

# CANE BREEDING AND IMPROVEMENT

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**IMPROVEMENT of crop plants commenced with primitive people at least 12 000 years ago, with most crops being moulded very much to the forms seen today by these early plant breeders. They domesticated wild species, selecting plants with characteristics suited to more stable cultivation rather than to a hunting-gathering way of life. Indeed agriculture, based on these improved plant types, contributed significantly to the development of civilisation.**

The first improvements in sugarcane resulted from the selection of sweeter, less fibrous types suitable for chewing. Brightly coloured or striped varieties were also selected and grown in gardens. According to George Muller: 'From its mythical origins and early sources up to its present-day production and worldwide utilisation, cane sugar has played its role in the history of human culture and world economy. The cultivation of sugarcane provides an outstanding example of the spread, the development and the breeding of a valuable plant and of its processing from the most primitive beginnings to a state of near perfection.'

## **HISTORY OF CANE BREEDING IN AUSTRALIA**

In the early days of sugarcane production in Queensland, the principal variety grown was

Bourbon, also known as Otaheite, which had been introduced from Mauritius. In 1873, this variety was attacked by 'rust', which became so widespread that Bourbon had to be replaced by rust-resistant varieties. These were the three Cheribon varieties, known in Queensland as Rappoe (or Rose Bamboo), Striped Singapore and Meera.

In 1899, gumming disease swept the fields and this brought about another varietal revolution with the extensive planting of D1135. This was one of a number of varieties introduced from Demerara in Guyana by Young Brothers of Fairymead Plantation, Bundaberg, and was known as the 'Fairymead Cane'. With the rapid expansion of the industry northwards during the early 1900s and the greater diversity of soil types and climatic conditions, the demand for new varieties increased. New Guinea, a centre of

origin of the noble canes (*Saccharum officinarum*), became a source of numerous collections from 1875 to 1957.

From all the expeditions, by far the most important commercial variety obtained was Badila, collected by Tryon in 1896. However, several varieties were most valuable from a breeding perspective. Easterby lists 470 clones introduced into Australia between 1847 and 1914. Varieties such as Meera, Mauritius Gingham, Malabar, Rose Bamboo, Striped Singapore, Daniel Dupont, Black Java, Tanna, Lahaina, NG16, Mahona, Goru, Black Innis and Petite Senneville all played a part and contributed in varying degrees to the industry's progress.

Two events were landmarks in more recent genetic improvement of sugarcane: the discovery of sexual reproduction (Parris, 1858 in Barbados; and Soltwedel, 1885 in Java), and interspecific hybridisation (Soltwedel, Jeswiet and van Harreveld). It was not until 1888 that workers in Java and Barbados independently realised that sugarcane seedlings were a source of variation that could be exploited. Subsequently, the Dutch breeders in Java were faced with a serious outbreak of serah disease, and all noble clones (*Saccharum officinarum*) appeared to be susceptible. Crosses with Kassoer, later found to be a natural hybrid between *S. officinarum* and *S. spontaneum*, produced clones combining serah resistance with other desirable characteristics. Jeswiet recognised the high yielding potential of these hybrids and developed a systematic 'nobilisation' of progeny by backcrossing to *S. officinarum* to improve sugar content. These hybrids formed the genetic basis of many sugarcane breeding programs today, and culminated in the release of POJ2878 in 1921. It became a significant variety in Java, Australia, and many other countries and is in the pedigree of almost every commercial variety in production in Australia today. It was known locally as 'Wondercane'.

### Cane breeding in Australia

In 1889, the Queensland Acclimatisation Society began to raise seedling canes, but it was not until 1900 that any marked degree of success was achieved and appreciable numbers of seedlings were raised. This early breeding consisted of collecting arrows in the field and germinating the fuzz. Thus, only the female parent of any seedling was known. Until 1907, the society raised about 1805 sugarcane seedlings, the best known of which was Q813, while Q855 and Q1098 were also grown to a limited extent. Q1098 later achieved importance as the male parent of Q28.

The Colonial Sugar Refining Company also began raising seedlings in 1901 at Hambledon near Cairns. Although this work was discontinued in 1905, the varieties HQ285, HQ409 and HQ426 were selected. Each of these achieved some prominence as a commercial variety and HQ426, also known as Clark's seedling, was still listed as an approved variety in 1961. The Bureau of Sugar Experiment Stations did not possess an experiment station north of Mackay during this period and this was too far south to be a satisfactory centre for seed production. In 1921, with the development of a new experiment station at South Johnstone, seedling production became an important feature of the station activities. The 'SJ' seedling canes became known in the industry, and SJ4 and SJ16 were important varieties developed there.

Both foreign and local varieties have been important, in particular to help control the many diseases that have plagued the sugar industry during the twentieth century. POJ2878 became a commercial variety in 1933 and revitalised the industry in the south. Other foreign varieties to have dominated the Queensland industry include Badila and NCo310.

The development of locally bred varieties from controlled pollination began in earnest in the 1930s. The Bureau of Sugar Experiment



Stations (BSES) varieties were designated as 'Q'. One of the early Q varieties, Q28, was first crushed in 1943. It revolutionised cane growing in the Mackay region, becoming the third most important variety in the state in 1948 when 923 000 t were harvested. One of the most important CSR varieties was Trojan, bred in 1933. Apart from its value as a commercial variety, it was a major parent in the CSR program and was the female parent of 17 'Q' varieties from Q63 to Q106. Other dominant locally bred varieties include Q50, Q117, and Q124 (BSES) and Pindar (CSR).

There have been a number of highly successful crosses that have resulted in many commercial varieties. Three of the most notable have together produced 31 Q canes—Trojan × Co475 (11), NCo310 × 54N7096 (11), and 58N829 × 66N2008 (9).

In 1998, 96 cane varieties were delivered to Australian mills (excluding Western Australia), 74 in Queensland, 34 in New South Wales, with 12 varieties in common to both areas. Of the 96 varieties, 65 were bred by BSES, 18 by CSR, while 11 were of foreign

origin. The origin of varieties delivered to Queensland mills since 1942 (Figure 1) shows the high reliance the industry had on foreign varieties up until 1950, particularly with varieties like Badila, POJ2878 and Co290. A resurgence in foreign varieties occurred when NCo310 dominated the industry during the 1970s to mid 1980s. However, since the mid 1980s, BSES-bred varieties have gradually assumed dominance, while both foreign and CSR-bred varieties have steadily declined in importance.

