BSES Limited



MANUAL OF PROCEDURES FOR THE CONTROL OF BSES LIMITED VARIETIES: VARIETY AUDIT, DNA FINGERPRINTING AND PLANT BREEDER'S RIGHTS

by

Michael Cox

Program Leader, Plant Improvement MN04002

Contact:

Mike Cox BSES Limited Private Bag 4

Bundaberg DC Q 4670
Phone: 61 7 4132 5200
Fax: 61 7 4132 5253
Email: mcox@bses.org.au

CONTENTS

	Page No
1.	INTRODUCTION 1
2.	PROCEDURES 1
	2.1 Overview
	2.2 Selection Meeting
	 2.3 Variety audit 2.4 Variety Approval Process 2.4.1 Regional Productivity Advisory Committee (RPAC) Approval
	2.4.1 Regional Productivity Advisory Committee (RPAC) Approval
	2.4.2 Q applications and Maximum Propagation applications
	 2.4.2 Q applications and Maximum Propagation applications 2.4.3 BSES Limited approval
	2.4.4 Formal notification of Distribution Agents
	2.4.5 Queensland Department of Primary Industries and Fisheries approval
3.	DNA FINGERPRINTING
	2.5 Small scale evaluation trials DNA FINGERPRINTING 3.1 Methodology 33
	3.2 Database
	3.3 Variety uniqueness 4
4.	PLANT BREEDER'S RIGHTS (PBR)
••	4.1 General
	4.2 Qualified Persons
	4.3 Comparative trials 5
	4.4 Data collection 5
	4.5 Part 1 application
	4.6 Part 2 application
	4.7 Notification and Autocorrect use of PBR symbol
	4.8 PBR status
5.	APPENDIX 1 – OVERVIEW OF VARIETY ACCELERATION, PROPAGATION
	PROTECTION AND RELEASE
6.	APPENDIX 2 - APPLICATION FOR FIRST TRANSFER OF CLONE
7.	APPENDIX 3 - GUIDELINES FOR LEAF SAMPLING FOR DNA
	FINGERPRINTING 12
8.	APPENDIX 4 - EXAMPLE MEMORANDUM FROM CEO TO VARIETY
	OFFICER RE Q NUMBER ALLOCATION 14
9.	APPENDIX 5 - EXAMPLE LETTER FROM CEO TO DISTRIBUTION AGENT
	RE ACCELERATED (UNRELEASED) VARIETIES 15
10.	APPENDIX 6 - PROTOCOL FOR ASSESSING DISEASE RESISTANCE OF
	SUGARCANE CULTIVARS BEING CONSIDERED FOR APPROVAL 17
11.	
	GENERAL (QDPI&F) AND DISEASE RESISTANCE REPORT 20
12.	APPENDIX 8 - APPLICATION TO CONDUCT A SMALL-SCALE
	EVALUATION TRIAL ON AN UNRELEASED CLONE 25
13.	APPENDIX 9 – INSTRUCTIONS FOR DATA COLLECTION IN
	COMPARATIVE TRIALS 28

1. INTRODUCTION

BSES Limited has been protecting its varieties through Plant Breeder's Rights (PBR) since 1995. All varieties from Q163⁽¹⁾ onwards (except Q164) are or will be protected. In 2001, growers were asked to sign a PBR 'Licence Agreement' as well as a 'Service Fee Agreement'. Growers who signed both were exempted from paying royalties on cane delivered to sugar mills while growers signing the PBR 'Licence Agreement' but not the 'Service Fee Agreement' could be charged royalties. Any grower not signing a 'Licence Agreement' who grows a PBR variety is in breach of the PBR Act (1994) and is liable to fines as well as action by BSES Limited.

Plant Breeder's Rights (PBR) is designed to protect the industry investment in plant breeding. Currently it protects the equity of growers and millers continuing to support plant improvement through the 'Service Fee'. However, different funding arrangements in the future may increase the importance of PBR to BSES Limited. Thus, it is imperative that the procedures described in this manual are rigorously followed to ensure that PBR is not compromised and can, if necessary, be defended in court.

There are three key elements in these procedures. The first involves a 'Variety Audit' system that provides a quality assurance mechanism to the varieties that BSES Limited releases to the sugar industry. The second is designed to strictly control unreleased varieties prior to protection so that PBR is not subsequently compromised. This may involve third parties such as Distribution Agents and/or growers. The third involves the actual mechanics of describing varieties and going through the application processes to be granted PBR on varieties.

2. PROCEDURES

2.1 Overview

An overview of variety acceleration, propagation, protection and release is presented diagrammatically in Appendix 1. The sequence of events and procedures to be followed to meet the requirements are detailed in the following sections.

2.2 Selection Meeting

Selection meetings are held annually in each region, as early as possible in the year after the previous season's data are analysed and the database updated. This is necessary to ensure that Distribution Agents are formally notified of propagation and distribution decisions and that appropriate approvals are obtained. This must occur in a timely manner so as not to hold up normal distribution arrangements.

Plant Improvement staff from the region, the responsible Pathologist, the Area Development Manager, extension staff and the Program Leader, Plant Improvement should attend the meeting. Representatives of the Distribution Agents or Industry (eg cooperators) may also be invited to attend, depending on circumstances.

The selection meeting participants will decide the following:

- a. New accelerated clones to be given to the Distribution Agent (currently North, Burdekin, South) or to be propagated by BSES Limited (currently Herbert and Central). Usually a maximum of 5 or 6 clones are involved.
- b. Action on all previously accelerated clones, including release, maximum propagation, holding and discard.

2.3 Variety audit

A Variety Audit system has been established as a Quality Assurance measure for all accelerated clones. An 'Application for <u>first</u> transfer of clone to a BSES Distribution Agent for propagation' form (BSES Limited Form 21 10/03) must be completed at the time of transfer. A copy of this form will be available on the Intranet to download (provide site when established). A copy is provided in Appendix 2. Leaf sampling methods for DNA extraction and fingerprinting are provided in Appendix 3.

