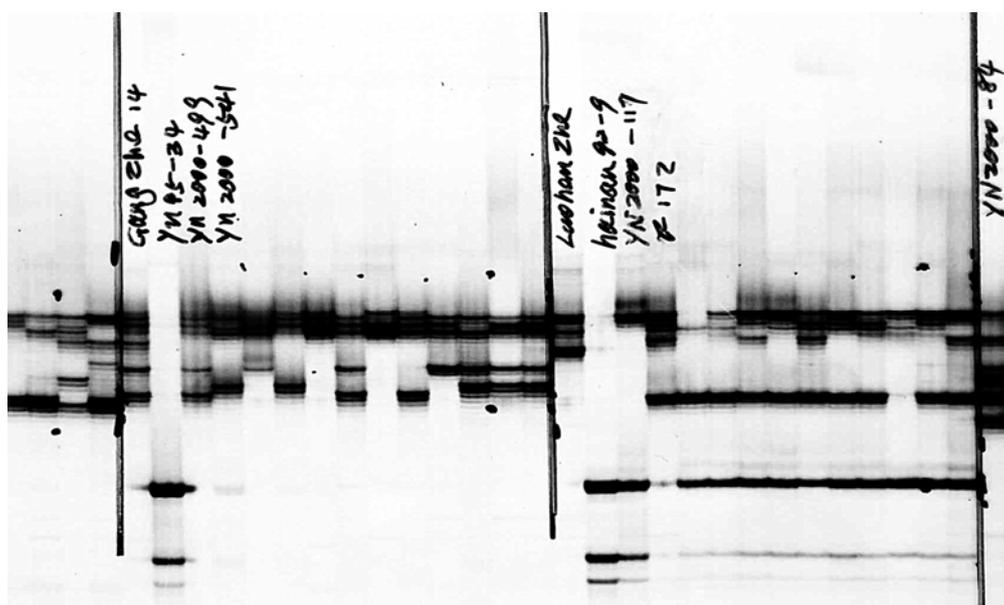


Fig. 1—Examples of checking progeny from crosses involving *Erianthus* using DNA markers. These are results from a single micro-satellite primer (one of three used routinely for checking), showing two groups of parents and progeny. The group on the left shows grandparent Guangzhe 14 (*S. officinarum*) which was crossed with YN95-34 (*Erianthus arundinaceus*) to produce YN2000-499, which in turn was crossed with YN2000-341 to produce putative BC1 progeny, a random sample of which is shown to the right. There is no evidence of bands characteristic of the *Erianthus* grandparent in YN2000-499, which appears to arise from selfing of Gangzhe 14, and therefore no evidence of BC1 progeny being derived from *Erianthus*. By contrast, the group on the right shows grandparent Luohouzhe (*S. officinarum*) which was crossed with Hainan92-84 (*E. arundinaceus*; note: this was mistakenly written as HN92-9 in the image above) to produce YN2000-117, which was crossed with commercial cultivar F172 to produce BC1 progeny, a sample of which are shown to the right. In YN2000-117, bands specific to the *Erianthus* parent are shown, and these are also apparent in the BC1 progeny, providing evidence of these being derived from *Erianthus*.

Table 1—Numbers of crosses made for different types of crosses, showing numbers of crosses with validated true hybrids (ie. not arising from self pollination or pollen contamination). 'Commercial' refers to commercial cultivar or elite parent used in commercial breeding program.

Type	Not examined to date	Tested and no true hybrids	True hybrids present	Total made
<i>S. officinarum</i> × <i>Erianthus arundinaceus</i>	3	7	10	20
COMMERCIAL × <i>Erianthus arundinaceus</i>	0	13	4	17
<i>S. officinarum</i> × <i>E. rockii</i>	0	0	2	2
COMMERCIAL × <i>E. rockii</i>	0	1	0	1
F1 <i>Erianthus arundinaceus</i> × COMMERCIAL	5	8	6	19
BC1 <i>Erianthus</i> × COMMERCIAL	35	9	24	68
COMMERCIAL × F1 <i>E. rockii</i>	3	0	0	3
<i>S. officinarum</i> × <i>S. spontaneum</i>	4	3	15	22
COMMERCIAL × <i>S. spontaneum</i>	0	0	25	25
COMMERCIAL × F1 <i>S. spontaneum</i>	6	5	14	25
Grand total	56	46	100	202

In all of the crosses made, half the seed was retained for use in China, and half imported into Australia following strict quarantine protocols. Clones generated from these crosses are now undergoing selection and use in further backcrosses to high-value parents in sugarcane breeding programs in both countries.