### Yield improvement

Since 1942, cane production in Queensland has risen from less than 5 to over 37 Mt in 1998, while the harvested area has increased from 96 000 ha to more than 380 000 ha (Figure 2). Cane yield (Figure 3a) over this same period has increased from under 50 t/ha to over 95 t/ha, almost a doubling of yield. The rate of increase in cane yield averaged 0.75 t/ha/year over this 57-year period. The average CCS (commercial cane sugar) for Queensland (Figure 3b), however,

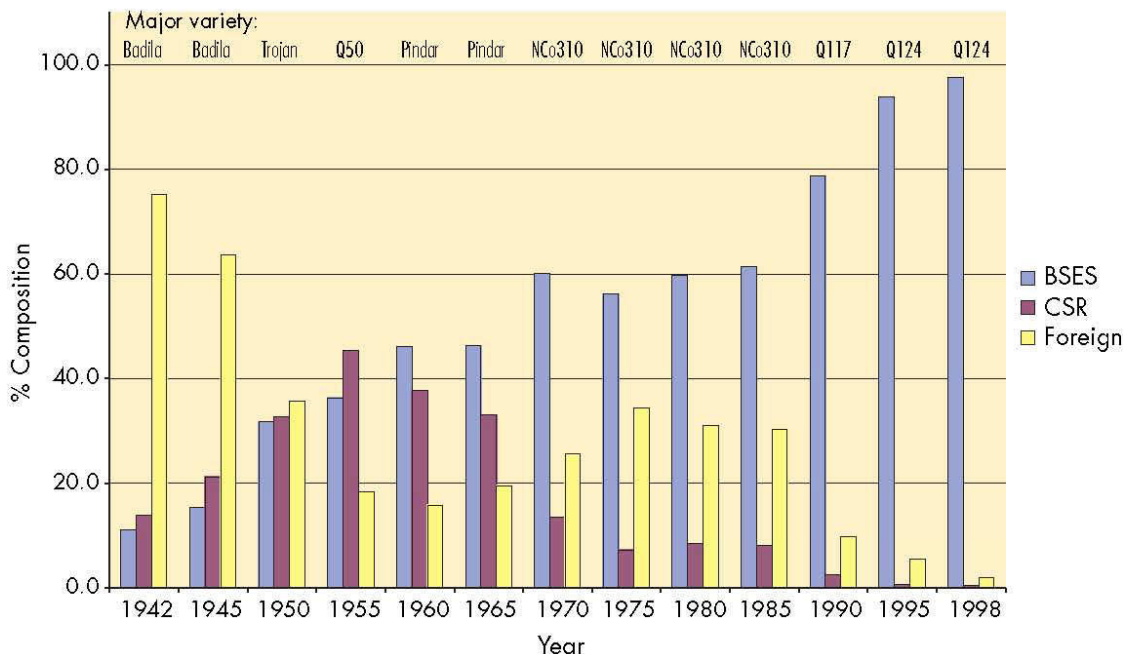


Figure 1. Varietal Composition Queensland 1942-98.

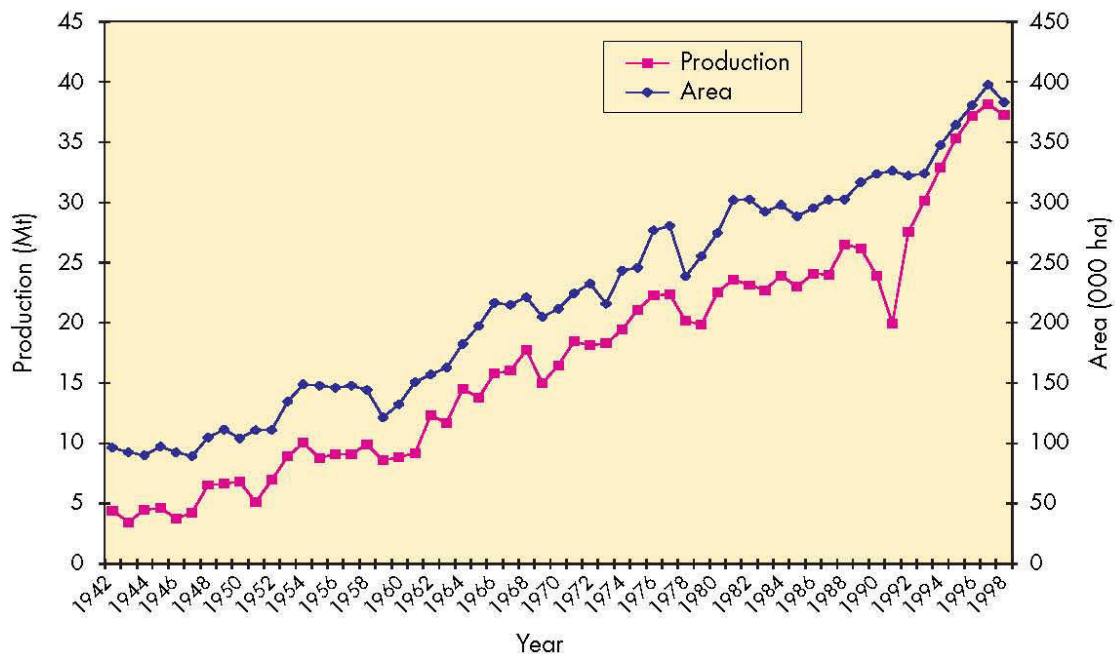


Figure 2. Cane Production and Area Queensland 1942-98.

was highly variable, showing a slight decline ( $-0.014$  units/year). The CCS prior to the 1970s, when full implementation of mechanical harvesting occurred, was mostly above 14 (75, 50, and 80% of years for the decades of the 1940s, 1950s, and 1960s, respectively). Since then, CCS has been greater than 14 in only 34% of years (60% in the 1970s, 10% in the 1980s, and 38% in the 1990s). Sugar yield (Figure 3c) has also improved, averaging just under 0.1 t/ha/year. Using 5-year moving mean data, the average percent increase per year was 2.1% for cane yield,  $-0.1\%$  for CCS, and 1.8% for sugar yield. The Queensland sugar industry improved yields by 1.9% from 1948 to 1975, and plant breeding was likely to have contributed about half of this increase. Clearly, both cane and sugar yields have continued to increase at about the same rate, and this is despite much of the recent expansion being on to more marginal land.

### Disease resistance

Apart from productivity improvements, many varietal changes have occurred to counteract disease problems. For example, while POJ2878 was partly responsible for the elimination of gumming disease in Bundaberg and Isis, it was later found to be highly susceptible to both Fiji disease and downy mildew. The variety Co290, released in 1935, had some resistance to these diseases and was crossed with POJ2878. This resulted in a highly successful cross from which the varieties Q47, Q49, Q58, Q61 and Q68 were derived, the first two combining resistance to all three diseases.

