SOUTH AFRICAN SUGARCANE RESEARCH INSTITUTE

Plant Breeding Crossing & Selection Programmes

PLANT BREEDING CROSSING & SELECTION PROGRAMMES

Background

The first sugar produced in South Africa in 1852 was derived from varieties of noble cane (*Saccharum officinarum* sp). South Africa was dependent on regular imports of new varieties because over time the foreign varieties became susceptible to local diseases such as smut and mosaic. Importing the variety, Uba (*S. sinensis*) in the 1880s gave growers a respite, because Uba was resistant to mosaic. It ratooned better than previous varieties, and each stool produced many stalks. Although Uba was pleasing to the growers, the millers did not favour it, as it was high in fibre and low in sucrose and purity. Growers were nevertheless urged by government to plant only Uba, to eliminate other sugarcane diseases from the industry. This strategy proved less than ideal around 1915 when Uba was found to be infected with streak disease, leaving the sugar industry in a crisis with no new varieties available to replace Uba.

In 1925, it was decided that an experiment station would be established at Mount Edgecombe, with the major objectives of importing, testing and releasing new

SASRI was established in 1925.

SASRI plant breeders select parent varieties that will be planted in the glass-house and photoperiod house.

varieties. Newly appointed researchers tried making crosses, but no fertile seed was obtained. Later, true seed was imported from several countries, and the batch that was received from India in 1938 produced the renowned NCo310 (N=Natal, Co=Coimbatore, India) a major variety in South Africa from 1965 until 1995 including a number of other NCo varieties. The successor to NCo310 was NCo376, which was widely grown in South Africa between 1965 and 1995.

In 1945, Dr Peter Brett (a SASRI Plant Breeder), found that the absence of viable seed in crosses was due to pollen being infertile, a problem that could be overcome by keeping flowering sugarcane stalks in temperatures above 20°C. Heated glasshouses were constructed and fertile seed was obtained from crosses. However, breeding progress at Mount Edgecombe (which lies at a latitude of 30°S) was limited by variable flowering in parent varieties. In further experiments, Dr Brett developed procedures to induce flowering, even in shy-flowering varieties, by exposing the cane to day-lengths that occur in countries where flowering is profuse. In 1966, a large glasshouse was built, and later in 1971 a photoperiod

house was constructed, both with controlled heating, which enabled breeders to make crosses between a wide range of parents. Today, most flowers used in crosses are initiated in the glass and photoperiod houses.

CROSSING PROGRAMME

Parent selection

Each year SASRI plant breeders select parent varieties that will be planted in the glasshouse and photoperiod house to obtain flowers for crossing. For breeding purposes, the sugar industry is divided into five agro-climatic regions (see Research stations on page 12). Parent varieties for each region are chosen using a number of criteria, such as high sucrose yield, desirable agronomic traits such as good ratooning, disease resistance and resistance to the eldana borer. Imported varieties are also used as parents and wild germplasm is included to widen the genetic base of the sugarcane breeding population and provide novel sources of disease resistance and other important traits. Species of wild germplasm include the vigorous, low sucrose *S. spontaneum* and the poor-growing, but high sucrose *S. officinarum*.

The facilities accommodate around 50 parent varieties for each agro-climatic zone, as well as another 50 parent varieties for special crosses (e.g. for eldana, smut and rust resistance or introgression of wild germplasm).

The heated glasshouse at SASRI was constructed to obtain crosses from fertile pollen.

Cane is planted into canisters on trolleys or "trains".

Flower initiation

There are five photoperiod treatments in the photoperiod house and three in the glasshouse. Each parent variety is allocated to a particular photoperiod treatment that will determine the approximate flowering date of the variety and whether it could produce fertile pollen or not. In September each year, cane is planted as single buds in transplant trays. Six weeks later the plants are transferred to a compost and vermiculite mixture in metal canisters in racks which are mounted on trolleys. The cane is watered and fertilised regularly and kept outdoors until flower initiation treatments commence in February. At this time, trains are moved to the heated facilities each night, and moved out in the morning. The temperatures are kept above 20°C to ensure pollen fertility. The day-lengths are artificially altered to stimulate flowering. Due to the greater control of day-lengths, the photoperiod house is used to promote flowering in shy-flowering varieties and to increase the number of flowers with fertile pollen (males).