2.4 Variety Approval Process

2.4.1 Regional Productivity Advisory Committee (RPAC) Approval

If the selection meeting identified clones to be released or maximum propagated in a region, the Area Development Manager should arrange a meeting of the Regional Productivity Advisory Committee (RPAC) as soon after the selection meeting as possible, to consider these varieties. The responsible Breeder and/or Variety Officer should present all the information (productivity, disease resistance, sugar quality and fibre quality) to the RPAC. The Area Development Manager should notify the Program Leader of Plant Improvement of the RPAC decision – ie whether or not the selection meeting decision was endorsed. If the RPAC decides not to endorse this decision, the Program Leader should be notified immediately (preferably while the RPAC is still meeting).

Note: The role of RPAC is currently under review and a different body or system may operate in the future.

2.4.2 Q applications and Maximum Propagation applications

The Variety Officer will prepare Q applications for all clones the selection meeting decided should be released. This should be done as soon as possible after the selection meeting, with no need to wait for endorsement from the RPAC. The Q application should be sent to the Program Leader and Pathologists located at Tully (currently Rob Magarey) and Woodford (currently Barry Croft). Any Maximum Propagation applications should also be prepared once any Q applications have been completed (timeliness is not as critical as Q applications). These should also be sent to the Program Leader and both Pathologists (see above).

2.4.3 BSES Limited approval

Once the RPAC has approved the release and the Program Leader and Pathologists have signed off, the Program Leader will forward the Q application to the CEO (copy to Manager R&D) together with a draft memorandum from the CEO to the Variety Officer advising of the Q Number allocated to the variety. The memorandum from the CEO

should be copied to all plant breeders, the Area Development Manager, appropriate extension officers in the region, pathologists at Tully and Woodford and to the person responsible for updating SPIDnet (Plant Improvement database). A copy of an example memorandum is given in Appendix 4.

2.4.4 Formal notification of Distribution Agents

Once BSES Limited approval has been given or, if there are no varieties to be released, as soon as possible after the selection meeting, the Distribution Agents should be formally notified of the new accelerated clones and action on all previous accelerated (unreleased) clones, including clones to be released, maximum propagated, held or discarded. This should be drafted by the Program Leader, Plant Improvement and sent out under the CEO's signature. An example letter is shown in Appendix 5.

2.4.5 Queensland Department of Primary Industries and Fisheries approval

Once all varieties to be released in a given year have been allocated Q numbers, a letter should be drafted to the Director-General, Department of Primary Industries and Fisheries (QDPI&F) requesting approval of the varieties under the Sugar Industry and Other Legislation Amendment Act 2003. This should be drafted by the Principal Pathologist (Woodford) under the CEO's signature. The Disease Resistance Reports for each variety should be attached to the letter. The 'Protocol for Assessing Disease Resistance of Sugarcane Cultivars Being Considered for Approval' is shown in Appendix 6 and an example covering letter to the Director-General (QDPI&F) and Disease Resistance Report are shown in Appendix 7.

2.5 Small scale evaluation trials

Any trials outside of conventional plant breeding trials (Final Assessment Trials, FAT) containing unreleased clones and planted off a BSES Limited experiment station must be approved before planting. This includes strip trials or other larger scale trials. Propagation for these trials, where it is done on a grower's farm, also requires approval of the breeder and the Program Leader. The approval form (BSES Limited Form 20 10/03) is shown in Appendix 8 and is also available on the BSES Intranet. Approval is required from the Program Leader, who will also seek an opinion from the Plant Breeder's Rights Office that such trials do not compromise the rules on 'Prior Sale'. In addition, a 'Grower Agreement' must be signed by the grower, prohibiting the propagation or sale of plants from this trial.

3. DNA FINGERPRINTING

3.1 Methodology

The procedure for collecting and sending leaf samples for DNA fingerprinting is provided in Appendix 3. The laboratory procedures related to DNA fingerprinting must be conducted by trained laboratory staff only, using established protocols. Experiments must

be performed in a controlled and precise manner, and recording of all experiments must be accurate and easily interpreted.

DNA fingerprinting should be conducted with at least four microsatellite primer pairs. For each microsatellite primer pair, a negative control (no DNA) and at least two or three controls (example DNA profiles) must be included in each test, along with a suitable size ladder.

3.2 Database

The DNA profile information should be interpreted by two trained personnel and the information recorded in the DNA fingerprint database. When the new information has been recorded into the database it should then be checked to see if the new variety matches the DNA profile of any other variety already in the database.

3.3 Variety uniqueness

Ideally, new varieties should be sampled from at least two locations for DNA fingerprinting (see Appendix 2). If the samples from the different locations do not have identical DNA profiles, then a mix-up has occurred either in the field or in the laboratory, and the whole DNA testing procedure must be repeated. If the DNA profile of the new variety matches something already in the database, the whole procedure must also be repeated to confirm the result. If the DNA profiles of the samples from different locations are identical, and do not match any other DNA profile in the database, then the new variety can be considered unique.

4. PLANT BREEDER'S RIGHTS (PBR)

4.1 General

Applications for PBR to the Plant Breeder's Rights Office (PBRO) will be made for all varieties released by BSES Limited, as well as those jointly released by BSES Limited and CSR. This will mean that a Part 1 application must be submitted a maximum of 12 months after the first sale of the variety (generally first distribution). Each variety should be planted into a comparative trial in the year it undergoes maximum propagation. This means that a Part 1 application can be submitted in the same year as the first release, well within the 12 month limit. If the situation arises where a variety is set for release and is not available in a comparative trial for description, either a Part 1 application can made from observations in other plots (and then planted into a comparative trial for description and submission of a Part 2 application the following year) or distribution will have to be delayed.