Q90 was a highly successful variety in north Queensland but succumbed to common rust, discovered in October 1978. It had become a minor variety by the mid-1980s. Fiji disease reached epidemic proportions in the southern region (and, to a lesser extent in the central region) following

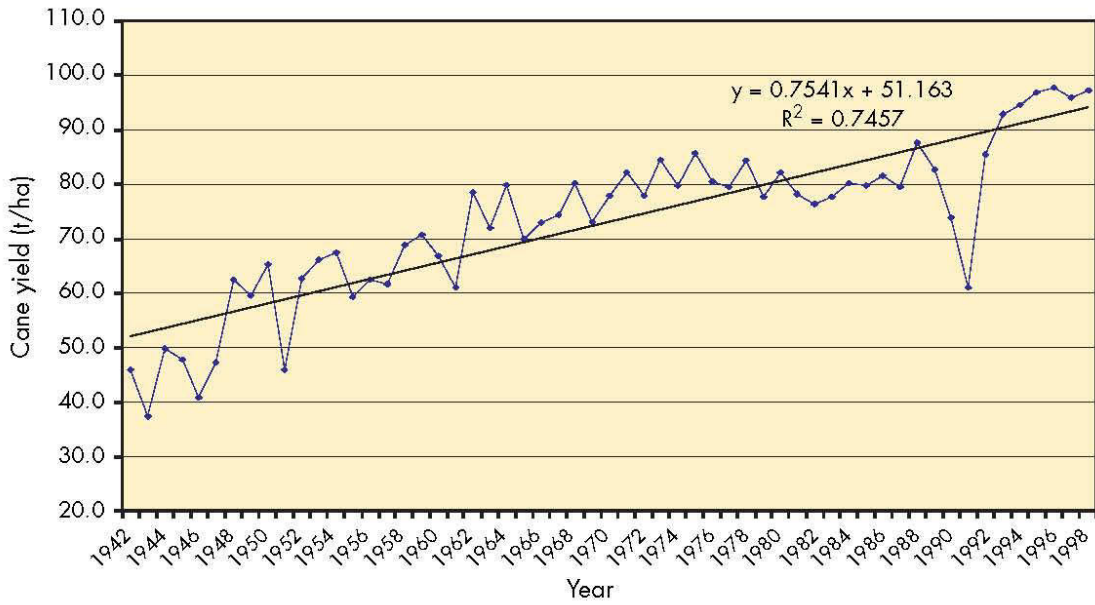


Figure 3a. Cane Yield Queensland 1942-98.

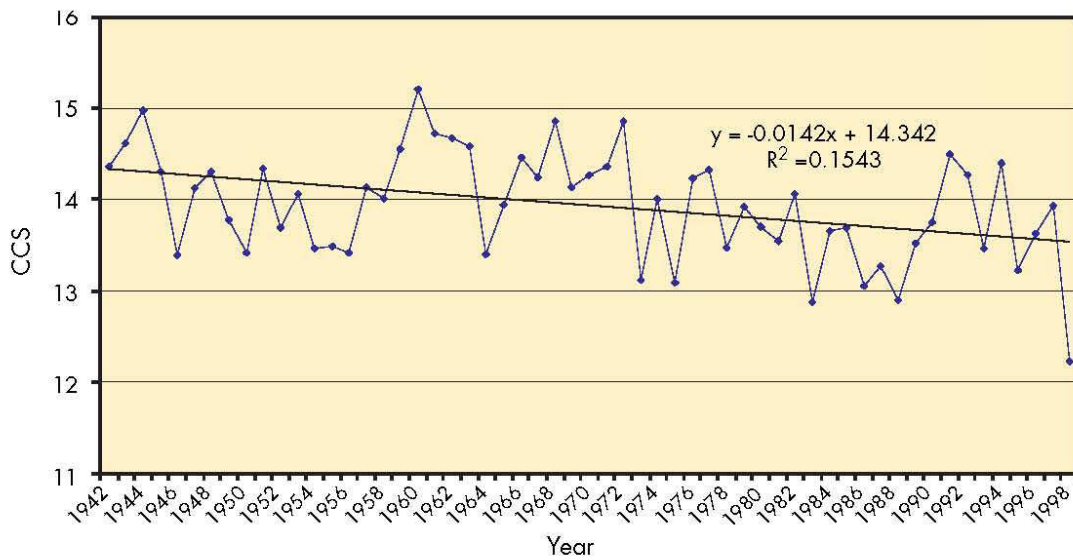


Figure 3b. CCS Queensland 1942-98.



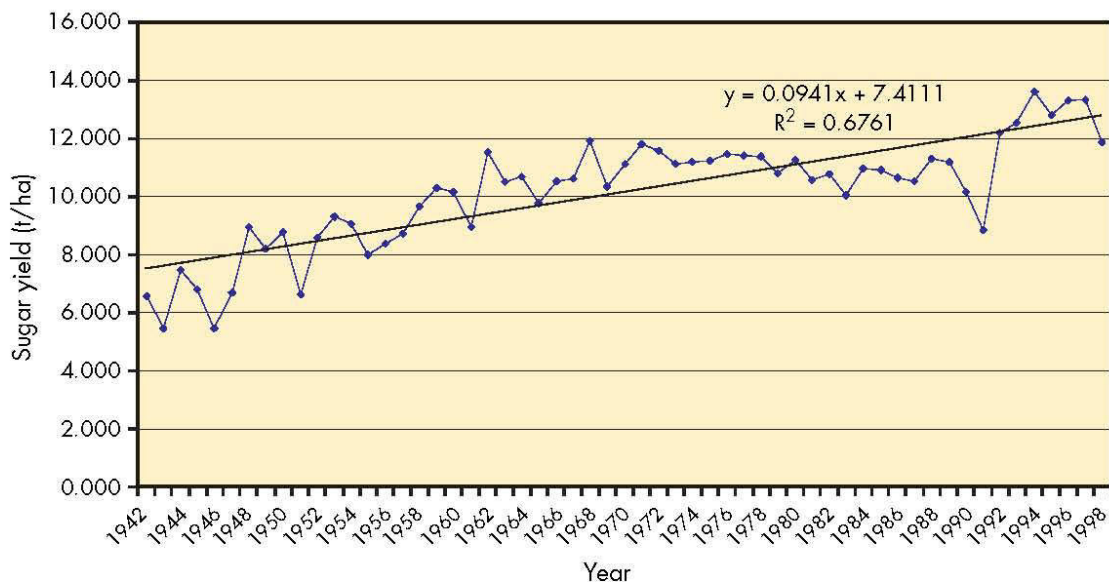


Figure 3c. Sugar Yield Queensland 1942-98.

its rediscovery in April 1969. This region's major variety, NCo310, which accounted for almost 80% of the south Queensland crop at its peak (over 90% in some mill areas), was highly susceptible. The varieties Q87 and CP44-101 became available to the industry and showed acceptable resistance to Fiji disease, although diseased stools were found in localities with heavy infection pressure. Four varieties with better resistance were released in 1979 (Q108, Q109, Q110 and Q111), with Q110 becoming an important variety in the south. A major variety in the Mackay and Burdekin regions was Q63. Its susceptibility to leaf scald resulted in its removal as an approved variety in the Mackay (1974) and Burdekin (1978) regions, being replaced by the slightly more resistant Q96.

