Micropropagation for Quality Seed Production in Sugarcane in Asia and the Pacific

Food and Agriculture Organization of the United Nations (FAO)
and the
Asia-Pacific Consortium on Agricultural Biotechnology (APCoAB)
Asia-Pacific Association of Agricultural Research Institutions (APAARI)

Cover:

Top row (left to right): Highly enlarged sugarcane apical meristem with a leaf primodium. Multiple shoot formation. Rooting of plantlet.

Bottom row (left to right): Well established seedlings ready for field transfer. 180 days after field planting. Breeder seed plot.

MICROPROPAGATION FOR QUALITY SEED PRODUCTION IN SUGARCANE IN ASIA AND THE PACIFIC

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FOREWORD

Sugarcane is globally an important source of commercial sugar accounting for nearly 70 per cent of the world sugar production. Besides being a source of other products like animal feed, antibiotics, particle board, biofertilizer and raw material for generating electricity, sugarcane has lately emerged as an important base material for bioethanol production. In view of the depleting petroleum reserves, most countries are seeking alternative plant-based renewable fuel resources. Biofuel production has doubled in the past five years and is likely to double in the next five years. In Brazil, a dominant producer of bioethanol, nearly 90 per cent production is from sugarcane. India, Philippines, China, Thailand, Indonesia, Vietnam and Myanmar are some of the countries in the Asia-Pacific region with major interest in sugarcane-based bioethanol production.

Although nearly 20 countries in the Asia-Pacific region grow sugarcane on a commercial scale, the total production in these accounts for only about 44 per cent of the total world production of 1,387.8 million tonnes. Moreover, the average sugarcane yield of 56.7 t/ha in this region is far below the world average of 68 t/ha. Sugarcane, being propagated vegetatively, has a low 1:6 to 1:8 seed multiplication rate due to which seed production of newly released varieties is invariably slow. Further, the seed accumulates diseases and pests during several cycles of field production. Hence, non-availability of disease-free, true to type planting material is a major constraint in improving sugarcane productivity.

Development of tissue culture technology for rapid multiplication of disease-free planting material has been an important step towards quality seed production in sugarcane. Australia, India and the Philippines in the Asia-Pacific region have already applied this technology for commercial seed production and the benefits have become evident through rapid multiplication and distribution of elite varieties and increased sugarcane production. The experiences of these countries would be of considerable benefit to all those who are in the process of adopting micropropagation for their seed production programs.

The Asia-Pacific Consortium on Agricultural Biotechnology (APCoAB) was established in 2003 under the umbrella of Asia-Pacific Association of Agricultural Research Institutions (APAARI) that has been promoting appropriate use of emerging agri-technologies and tools in the region. One of the activities of APCoAB is to bring out status reports of biotechnological applications that have proved useful to the farmers and other stakeholders in the region. The present publication "Micropropagation for Quality Seed Production in Sugarcane" is fourth such publication in this series. It provides detailed information on micropropagation and related field propagation techniques standardized at the Sugarcane Breeding Institute, Coimbatore, India and applied for large-scale seed production in

several parts of the country. Success stories of sugarcane micropropagation in other Asia-Pacific countries have also been included along with suggestions for enhancing technology adoption by resource poor farmers. It is my hope that this publication will be of use to the seed industry, researchers and policy planners in the region in evolving efficient seed production system for faster coverage under improved varieties of sugarcane.

(Raj Paroda)
Executive Secretary, APAARI

ABBREVIATIONS AND ACRONYMS

APAARI	Asia-Pacific Association of Agricultural Research Institutions
APCoAB	Asia-Pacific Consortium on Agricultural Biotechnology
AST	aerated steam therapy
BAP	6-benzyl amino purine
BSES	Bureau of Sugar Experiment Station, Brisbane, Australia
CCS	commercial cane sugar
CD	critical difference
CMRTD	Consortium on Micropropagation Research and Technology Development
Co	Coimbatore-Sugarcane Breeding Institute
CoC	Coimbatore-Cuddalore
CoH	Coimbatore-Haryana
CoS	Coimbatore-Shahjahanpur
CoSi	Coimbatore-Sirugamani
CoJ	Coimbatore-Jalandhar
DBT	Department of Biotechnology, Government of India
DAC-ELISA	Direct Antigen Coating-ELISA
DAS-ELISA	Double Antibody Sandwich-ELISA
EDTA	ethylenediaminetetraacetic acid
ELISA	enzyme-linked immunosorbant assay
FAO	Food and Agriculture Organization of the United Nations
ha	hectare
HEPA	high efficiency particulate air
IBA	indole-3-butyric acid
ICAR	Indian Council of Agricultural Research
LDM	liquid differentiating medium
MC	meristem tip culture
MS	Murashige and Skoog
MCB	methyl benzimidazole-2yl-carbamate
mg/l	milligrams/litre
mt	million tonnes
NAA	a-napthaleneacetic acid
NPK	nitrogen-phosphorus-potash
PCR	polymerase chain reaction
pH	hydrogen ion concentration
psi	pounds per square inch

R&D	research and development
RT-PCR	reverse transcription polymerase chain reaction
SARP	Sugarcane Adaptive Research Programme
SBI	Sugarcane Breeding Institute, Coimbatore, India
SE	standard error
SCMV	*Sugarcane mosaic virus*
SCYLV	*Sugarcane yellow leaf virus*
w/v	weight/volume
t	tonnes

CONTENTS

1. INTRODUCTION

Sugarcane is one of the most efficient converters of solar energy into sugars and other renewable forms of energy. The plant was domesticated by the Polynesians (Brandes, 1958) for its sweet stem, but presently it has emerged as a multipurpose crop providing not only sugar but also a series of value added products such as paper, ethanol and other alcohol derived chemicals, animal feed, antibiotics, particle board, bio-fertilizer and raw material for generating electricity. Global sugar consumption has been increasing at a steady rate of 2 per cent per annum (http://www.illovo.co.za/worldofsugar/internationalSugarStats.htm). Ethanol has emerged as a key product from the sugarcane industry globally. With ever increasing oil prices, more and more countries are encouraging plant-based ethanol production as an environment-friendly fuel.

In a typical sugar mill, 100 tonnes (t) of sugarcane on an average produce 10 t of sugar, 4 t of molasses from which ethanol is produced, 3 t of press mud which is converted into biofertilizer, 30 t of bagasse used for cogeneration of power to yield 1,500 kW electricity and for manufacturing paper. Apart from these, about thirty t of cane tops and leaves are generally left in the field, which through recycling further add to the economic value of the crop. Sugarcane, thus, plays a major role in the economy of sugarcane growing areas and, hence, improving sugarcane production will greatly help in economic prosperity of the farmers and other stakeholders associated with sugarcane cultivation.

About 20 countries in the Asia-Pacific region grow sugarcane on a commercial basis contributing 608.37 million tonnes (mt) to the world production of 1,387.78 mt (Table 1). However, sugarcane yields vary widely across the region, ranging from 17.1 tonnes/hectare (t/ha) in Cambodia to 91.97 t/ha in Australia with an average yield of 56.66 t/ha compared to the world average of 67.98 t/ha. Most of the sugarcane farmers in this region are small and confronted with problems of low cane yields due to poor quality seed, low fertilizer inputs, prevalence of diseases and pests, lack of proper irrigation facilities, untimely harvests and several other local constraints. The limited cultivable area available for expansion and continuing conversion of agricultural land for non-agricultural purposes necessitate that production increase comes mainly from increase in per hectare yields. Improved agronomic practices, use of required quantity of fertilizer at appropriate time, better irrigation facilities, comprehensive disease and pest management packages and regular development of improved varieties are the necessary inputs required for improving sugarcane production and productivity. Besides, availability of disease and pest-free, true to type planting material is an important prerequisite for achieving the desired yield improvement. Sugarcane, being a vegetatively propagated crop, has a low 1:6 to 1:8 seed

Table 1. Sugarcane area and production in Asia-Pacific during 2006

Country	Area (1000 ha)	Production (mt)	Yield (t/ha)
Australia	415.00	38.17	91.97
Bangladesh	157.20	6.42	40.86
Bhutan	0.41	0.01	31.22
Cambodia	8.30	0.14	17.08
China	1,220.00	100.68	82.53
India	4,200.00	281.17	66.95
Indonesia	370.00	30.15	81.49
Iran, Islamic Rep of	63.40	5.53	87.23
Japan	23.00	1.25	54.35
Laos	7.20	0.24	33.33
Malaysia	12.00	0.90	75.00
Myanmar	140.00	7.30	52.14
Nepal	62.06	2.46	39.68
Pakistan	907.30	44.67	49.23
Papua New Guinea	8.50	0.45	52.94
Philippines	392.28	24.35	62.06
Sri Lanka	21.58	1.14	52.67
Thailand	936.23	47.66	50.90
Viet Nam	285.10	15.68	54.99
Total Asia-Pacific	**9,229.55**	**608.37**	**56.66**
World	**20,413.66**	**1,387.78**	**67.98**

Source: http://faostat.fao.org/ (accessed 28 August 2007)

multiplication rate. Hence, non-availability of quality seed material is one of the major problems faced by farmers in developing countries. Further, the bulky cane cuttings used for planting as seed harbor many pests and diseases thereby decreasing cane yield and quality drastically. Accumulation of diseases over vegetative cycles leads to further yield and quality decline over the years. In fact, poor quality seed is a major constraint in sugarcane production.