4.2 Qualified Persons

The PBR Office registers and certifies Qualified Persons (QPs) who are able to lodge PBR applications. This usually requires annual attendance at QP Workshops that are run by PBRO and payment of an annual fee. **BSES Limited must maintain at least two, and preferably three, Qualified Persons at all times.** These will usually be plant breeders,

although there is no reason other experienced staff cannot hold this position. This will ensure obtaining PBR is not compromised in the event of staff loss. One of the QPs will be designated as the BSES Limited staff member responsible for PBR, including developing budgets, coordinating planting of comparative trials and submitting applications to PBRO. Currently Dr George Piperidis holds this position (BRPBR), and he and Drs Mike Cox and Xianming Wei are QPs.

4.3 Comparative trials

Each year, the Program Leader, Plant Improvement, will inform the breeder responsible for PBR (BRPBR) the clones that will undergo maximum propagation that year. The BRPBR will contact the Variety Officer to determine the varieties to be used as comparators in the trial. Comparators will be varieties of 'common knowledge' that have similar states of expression to the new variety for the following 'grouping characters': unexposed internode colour; internode shape, waxiness and bud shape (longest internode); and auricle shape (underlapping or overlapping).

The comparative trials should be planted at Meringa and/or Mackay in June or July each year. The new varieties, the comparator varieties and, where available, the parents of the new varieties should be included. The trial should also include at least five 'standard' or 'example' varieties, over and above those already included as comparators and/or parents, from the following list:

BN81-1394

H56-752

O117

Q121

Q124

Q136

Q138

O152

Q170^(b)

Q179^(b)

Q186^(b)

RB72-454

The final list of varieties to be planted in the trial will be provided by the BRPBR. The trial is a randomised complete block design with three replicates, with plots single row x 10 m in length. The trial should be planted in June or July each year to allow a 10 month growing period before the descriptions are taken.

4.4 Data collection

A total of 34 qualitative and 10 quantitative characteristics are evaluated or measured. The characteristics conform to UPOV Technical Guidelines for sugarcane (http://www.upov.int/). A number of field observations are recorded prior to the main data collection ie. before any stalks are cut from the trial. These observations are recorded on the Field Observation Sheets (Appendix 10), and should be made on all varieties in all three replicates, preferably in teams of two with each team recording from one replicate.

Each variety (new variety, comparator, parent, or standard) is processed in turn, with 12 stalks randomly collected from each replicate. It is essential that the stalks are collected at random and not just from the outer stools of the plot. The stalk bundles should be labelled clearly with variety and replicate. Ten stalks from each replicate are used for quantitative measurement and the other two stalks per replicate put together for qualitative trait recording. Detailed instructions are provided in Appendix 9.

(??Attach all recording forms or give directory information where kept. Eventually this will all be put into SPIDnet and can use intranet address).

4.5 Part 1 application

A completed Part 1 application is the first step in applying for Plant Breeders' Rights. It includes general information about the Applicant, the new variety, and the origin and breeding procedure used to generate the new variety. Detailed instructions for completing a Part 1 application can be found on the PBR website (go to http://www.affa.gov.au/ and select "Plant Breeders Rights" in the "Search by specialist area" dropdown menu).

When the application is completed, it should be sent to the PBRO along with the application fee (currently \$300/application), and a photograph of the new variety. The applicant is notified by the PBRO when the application has been received. The application is then reviewed by the PBRO and when all details are confirmed the variety receives the status of 'Accepted', which means that the variety has 'provisional protection'.

4.6 Part 2 application

A Part 2 application should be submitted within 12 months of an application being 'accepted' by the PBRO. If a Part 2 application cannot be submitted within this period, an extension must be sought from the PBRO within the 12 months. Failure to submit either a Part 2 application or an extension form will result in the application being withdrawn, and PBR will not be granted. (PROVIDE A LINK TO AN APPLICAION ON THE INTRANET

A Part 2 application includes all of the descriptive data from the comparative trial, and must be submitted by a Qualified Person. The application must also be accompanied by the examination fee (\$1400/variety for single applications, or \$800/variety from an authorised Centralised Testing Centre). The description and comparative photograph of the new variety is then published in the *Plant Varieties Journal*, followed by a six-month period to allow for objections or comments. On completion of all the requirements, and resolution of any objections, the applicant receives a Certificate of Plant Breeders Rights.

4.7 Notification and Autocorrect use of PBR symbol

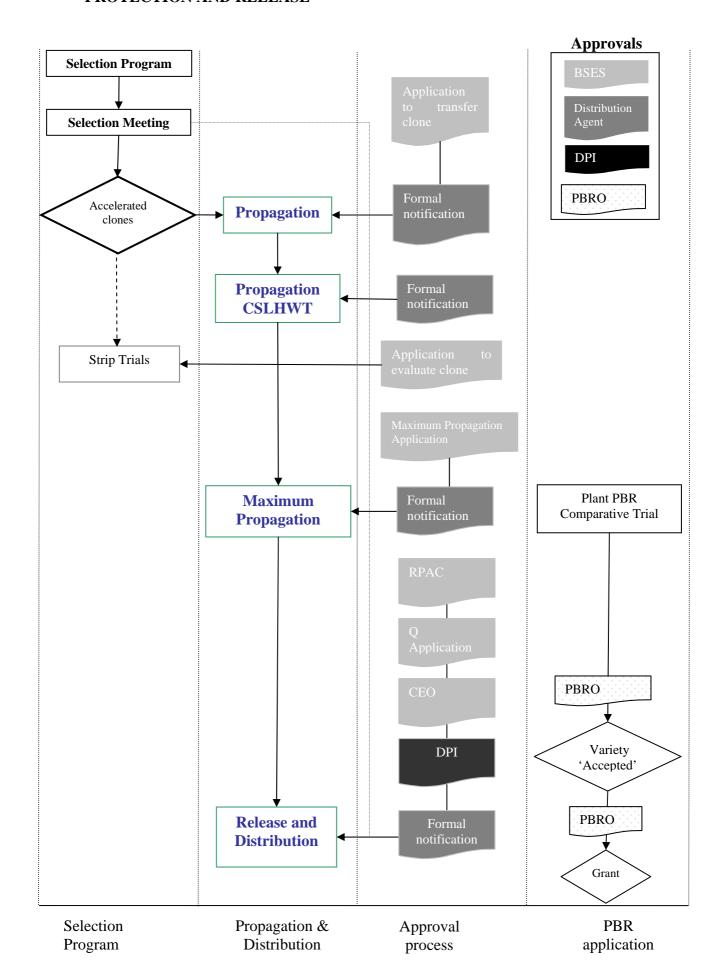
As mentioned previously, a variety becomes protected by PBR when it is "Accepted" by the PBRO. When this occurs an email should be sent to everyone at BSES (and others) to inform them that the PBR symbol should be used in any documentation relating to the new variety. At the same time, an autocorrect document with the newly "Accepted"