The incursion of sugarcane smut into the Ord region of Western Australia in 1998 has led to a massive screening program for resistance. While the disease has not yet been found in east-coast sugar regions, the implications of this disease are already being felt. One of the highly susceptible varieties is Q117 and the threat of this disease will

undoubtedly lead to a reduction in its planting.

In 2000, a new strain of orange rust attacked Q124 and caused very serious losses of yield and sugar content. There is ample resistance to the new strain, so new varieties should solve this problem relatively quickly.

During the past 100 years, breeding and selection techniques have improved dramatically. Most of these improvements have been underpinned by research and, more recently, developments in technology have had a major impact.

### CANE IMPROVEMENT PROGRAM

Any plant improvement program consists of two main aspects—the creation of genetic variability (usually through crossing) and discrimination within this variability (selection). Infusion of new germplasm through the introduction of varieties is also a critical component. The plant breeding cycle is shown in Figure 4. The two main elements of sustained success in plant breeding are the release of new improved varieties and the continuous improvement of the breeding

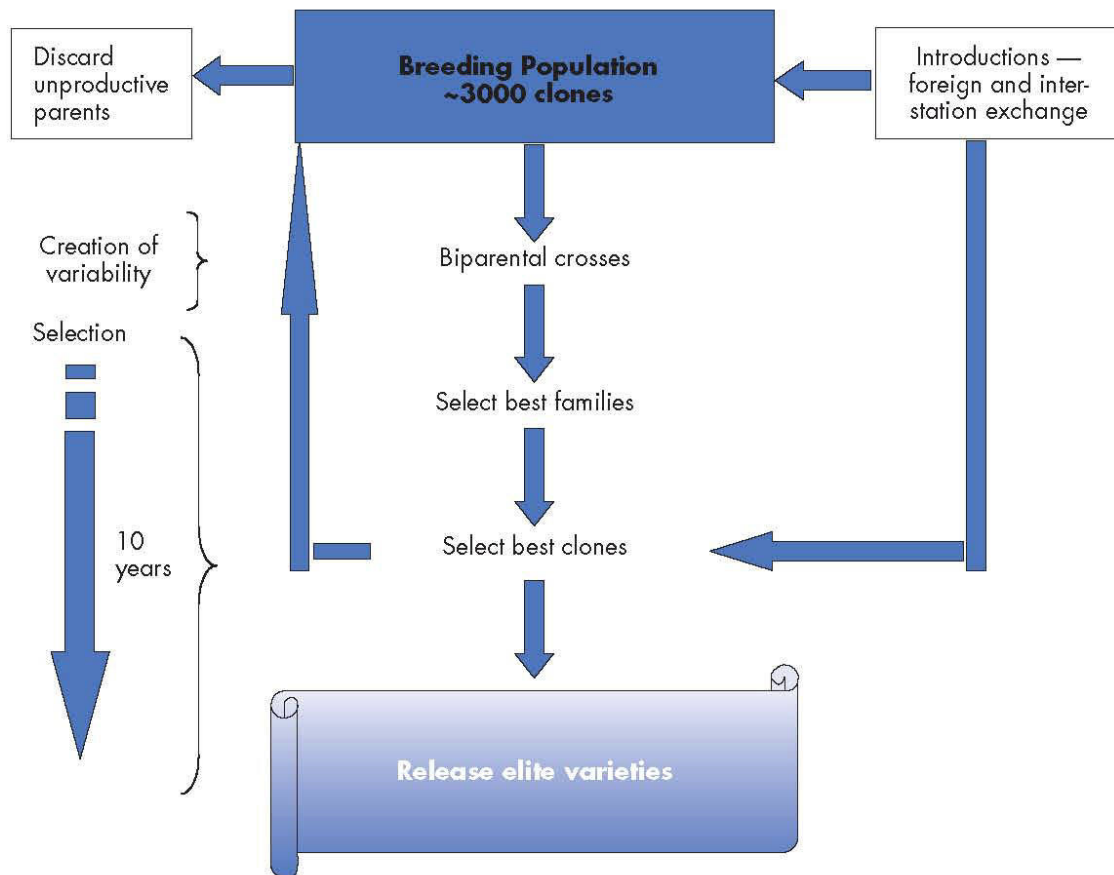


Figure 4. Cane Breeding Cycle.

population. Release of new varieties occurs when there is an effective breeding and selection program. Population improvement is attained through rapid recycling of elite clones from the selection program, introduction of new clones (both foreign and from other selection programs), and by discarding unproductive parents. Thus, the breeding population must be highly dynamic.

### Crossing

Meringa is now the major breeding station for BSES, while Macknade has been the centre for CSR (CSR is winding down its cane improvement program). At Meringa, the parent population consists of over 3000 clones which are grown in the field each year. Five groups of clones are grown in

flowering blocks: Q varieties; elite clones from the northern, Herbert, Burdekin, central, southern, and New South Wales selection programs; elite clones from other Australian programs; foreign introductions; and basic germplasm (*S. officinarum*, *S. spontaneum* and related genera and species).

The main crossing season at Meringa is from mid-May until mid-July, but flowering in the field is highly variable from year to year. On average, 35% of clones flower, but this ranges from low (16% in 1993) to reasonably high (66% in 1984). Initiation of flowering occurs between mid-February and mid-March, mainly in response to shorter days (photoperiod). Other environmental factors during this period that determine the amount of flowering include moisture and



temperature. Irrigation to minimise stress improves flowering, but high temperatures during initiation (e.g. several days above 32°C) reduce flowering.

#### **Routine crossing and seed production**

A census of the breeding population is conducted to determine which clones are flowering (usually twice weekly). Several florets of each flowering clone are collected to conduct a pollen test. This test, using a projected microscope image of pollen cells stained with iodine, determines if a clone is male (high pollen fertility) or female (low pollen fertility).

These data are entered into a computer. A crossing program is then run to determine the optimal biparental combinations that could be made at that time. This program accesses database information on each clone's previous performance as a parent, its agronomic performance, and its disease resistance. For example, crosses will not be chosen between two clones that are both susceptible to an important disease.

Flowering stalks of clones selected for crossing are cut and labelled, and carefully transported to the crossing shed. Stalks of the female and male clones are placed into buckets containing a solution of a dilute mixture of phosphoric and sulfurous acids with small amounts of hydrochloric, nitric and sulfuric acids, that preserves the stalks and provides nutrients. The flowers of these clones are contained within a bag (lantern), made of a finely woven muslin cloth to prevent pollen contamination (Figure 5). The crossing solution is replenished regularly.