Development of tissue culture technology for rapid multiplication of disease-free planting material has greatly facilitated mass production of quality seed in sugarcane. A number of micropropagation techniques have been adopted successfully by farmers and industry in some sugarcane growing countries of Asia-Pacific, e.g. India, Australia and the Philippines. This publication details mainly the micropropagation technology for sugarcane seed production developed at Sugarcane Breeding Institute, Coimbatore, India (SBI) and adopted in several states of the country. Success stories of impact of micropropagation technology, integration of micropropagation with conventional seed production system and suggestions for future strategies are included in the publication.

2. SUGARCANE SEED PRODUCTION

Sugarcane stem used for production of seed is composed of a series of nodes and internodes (Figure 1). Each node has a leaf, in the axils of which a bud is located. The bud has a dormant apical meristem well protected by several tightly clasping bud scales. Besides the bud, the node possesses a root band zone bordered by a growth ring. The root band contains one to several rows of root primodia which produce roots when the cane cuttings are planted. The growth ring is an intercalary meristem located immediately above the root band. Cane cuttings with one, two or three buds, known as "setts", "seed canes" or "seed pieces" are used as seed. In some instances, buds scooped out of the cane with a bud-chipping machine are used for raising the seed nursery.

For raising a healthy sugarcane crop, setts should be harvested from 7 to 10 months old crop which is totally free of diseases and pests. The setts should be healthy and must have high moisture content. The buds should be dormant and the canes used to obtain seed setts must be free from rooting at the nodes, splits on the internodes and other damages.

2.1 SEED SETT PREPARATION

In India, seed setts are prepared manually. Seed canes are harvested and dry leaves removed manually to avoid any damage to the buds. Canes are cut with a sharp knife into setts containing two or three buds each. Sett-cutting machines are now available making the process more efficient (Figure 2).

The cut ends of seed setts become easy entry points of many disease causing microbes, leading to sett rotting and damage to the buds and root primodia. Soaking the setts for 5 to 10 minutes in 0.1 per cent solution of a systemic fungicide such as methyl benzimidazole-2yl-carbamate (MBC) just before planting is recommended to ensure protection.

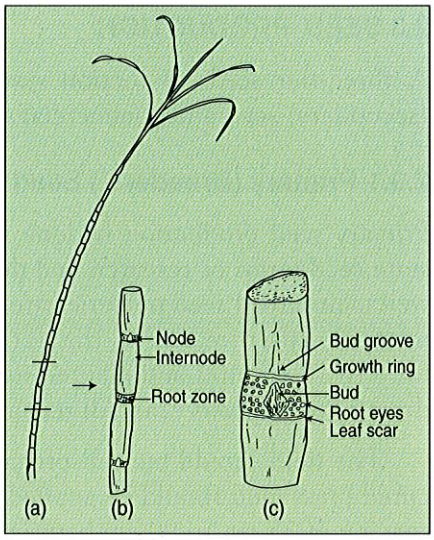

Figure 1. Sugarcane seed cane and seed setts. (a) Seed cane. (b) Three-budded seed sett. (c) Single-budded seed sett.

2.2 HEAT TREATMENT OF SETTS

Sugarcane setts may harbor a host of diseases such as sugarcane smut, red rot, grassy shoot, ratoon

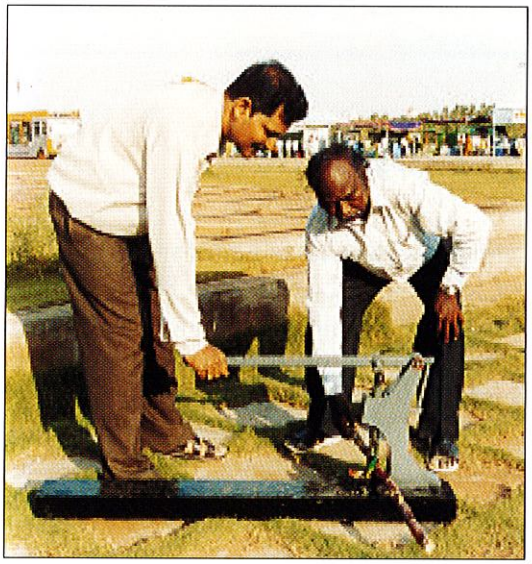

Figure 2. *Cutting machines used for making seed setts.*

stunting, sugarcane mosaic and yellow leaf. Also, scale insects and borers present on the setts can cause heavy damage to the new crop. Heat treatment of setts helps in getting rid of several diseases and pests.

There are four types of heat therapies: (1) Hot water: setts are immersed in water maintained at 50°C for two to two and a half hours. Often, fungicides are mixed in hot water to eliminate smut disease. (2) Hot air: dry heat produced by electric heaters placed at different points in the heating chamber is circulated with a fan. Temperature is maintained at 56°C and the seed is treated for eight hours. (3) Moist hot air: steam is injected into the treatment chamber for four hours maintaining the temperature at 54°C. (4) Aerated steam: steam is mixed with air in 1:4 proportion and forced into the treatment chamber through small holes. The treatment is given for one hour at 50°C.

When applied properly, heat therapy eliminates ratoon stunting disease, grassy shoot disease, sugarcane smut disease, and also seed borne insect pests.

2.3 SEED PRODUCTION

A three-tier seed production system comprising breeders' (primary) seed, foundation (secondary) seed and commercial seed production as detailed below is ideally followed.

2.3.1 Primary (Breeders') Seed Production

Primary seed production is done in scientifically supervised farms of research stations, state seed farms or research and development (R&D) farms of sugar industry. Setts from well maintained seed nurseries are given heat treatment by any one of the above detailed methods. After treatment, the setts are soaked in a fungicide solution (0.1 per cent MBC) for 5 to 10 minutes and planted in a well-prepared field, where sugarcane was not grown during the previous year. All recommended agronomical practices are followed.

The field should be well-prepared and organic manure such as farm yard manure or cured press mud should be applied at the rate of 25 to 30 t/ha 15 days before planting. A spacing of 75 cm to 90 cm between rows is recommended. A slightly higher seed rate of 75,000 two-bud setts is recommended for raising breeders' seed (primary seed) to compensate for germination loss due to heat therapy. For foundation and certified seed nurseries, a seed rate of 60,000 two-bud setts is adequate for obtaining a good stand.

Early application of a higher dose of fertilizer comprising 250-300 kg nitrogen, 75 kg phosphorus and 75 kg potash/ha is recommended for achieving rapid growth. The fertilizer may be applied in three split doses at 30 days, 60 days and 90 days after planting. Irrigation once in a week for loamy soil and every 10 to 12 days for heavy clay soil is adequate.

Monitoring of the seed nursery is done at least three times during the crop growth. First inspection is done at 45 to 60 days after planting to detect off-types and to remove plants infected with designated diseases and pests. The second inspection is done at 120-130 days after planting to check for off-types, designated diseases and pests. The third inspection is done 15 days prior to harvesting of canes to check the general condition of the canes as seed. The crop is harvested at 7 to 10 months and used for planting foundation seed (secondary seed) nursery. The multiplication rate is around 1:6 to 1:7, lower than the normal multiplication rate of 1:7 to 1:8 due to slightly lower germination as a result of heat treatment of setts.

2.3.2 Secondary (Foundation) Seed Production

Setts from primary seed nursery are used for planting secondary seed nursery. All the required agronomic practices are followed and the seed plots are inspected at regular intervals for prescribed standards (*Annexure I*). The crop is harvested at 7 to 10 month age and setts are used for planting commercial seed nurseries.

2.3.3 Commercial Seed Production

Setts obtained from foundation seed crop are used for planting commercial seed nurseries. Commercial seed plots are laid in farmers' fields identified for the purpose and distributed throughout the operational area of the sugar mill. This practice avoids transport of bulky seed to long distances. The seed plots are inspected as per seed certification standard (*Annexure I*). The crop is harvested at 7 to 10 month age and the cane is supplied as commercial seed. Care is taken to ensure that the buds are intact during transportation. The commercial seed thus produced can be propagated for about 4 to 5 years. Seed replacement with fresh commercial seed is done only after 4 years (Sundara, 2000).