varieties, and instructions to install the autocorrect function should be sent so that the PBR symbol is automatically inserted whenever a protected variety is written in Microsoft® Word.

4.8 PBR status

The PBR status of all sugarcane varieties can be accessed through the 'PBR Database Search' button on the PBR website (see Section 4.5). This information is available for download and can be copied into a spreadsheet for personal use.

5. APPENDIX 1 – OVERVIEW OF VARIETY ACCELERATION, PROPAGATION, PROTECTION AND RELEASE



APPENDIX 2 - APPLICATION FOR FIRST TRANSFER OF CLONE



Application for <u>first</u> transfer of clone to a BSES Distribution Agent for propagation

Sugarcane for the future		propagation			
	File	Number: 30	3-0301		
Clone:					
BSES Officer:					
Distribution agent:					
Region:					
Mill Area:					
Location (include GPS coordinat	es)				
Proposed date of transfer:	,				
Location proposed as source (fare Disease ratings if available:	m and block number):				
Fiji disease	leaf scald	mosaic	Pachymetra		
Orange rust	Common (Brown) rust		Yellow spot		
Other diseases noted (eg red strip	pe top rot, eye spot)				
Previous History:					
	20	20	20		
Location (Block)					
Plot description (eg Prop/FAT)					
Crop Class					
Row-plot					
LHWT (Y/N)					
CSLHWT (Y/N)					
Inspection for diseases and mutants (Y/N)*					
Disease or abnormality found?					

In gathering this information, inspections must have been made when the cane was between knee and chest height to the TVD and again when in older crop (6-12 months, depending on district and accessibility) and no disease or genetic abnormalities were found. In addition four rows either side of the proposed plot should have been inspected.

Elisa test result (+/-) & No.

farm or immediately neighbouring farms)

Comments (eg diseases known to be present on the proposed source

samples

Clone identification

 A visual comparison of cane in the source plot, all current trial plots and other BSES propagations should be made. (Please specify locations, row-plots and trial codes, where appropriate)

Location	Trial code	Row-plot

2. Leaf samples should be taken from the source plot (to be used to plant the first and any subsequent Mother Plot), at least one current trial plot and other BSES holding plot for DNA testing.

Location	Trial code	Row-plot

Results: Please attach email with results of DNA fingerprinting of these samples.

Results:

Comments:

Note: As necessary, subsequent DNA fingerprinting will be done:

- PBR comparative trial
- Source to plant variety into ASCGRC
- Quarantine glasshouse
- Any CPPB plots as required

Please email this form to:

Mike Cox: mcox@bses.org.au
Barry Croft: bcroft@bses.org.au
Records: records@bses.org.au

Please also provide a copy to each CPPB that receives this clone

Insert copy of email here:

7. APPENDIX 3 - GUIDELINES FOR LEAF SAMPLING FOR DNA FINGERPRINTING

- About 2 or 3 of the topmost leaves are needed, sampled from a single stalk or stool.
 Note: sample only from healthy, disease-free plants, and minimise the amount of contact between yourself and the leaves.
- Write the name of the clone/variety on a couple of the leaves (at the end, not the middle) with a waterproof pen, roll them together and secure with an elastic band.
- Wrap the leaf samples in moist paper towel and then enclose in a clip-lock plastic bag.
- The parcel should be sent by overnight courier, addressed to:

Celine Frere/George Piperidis BSES Limited 50 Meiers Rd Indooroopilly Q 4068

- Also, send an email to Celine Frere (cc. to George Piperidis) the day that the samples
 are sent so that we know when to expect them.
- The form on the following page should also be filled out for each sample (one form for each sample or variety) and returned to Celine Frere/George Piperidis.

APPENDIX 3 Continued- LEAF SAMPLES FOR DNA FINGERPRINTING

Date sent :	
Specimen of:	
Variety:	
Crop/Age:	
Farmer:	
Location:	
Soil Type :	
Any further particulars :	

Signature :....

8. APPENDIX 4 - EXAMPLE MEMORANDUM FROM CEO TO VARIETY OFFICER RE Q NUMBER ALLOCATION

SD

Ross McIntyre, Variety Officer, Meringa Chief Executive Officer 08 November 2005 O number for 89N95

304-0500/MCC

I wish to advise that I have allocated Q219 to 89N95. Approval to grow Q219 is being sought from Department of Primary Industries and it may be distributed in Babinda region once this has been received. I will notify you as soon as we receive this advice.