After 2 weeks, the pollinated female stalks are set aside for maturing, still in crossing solution. After a further 2 weeks, panicles bearing seed are dried in open-weave terylene bags using cold dehumidified conditions in an insulated chamber at 11–13°C and 15–25% relative humidity. This dries the seed to 4.5% moisture in about 4 days, ideal for long-term storage. Seed is then stored in sealable aluminium foil bags and

stored in deep-freeze conditions (<-20°C) until required for germination.

Depending on the amount and intensity of flowering, between 800 and 1800 new cross combinations from the field are made each year. Germination tests are conducted and sufficient seed to give approximately 200 seedlings is packed into each bag. The breeders responsible for each selection program choose appropriate crosses for their region from the stored seed list. Requested seed is shipped to each experiment station. These are germinated, potted, and, finally, transplanted to the field.

Crossing methods and seed production procedures have improved dramatically since the first controlled pollinations. Research into better materials for crossing lanterns resulted in the use of a finer-weave muslin that was effective in keeping out stray pollen. This work casts some doubt over the parentage of varieties produced from crosses made prior to this. Cold dehumidification technology for the drying of seed has been a marked advance over forced-air drying at 36–38°C, improving both germination and longevity of seed. Similarly, the universal use of improved germination cabinets since 1996, with good control of temperature and light, has seen a significant improvement in germination of seed (fuzz).

Development of computerised breeding records has improved the operational efficiency of the crossing program and has undoubtedly meant superior crosses have been made. The system summarises the vast amount of agronomic, disease and breeding-performance data available on each clone and uses this information to predict the likely performance of a cross between two parents. More recently, sophisticated statistical techniques, such as Best Linear Unbiased Predictors, have provided an even better estimate of parental value than the previous empirical system. Use of these methods has depended on the dramatic increase in the power of computers.



Poor flowering in the field has been one of the greatest limitations to the genetic improvement of sugarcane. To improve flowering, a photoperiod facility was commissioned at Meringa in 1986. While the Meringa facility was the first in a tropical location, it was based partly on a South African design. Since 1996, when the Meringa facility began production of routine crosses, between 150 and 300 crosses have been made each year. In 1997, a photoperiod glasshouse was constructed in Bundaberg to facilitate crossing for the central, southern and New South Wales selection programs (Figure 6). These regions are isolated from Meringa by a quarantine barrier, resulting in a delay in the use of elite clones of at least 3 years. Some experimental work has been necessary to fine-tune the optimal conditions for flowering in this sub-tropical region, but the first 22 crosses were made in 1998. Construction of a second photoperiod facility at Meringa commenced in 1999.

These facilities have changed the way breeders cross sugarcane. Until now, breeders have been limited to combining clones that happen to be flowering at the same time, and many desirable crosses may

never be possible. The photoperiod facilities offer the opportunity to synchronise flowering of most clones by manipulating initiation times, environmental parameters, or both. This allows the breeder greater choice in the crosses to be made. The industry has made a large capital investment in this technology, and it indicates the importance of breeding in varietal improvement.



**Figure 6.** *Sugarcane flowering in a photoperiod house at Bundaberg.*



**Figure 5.** *Crosses erected in pollen-proof lanterns in a crossing shed.*

### **Selection**

Selection programs have evolved as sugarcane breeders' knowledge through research has improved and as new technology has been adopted. Some of the recent major improvements are summarised below.

Mechanisation has been a major boon to selection programs. Mechanical planting of variety trials, albeit with whole-stick planters, has saved considerably on labour compared with older hand-planting methods. It has allowed a much larger trial program to be implemented, both in terms of numbers of varieties and locations. Mechanical transplanting of seedlings has had similar effects, particularly since the advent of family



selection (see below). Weighing of trials using the sampling method (hand harvesting, weighing and counting of stalks) imposed severe restrictions on the number of plots that could be handled in selection programs each year. Much selection was based on visual selection for yield, often associated with measurement of brix (total soluble solids, related to CCS). Only in advanced trials were clones weighed and CCS measured. Since the development of mobile weighing machines in Australia in the mid 1980s, clones are now mechanically harvested with commercial machines and weighed at all stages of selection. This collection of objective, rather than subjective, yield data has improved the efficiency and effectiveness of selection programs and has led to the release of superior varieties since the late 1980s.

Another spin-off from the development of mobile weighing machines has been the ability to implement family selection. Family selection is now routinely used in all selection programs in Australia and it is efficient in terms of labour use and highly effective in terms of genetic gain. Prior to family selection, large seedling populations were visually selected for yield, a trait that has very low heritability on a single-plant basis (e.g.  $\leq 0.17$ ) but reasonable heritability on a family-mean basis (e.g. 0.75). This meant that fairly liberal selection was necessary and genetic gains were low. In family selection, replicated family plots, consisting of 20 to 45 seedlings (all genetically different) are harvested and sampled for CCS. Only families identified as having high sugar yield in the plant crop then undergo further selection. Individual clones are selected from these superior families using visual selection (and sometimes brixing) in the first-ratoon crop. This combination of family and individual selection has led to a higher proportion of elite clones in the next stage of selection.

Currently, selection programs are conducted in the northern, Herbert, Burdekin, central, southern and New South

Wales regions of the Australian sugar industry. In recent times a combined southern Queensland-northern New South Wales selection program has been implemented. Selection programs vary slightly in each region, but the basic approach is very similar.

Selection programs start with the planting out of seedlings from appropriate crosses; this is the only time that sugarcane is not vegetatively propagated. The large numbers of seedlings, up to 35 000 for each selection program (approximately 150 000 in total), undergo selection through different stages over the next 10 to 12 years. In the first clonal stage, when large numbers of clones are involved, selection is based on small plots, little or no replication, and few sites. Large numbers of clones are discarded and about 100 elite clones are finally tested in larger replicated plots grown over a number of sites throughout the particular region. As elite clones are identified, propagation begins so that, as the final results become available, there is sufficient planting material for distribution to growers. During this period, a series of agronomic assessment trials are also conducted to provide information on the likely target environments for the new variety and how best to manage it. A modern selection program is shown in Figure 7.

Shortening the selection program from 13–15 years to 10–12 years has been another improvement that has seen quicker delivery of varieties to the industry. From as many as six stages, most selection programs now have only three—family assessment, clonal assessment, and final assessment.