Targeted research trials were carried out on a range of progeny derived from *S. spontaneum*, and some methods and results in this study were reported in detail by Wang *et al* (2008). This study provided a preliminary evaluation of performance of a range of clones derived from diverse *S. spontaneum* clones when crossed with *S. officinarum* or sugarcane clones, as well as some basic statistical parameters that should guide the design of optimal early-phase selection among similar populations in the future.

Overall, the high genetic variation and broad-sense heritabilities (>0.7) observed among progeny for all traits suggests that large gains from selection in these populations could be easily achieved with limited replication (1 or 2 replicates) in small plot trials. For most biomass composition traits, the variation among the families comprised a substantial proportion (over 40%) of the genetic variation.

A selection system involving such initial selection of high-performing families for biomass composition traits, followed by selection among large populations within the best families, would represent an efficient method. Initial selection among families based on measuring a limited random sample of say 10 to 20 clones per family should be effective. For both cane yield and stalk biomass yield, the relatively smaller size of the among-family component suggests that family selection would be limited in effectiveness, and individual clonal selection within families would be important from early stages of selection.

Mid-parent values in an independent trial predicted biomass composition traits (brix, pol, fibre, dry matter %) among the progeny families reasonably well (generally $r > 0.6$), but less so for cane and biomass yield ($0 < r < 0.4$), suggesting the importance of non-additive genetic variance for yield traits. For biomass composition traits, moderate to high (>0.5) correlations were observed between the female (sugarcane or *S. officinarum*) parent and mean progeny performance, but not between the male (*S. spontaneum*) parent and progeny performance, emphasising the high importance of using high-performing female clones in crosses with *S. spontaneum*. For cane yield components of stalk number and stalk weight, correlations with progeny performance were high for both female and male parents.

Pol and purity levels in these progeny clones, as expected, were overall much lower than the commercial cultivars included in the trials for comparison, but some clones performing at levels similar to the commercial cultivars were also identified. For cane yield and biomass yields, clones with considerably higher biomass levels relative to commercial sugarcane were identified. However, as emphasised above, results relating to cane and biomass yields should be interpreted cautiously at this stage, since competition effects are important in the small plots used in this study, and further evaluation in large plots is required to substantiate these results. Nevertheless, the performance levels in biomass coupled with large predicted gains from selection suggest that some clones generated from crosses between sugarcane and *S. spontaneum* could offer opportunities for providing high yielding biomass crops. In addition, based on prior knowledge about the contribution of *S. spontaneum* to sugarcane breeding, it may be expected that strong ratooning ability and adaptation to a range of environmental stresses may be expected to be a feature of some first cross progeny involving *S. spontaneum*. These traits were not tested in the current study, but should be a priority for further evaluation.

Use of DNA markers for introgression breeding

A large effort was devoted toward determining if and how DNA markers may be used to identify favourable or unfavourable genes or QTL in exotic germplasm, which could be used in subsequent breeding and selection cycles. Scoring the AFLP gels for the large numbers of markers required for QTL mapping in sugarcane was extremely time consuming and prone to error variation, and any future marker work should benefit from a more automated marker system such as DArT (Jaccoud *et al.*, 2001). Several case study populations derived from *S. spontaneum* and *Erianthus arundinaceus* were studied. One population and some results are briefly described here to illustrate the approach used. This population was derived from the cross between ROC25 (a commercial cultivar bred in Taiwan province) × YN02-356. The latter clone was selected from the cross Co419 (Indian commercial cultivar and parent) × YN75-1-2 (a *S. spontaneum* clone collected from the wild in Yunnan province).

It was of particular interest to determine if favourable QTL could be identified from the wild *S. spontaneum* clone, which could then be selected for or against in further breeding cycles. A sample of 300 clones from this BC1 population was evaluated in a trial in Australia in very small plots, with each clone being planted into 4 replicates, arranged in a randomised complete block design with individual plots being a single row × 4 m long. Measurements were made on brix in juice, pol in juice, fibre, CCS, stalk weight, stalk number, cane yield (from product of prior two) and biomass yield. The population of clones was also screened with approximately 40 AFLP primer pairs and 12 SSR primer pairs in labs at YSRI and CSIRO.