The time taken to flower, from the time that day-lengths reach 12.5 hours,

varies between 90 and 110 days. The number of stalks producing flowers varies between 50% and 70%, and depends on season and management practices. While flowers from the field rarely have fertile pollen, the photoperiod house treatments produce more pollen-fertile flowers than the glasshouse treatments.

Trains of cane are moved into the heated photoperiod house each night, and moved out in the morning. The photoperiod house is used to promote flowering in shy-flowering varieties and increase the number of flowers with fertile pollen (males).

Crossing

Upon flower emergence, the stalks in metal cylinders are moved from trolleys and placed on racks in the glasshouse. When the first florets open, anthers with pollen are collected and stained with an iodine solution as an indicator of pollen fertility. Flowers with no pollen grains, unstained or less than 30% stained pollen grains, are designated as females. Flowers with a pollen stain greater than 30% are designated males. Once the fertility levels of the flowers are known, the breeders decide which crosses to make. This process is a critical stage in the breeding programme, and utmost care is taken in deciding which combinations to make. Thereafter, each cross is set up in a separate compartment within the glasshouse. Male flowers are placed above female flowers and are manually shaken by a technician in the mornings to encourage the release of pollen. During crossing, the minimum temperature is kept above 20°C and humidity levels are maintained above 70% to ensure good pollen viability and seed set. After 14 days, when the shedding of

Crossing is done in separate compartments within the glasshouse.

pollen ceases, males are discarded and females are moved to a ripening area.

At Mount Edgecombe, flowering and crossing take place between May and August. Approximately 1 700 crosses were made in 2002, with the 4-year average being 1 600. The amount of viable seed being produced is around 350 seeds per flower or 70 seeds per gram of fuzz.

Seed storage

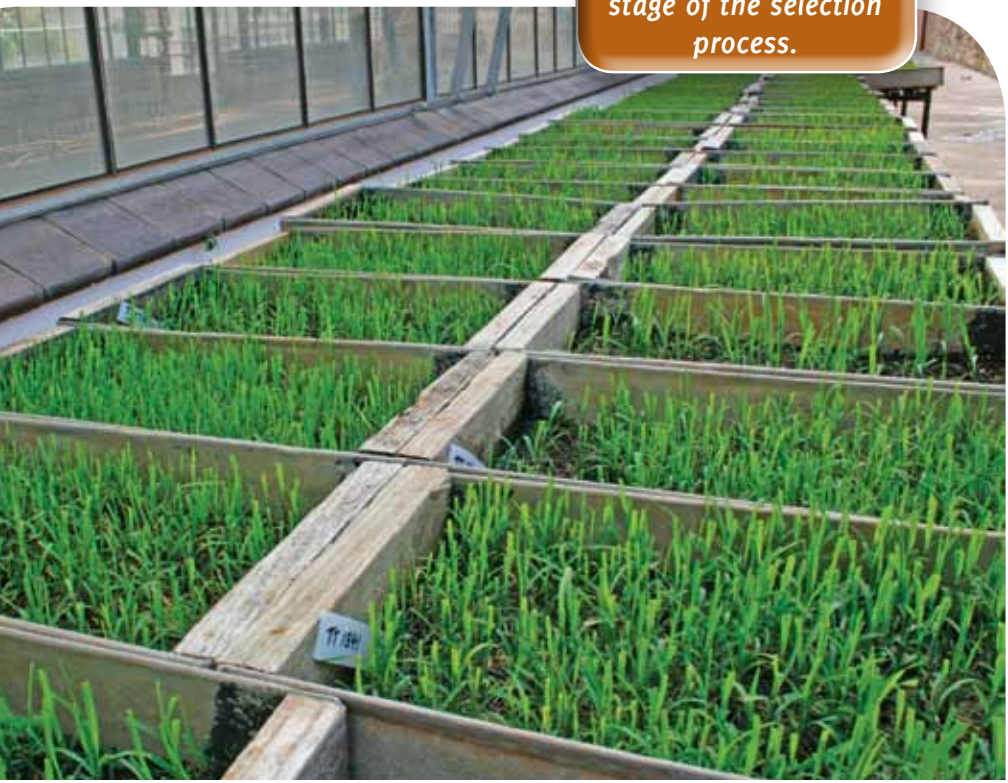
Flowers are harvested and dried when the topmost part of the flower begins to fluff up into a fuzz, which is a sign that the seed is mature. The fuzz and the seed it contains are dried for 24 hours at 30°C. A sample of seed is taken for a germination test and the remaining seed is placed in a plastic sachet and sealed, with each cross in a separate sachet. The seed is stored on racks in a cold room until required, and is kept at a temperature of 20°C. Viability of seed at this temperature is about 10 years.