Measurement of juice quality components (brix, pol, CCS) as well as fibre is an integral part of the selection program. Large numbers of samples are handled through each of the laboratories, often with more than 20 000 samples being processed each year. From time-consuming methods using cylinder brix and manually read polariscopes, the modern juice laboratory has developed into one with automatic data acquisition from barcoded labels and use of instruments to measure

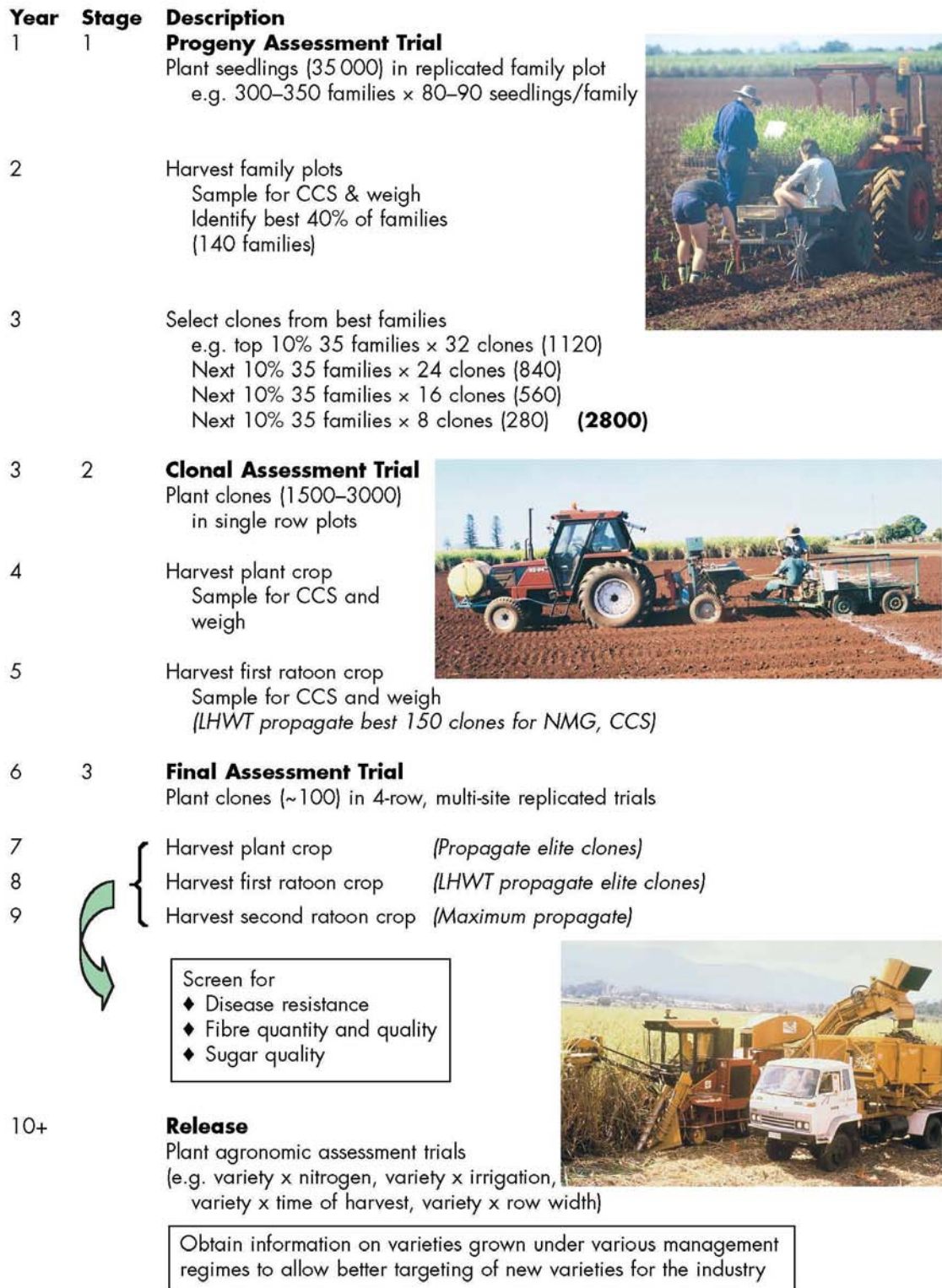


Figure 7. Sugarcane Selection Program.



refractometer brix, pol and conductivity. More recently, the development of near-infrared-spectroscopy technology using fibrated cane samples has allowed the simultaneous measurement of quality traits, such as brix, pol, CCS, moisture and fibre content. Sample delivery and data acquisition have also been automated.

A major objective of the selection programs is to improve sugar yield per hectare. Cane yield is estimated from mechanically harvested and weighed plot yields. Because of the economic importance of CCS in the cane payment formula, CCS is given high importance. Clones with low CCS are discarded from the program whatever the cane yield. Net merit grade (NMG), an economic index of productivity, is calculated to give a relative estimate of sugar yield with an adjustment for CCS that reflects the industry benefit for high CCS. However, while improved productivity (cane yield and CCS) is one of the major objectives of the selection program, incorporation of many other important traits is crucial to the development of useful varieties. Some undesirable agronomic characters are scored in an appearance-grade rating, which is subsequently used to penalise the net merit grade of a clone. Such traits include flowering, suckering, side-shooting and lodging. The mean of the standard commercial varieties in the trial is set at 10, so clones with NMG >10 are superior to the standards. For example, a NMG of 12 indicates that the clone has an economic worth 20% greater than the mean of the standards.

Increases in cane production have put strains on milling capacity in some areas and research has identified the potential to breed for high early-season CCS as a way of extending the harvesting-season length. Breeding and selection for this trait are now significant parts of the core program in each region.

Disease resistance is of major importance to the industry and clones are screened for

resistance to several important diseases. These include Fiji disease and sugarcane mosaic virus (viruses), leaf scald (bacterium), and red rot and pachymetra root rot (fungi). This screening of clones for resistance is conducted at the pathology farm at Woodford, which is located outside major sugarcane areas, or, in the case of pachymetra root rot, in a special glasshouse in Tully. Resistance to other diseases, such as common rust and yellow spot, is recorded through field observation. Since 1998, when sugarcane smut was first found in the Ord River region of West Australia, a cooperative screening program with Indonesia has been established for this disease. Information is now available on which commercial and near-commercial varieties have resistance to this disease. Resistance data on parent varieties will be used to improve the frequency of resistance in the progeny of crosses.

Milling quality of the cane is another factor considered in the selection program. Fibre quantity is routinely measured in advanced trials and varieties with excessively high or low fibre are rejected. In addition, small-scale tests that predict the performance of varieties in the mill are conducted on all varieties being propagated for potential release. These tests measure short fibre, impact reading (effect through the rollers), and shear strength (effect through the shredders). The limits for these traits to provide acceptable milling quality are <80%, between 0.3 and 0.8, and between 10 and 38, respectively.

Database development has been crucial to handle the large amount of information collected on thousands of clones in the various selection programs. A Microsoft Access® 97 database, called SPIDS (Sugarcane Plant Improvement Database System) contains all the productivity data (cane yield, CCS, sugar yield, net merit grade) and trial information of families and clones in all stages of selection in all regions. Within this database, there are links to other databases containing disease ratings, high early sugar ratings, and sugar quality information.