Despite the relative sparseness of existing linkage maps, a number of important associations between markers and trait performance were apparent (Table 2). The effects for cane yield are particularly interesting, with these effects representing potentially important effects in a sugarcane breeding program. These results have provided further impetus to utilise selected clones from this population and linked markers for further breeding efforts, as discussed below.

Table 2—Three largest marker/QTL effects for cane yield and brix in the ROC25 × YN02-356 population in Australia.

Marker	P value	Effect	R ²
Accctc58	<0.00001	−39.4 t/ha cane yield	13
Accctc56	0.00002	+27.1 t/ha cane yield	6
Accctc51	0.0001	+24.4 t/ha cane yield	5
Cir56a	0.00017	+0.7 units brix	5
Accctc60	0.001	+0.6 units brix	4
Cir36d	0.001	+0.6 units brix	3

Genotype × environment interactions between countries

A common set of 26 clones was evaluated in multi-environment trials within both Australia and China (24 environments in Australia and 10 environments in China), with trial design and other procedures used described by Jackson *et al.* (2007).

Despite some major differences among trial environments between China and Australia for climate (especially temperature and solar radiation), and for some diseases, moderate to high genetic correlations between clone performance in China and Australia were observed for sugar content and cane yield. The average genetic correlation between environments in China versus Australia was 0.75 for CCS and 0.65 for cane yield. This is consistent also with results found with families derived from *S. spontaneum* where common families were evaluated in Australia and China and good genetic correlations between countries were observed (reported in Wang *et al.*, 2008). These results are the first to our knowledge documenting genotype × country interactions. The results indicate that selection results in China are relevant to Australia and vice versa. Therefore, ongoing exchange of data, seed and selected clones could be mutually beneficial. This has led to a draft collaborative agreement between institutes in China and CSIRO/BSES for ongoing exchange of data and seed.

Further work and recommendations

Arising from the collaborative program of work, the following activities are planned or recommended:

- Further and more accurate evaluation of promising clones derived from *S. spontaneum* and *Erianthus* in both Australia and China, and further crossing of selected and verified (verified with DNA markers) clones to high value commercial parents.
- Initial selection among families and clones in the above program should be based on phenotype, without marker assisted selection. When a small number (2–3) of the best parents and bi-parental populations are identified, then QTL mapping should be done on one or more high value populations derived from new germplasm sources. Markers linked to favourable QTL introgressed into mainstream breeding programs may then be used in ongoing marker-assisted selection in materials along with other markers already used in the core program.
- There is interest in exploiting the genetic material generated in this collaborative project for emerging energy production systems in both countries. The relative value of fibre and other traits should be reviewed further with a view to informing breeding objectives. However, for the purposes of initial selection, a value of fibre relative to brix is suggested as 40% (ie. 1% increase in fibre content in fresh cane is worth 0.40 of the value of a 1% increase in CCS and brix content). This initial assumption is based on likely biofuel yields from sugars versus fibre and costs of conversion and assumed price parity between sugar and biofuel in the future. Other traits also need to be reviewed to determine optimal selection indices.
- To continue to exchange data and germplasm arising from the project efforts to date between China and Australia, in order to achieve mutual advantage from each other's efforts in sugarcane breeding in the future.