Seed being sown from the top most part of the flower known as fuzz.

SELECTION PROGRAMME

The main goal of this programme is to select varieties suited to the major agro-climatic regions of the sugar industry. To achieve this, selection is carried out at six SASRI research stations, one located in each of the five strategic areas of the industry except in the Midlands, which has two. The first four stages of the selection programme are established on these research stations, with each research station receiving clones that have been produced from crosses made with parents specifically adapted to that region. At stage 5 the top clones from each region are exchanged between research stations so that they can be evaluated in a number of environments. It takes between 11 and 15 years from the seedling stage to the release of a new commercial variety.

Seedlings are grown for 7-9 months, after which the weakest seedlings are discarded and the stronger ones advance to the next stage of the selection process.



Stage 1: Seedlings in terraces

The selection programme starts with seedlings raised in the glasshouse at Mount Edgecombe. About 250 000 seedlings are raised from true seed each year, 50 000 for each region (see flow-chart on page 8). Breeders select crosses from the seed store for selection at the various research stations. Seed sowing takes place in January each year at Mount Edgecombe. Each cross is sown in a separate box by spreading the fuzz evenly over the surface of a mixture of peat moss and river sand. The fuzz is lightly covered with peat moss and watered, then placed in a heated glasshouse at 30°C. Germination occurs within three days. The seedlings are watered frequently and are fertilised weekly. Five days after sowing, the seedlings are counted and moved outside the glasshouse for steady lengthening periods until they are hardened off. When the seedlings are 3-5 cm tall, they are transplanted to airbricks in a nursery. The size of the area occupied by each seedling is 640 cc (8 x 8 x 10 cm).

Seedlings are planted into airbricks in a nursery when they are 3-5 cm tall.

Crosses are divided into groups (or replications) to enable the groups of seedlings to be planted in randomised units thereby minimising variation due to soil and moisture. This planting layout in the nursery will remain the same for the next two stages of the selection programme. Seedlings are allowed to grow for seven to nine months. The weakest seedlings are discarded and approximately 66% are advanced to the next stage, where the miniature stalks (setts) are cut to 60 cm in length and planted in the field as Stage 2.