### RELEASE OF NEW VARIETIES

The aim of the breeding and selection program is the release of a new variety. The chief executive officer of BSES gives the final approval for the release of new varieties, taking into account the overall benefit to the industry. For BSES-bred varieties, a 'Q' number is then assigned. Release of new varieties involves extensive consultation between the cane breeding and improvement team, extension officers, pathologists and sugar industry representatives from both growing and milling sectors. Following an annual selection meeting in each region involving the cane breeding team and extension officers (and sometimes a small number of growers), all information on promising clones is collated and presented to a meeting of local industry representatives. This meeting then makes a recommendation whether or not to proceed with maximum propagation or release of each of these clones. The variety officer then prepares an application for maximum propagation or release which collates all productivity data from trials, disease resistance ratings, fibre content and quality, and agricultural characteristics (e.g. germination, ratooning ability, stool habit, etc). The SPIDS database has a facility to collate this information and prepare the report. Also provided is the parentage, a general description of the variety, a recommendation on suitable soils/areas for growing, and a history of the planting material, including hot-water treatment.

In most cases local Cane Protection and Productivity Boards (CPPB) are involved in the propagation of new varieties and provision of clean planting material to the industry. To get new varieties to the growers as early as possible, most programs have a system of accelerated variety propagation. Breeders identify up to about six promising clones each year to give to CPPBs to start propagating. In the following year, as more information becomes available, some of these are propagated further and some discarded.

In this way, by the time a decision is made to proceed to maximum propagation, sufficient quantities of clean planting material are available for distribution to growers. All cane comes from a long-hot-water-treated source and samples are taken to ensure ratoon stunting disease (RSD) is not present. The test for this bacterial disease that can infect planting material is done using ELISA, which is an antigen test for a specific bacterial protein.

The year after a variety is released, it is placed on an 'Approved Variety List' that is published each year for each mill area. Varieties for planting and ratooning, and for ratooning only are given, so varieties can be progressively taken off these lists as well as added.

Since 1997, BSES has been protecting its varieties under the *Plant Breeders Rights (PBR) Act*. At the time of writing, 18 'Q' varieties have been granted full or provisional protection. These varieties are: Q163<sup>®</sup>, Q165<sup>®</sup>, Q166<sup>®</sup>, Q167<sup>®</sup>, Q170<sup>®</sup>, Q171<sup>®</sup>, Q172<sup>®</sup>, Q173<sup>®</sup>, Q174<sup>®</sup>, Q175<sup>®</sup>, Q176<sup>®</sup>, Q177<sup>®</sup>, Q178<sup>®</sup>, Q179<sup>®</sup>, Q180<sup>®</sup>, Q181<sup>®</sup>, Q182<sup>®</sup>, Q185<sup>®</sup>. To be granted protection under the PBR Act, the variety must be shown to be distinct from all other varieties of common knowledge, and be uniform and stable. In addition, a declaration that the variety was developed through breeding is required. Detailed descriptions of what each new variety looks like and the most similar varieties are made to demonstrate distinctness.

Varieties with PBR protection must be made freely available to other organisations for research or breeding purposes. However, they can only be grown commercially with permission of the holder of the PBR. The main reason for protecting new varieties is to safeguard the large industry investment in varietal development. In the future, it may support cane breeding and improvement, and many closely associated disciplines, through the collection of royalties on the harvested product.



### CONTRIBUTION OF MOLECULAR BIOLOGY

Since the invention of recombinant DNA technology (genetic engineering) in the early 1980s, the science of molecular biology has made significant contributions to numerous fields including medicine, food technology, pharmaceuticals and agriculture. In many instances the term biotechnology is used instead of molecular biology (literally the biology of molecules), although in its original definition, biotechnology referred to the production of valuable products by fermentation. Present use of the term usually implies products resulting from the use of recombinant DNA technology and includes insulin, some modern vaccines, and genetically engineered crops and animals. Besides genetic engineering, there are other extremely useful applications of molecular biology in crop improvement. The development and application of molecular markers to tag genes or traits during breeding programs allows for the early selection and evaluation of superior selections, resulting in faster overall genetic gain and selection for specific characters. The power of molecular biology is also being applied to the very sensitive and specific detection of pathogens in germplasm. This is especially important in screening quarantined germplasm for the presence of diseases not in this country (e.g. strains of sugarcane mosaic virus) or to prevent the spread of diseases (e.g. Fiji disease) between canegrowing regions.

#### Genetic engineering of sugarcane

Sugarcane is a genetically complex, interspecific hybrid with a number of characteristics which slow progress by conventional breeding. Some agronomically elite clones are susceptible to pests and pathogens, limiting or restricting their exploitation in the industry. One example is Q124, a highly productive variety that is susceptible to Fiji disease. In areas where Fiji disease virus is prevalent, this susceptibility has resulted in limited use or withdrawal of

this variety. Fiji disease virus can not be eradicated. So, retaining the elite agronomic characters of Q124 by crossing with a resistant variety in a breeding program and then selecting for resistant progeny with the agronomic performance of Q124 would take 10-12 years and considerable resources. An alternative approach is to add a gene for resistance to Fiji disease virus directly to Q124. While the outbreak of orange rust in Q124 in 2000 makes development of Fiji disease resistant Q124 less important, the resistance genes being developed can be used to engineer resistance into any suitable cultivar. While the feasibility of sugarcane genetic engineering for pest and pathogen resistance has been proven, the time frame to screen and select transgenic plants is close to the 10-12 years required in the conventional approach. However, as the technology is rapidly improving, the speed of production of transgenics will increase.

Genetically engineered sugarcane is developed by the insertion of DNA coding for a particular gene into a sugarcane cultivar growing in tissue culture and selecting for cells with recombinant DNA containing the new gene. The DNA is inserted using a microprojectile gun that propels the genetic material into the growing cells with sufficient force to break through the cell wall and the membrane surrounding the nucleus. In a proportion of cells, this new DNA is incorporated into the existing DNA of the plant and the new gene may be expressed. Using specific media, plantlets are then regenerated from these cells and can eventually be propagated normally. Strict regulation of the growing of these transgenic plants is enforced by the Genetic Manipulation Advisory Committee, both in the glasshouse and in the field.

Genes have been identified or developed for application in sugarcane genetic engineering. There has been considerable focus on pest and pathogen resistance, although genes to influence other traits are being investigated. For example, genes



regulating sugar metabolism and colour are currently being researched.

In sugarcane, resistance to leaf scald and sugarcane mosaic virus has been achieved through genetic engineering. The concept of pathogen-mediated resistance to viral diseases has been demonstrated in a number of crops. This occurs when a gene from the pathogen is inserted into the host, and prevents the disease from developing. In sugarcane, the gene for the coat-protein of sugarcane mosaic virus has been successfully inserted into susceptible sugarcane varieties and transgenic plants are resistant in glasshouse and field tests. Similarly, a number of target genes from the more complex Fiji disease virus have been developed and transgenic varieties are being produced.