In publicity about Q219, you should ensure that growers are clearly advised that 89N95 may produce severe side-shooting under certain conditions and that it is only recommended for the Babinda Mill area. It is not recommended for the Herbert region. An application for Plant Breeder's Rights will be made in the next few months, well within the 12 month 'Prior Sales' limit.

E S Wallis Chief Executive Officer

Copy to: Dr Mike Cox, Program Leader, Bundaberg

Dr Nils Berding, Principal Research Scientist, Meringa.

Dr Rob Magarey, Principal Scientist, Tully Allan Rattey, Research Scientist, Burdekin Dr Xianming Wei, Research Scientist, Mackay Barry Croft, Principal Scientist, Woodford

Dr George Piperidis, Research Scientist, Indooroopilly

Greg Shannon, Regional Manager, Ingham David Calcino, Regional Manager, Meringa David Wallis, Extension Officer, Innisfail

9. APPENDIX 5 - EXAMPLE LETTER FROM CEO TO DISTRIBUTION AGENT RE ACCELERATED (UNRELEASED) VARIETIES

303-0301/MCC

21 May 2004

Mr George Bugeja Productivity Coordinator Innisfail-Babinda Cane Productivity Services PO Box 77 MOURILYAN QLD 4858

Dear George

Further to my letter of 7 November 2003 regarding the propagation and distribution of BSES Limited varieties, I am formally notifying you of the new accelerated clones to be propagated this year and of the specific action for all previous unreleased varieties still in your plots. These decisions follow the selection meeting held at BSES Meringa on 6 April 2004 and confirmed at the northern RPAC meeting on 7 May 2004.

- 1. The new accelerated varieties for 2004 are 95N1661 and 95N1882. Approximately 50 stalks of each of these varieties will be made available for you to propagate and you should liaise with Ross McIntyre to arrange this at your convenience. The cane (ex Sues) will need to be cold soaked long hot water treated and BSES Limited will arrange this. Each of these varieties will be DNA fingerprinted at the time of transfer.
- 2. With regard to the accelerated varieties currently held by you, the following action should be taken:
 - a. 87N1279 (Q217), 89N1659 (Q218) and 89N95 (Q219) should be distributed in 2004, subject to approvals by the Department of Primary Industries as well as my final approval. At this stage we do not foresee any impediments to their release and I will formally notify you prior to distribution. You should ensure that growers are clearly advised that 89N95 may produce severe side-shooting under certain conditions.
 - b. 92N158 should be maximum propagated in 2004 for possible release in 2005. Sufficient cane should be planted to allow a possible distribution in 2005.
 - c. 92N19 should be held in your plots in 2004 to allow further assessment of fibre quality. If satisfactory, it may be maximum propagated in 2005 for possible distribution in 2006
 - d. The following varieties have been discarded and should not be propagated further
 - i. 92N1234
 - ii. Any other unreleased varieties held by you not listed above

If you require any further information or clarification, please contact either Dr Nils Berding or Ross McIntyre.

Thank you for signing my letter and agreeing to the formalisation of arrangements for handling varieties prior to release.

Yours sincerely

E S Wallis

Chief Executive Officer

Copies to:

Dr Ross Gilmour, Manager - Research and Development, Indooroopilly David Calcino, Regional Manager - North, Meringa Dr Nils Berding, Principal Scientist, Meringa Dr Mike Cox, Program Leader - Plant Improvement, Bundaberg Ross McIntyre, Variety Officer, Meringa David Wallis, Extension Officer, Innisfail Dr Rob Magarey, Principal Pathologist, Tully Barry Croft, Principal Pathologist, Woodford

10. APPENDIX 6 - PROTOCOL FOR ASSESSING DISEASE RESISTANCE OF SUGARCANE CULTIVARS BEING CONSIDERED FOR APPROVAL

DRAFT August 26, 2002 B J Croft

Background

Host plant resistance is the main strategy for disease management in the Queensland sugar industry (Croft *et al.* 2000). An important component of this strategy is the ability to control which cultivars can be grown in a region. At present, management of the choice of cultivars is controlled by government regulation. This prevents the growing of a disease susceptible cultivar by a small number of growers where the financial impact of growing the cultivar will not be restricted to this group of growers but will be incurred by all growers in a local region. The action of a small number of growers to grow susceptible varieties is neither a fair nor reasonable economic consequence for the growing and milling sectors in the district. Because sugarcane is a perennial crop, the industry cannot quickly respond to a disease outbreak and replace susceptible cultivars with resistant cultivars. Disease epidemics can have serious long-term economic consequences for a district.

The diseases controlled by regulation are characterised by the following common features:

- They cause severe yield losses.
- They can spread from farm to farm by insects, wind or by contaminated machines.
- They are systemic diseases that are carried in diseased cuttings and into regrowth crops.
- Resistant cultivars are available for their control.

Currently, Fiji disease, leaf scald and sugarcane mosaic disease are controlled in this way. If a small number of growers plant cultivars susceptible to these diseases, they can affect the wider cane growing community for a considerable period.

Control of these diseases by other management options such as monitoring crops, removing diseased plants or crops and providing disease-free planting material has been attempted in the past as an interim option until resistant cultivars were available. During the Fiji disease epidemic in the Bundaberg region in the 1970s a large program was conducted that involved up to 45 men carrying out inspections and the supply and transport of up to 37,000 tonnes of seed cane from outlying areas where the disease pressure was low (Egan and Toohey 1977, Egan and Fraser 1977). This scheme placed a huge economic burden on the region. It is unlikely that a program of this magnitude could be conducted again because of labour costs.

The following sections outline the procedures and terminology used to assess if a cultivar should be made available for each of the sugarcane pest quarantine areas.

Procedure for approval of sugarcane cultivars

1. Rating Scale

The International Society of Sugar Cane Technologists recommends that all disease ratings be expressed on a 1-9 scale where 1 is highly resistant and 9 is highly susceptible as described in Hutchinson and Daniels (1972). This international rating system has been used in Australia for many years by both the BSES and the CSR breeding programs. If a variety has several ratings for a disease, a mean rating is calculated.