The setts from commercial seed plots are supplied to the sugarcane farmers generally by the cane development department of the sugar mills. While the system of seed production and distribution works satisfactorily at some places, at several others one or more stages of the system are impaired and the seed production is affected. Thus, a large proportion of the farmers in most of the developing countries still use traditional, poor quality seeds resulting in poor yields.

3. MICROPROPAGATION FOR SUGARCANE SEED PRODUCTION

Micropropagation is a methodology through which plants are multiplied rapidly by aseptic culture of meristematic regions under controlled nutritional and environmental conditions (*Annexure II*). When disease-free material is used as the source of explant or the explants are heat-treated to eliminate diseases, the resultant micropropagated plants are disease-free and healthy. Micropropagation also helps in the rapid seed multiplication of newly released varieties which is of particular importance in sugarcane where, as mentioned earlier, the normal seed multiplication rate is very low.

A number of micropropagation techniques suitable for commercial seed production in sugarcane have been reported. Apical meristem culture was used by Coleman (1970) and Hendre *et al.* (1975) to obtain sugarcane mosaic virus free plants. Axillary bud culture was applied successfully by Sauvaire and Galzy (1978) to produce true to type clones in many sugarcane varieties. Hendre *et al.* (1983) standardized an apical meristem culture technique for rapid multiplication of mosaic virus-free plants of variety Co 740. Sreenivasan and Jalaja (1981) standardized micropropagation technique based on the use of apical meristem with two or three leaf primodia (meristem tip) as the explant. The latter can be excised without the aid of a microscope and the success rate of organogenesis is quite high. The number of plantlets produced from one shoot tip in 372 days can be as high as 180,000. The micropropagated plants are remarkably uniform except for rare off types showing some color changes, the latter can be rouged in the first generation itself. This meristem tip culture technique that has been widely adopted for commercial sugarcane seed production in India is detailed in the following pages.

3.1 MERISTEM TIP CULTURE

In a growing sugarcane plant, the apical meristem is located at the tip of the stem surrounded by developing leaves and leaf sheaths. Meristems are also located in axillary buds which are dormant as long as the apical growing point is functional. Both the apical and the axillary buds are used for initiating meristem tip cultures. The shoot meristem measures approximately 0.1 mm in diameter and 0.25 mm to 0.30 mm in length and can be exposed by carefully removing the surrounding leaf sheaths. The meristem remains in an active state during the vegetative growth phase and the meristem cells are in a permanent embryonic state. The cells of the meristem are genetically highly stable and, hence, the plants produced from them are generally identical to the donor plants, except for the occurrence of rare mutations (Hendre *et al.*, 1983; Sreenivasan and Jalaja, 1992).

3.1.1 Media

Media based on Murashige and Skoog (1962) and White (1963) are used for meristem tip culture. Minor modifications with addition of vitamins, hormones and sugars are generally made in the medium by different laboratories to suit their needs. The standard media used at SBI and which have proved successful for micropropagation of 20 sugarcane varieties (Table 2) are given in *Annexure III*.

Table 2. Salient features of 20 sugarcane varieties micropropagated at Sugarcane Breeding Institute, Coimbatore, India

S.No.	Variety	Year of selection	Salient features
1	Co 419	1933	A high yielding, mid-late variety of tropical India
2	Co 740	1949	A high yielding, high sugared variety of tropical India, good ratooner and drought tolerant variety
3	Co 6907	1969	A high sugared variety of coastal Andhra Pradesh state
4	Co 7219	1972	A high yielding, high sugared variety of Maharashtra state
5	Co 7717	1977	An erect, high yielding variety with moderate sugared, subtropical variety
6	Co 8014	1980	A high yielding, mid-late variety, normally non-flowering, grown in northern Karnataka and Maharashtra states
7	Co 8021	1980	A mid-late maturing, smut resistant variety of tropical India
8	Co 8122	1981	A mid-late maturing, water logging/flood resistant variety for tropical India
9	Co 8208	1982	A high yielding, high sugared, good ratooning variety released for Tamil Nadu state
10	Co 85007	1985	A high yielding, heavy tillering drought tolerant variety suited for Madhya Pradesh state
11	Co 85019	1985	A high sugared, high yielding variety for Tamil Nadu and Karnataka states
12	Co 86010	1986	A high yielding, high sugared variety released for general cultivation in Tamil Nadu state
13	Co 86032	1986	A high yielding, high sugared variety for tropical India
14	Co 86249	1986	A high yielding, early maturing variety for tropical India
15	Co 87025	1987	An erect cane, suitable for mechanical harvesting
16	CoC 671	1967	A high yielding, high sugared variety, early maturing which retains sugar for longer periods without deterioration. Cultivated throughout tropical India
17	CoC 86062	1986	An early maturing, high yielding variety of Tamil Nadu state
18	CoC 90063	1990	A high yielding, high sugared variety of Tamil Nadu state
19	85 R 186	1985	A drought tolerant high yielding erect variety from Rudrur, Andhra Pradesh, India
20	CoJ 64	1971	An early maturing, high sugared variety of subtropical India

3.1.2 Methodology

The methodology of sugarcane micropropagation involves the following steps:

1. Collection and sterilization of shoots
2. Preparation of tops for shoot tip culture
3. Inoculation of meristem tips
4. Multiplication of shoots
5. Transfer of shoots to rooting medium
6. Hardening of plantlets
7. Field planting of plantlets
8. Commercial seed production

3.1.2.1 Collection and Sterilization of Shoots

Sugarcane varieties selected for micropropagation should have accompanying morphological description to enable verification of varietal characteristics during different stages of seed production. The nursery crop meant for harvesting of shoots for culture is raised from heat-treated setts in a field where sugarcane crop has not been grown during the previous season. The nursery should be inspected and certified by pathologists from accredited laboratories for freedom from diseases such as grassy shoot, phytoplasma, sugarcane mosaic virus, sugarcane yellow leaf virus, ratoon stunting disease, leaf scald, smut and red rot by using DAC-ELISA or DAS-ELISA methods. In addition to ELISA, PCR techniques (RT-PCR) for SCMV and SCYLV may be used for the detection of the pathogen. Care also needs to be taken to protect the nursery from secondary infection of these diseases by growing the crop under insect proof nets and application of insecticides as and when required.

Shoot tip explants can be obtained from three sources: (a) tops of actively growing canes (Figure 3a), (b) elongating axillary shoots from the decapitated shoots, and (c) dormant axillary buds (Figure 3b). In our experience, the best explant is the shoot tips from actively growing sugarcane tops. For best results, harvesting of shoot tips is done 120 and 180 days after planting and one to two days after a good irrigation of the nursery.

3.1.2.2 Preparation of Tops for Shoot Tip Culture

The surrounding leaf sheaths of sugarcane tops are carefully removed one by one until the inner white sheaths are exposed. The tops are sized to 10 cm length by cutting off at the two ends, locating the growing point somewhere in the middle of the top. Seven to eight such prepared tops are placed in a 2-l flask containing soap solution. The tops are washed for five minutes to remove the wax on leaf sheaths and later rinsed four to five times with distilled water until the soap solution is completely washed out. A quick rinse is then given in 70 per cent ethyl alcohol for 1 minute. The alcohol is poured off and the tops rinsed in

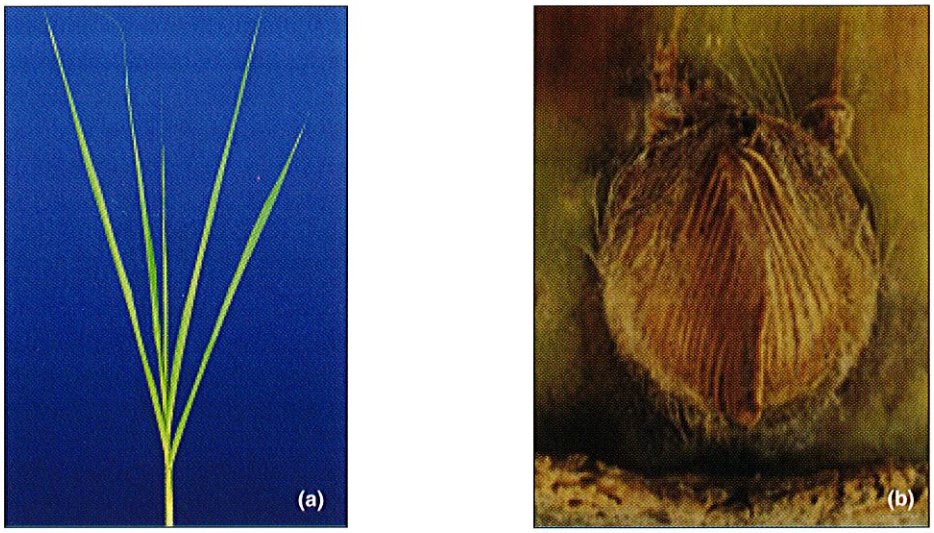

Figure 3. *Sugarcane explant sources. (a) Tops with growing points. (b) Dormant axillary bud.*

sterile distilled water until the smell of alcohol is totally removed. A 10 per cent solution of sodium hypochlorite (4 per cent w/v available chlorine) is then poured into the flask to immerse the tops completely. The flask is closed with aluminum foil to prevent the escape of chlorine gas and shaken at 50 rpm or hand-shaken vigorously for about 20 minutes. The flask is moved to a laminar flow chamber where the solution is poured out and the material is washed four to five times with sterile distilled water until the chlorine smell is completely eliminated. The material is now ready for dissection and inoculation.