Canegrubs are important pests of sugarcane in Australia, and there is heavy reliance on one group of chemicals to offer protection against damage. Natural resistance to this pest in sugarcane appears to be limited. Genes have been identified with potential to provide some level of resistance. Transgenic sugarcane varieties with genes from potato (PinII) and snowdrop (GNA lectin) have been produced and, in recent glasshouse screening trials, some of these appear to have enhanced resistance. This result demonstrates the potential of plant resistance to contribute to the control of canegrubs. However, transgenic canegrub resistant sugarcane will not be the solution to this pest problem. Rather, transgenic resistant plants will make an important contribution to the integrated pest management package by providing a component currently missing, namely plant resistance.

It appears unlikely that any commercial, genetically engineered varieties will be available to the sugar industry before at least 2005. Significant progress has been made since the early 1990s, when a sugarcane transformation system was first developed, although there is still a considerable amount of research necessary to move current

transgenics from limited glasshouse and field trials to commercial production.

### **Molecular markers**

Genes have precise and defined physical locations on chromosomes and this physical location can be identified by molecular markers. Markers can be thought of as signposts identifying important genes or traits, which can be exploited in breeding programs. Most higher organisms have 50 000–100 000 genes. If a gene is visualised as a house in a city of 100 000 houses, then each gene (house) has a unique physical address such as number on a street, but also has a general neighbourhood address such as the street, as well as a suburb address. In this analogy, the city is the genome, the suburbs chromosomes and the street neighbourhoods are regions within each chromosome. Because of the way genes are reassorted during meiosis, often all that is required to tag a gene is its general street neighbourhood address, not its actual street number. There are numerous and various types of molecular markers including RFLP (random fragment length polymorphism), AFLP (amplified fragment length polymorphism), EST (expressed sequence tag), SSR (simple sequence repeats, also known as 'microsatellites'), RAPD (random amplified polymorphic DNA) and DAF (DNA amplified fragment). All these types of markers can, in general, be applied to assist in breeding and selection, although some are better suited for particular purposes than others.

### **Fingerprinting**

In much the same way that a person can be identified by a unique physical fingerprint, plants, animals and humans can also be identified by a unique molecular fingerprint of their DNA. This technology is used in forensic pathology and paternity cases, although in plants its principal application is to assist in the management of germplasm and variety collections. At Meringa Sugar Experiment Station there are over 3000 accessions in the germplasm collection.

Fingerprints of all these accessions reduce the possibility of mislabelings during replanting or distribution, and indicate whether there are multiple accessions of the same variety under different names, or if two accessions with the same name in different collections are different. Fingerprinting would also be valuable in ensuring that distributions of key germplasm to different centres were consistent, especially in instances where varieties are difficult to distinguish and differentiate by their physical characteristics.

### *Introgression*

There are valuable genes, such as drought tolerance, in wild germplasm related to commercial sugarcane. However, bringing these genes into breeding populations by conventional breeding methods is difficult. Part of this difficulty is due to sexual incompatibilities between the plants in different genera, but identification of true hybrids from plants arising from a 'selfed cross' is also difficult and has considerably slowed progress in this important area. Marker technologies to tag and differentiate whole chromosomes have been developed and, by applying this method to the chromosomes of potential hybrids, the true hybrid status of the plant can be determined relatively quickly. A true hybrid will contain chromosomes from both parents, whereas a self will only contain chromosomes from the female parent.

### *Gene flow/breeding*

The current sugarcane breeding system tests for performance through field trials. By using a marker-based approach, the likely performance of a set of seedlings from a cross can be determined with sufficient probability to allow selection for elite genotypes 'in the test tube'. Thus, if particular markers are present in the good ratooning parent, and in a selection of its seedlings, then there is a high probability that those seedlings will have good ratooning ability. The ability to use

molecular markers to select seedlings will allow better use of resources to screen for important traits with low genetic heritability, such as some disease resistances, as well as permitting more crosses to be evaluated.

### *Genetic diversity*

It has long been recognised that crosses between parents that are more distantly related will generally produce more vigorous offspring than crosses between closely related parents. This is referred to as hybrid vigour. Markers provide a method to measure the degree of genetic relatedness between potential parents and allow for 'wide' crosses to be made. This allows more rational and defined use of parents in breeding programs to produce more robust seedlings for evaluation.

### *Sourcing genes*

Molecular markers can allow access to the genes responsible for a particular trait of interest, such as resistance to leaf scald. Some marker technologies, such as ESTs, will permit accurate (number on the street) identification of the precise physical location of the gene. The gene can then be cloned (copied in the laboratory) and transferred by genetic engineering to another variety. Obtaining the precise location of the gene means that, rather than a 'street neighbourhood address', we now have the 'exact house number' of the gene. This gene can be used as a perfect marker, rather than as a general marker, used in the applications identified above.

### *Screening germplasm*

Another of the powerful applications of molecular biology to sugarcane breeding and improvement is the sensitive and highly specific screening of germplasm for the presence of disease-causing pathogens. Tests based on either a DNA probe or PCR (polymerase chain reaction) have been developed to specifically screen for Fiji disease virus, sugarcane mosaic virus, sugarcane bacilliform virus, sugarcane yellow



leaf virus, leaf scald, ratoon stunting disease, gumming disease and smut.

Some of these tests are in the process of being routinely applied to screen both domestic and international germplasm. By lowering the threshold of detection, these tests are improving the security of the quarantine system and increasing the benefits associated with sourcing new, but uninfected germplasm.

### SUMMARY

Breeding of sugarcane in Australia has been successful in improving productivity and maintaining stability of production throughout the 1900s. Although it is difficult to objectively measure the contribution of plant breeding to increased yield, it is likely to be at least 1% each year for at least the last 60 years of this century. Cane breeding has certainly saved the sugar industry from the ravages of disease on many occasions, with gumming disease, leaf scald, Fiji disease and common rust serving as good examples. Basic approaches to cane breeding and improvement were used early this century, but today, scientific knowledge and developments in technology have both had major impacts on the methods used to develop new varieties. Research has identified far more effective ways of structuring breeding and selection programs and these are now much larger and handled more efficiently. It is now unthinkable to imagine running a breeding and selection program without access to a computer to generate plans and labels, keep records and analyse data. Equally, it would be impossible to run a selection program without the provision of commercial harvesters and mobile weighing equipment. Breeding for other traits, such as pest resistance, better sugar quality or alternative products, is receiving increased attention. The question is whether the genetic improvement realised in the twentieth century can be sustained into the twenty-first century, and what part some of the emerging technologies will play in the

future. While genetic engineering and molecular markers are likely to be useful tools for the plant breeder, traditional plant breeding methods based on quantitative genetic theory will remain the major delivery system of improved varieties well into the future.

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