2. Specified Pests and Threshold Ratings

The list of specified diseases and the disease thresholds have been agreed to by industry representative groups.

Approval is currently for resistance to the following diseases and pests:

Leaf scald caused by *Xanthomonas albilineans*Fiji leaf gall (formerly known as Fiji disease) caused by Fiji disease virus
Sugarcane mosaic caused by sugarcane mosaic virus

Cultivars will not be approved if their overall rating exceeds the values shown below for each quarantine district when measured by the standard procedure described in section 3:

SPQA 2 Coen to Townsville

Leaf scald 7

SPQA 3 Townsville to Bowen/Collinsville

Leaf scald 7

SPQA 4 Bowen/Collinsville to Rockhampton

Fiji disease 6*
Leaf scald 7

*Note: An application has been made to QDPI&F to increase this threshold rating to 7

SPQAs 5 and 6 Rockhampton to Howard and Howard to the NSW Border

Fiji disease 6 Leaf scald 7 Mosaic 7

The list of diseases and the thresholds will be reviewed every five years or as required by a group comprising members of industry, government, research groups and independent experts. BSES Regional Planning Advisory Committees (RPAC) could form the basis of this review group.

3. Rating method

Clones will be rated relative to a set of standard clones of known reaction for that disease. A minimum of six standards will be included in each screening trial. The standards must include at least two resistant cultivars, two cultivars of intermediate reaction and two susceptible cultivars. The ratings for the standard cultivars must be generally accepted by sugar industry research groups and the industry based on previous experimental and field experience. The correlation between the rating of the standard cultivars and the disease level measured in the trials used to assess the cultivar under consideration must be statistically significant at a probability of < 0.05.

The rating of cultivars will be determined by comparing the reaction of the cultivar in an acceptable trial with the standard cultivars in that trial. The regression equation for the measured level of disease for each standard cultivar (y) and the rating of the standard cultivars (x) is calculated using the following formula:

$$Y = mx + c$$
 (1)

where m is the slope of the line and c is the intercept.

From (1) the rating of the test cultivars can be expressed as:

$$x = (y-c)/m$$

The cultivar must be rated in at least two acceptable screening trials for Fiji leaf gall and leaf scald and one trial for mosaic and the overall rating will be compared to the threshold levels in section 2. The overall rating will take into consideration the accuracy of the trials.

References

- Croft, B J, Magarey R C and Whittle P (2000) Disease Management In (Hogarth D M and Allsopp P G, eds) Manual of Cane Growing. BSES Brisbane.
- Egan B T and Fraser T K (1977) The development of the Fiji disease epidemic. Queensland Society of Sugar Cane Technologists 44: 43-48.
- Egan B T and Toohey C L (1977) The Bundaberg approved plant sources scheme. Queensland Society of Sugar Cane Technologists 44: 55-59.

Hutchinson, P B and Daniels J (1972) A rating scale for sugarcane characteristics. International Society of Sugar Cane Technologists 14:128-131.

11. APPENDIX 7 - EXAMPLE COVERING LETTER TO THE DIRECTOR-GENERAL (QDPI&F) AND DISEASE RESISTANCE REPORT

SD 304-0200/BJC/PGA/J Varghese, D-G, DPI&F, Approval of sugarcane varieties

25 May 2004

Mr J Varghese Director-General Department of Primary Industries and Fisheries GPO Box 46 BRISBANE Q 4001

Dear Jim

Re: Approval of sugarcane varieties (Sugar Industry and other Legislation Amendment Act 2003).

The Sugar Industry and Other Legislation Amendment Act 2003 allows for the Chief Executive of DPI&F to approve sugarcane varieties for growing in the various sugarcane pest quarantine areas in Queensland.

BSES currently has three varieties that it would like considered for approval. These varieties have been discussed with industry groups who are supportive of the decisions. Attached are reports on the disease resistance of the varieties to support the applications.

BSES hopes to release the three varieties Q217, Q218 and Q219 to growers in spring and we would appreciate your timely consideration of these applications. In future years we will attempt to coordinate the submission of applications for approval of varieties so that they can be processed in one batch each year. Unfortunately in this the first year of the new system we have been unable to coordinate this request with an earlier request for approval of varieties.

If you have any questions about the process of approval of sugarcane varieties or need any further information to expedite the approval process please contact Dr Peter Allsopp at BSES Indooroopilly (telephone 3331 3316).

Yours sincerely

E S Wallis Chief Executive Officer

Attach

Application for Approval of Sugarcane Cultivar

Q217

Organisation requesting approval: BSES Limited

Contact Person: Mike Cox **Phone:** 07 4132 5200

Fax: 07 41325253

Email: MCox@bses.org.au

PBR owner of cultivar: BSES Limited

Clone: Q217 Alternative name: 87N1279

Approval requested for:

Sugarcane Pest Quarantine Area 2 Coen to Townsville

Relevant Specified Diseases and threshold levels of resistance:

Leaf scald rating 7 or less

Data to Support Application

Leaf scald

Screening Trial 1: LS154

Location experiments conducted: Woodford

Organisation conducting experiment: BSES

Date trial planted: 3/10/02

Replication: 4 reps

Inoculum source and transmission method: Juice from leaf scald infected stalks, applied by paint

brush to freshly cut leaves of the test plants.