The same sterilization procedure may be followed when using axillary shoots or dormant axillary buds as explants. The outer scale leaves are removed after wiping them with 70 per cent ethyl alcohol. The material is further processed through sterilizing solution as detailed above and washed and kept for dissection and inoculation. However, the sterilized material should not be kept in hypochlorite solution or sterile distilled water for too long to avoid excessive softening of the material.

3.1.2.3 Inoculation of Meristem Tips

The explants (apical shoots/axillary shoots/dormant axillary buds) are picked carefully with sterilized forceps and placed in a sterile Petri dish. Using a fine forceps and scalpel, which are flamed and cooled every time after use, the outer leaf sheaths are removed one by one. Initially, three to four longitudinal slits are given superficially with the scalpel. By giving superficial transverse cuts at the base, the leaf whorls are removed carefully without exerting pressure on the internal tissues. The process is repeated until the apical dome with two to three leaf primodia is exposed (Figure 4a). This process should be done very carefully to avoid damaging the apical dome. After excising the apex with a sharp blade,

the explant is transferred immediately on a filter paper support (Figure 4b)immersed in the Shoot Apex Medium (*Annexure III*). The filter paper support is initially kept well above the surface of the medium. At the time of inoculation, it is slightly pushed down into the medium so that the latter is just above the surface of the support. This is done to avoid excessive softening and disintegration of filter paper while ensuring availability of the medium to the explant. The inoculated tubes are kept in the culture room under light (2500 lux) at 26°C. Due to phenolic exudates, the filter paper support gets discolored at the place of contact of the explants which hinders the absorption of nutrients resulting in its drying. Shaking the tubes gently without opening the caps changes the position of the explants and avoids the problem. After one week, the explants are transferred to fresh medium over filter paper supports. In case of further browning, another transfer to fresh medium is carried out. Initially, the growth is slow and it takes about 30 to 45 days for new shoots to appear (Figure 4c).

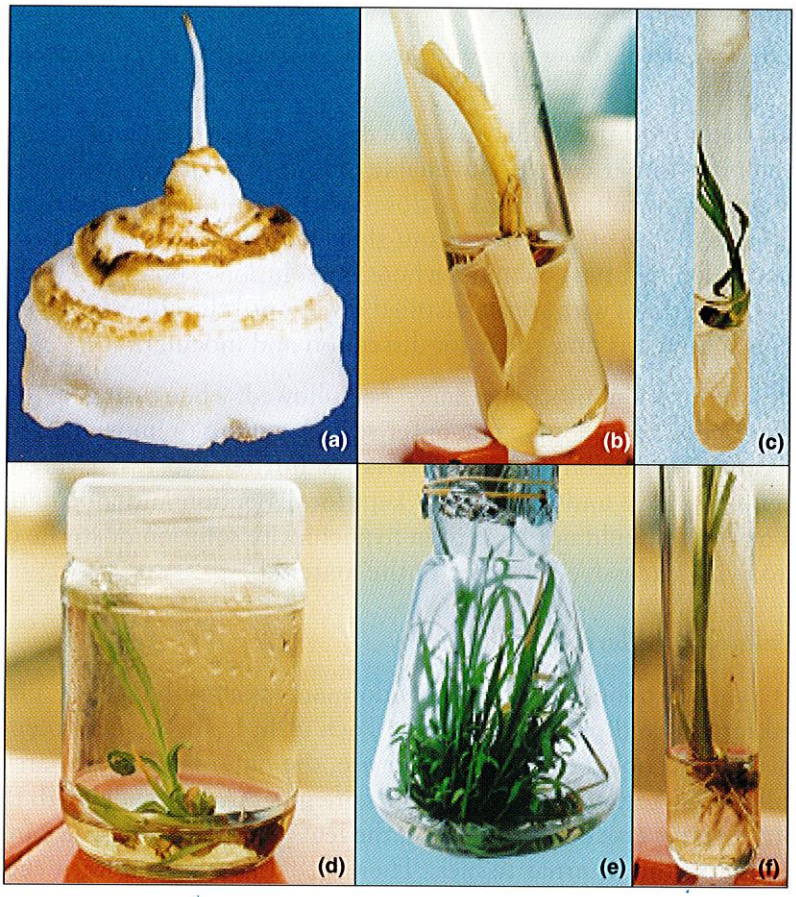

Figure 4. *Stages in sugarcane micropropagation. (a) Highly enlarged sugarcane apical meristem with a leaf primodium. (b) Developing explant on filter paper support. (c) Developing miniature shoot. (d) Axillary shoot development. (e) Multiple shoot formation. (f) Rooting of plantlet.*

3.1.2.4 Multiplication of Shoots

The developing shoots are transferred to fresh containers with liquid differentiating medium (LDM) (*Annexure III*). Shoot multiplication follows soon after (Figure 4d, e) and the process is repeated every 15 to 30 days depending upon the rate of multiplication, which may differ with the variety.

The concentration of 6-benzylaminopurine (BAP) in LDM may need to be adjusted depending upon the growth of shoots. Initially, 6 mg/l 6-BAP is used for quick shoot multiplication. Before transfer to rooting medium, the bigger shoots are subcultured once or twice in LDM devoid of 6-BAP. The rest of the smaller shoots are transferred to LDM containing 0.25 mg/l 6-BAP. Rarely, some varieties may not multiply in medium containing 6 mg/l of 6-BAP. For these, a range of 6-BAP concentrations from 0.25 mg/l to 6 mg/l need to be tried, which may later be reduced to 0.25 mg/l. Some cultures may show a ball like appearance due to excessive multiplication of shoots; this can be avoided by addition of either 0.5 mg/l of gibberellic acid or by elimination of 6-BAP from the next two or three subcultures. The use of gibberellic acid may, however, lead to inhibition of rooting in some varieties.

Subculturing of shoots in LDM is done after 15-20 days. However, if very rapid multiplication is observed, subculturing may be required once in 7 to 8 days. Similarly, if there is depletion of the medium, phenolic exudation or drying of leaves, subculturing is done at more frequent intervals. Further, it is better to restrict the number of plantlets to 25-30 per flask as crowding may result in the production of unhealthy, lanky plants.

3.1.2.5 Transfer of Shoots to Rooting Medium

Only well-grown shoots with three to four leaves should be transferred to rooting medium. Dry leaves are removed and green leaves trimmed at the tips. While separating, care is taken not to damage the basal portion of the shoots from where the roots emerge. Groups of five to six shoots are placed in bottles containing Rooting Medium (*Annexure III*). Roots are formed within 15-25 days and once good root development has taken place (Figure 4f), the plantlets are transferred to polybags/planting trays. If no rooting is observed, 0.75 mg/l indole-3-butyric acid (IBA) is added to the medium. In case new shoots emerge from the basal region after transferring the plantlets to the rooting medium, it is a sign of excessive 6-BAP in the plant tissues. Sometimes, the carryover effect of 6-BAP is exhibited even by the field grown plants which produce large numbers of tillers with low stalk diameter. Such plants will be unacceptable for seed production or commercial cane production. The problem can be avoided by subculturing two or three times more in LDM devoid of 6-BAP before transferring to the rooting medium.

3.1.2.6 Hardening of Plantlets

Plantlets with well developed shoots and roots are taken out of the culture bottles and thoroughly washed with water to remove all traces of the medium. The plantlets with

slightly trimmed roots and leaves are sown in polybags/planting trays containing a mixture of separately sieved river sand, silt and well decomposed press mud or farm yard manure in a 1:1:1 ratio. The sown plantlets may be kept in mist chamber (Figure 5a) or under shade; in the latter case, humidity is maintained by covering the bags/trays with polyethylene sheets on appropriate supports for 10 to 14 days or until the first new leaves emerge. During this period, watering is done as per requirement while taking care to avoid excess watering. A 1 per cent NPK spray once in a week after establishment of the plants boosts initial growth. The plants will be ready for field planting after 45 days (Figure 5b).