Stage 2: Mini-lines

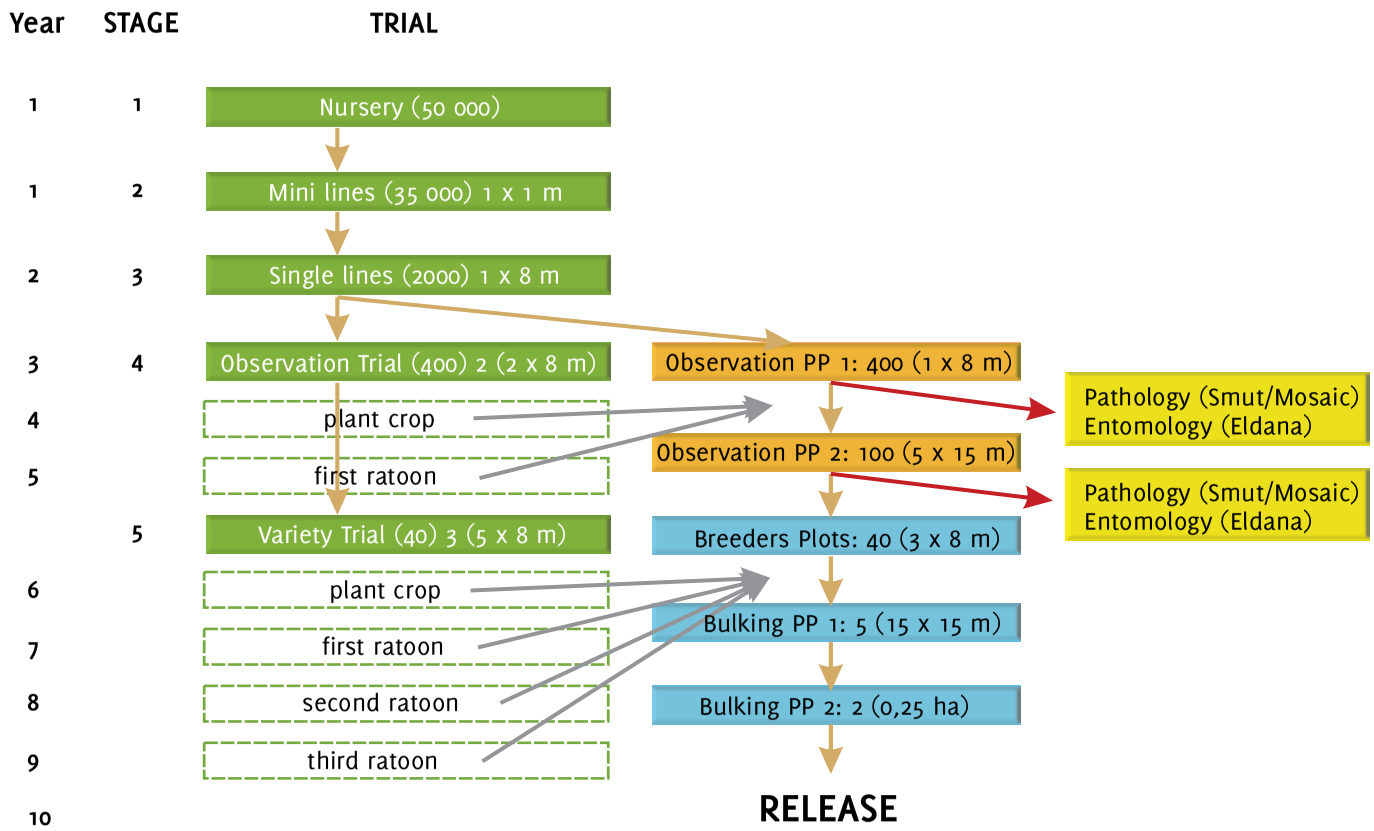
The setts selected from the nursery for advancement to this stage are planted individually as one metre plots, one metre apart in the row. The row widths in all trials vary from 1.0 to 1.4 m, depending on the research station. Every third row is skipped to allow sufficient room for the mini-lines to be examined for major diseases, as well as for selection to Stage 3.

Selection at the mini-lines stage is based on visual assessment, freedom from disease and family values for yield, sucrose content, diseases and pests. This is obtained by determining the following:

- Sucrose content in a sample of one stalk from each of a sample of 20 plots,
- Yield estimates based on family yield components (stalk numbers, height, diameter) from a sample of 20 to 50 plots,

Selection at the mini-lines stage is based on visual assessment, freedom from disease and family values for yield, sucrose content, diseases and pests.

SELECTION PROGRAMME - 12 MONTH CYCLE



- Disease scores, and
- Eldana status on random plots within a family.

Selection to Stage 3 is biased towards high cane yield, high sucrose and disease and pest resistant families. Approximately 11% of the best mini-lines are chosen for the single line stage.

Stage 3: Single-lines

Ten to twelve stalks of each clone selected from the mini-lines stage are planted in 8 m rows in the single line stage (See page 11). It is at this stage that each clone receives a unique number: the year in which the line was planted, the site and its location (number) in the trial, e.g. 98F2225. Periodic disease inspections are carried out, and when the cane is at harvesting age, a 12-stalk sample is taken from each line for sucrose analysis. Cane yield estimates using measurement of yield components are calculated. Cane yield estimates and the sucrose content (analysed from the mill room) are used to calculate the sucrose yield estimates. Due to small plots, large trial size and non-replication of individuals, field variability has a large effect on the performance of each line. The data is therefore adjusted for spatial trends across the trial. Selections to Stage 4 are made on the combined information from yield estimates, disease and pest data and visual evaluation of the genotypes in the field for yield components, absence of disease and other important agronomic traits.

Periodic disease inspections are carried out. A 12-stalk sample is taken from each line for sucrose analysis when cane is at harvesting age.