Date trial rated: 20/10/03

Rating method: Weighted percent infected stalks, weighting based on severity of symptoms. The rating

scale and the formula for the weighted percent infection is as below:

Severity scale

F = few pencil lines

M = many pencil lines

W = wilted and/or chlorotic

D = dead

Formula

Weighted per cent infection = $((F\times1 + M\times2 + W\times3 + D\times4)/\text{ total stalks}\times4)\times100$

Cultivars and their ratings

Cultivar	Rating	Arcsin % infection
Standards		
1.Q44	9	64.8
2.Q63	6	27.0
3.Q68	1	9.8
4.Q87	7	23.2
5.Q96	5	18.5
6.Trojan	3	28.3
7. Q124	1	9.0
Proposed approved cultivar		
87N1279	2	11.0

Correlation coefficient and Probability of significance: r = 0.80 Prob.<0.05

Regression equation: y = 5.30x + 0.825

Screening Trial 2: LS151

Location experiments conducted: Woodford

Organisation conducting experiment: BSES

Date trial planted: 11-12/10/01

Replication: 2 reps

Inoculum source and transmission method: Juice from leaf scald infected stalks, applied by paint

brush to freshly cut leaves of the test plants.

Date trial rated: 12-18/11/02

Rating method: Weighted percent infected stalks, weighting based on severity of symptoms. The rating

scale and the formula for the weighted percent infection is as below:

Severity scale

F = few pencil lines

M = many pencil lines

W = wilted and/or chlorotic

D = dead

Formula

Weighted per cent infection = $((F\times1 + M\times2 + W\times3 + D\times4)/\text{ total stalks}\times4)\times100$

Cultivars and their ratings

Cultivar	Rating	Arcsin % infection
Standards		
1.Q44	9	62.2
2.Q63	6	61.7
3.Q68	1	7.4
4.Q87	7	56.7
5.Q96	5	48.1
6.Trojan	3	30.6
7. Q124	1	7.0
Proposed approved cultivar		
87N1279	1	3.8

Correlation coefficient and Probability of significance: r = 0.94 Prob.< 0.01

Regression equation: y = 7.90x + 1.86

Screening Trial 3: LS143

Location experiments conducted: Eight Mile Plains

Organisation conducting experiment: BSES

Date trial planted: 1/9/97

Replication: 2 reps

Inoculum source and transmission method: Juice from leaf scald infected stalks, applied by paint brush to freshly cut leaves of the test plants.

Date trial rated: 12-18/11/02

Rating method: Weighted percent infected stalks, weighting based on severity of symptoms. The rating scale and the formula for the weighted percent infection is as below:

Severity scale

F = few pencil lines

M = many pencil lines

W = wilted and/or chlorotic

D = dead

Formula

Weighted per cent infection = $((F\times1 + M\times2 + W\times3 + D\times4)/\text{ total stalks}\times4)\times100$

Cultivars and their ratings

Cultivar	Rating	Arcsin % infection
Standards		
1.Q44	9	86.3
2.Q63	6	56.4
3.Q68	1	0
4.Q87	7	82.5
5.Q96	5	35.9
6.Trojan	3	46.8
7. Q124	1	0
Proposed approved cultivar		
87N1279	1	0

Correlation coefficient and Probability of significance: r = 0.93 Prob.< 0.01

Regression equation: y = 10.86x - 5.65

Summary of Disease Ratings

Disease	Overall rating	Number of trials	
Leaf scald	1.3	3	



		Clone name:	
NTERNAL APPROVAL			
Fo: Program Leader, Plant I request permission to conduct		al(s) listed on the following page	
Variety Officer:			
	(Name)	(Signature)	(Date)
Plant Breeder:			/
	(Name)	(Signature)	(Date)
Application received:			
	(Date)		
Application sent to Plant Bro	eeder's Rights Office to confi	rm small-scale evaluation trials listed	do not constitute 'Prior Sale':
Date sent:			
Date of reply:			
Result:			
State if email on file)			
Application is approved/not	approved		
Program Leader:			//
	(Name)	(Signature)	(Date)
Variety Officer and Plant Br		, 	
	(Da	te)	

BSES Limited – Form 20 10/03

25



Application to conduct a small-scale evaluation trial on an unreleased clone

(File No.: 304-0009)

01		
Clone	name:	

Trial details	Grower 1	Grower 2	Grower 3	Grower 4	Grower 5
Trial ID					
Grower name					
Grower ABN					
Region					
Mill					
Farm/Block					
Area of clone (ha) 1					
Area of trial					
Other varieties					
Year planted					
Expected tonnage (t) ¹					
Milling: Deliver to mill (Y/N) If yes, which years?					

¹ Refers to clone listed at top



Application to conduct a small-scale evaluation trial on an unreleased clone

(File No.: 304-0009)

Clone	name:					

GROWER AGREEMENT

With regard to the clone listed above, to be planted in a small-scale evaluation trial on my farm, I agree to the following conditions:

- 1. I will not propagate further planting material on any farm unless I have written permission from BSES.
- 2. I will not provide planting material to any third party for the purpose of propagating
- 3. The cane will only be harvested and delivered to a mill as instructed by BSES
- 4. If instructed by BSES, I will plough out the trial

Grower: _			/ /
_	(Name)	(Signature)	(Date)
Witness:			1 1
_	(Name)	(Signature)	(Date)

13. APPENDIX 9 – INSTRUCTIONS FOR DATA COLLECTION IN COMPARATIVE TRIALS



GENERAL

Saccharum spp. hybrid is a perennial grass and complex polyploid and aneuploid.

Breeder: BSES Limited

<u>Terms</u>: TVD = top visible dewlap, ULP = underlapping, OLP = overlapping,

DUS = distinctiveness, uniformity & stability

UPOV = International Convention for the Protection of New Varieties of

Plants

Polyploidy = if original plant is 2n, then polyploids are 3n or 4n etc.

An euploidy = extra or loss of one or more chromosome 2n + 1 or 2n - 1

PBR trials:

Randomised complete block design with single row 10m plots and three (3) replicates. Each test variety needs to have at least two (2) comparators and parents if available and appropriate. The cane in each plot needs to be planted accurately to the 10m line and the gaps rotary hoed or sprayed out. Each plot should be pegged or labelled and at an appropriate time, checked by the variety officer.