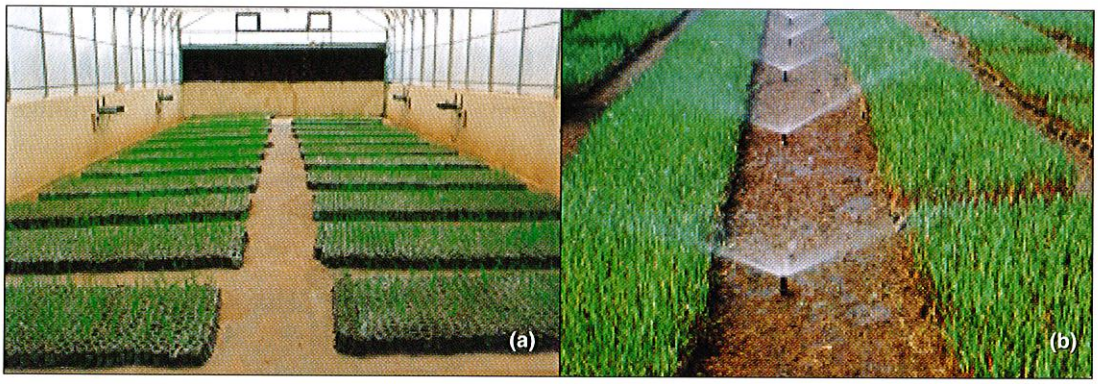

Figure 5. *Hardening of plantlets. (a) Plantlets in mist chamber. (b) Well established seedlings ready for field transfer.*

In addition to the above mentioned soil mixture, various types of rooting mixtures using moss, coconut coir pith, sugarcane bagasse from sugar factories and vermicompost are used to prepare good rooting media. Similarly, instead of polybags, various types of portable trays made of polythene, fibre and board are extensively used for planting tissue culture raised plantlets.

3.1.2.7 Field Planting of Micropropagated Plants

The field in which the hardened plants are to be grown is kept fallow during the previous year to reduce soil-borne diseases. After deep ploughing, harrowing and leveling, organic manure is added @15-20 t/ha. Six meter long furrows are opened at a row-to-row distance of 90-150 cm. Pits of 10 cm depth and diameter are dug at 60-100 cm distance and a basal dose of fertilizer (75 kg P_2O_5+100 kg N+40 kg K_2O/ha) is applied in the pits and mixed with the soil (Sundara and Jalaja 1994). For facilitating drip irrigation, planting is done in paired rows with 90 cm distance between rows and 180 cm distance between pairs of rows. The hardened plants are brought to the field after light trimming of the leaves and planted one per pit without disturbing the root-soil mass. The field is irrigated immediately after planting and the next irrigations are given on the third day. Subsequently, weekly irrigation is given until the first new leaf emerges which is an indication of successful establishment. First dose of fertilizer (100 kg N+ 40 kg K_2O/ha) is applied at 45 days and second dose of

same composition at 90 days followed by a good earthing up. Off types, if any, are rouged out at 180 to 200 days of planting.

Establishment of the tissue culture raised plants in the field is above 95 per cent if proper maintenance and irrigation schedules are followed (Figure 6). The crop growth is uniform with synchronous tillering and freedom from diseases and pests. The ratoons are excellent, without any gaps and ratoon yields are equal or better than the main crop yields (Figure 7). Canes from the ratoon crop, however, should not be used for seed production.

3.1.2.8 Commercial Seed Production

The canes produced from the field grown, micropropagated plants are regarded as primary seed (breeders' seed) in relation to the three-tier seed production system as detailed earlier. These canes are cut into two-budded or three-budded setts to raise the secondary seed (foundation seed) nursery and the seed from latter is used to raise commercial seed plots. These seed plots should preferably be located near the area where the commercial crop is to be raised so as to minimize transportation costs and damage during transit. A change of seed is required once in four years.

Studies were carried out over several years at SBI on multiplication rate achieved through micropropagation and performance of micropropagation-raised crop. Table 3 details plantlet multiplication at different stages of micropropagation and the duration of each stage. Accordingly, sufficient number of seedlings to cover 14 ha field area are produced in about one year.

Studies have further shown that the micropropagation-based crop has prominently better germination, tillering, cane yield, and juice content and quality than the conventionally raised crop (Table 4). Heat therapy of setts also helps in improving crop and cane juice yields. The studies also revealed no detectable variations in botanical characters of micropropagation raised plants, though during the first year a few color changes in pigmented varieties and an occasional increase in tillering with a slight reduction in stalk

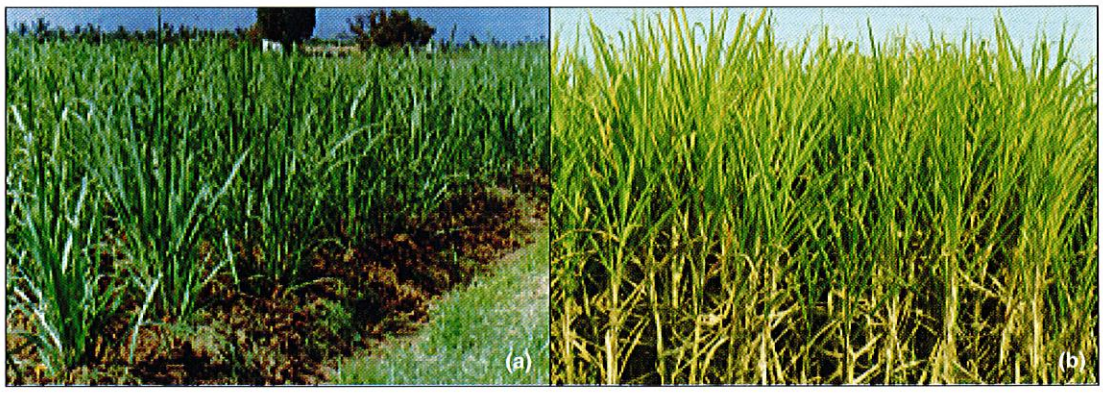

Figure 6. *Field performance of micropropagated plants. (a) 120 days after planting. (b) 180 days after planting.*

Figure 7. Breeder seed plots. (a) Co 86032. (b) Co 86249. (c) Co 87025. (d) Excellent tillering.

Table 3. Rate of plantlet multiplication in sugarcane through micropropagation

Stage	Number of shoots	Ratio	Number of days in each stage	Total number of days
Meristem tip inoculation	1	–	15	15
Shoot growth	1	–	21	36
Shoot multiplication cycle 1	1	–	21	57
Shoot multiplication cycle 2	6	1:6	45	102
Shoot multiplication cycle 3	36	1:6	45	147
Shoot multiplication cycle 4	216	1:6	45	192
Shoot multiplication cycle 5	2,160	1:10	45	237
Shoot multiplication cycle 6	21,600	1:10	45	282
Shoot multiplication cycle 7	216,000	1:10	45	327
Rooting	216,000 (approximately 200,000 after accounting for mortality)		45	372
Area covered	14 ha			

Table 4. Effect of aerated steam therapy (AST), meristem tip culture (MC) and additional NPK on growth and yield in sugarcane variety Co 740

Treatment	Germination (%) of three-bud setts	Tillering	Cane yield t/ha	Sucrose in juice (%)	Juice extraction (%)
Check	37.0	2.02	90.5	18.59	54.1
AST	46.0	2.41	105.3	18.82	60.3
AST + 25% extra NPK	44.3	2.56	107.6	18.40	61.5
MC	48.0	2.48	112.4	20.25	62.0
MC + 25% extra NPK	50.0	2.53	118.9	19.22	61.7
SE	1.7	0.07	2.6	0.41	1.2
CD (0.05)	4.6	0.21	6.8	1.22	3.6

Source: Sundara (1995)

diameter were observed. Most of the changes, however, occurred at similar frequencies as observed in the conventionally raised crop. The most common problem of excessive tillering and thin canes in the micropropagation raised seedlings (Sreenivasan and Jalaja, 1992; Sreenivasan, 1995) can be avoided by suitable corrections in the culture medium, as detailed earlier.