Stage 4: Observation trial

In Stage 4 the plots have two lines and there are two or more replications. A third plot of each clone, consisting of an 8 m row is established for use as initial propagation material. After harvesting the plant crop, pre-selection of approximately 100 clones is done and these clones are planted in 5 x 15 m plots to supply seed to Stage 5. The trial and propagation plots are inspected for diseases. When the cane is mature, observation plots are harvested, weighed and tested for sucrose content in the plant and first ratoon crops. Cane and sucrose yields are calculated and adjusted statistically for field and other variation. In the northern irrigated areas where smut is a problem, clones planted into Stage 4 trials are also planted into preliminary smut inoculation trials. Eldana is a serious problem in all coastal rain-fed areas, and eldana damage is therefore estimated in each plot from a 20-stalk sample taken at the time of harvest.

Trial and propagation plots are inspected for diseases.

Selections to Stage 5 are based on the trial sucrose yield and content data after a combined analysis of the plant and first ratoon crops, the propagation plot visual selection, and disease and eldana inspection results. Once again, for the yield data, emphasis is placed on the trial data where the plots were actually weighed, rather than visual observations in propagation plots.

Stage 5: Advanced variety trial

This is the final evaluation phase of the selection programme. Only the remaining clones, including clones from other research stations, are tested in advanced variety trials at each of the six research stations. Two or three additional trials are planted on co-operator farms near each research station to monitor clonal performance in different environments. Further disease and pest screening trials are established at this stage to confirm reactions to, amongst other, smut (natural infection), mosaic, leaf scald and eldana. The selection process is repeated after the results of the second ratoon become available. Combined data analysis is used to recommend varieties for release and to determine the areas recommended for release as well as determine the genotype by environment interaction of the released varieties.

Pests and diseases

The following is a list of diseases (and pests) that have been observed in sugarcane in South Africa, some of which can be quite severe and may cause significant yield losses. The pests and diseases that are most important in the South African sugar industry have been highlighted.

- Brown spot
- Brown stripe
- Chlorotic streak
- Eldana borer
- Eye spot
- Gumming
- Leaf scald
- Mites
- Mosaic
- Pokkah boeng
- Red rot
- Red stripe

Plant Breeding Selection - Research Stations

	Research station	Conditions represented
1	Pongola	Northern irrigated areas
2	Empangeni	Coastal high potential
3a	Gingindlovu (Long cycle)	Coastal average potential (18 months)
3b	Gingindlovu (Short cycle)	Coastal average potential (12-15 months)
4	Kearsney	Coastal hinterland
5	Bruyns Hill	Midlands (Humic soils)
6	Glenside	Midlands (Sandy soils)

- Rust • Thrips
- Ratoon stunting disease (RSD)
- Smut • Streak
- Yellow leaf syndrome

Some of these diseases and pests are specific to particular areas.

Millroom procedure

The Diagnostic and Analytical Resource Unit (DARU) at SASRI performs sucrose analyses on all plant breeding trials. Until 2001, 130 sucrose determinations were conducted per day using a saccharimeter (pol content), refractometer (Brix) and dry matter. About 240 samples were analysed when dry matter was not determined and only the quality of first expressed juice was determined (java ratio method). With the newly installed near infrared (NIR) analysis of shredded cane samples, about 410 samples can be analysed per day.