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Résumé

En 2002, un programme de collaboration en hybridation génétique et de recherche a été initié, ayant pour objectif l'utilisation du germoplasme sauvage provenant de Chine pour l'amélioration de la canne en Chine et en Australie. Quelques résultats et recommandations découlant de ce programme à ce jour, sont rapportés ici. Le programme avait comme objectif: 1. Caractériser la diversité génétique du *S. spontaneum* et *Erianthus arundinaceus* chinois. 2. Effectuer des croisements entre *S. spontaneum*, *Erianthus* spp. de Chine et la canne à sucre et évaluation préliminaire de la descendance. 3. Déterminer si et comment les marqueurs moléculaires peuvent être utilisés dans l'identification des régions du génome des clones sauvages avec des valeurs positives ou négatives et favoriser le programme d'introduction des gènes nouveaux à partir des cannes sauvages dans les cultivars commerciaux. 4. De quantifier les interactions génotype x environnement entre l'Australie et la Chine. Les résultats des études sur la diversité génétique ont montré un taux élevé de diversité génétique chez *S. spontaneum* et *Erianthus arundinaceus* en Chine. Cela constitue le fondement pour un échantillonnage ciblé et l'usage de ce matériel dans les programmes d'amélioration génétique ou pour constituer des collections de base. Deux cent deux croisements découlant d'une gamme de clones *S. spontaneum* et *Erianthus* ont produit des graines viables. Cent de ces croisements ont été authentifiés (à ce jour) comme étant des vrais hybrides en utilisant des marqueurs moléculaires. Ces résultats sont significatifs par rapport à l'*Erianthus* spp. et constituent (à notre connaissance) une première mondiale des hybrides authentifiés entre *Saccharum* x *Erianthus*, en dépit de beaucoup d'efforts dans le passé. Plusieurs cas d'études des populations dérivées du *S. spontaneum* et *Erianthus* ont été utilisées pour la cartographie des loci quantitatifs (QTL). Plusieurs QTL apparemment important pour le rendement en canne, ont été identifiés provenant de *S. spontaneum*. En se basant sur les résultats et l'expérience obtenue, une approche pour appliquer des marqueurs moléculaires aux fins d'hybridation par introgression est

recomendada. Un autre résultat significatif qui (à notre connaissance) est une première, est la documentation de l'interaction génotype x pays. Assez surprenant, des corrélations génétiques modérément élevées (>0.6) ont été obtenues entre la performance des familles et les clones entre les essais conduits en Chine et en Australie, en dépit des environnements divergents et une gestion différente. Ces résultats renforcent un besoin de collaboration continue entre la Chine et l'Australie par l'intermédiaire des échanges des résultats des essais de la sélection et du germoplasme.

UTILIZACIÓN DE CAÑAS SILVESTRES DE CHINA

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PALABRAS CLAVES: *Erianthus*, *Saccharum spontaneum*,
Introgresión, Mejoramiento Genético, Marcadores ADN.

Resumen

UN PROGRAMA colaborativo de mejoramiento e investigación comenzó en el 2002, procurando utilizar germoplasma silvestre de China para mejorar las variedades de China y Australia. Algunos resultados y recomendaciones que salieron de este proyecto hasta hoy se reportan aquí: El programa tuvo como finalidad de: 1. Caracterizar la diversidad genética de las especies Chinas de *S. spontaneum* y *Erianthus arundinaceus*; 2. Conducir cruza entre *S. spontaneum* and *Erianthus* spp. chinos y caña de azúcar cultivada así como una evaluación inicial de las progenies resultantes; 3. Evaluar si los marcadores del ADN pueden ser usados para identificar regiones del genoma de valor positivo o negativo de los clones silvestres, y asistir a los programas para introducir nuevos genes de las cañas silvestres a las cultivadas comercialmente; 4. Identificar las interacciones genotipo × ambiente entre Australia y China. Los estudios de diversidad genética mostraron un alto nivel de diversidad en *S. spontaneum* y *Erianthus arundinaceus* en China, y proveen las bases para identificar materiales claves y usar en cruzamientos futuros o formar colecciones núcleo. Se realizaron 202 cruza de un rango de clones de *S. spontaneum* y *Erianthus* con semillas viable y 100 de estos cruzamientos se verificaron como híbridos verdaderos usando marcadores moleculares (hasta hoy). Estos resultados fueron significantes en relación a *Erianthus* spp. mostrando por primera vez la existencia de híbridos fértiles entre *Saccharum* × *Erianthus* comparados con otros esfuerzos del pasado. Varios estudios de caso de poblaciones derivadas de *S. spontaneum* y *Erianthus* fueron usadas para estudios y mapeo de locus de Caracteres Cuantitativos (QTLs). Aparentemente, varios QTL importantes para producción de caña fueron identificados de *S. spontaneum*. Un método para aplicar marcadores moleculares en trabajos futuros de introgresión en caña de azúcar se recomienda basándose en las experiencias y resultados obtenidos. Otro resultado significativo (de lo que conocemos) fue el primer reporte documentado sobre la interacción genotipo × ambiente. Sorpresivamente, las correlaciones genéticas fueron de moderado a alto (>0.6) en el comportamiento de las familias y clones entre los experimentos en China y Australia. Todo esto a pesar las condiciones contrastes del ambiente y de manejo. Estos resultados soportan el trabajo colaborativo que está en marcha entre China y Australia a través de intercambio de germoplasma y resultados de los experimentos.