3.1.3 Quality Control

Quality control is essential to ensure that appropriate initial material is used for micropropagation, culture conditions are satisfactory and the identity of cultivar is maintained during the culture process. The following aspects have been emphasized for maintaining the quality of tissue culture raised sugarcane plants (Sinha, 2006):

1. *Genetic purity of source material:* The genetic purity of the variety to be micropropagated should be certified by the breeder/research organization identified for the maintenance of the variety.
2. *Source material:* The explant should be taken from vigorously growing healthy plants raised from heat-treated setts and grown under optimum moisture and nutritional conditions. The crop raised from micropropagated seedlings should not be used as source material.
3. *Accreditation of micropropagation laboratory:* Micropropagation laboratory should be accredited by an appropriate authority to ensure technical competence and satisfactory infrastructure.
4. *Micropropagation protocol:* Micropropagation protocol should ensure only minimal genetic changes. Shoot multiplication cycles should be restricted to avoid morphological variation.
5. *Seedling establishment:* The seedlings should be well-established in soil mixture with good root system and with 4 to 5 green leaves at the time of supply to user agencies.

6. *Disease indexing:* The micropropagation-raised plants should be indexed for freedom from viruses and virus-like diseases through ELISA, and molecular methods. Standard molecular techniques may be used to assess the genetic purity of plants.
7. *Seed production:* The micropropagation-raised seedling should be treated as breeders' (primary) seed. This seed should be further propagated through vegetative cuttings to produce foundation (secondary) seed and then commercial seed. Inspection of the field at the breeders' seed production stage must be done to remove any off types.
8. *Commercial seed:* Commercial seed thus produced should be used up to four years.

3.1.4 Technology Transfer

The sugarcane micropropagation technology developed and refined at SBI during 1981-1988 was initially utilized for institute level seed production. In 1989, the technology was incorporated as a component in Sugarcane Adaptive Research Project (SARP) for implementation in nine states of India over a period of five years. The project had the objective of demonstrating that an effective seed program including the use of micropropagation technology can play a significant role in increasing sugarcane productivity. The SARP provided an effective channel to popularize sugarcane micropropagation technology, utilize it for quality seed production on a large scale and train personnel involved in seed production (Jalaja, 1994). The program was implemented through following activities:

- A micropropagation laboratory was designed and established at SBI exclusively for large-scale clonal propagation of identified varieties. The experience gained was utilized to provide technical support for the establishment of micropropagation facilities at four locations comprising one state agricultural university and three sugar mills in two states.
- Twenty identified varieties were micropropagated and the rooted plants in polybags were supplied free of cost for seed production to 42 sugar mills located in eight states of India (Table 5). The management and workers of the mills experienced first hand the superior performance of micropropagated plants. As a result, considerable enthusiasm was generated among sugar mills to establish their own micropropagation facilities, for which Government of India provided financial support.
- The SBI continued to upgrade the micropropagation technology during the subsequent years laying emphasis on reducing the production cost of micropropagated plants. The Institute started supplying cultures in flasks at multiplication stage itself to users having appropriate facilities for subsequent laboratory and field culture (Table 6). This approach helped in reducing the cost and time of plant production.

  SBI also standardized an encapsulation technique for distribution of the micropropagated shoot initials (Jalaja, 2000).
- A total of 88 persons including technicians and university and college students from India and other countries were trained on sugarcane micropropagation. A practical

Table 5. Distribution of micropropagated seedling to sugar mills and other users in India by Sugarcane Breeding Institute, Coimbatore

State	Organization
Andhra Pradesh	K.C.P Sugars & Industries Corporation Ltd., Vuyyuru. K.C.P Sugars & Industries Corporation Ltd., Lakshmipuram. Shri Sarvaraya Sugars Ltd., Chelluru. The Andhra Sugar Mills Ltd., Tanuku. The Jeypore Sugar Company Ltd., Chagallu.
Gujarat	Sahakari Khand Udyog Ltd., Navsari. Shree Khedut Sahakari Khand Udyog Mandli Ltd., Bardoli. Shree Sayan Vibhag Sahakari Khand Udyog Mandli Ltd., Surat. Shri Chaltan Vibhag Khand Udyog Sahakarai Madli Ltd., Surat.
Haryana	The Haryana Cooperative Sugars Ltd., Rohtak.
Karnataka	Dakshina Kannada S.S.K. Ltd., Udupi. Devangere Sugar Company Ltd., Davangere.
Kerala	The Cooperative Sugars, Chittur.
Maharashtra	Shri Chatrapathi Rajaram SSK Ltd., Kolhapur.
Tamil Nadu	Arinğar Anna Sugar Mills, Thanjavur. Bannariamman Sugars Ltd., Erode. Cauvery Sugars and Chemicals Ltd., Tiruchirappalli. Dharani Sugars and Chemicals Ltd., Tirunelveli. EID (Parry) India Ltd., Nellikuppam. EID (Parry) India Ltd., Pugalur. G.R.D College, Coimbatore. Jawaharlal Nehru Cooperative Sugar Mills, Perambalur. K. R. Ramasami Cooperative Sugar Mills Ltd., Mayiladuthurai. Kothari Sugars and Chemicals Ltd., Tiruchirappally. Madura Sugars Ltd., Madurai. Main Biocontrol Lab., Chengalpattu. Ponni Sugars and Chemicals Ltd., Erode. Rajashree Sugars and Chemicals Ltd., Theni. S.V. Sugars Ltd., Kanchipuram. Sakthi Sugars Ltd., Erode. Shri Ambika Sugars Ltd., Cuddalore. Skathi Sugars Ltd., Unit II. Shivganga State Seed Farm, Paramakudi. The Amaravathi Cooperative Sugar Mills Ltd., Udumalpet. The Ambur Cooperative Sugar Mills Ltd., Ambur. The Madurantakam Cooperative Sugars Ltd., Madurantakam. The National Cooperative Sugar Mills, Madurai. The Vellore Cooperative Sugar Mills Ltd., Vellore. Thiru Arooran Sugar Mills, Thiruvarur. Tiruppathur Cooperative Sugar Mills Ltd., Vellur. Tiruttani Cooperative Sugar Mills Ltd., Tiruttani.
Uttar Pradesh	Ganeswar Ltd., Deoband.

manual on sugarcane micropropagation for the use of skilled technicians was prepared and distributed free of cost (Jalaja, 2001a).

During the implementation of SARP, long-distance transportation of seedlings established in polybags was found to pose serious problems. The seedlings used to get damaged and the transportation charges added significantly to the cost of production. This problem was mitigated by developing a strategy of transporting plantlets without soil in plastic containers. In this procedure, the rooted plantlets from culture vessels are washed thoroughly in water and excess roots and leaves are trimmed. The plantlets are carefully packed in plastic containers keeping them erect with moist filter paper or cotton at the base and sides of the containers. In this way, 1,000 to 1,500 plantlets can be packed in one container of 10 cm diameter and 15 cm height, and several such containers can be transported in polybags without damage. The plantlets remain fresh for 3-4 days if transported in air-conditioned coaches. At their destination, these are planted in polybags kept in polyhouses. The usual

Table 6. Distribution of plantlets in culture flasks to sugar mills and other users in India by Sugarcane Breeding Institute, Coimbatore

State	Organization
Delhi	Sheel Biotech, Greater Kailash.
Gujarat	Sahakari Khand Udyog Mandali Ltd., Navasari. Shree Chaltan Vibhag Khand Udyog Sahakari Mandli Ltd. Shree Khedut Sahakari Khand Udyog Mandli Ltd., Bardoli. Shree Madhi Vibhag Khand Udyog Sahakari Mandli Ltd., Surat. Shree Sayan Vibhag Sahakari Khand Udyog Mandi Ltd., Surat.
Karnataka	Maruti Organics, Bangalore.
Kerala	Sugarcane Research Centre, Kozinchanpara.
Maharashtra	Saubhagyakalpataru, Hinginghat.
Tamil Nadu	G.R.D. College, Coimbatore. Main Biocontrol Laboratory, Chingalpattu. Rajashree Sugars and Chemicals Ltd., Theni. Shri Aruna Sugar Mills, Cuddalore. State Research Farm, Bhavani.
Uttar Pradesh	Daurala Sugar Works, Meerut.

procedures for establishment and growth are then followed. This method also helps in *in situ* hardening and good establishment and acclimatization of seedlings to the local conditions.

3.2 SUGARCANE MICROPROPAGATION IN OTHER ASIA-PACIFIC COUNTRIES

3.2.1 Australia

The David North Plant Research Centre, Bureau of Sugar Experiment Station, Brisbane, Australia (BSES) developed a micropropagation technology in 1998-99, designated as SmartSett, for rapid clonal propagation of sugarcane (Geijskes *et al.*, 2003).

The SmartSett micropropagation technology involves the following steps:

- The immature leaf whorls used as explants are sliced and incubated in the dark at 25°C for 12 to 14 days on MS basal media containing growth regulators. Direct development of plantlet occurs.
- Developing plantlets are then transferred to MS medium without growth regulators and placed in a 12 h light cycle at 25°C.
- The medium is changed every two weeks.
- Separation of plantlets into small groups is made to reduce competition and to allow further growth.