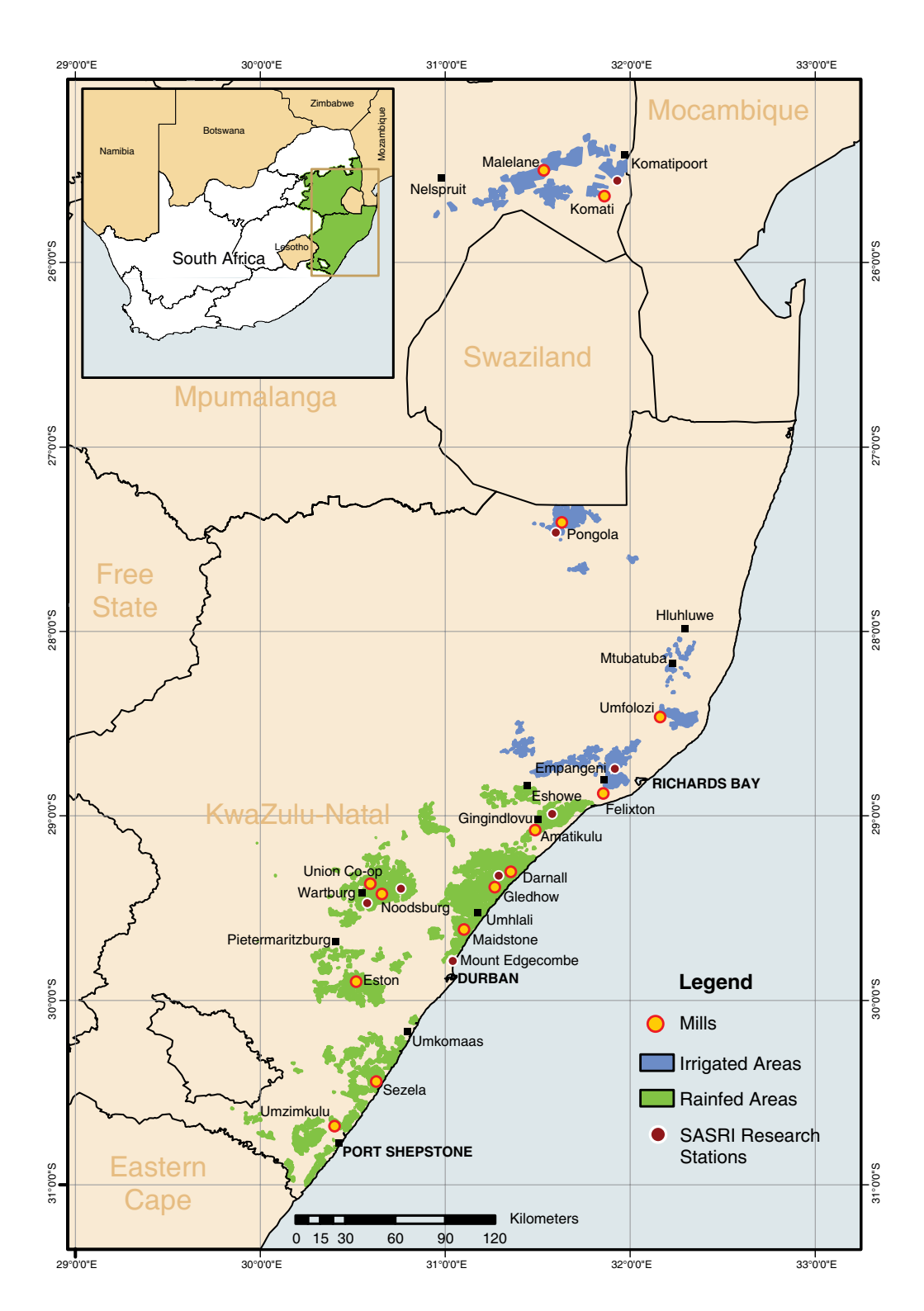
The millroom processes all plant breeding samples.

Bulking-up and release

At each research station, the best three to six varieties from Stage 5 yield and screening trials are promoted for further propagation to be considered for possible release. These bulking plots are planted with seedcane from propagation plots. Once testing is complete, one or two of the most promising varieties are sent to co-operators in those mill areas for which that variety will be recommended. This procedure is necessary so that the limited quantity of seedcane of a new variety (approximately 50 tons) can be increased on co-operator farms. The following year, the variety is released (if free from disease and pest susceptibility), and is given an N number. At the time of release, each large-scale grower receives a small amount of seedcane of the new variety, or it may be propagated by co-operators or seedcane schemes so that larger areas can be established. Thirty-two varieties, N22 to N53, have been released during the period 1990 to 2010.

Details of the selection programme

Selection stage	Clones per site/total	Trial design	Reps	Crops	Select rate %	Selection criteria		
Stage 1 Seedlings	50,000 x 5 250,000	Replication of families	Family	1	70	Family values, visual assessment and freedom from diseases		
Stage 2 Mini-lines	35,000 x 5 175,000	Replication of families		1	11	Family values for yield, sucrose, P&D		Freedom from naturally occuring diseases and pests, and from agronomic defects
Stage 3 Single-lines	4,000 x 5 20,000	Replication of families		1	10	Sucrose yield, P&D	High yield, sucrose, pest and disease	
Stage 4 Observation	400 x 5 2,000	Lattice, 2 x 8 m	3	2	10	Combined analysis for P + 1R		
Stage 5 Advanced Variety	40 x 5 150	Lattice, 5 x 8 m 5 trials	3	3		Combined analysis across sites & crops		
Bulking	1-2	Released Variety Trial						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Published by

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170 Flanders Drive, Mount Edgecombe, 4300

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Website: www.sugar.org.za/sasri

ISBN: 1-874903-36-0