Propagation of source material:

The varieties and their comparators need to be propagated the year before maximum propagated for release. This permits Part 1 of the PBR description to be submitted, and the variety protected by PBR, the year of the variety's release.

INSTRUCTIONS FOR PBR SAMPLING

Cutting samples:

- **Randomly** select 12 stalks from each plot.
- Do not top or strip the cane and be sure to cut the cane at ground level.

Laboratory measurements:

- Of the 12 stalks, 10 are used for quantitative measurements and 2 stalks from each replicate are bulked for descriptive measurements.
- Equipment check list:
 - > two pairs of digital (dial as a backup) vernier callipers.
 - > clear plastic ruler (have spares as they fade quickly).
 - > wooden rulers
 - > two retractable tapes
 - > two 10 x hand lens (a hand lens with an inbuilt light is also useful).
 - > a set of Field Observation Sheets (see Appendix 10) for each variety
 - > diagram cards.
 - > felt tip markers (suitable for writing on cane)
 - > sticky tape (wide and effective)

- > two good pair of secateurs
- > RHS colour charts
- > pencils, pens, rubbers
- > digital camera and tripod
- > display cloth
- > identification (ID)cards
- The **work bench** setup (see fig. 1 and fig. 2) needs to be long enough to lay the cane on. Usually two long tables butted together works well.
- Extra lighting will probably need to be rigged up.
- A vertical board needs to be attached to one end of the table where the cane butts are placed. This acts as a stop for the cane when measuring culm height.
- The two retractable tapes are attached with sticky tape to the table (both on the same side), one the full length of the table for measuring culm height and the other about half length at the cane top end to measure laminae and sheaths (see fig. 1).
- The best combination was found to be three people in the lab where one person records all the quantitative data while the other two do all the measurements. It was found that two in the lab was quite efficient with one either side of the table.
- The descriptive measurements can be efficiently dealt with by two people. Many of these are subjective and it is important the workers understand what they are looking for.

VERY IMPORTANT: It is absolutely essential the data is recorded in a clear and unambiguous way. To achieve this, the descriptive data should be recorded only on the specific files QANT DATA FILE, and DESCRIPTION TEMPLATE. (Eventually linked to intranet).

Photography:

- Cut the centre region of each stalk and make three per bundle. The stalks should be thoroughly stripped and longer than the field of view. Attach a label to one stalk in each bundle to prevent getting the bundles mixed.
- Each frame consists of one variety, a label, and a ruler.
- Lay the stalks horizontally on the display sheet (preferably ironed) with the variety name card below the cane (see fig. 3). <u>Butts to the left</u>. Place a ruler (yellow, or wooden ruler) on the sheet in the same direction as the cane.
- Position the cane to show its characteristics.
- Use ambient light but not direct sun to avoid shadows. The shots need to be taken directly overhead with a digital camera positioned on a tripod.
- It is important that all images are taken at the same distance and the same focal length so that they can be easily compiled at a later date.
- Take good quality digital images eg. 2560 x 1920 pixels

Field observations:

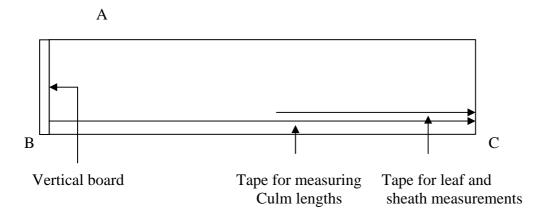
- Field observations are very subjective and therefore it is better if more than one person is involved.
- Field observations must be recorded before the main data collection ie. before any stalks are cut from the trial.
- Ideally, field observations should be made on all varieties in all three replicates, preferably in teams of two with each team recording from one replicate

Important note:

As there is considerable effort and travel involved in this work the possibility of losing the data by whatever means is of some concern. The data files should be copied onto two computers at the end of each day and also when all data has been recorded. The completed data files should also be copied onto a memory key.

Figure 1. PLAN VIEW OF TABLE

A, B and C are worker positions.



A strips cane, rates trash adhesion then passes cane to B and C plus records all data.

- B finds longest internode, measures internode lengths and diameters.
- C measures culm length, cuts cane below TVD leaf sheath then does all top measurements.

For two people only, A does above plus finds and measures longest internode.

B measures internode diameters, culm length and all top measurements.

Figure 2. SIDE VIEW OF TABLE

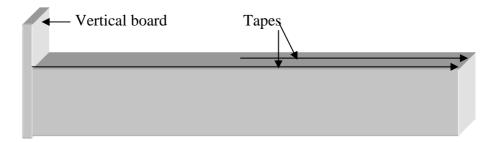


Figure 3. LAYOUT FOR PHOTO



- Butts to the left
- •
- Include ruler
- I D cards to the bottom
- Arrange canes to show details
- Frame horizontally.

		,

14. APPENDIX 10 – FIELD OBSERVATIONS RECORDING SHEET DESCRIPTIVE DATA FOR THE VARIETY:

<u>Date:</u>	Location:				
Comparative varieties:		_ Parentage: _	X	Seedling #:	

FIELD OBSERVATIONS

•	Growth habit	(erect/semi-erect/intermediate/semi-prostrate/prostrate)	Y
•	Leaf sheath (tr	ash) adherence (weak/medium/strong)	Y
•	Tillering	(weak/medium/strong)	Y
•	Suckering	(very few/few/medium/many/very many)	Y
•	Leaf canopy	(very sparse/sparse/medium/dense/very dense)	Y
•	Intensity of gre	een colour of leaf canopy (light/medium/dark)	Y
•	Leaf blade cur	rvature (straight/curved tips/arched/curved at base)	Y