After 10 to 12 weeks in culture, plantlets are acclimatized. The hardening of plantlets is done in seedling trays containing a mixture of 2 parts of peat: 2 parts of perlite: 1 part of sand. Before planting in this mixture, excess medium sticking on the plantlets is removed. The trays are kept in a glasshouse at high humidity under shade for one week. After another week, the seedlings are transferred to poly-tunnels for a further period of four weeks. Watering is done twice a day during this period. By applying this procedure,

seedlings are established up to 95 per cent. Airlifting of seedlings and onsite acclimatization helps in overcoming losses due to transportation stresses.

Trials conducted with SmartSett seedlings and plants produced through one-eye setts at harvest revealed that while there was some genotypic effect, the plants of the two groups could not be statistically differentiated (Mordocco, 2006). A yield of 101 t/ha and commercial cane sugar (CCS) of 15.17 per cent of SmartSett seedlings was comparable to the data from traditional sett propagated material of 104 t/ha cane yield and CCS of 15 per cent to 15.5 per cent despite the seedlings having been planted late in the planting season.

The limitation of the technology is reported to be the current cost of production of Australian $ 0.50 per plant. The major part of the cost derives from the labor required for transfer of the cultures on a two-week cycle. Process automation or semiautomation may reduce the cost in future.

Currently BSES is working to make SmartSett a reality for the industry within the next year. At present about eight hectare of SmartSett propagated seed is available (Mordocco, 2006).

3.2.2 Philippines

The Philippine Sugar Research Institute Foundation, Inc. has been promoting since 1998 a micropropagation technology based on the use of shoot tips as explants (http://www.bic.searca.org/news/2005/apr/phi/14.html). The explant containing culture vessels are placed inside a rotary shaker with continuous light. Initial shoots develop within 26 to 65 days which are separated and transferred to a fresh medium for shoot multiplication. Following two multiplication cycles at two-week intervals, rooting is induced in the plantlets. The plantlets are transferred to the nursery where rooted plantlets are placed in plastic trays using sterilized compost based media on sand boxes. The plantlets are placed under seedling sheds for two weeks, and transferred to an open rack for another four to six weeks. Finally, the plantlets are transferred to irrigated seedbeds. After six months of crop growth and following regular fertilization and maintenance schedules, the cane stalks are cut into seed pieces and distributed to farmers.

SUGARCANE ARTIFICIAL SEED

An artificial seed comprises meristematic tissue enclosed in a solid covering, a process called 'encapsulation' (Figure 8a). The covering made of polymer material is permeable to air and soluble in water. The procedure of encapsulation in sugarcane involves the following steps:

1. Production of micropropagules through shoot tip culture.
2. Separation of robust axillary shoots up to a size of 0.5 cm. Care should be taken not to damage the base of the shoots where the meristem is situated.
3. Encapsulation of shoots using a 3 per cent solution of sodium alginate prepared in distilled water or in MS medium. The micropropagules are dipped in this solution and placed in 2.5 per cent calcium chloride solution for 30 min with occasional agitation. The encapsulated micropropagules can be stored up to 20 days under culture room conditions. Neelamathi *et al.* (2007) have demonstrated that these can be stored in distilled water at 10°C for 60 days with good regeneration.

The encapsulated micropropagules can be regenerated when required by inoculating them on MS media supplemented with 1.07 mg/l kinetin and 0.5mg/l NAA at 25°C and under illumination for 16 hrs (Figure 8b). From this stage onwards regular micropropagation procedure is followed.

The encapsulated micropropagules have the advantage of easy transportability even to distantly located commercial micropropagation laboratories. However, the technology is not being used on a commercial scale since leading micropropagation laboratories are not providing this service currently.

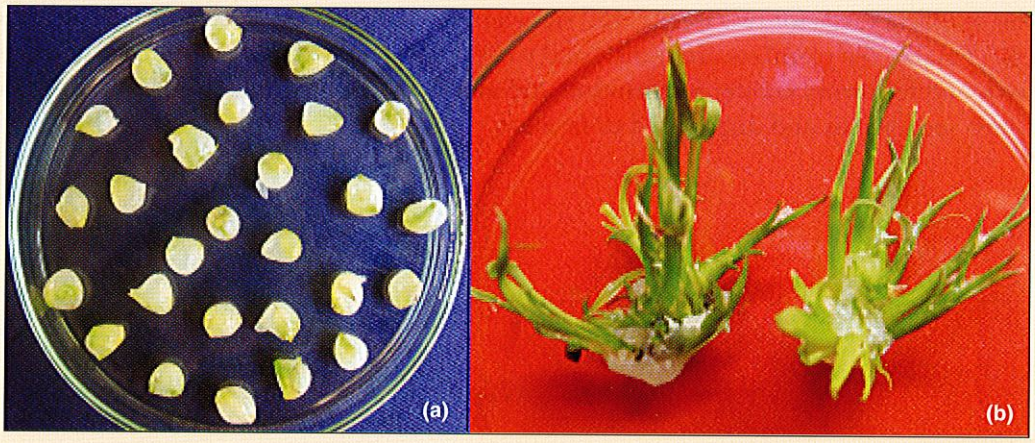

Figure 8. *Sugarcane artificial seed. a) Encapsulated micropropagules. (b). Shoot regeneration from encapsulated micropropagules.*

4. PROSPECTS OF SUGARCANE MICROPROPAGATION FOR QUALITY SEED PRODUCTION IN ASIA-PACIFIC

The sugarcane micropropagation and establishment protocols detailed in the previous chapters are standardized and tested for rapid mass production of healthy planting material and its field establishment. Compared to conventional seed production, the micropropagation-based seed production system developed at SBI enables 3-4 times greater area coverage (Table 7). Hence, the technique is highly desirable for rapid seed production of newly introduced varieties. Further, seed renovation of old, well adapted varieties through production of clean, disease-free material helps in restoring the original vigor and productivity of the varieties. The technology will also provide an opportunity to implement a well-programmed varietal scheduling for maintaining high recovery throughout the season (Jalaja, 2001).

These advantages of micropropagation-based seed production are well appreciated and the technology has been adopted with success in several countries of the Asia-Pacific region.

Table 7. Seed production schedules in sugarcane through conventional and micropropagation methods

Conventional method	Micropropagation method
Heat treated setts	**Heat treated setts**
Breeders' (primary) seed nursery	Micropropagation through shoot tip culture
Area covered: 1 ha Duration: 7 to 10 months Rate of multiplication: 1:6	Rate of multiplication: 1:200,000 Duration: 12 months
Secondary (foundation) seed plots	**Secondary (foundation) seed plots**
Area covered: 6 ha	Area covered: 14 ha
Duration: 7 to 10 months Rate of multiplication: 1:6 to 1:7	Duration: 7 to 10 months Rate of multiplication: 1:10
Commercial seed plots	**Commercial seed plots**
Area covered: 42 to 70 ha	Area covered: 140 ha
Duration: 7 to 10 months Rate of multiplication: 1:6 to 1:7	Duration: 7 to 10 months Rate of multiplication: 1:10
Commercial seed	**Commercial seed**
Area covered: 294 to 490 ha	Area covered: 1400 ha

4.1 SOME SUCCESS STORIES

4.1.1 India

The advantages of the micropropagation technology for quality seed production are now well appreciated by the sugar industry in India. Several sugar mills, research organizations, agricultural universities and private entrepreneurs have set up facilities for sugarcane micropropagation. The Department of Biotechnology, Government of India (DBT) has constituted a Consortium on Micropropagation Research and Technology Development (CMRTD) to provide the necessary know-how to interested users in India. The Government of India also provides financial assistance to various organizations to set up commercial micropropagation facilities. Quality seed produced through micropropagation is being used in major sugarcane growing states, Punjab, Haryana, Uttar Pradesh, Gujarat, Maharashtra, Andhra Pradesh, Karnataka and Tamil Nadu.

Sugarcane micropropagation on a commercial scale in the state of Tamil Nadu was initiated in early 1990 following heavy mortality due to the outbreak of red rot in the widely grown varieties, CoC 671 and CoC 92061. In coastal areas of Tamil Nadu where the problem was more severe, tissue culture raised plants of resistant varieties were used. During 1995-96, CoC 90063, a newly released, red rot resistant variety, was multiplied in about 23 ha using micropropagation-raised seedlings. Subsequently, several new varieties such as Co 86010, Co 85011, CoSi 95071 and CoSi 95076 were similarly multiplied and inducted in the seed production system. Presently, a number of sugar factories in Tamil Nadu meet their seed requirements from micropropagated plants. Prominent among these are the Rajashree Sugars and Chemicals group of industries and EID Parry (India) Ltd.

The Rajashree Sugars and Chemicals Limited Teni, Tamil Nadu established a sugarcane micropropagation laboratory in 1998 under a consultancy program with SBI. The current plant production capacity of the laboratory is 40,000 plants per month (Lakshmanan, 2006). Tissue culture raised plants at pre-hardening stage are transported in containers to the mill farms located in various sugarcane growing zones. The plants are potted and hardened at the mill farms or in specially selected and trained farmers' fields. Canes obtained from these plants are used for raising primary seed which is multiplied through two cycles to yield commercial seed. The entire area planted at the mill farms comprising 9,700 ha is planted with seed produced through micropropagation. An increase in cane yield of 4.84 t/ha over the conventionally raised crop has been recorded. The cost of micropropagation-based seed production is US$ 0.05 per seedling.

The micropropagation-based seed production technology is also widely accepted by the farmers who have obtained higher seed yields; an average of approximately 0.9 million two-budded setts per hectare using micropropagated plants as against 0.7 million two-budded setts obtained from conventionally raised material (Lakshmanan, 2006). Multi-ratooning in micropropagation-raised crop, due to absence of sett-borne diseases, has also been recorded. Another major advantage of adopting micropropagation was the faster

introduction of three newly identified varieties, Co 92012, Co 93001 and Co 94010 which otherwise would have taken several years for reaching the stage of commercial cultivation (Lakshmanan, 2006).

Much progress in adoption of sugarcane micropropagation technology has been made by the state of Maharashtra where sugarcane micropropagation facilities have been developed in both private and public sectors. The largest facility having a capacity to produce two million micropropagated seedlings per annum has been set up by Vasantdada Sugar Institute, Pune established by the sugarcane-growing members of the cooperative sugar mills in Maharashtra state. The Institute distributes more than a million hardened seedlings every year to farmers for breeders' seed production (Table 8). The Institute has also developed complete package of practices for producing commercial seed through the three-tier nursery program using tissue culture seedlings. The institute has drawn up programs to cover the entire sugarcane growing area in Maharashtra with tissue culture seedlings in four year cycles for which the sugar industry and sugarcane farmers are showing considerable enthusiasm (Nerkar, 2006; Tawar, 2006).

Table 8. Sugarcane micropropagated seedling production and supply by Vasantdada Sugar Institute, Pune, India

Year	Production	Distribution
1998-1999	109,789	51,250
1999-2000	281,627	98,245
2000-2001	529,599	367,489
2001-2002	1,621,216	844,835
2002-2003	2,424,441	1,181,681
2003-2004	1,384,208	1,074,058
2004-2005	1,980,274	1,093,311
2005-2006	1,921,050	1,386,980
Up to December 2006	1,568,000	1,025,000
Total	**11,820,204**	**7,122,849**

Source: Tawar (2007)

Following procedures are adopted to ensure quality commercial seed production from tissue culture raised plantlets:

- Specialist breeders provide certified nucleus seed material of sugarcane varieties to be propagated through tissue culture.
- Inspection of the nucleus seed material for freedom from disease and pest incidence is done before planting.
- The nucleus seed undergoes hot water treatment and is planted in the designated and well-maintained field at the campus.
- Monthly inspection is done to monitor the seed plot nursery.

- Random monthly checks are carried out in tissue culture laboratory for freedom from contamination.
- Random testing of tissue culture raised plants is done for genetic fidelity, using polymerase chain reaction (PCR).
- The tissue culture raised plantlets are labeled batch-wise to monitor their production, supply, and nursery and field performance.
- Soil used in greenhouse is tested for freedom from nematodes.
- Inspection of plantlets in greenhouse and hardening facility, and disease control measures, whenever required, are undertaken regularly. Multiplex PCR based tests are conducted for grassy shoot and sugarcane mosaic diseases.
- Well-planned field maintenance schedules are followed, including application of fertilizer and weed control measures as per the recommended package of practices.

In the state of Gujarat, initially three cooperative sugar mills established micropropagation facilities with the help of SBI after sugarcane production was badly affected by red rot. Disease-free seed material of CoC 671, the popular sugarcane variety of the area, and other varieties resistant to red rot was rapidly produced through micropropagation. Gujarat is now free of red rot epidemic. Currently, the Navasari Regional Centre of the Gujarat Agricultural University produces 60,000 micropropagated plants per year, sufficient to plant six hectares of breeders' seed and distribute the same to farmers to produce 600 ha of commercial seed which would cover 6,000 ha of commercial sugarcane production area (Patel, 2006). Tissue culture laboratory at Shree Chaltan Vibhag Khand Udyog Sahakari Mandli Ltd., Chaltan produces about 100,000 micropropagation-raised seedlings per year and supplies these to farmers for producing breeders' seed. Sree Khedut Sahakari Khand Udyog Ltd., Bardoli produces 50,000 seedlings per month; along with those obtained from other sources, about 95 ha of breeders' seed plots are raised every year from micropropagated plants. The cost of tissue culture raised seedlings from these laboratories ranges from US$ 0.11 to US$ 0.18 per seedling.

Tissue culture laboratories have been established with the financial assistance of Punjab State government in four sugar mills of Punjab Sugar Federation. The total production capacity of these mills is 500,000 seedlings per year, sufficient to plant approximately 40 ha of breeders' seed. Tissue culture raised seedlings are sold to the farmers at a subsidized rate to promote the use of technology.

The Haryana Agricultural University, Hissar and Haryana Sugar Federation have set up sugarcane micropropagation facilities for rapid multiplication of newly released varieties like CoH 92, Co 89003, CoS 8436, CoS 96268, CoH 56 and CoH 99. During the past five years, the Haryana Cooperative Federation has grown two million micropropagated plants to cover about 200 ha of seed nursery. The Haryana Sugar Federation has now set up its own micropropagation laboratory with a capacity of one million seedlings per year to meet the growing seed demand.

Five sugar mills in the state of Uttar Pradesh had also established micropropagation facilities. However, one major laboratory was closed down because adequate attention was not given to micropropagation protocol.

Besides the micropropagation facilities developed by the sugar industry, several other tissue culture laboratories in India produce sugarcane seedlings on a commercial scale. One such facility, Growmore Biotech, Hosur, Tamil Nadu produces between two million to three million seedlings per year, with a program to raise production to 10 million seedlings. The plants are delivered at the doorstep of farmers at a cost of US $ 0.07 to US $ 0.08 per plant; setts produced from 250 micropropagated plants are sufficient for planting one acre (0.405 ha) field area in seven months (Barathi, 2006). This scheme is reported to have become popular with the farmers.

4.1.2 Other Asia-Pacific Countries

In Australia, orange rust disease was first reported during January 2000 in the varieties cultivated in 89 per cent of the central region of the Australian sugarcane belt (Mordocco, 2006). There was an urgent need to replace 90,000 ha of area with orange rust resistant clones. Q 205 was resistant to orange rust disease and was an agronomically best-suited clone for the zone. Q 205 was released for cultivation in 2002 but sufficient quantity of planting material for distribution to the farmers was not available at that time.

Through conventional method of multiplication one stalk of cane produces 10 to 20 plants if whole cane is planted and 5 to 10 plants if setts are planted. This was too slow for an immediate replacement of existing orange rust susceptible varieties with resistant clone Q 205. The SmartSett process is fast with seedlings ready for planting in 12 to 14 weeks. In two years, 2001-02, about 10,000 seedlings of clones Q196 A and Q 205, and seedling selections 85N 1205 and 87A 1413 were produced for planting in the central region. In the year 2003, a scale up of up to 50,000 plants at a time had been possible. This helped the sugar industry to rapidly saturate the area with disease-resistant varieties which through the conventional system would have taken two to three years.

Sugarcane micropropagation in the Philippines was initiated in 1991 soon after some of the scientists were trained in micropropagation technique at Hawaiian Sugar Planters' Experiment Station, Hawaii (Barredo, 2006). The Philippine Sugar Research Institute Foundation, a private initiative of several stakeholders in the sugar industry realized the significance of this tool in sugarcane improvement and is now providing support for the whole Philippine sugar industry. The new high yielding varieties are micropropagated and shipped to different mill districts through couriers in boxes each containing 5,000 plants to be delivered within 24 hours. During the first five years (1998-2003) more than four million plantlets were distributed to 26 Mill District Coordinating Councils throughout the country (http://www.bic.searca.org/news/2005/apr/phi/14.html). Each recipient center grows these plants in nurseries before transplanting to the field. The new varieties are thus distributed very rapidly for adoption. Philippine government's Sugar Regulatory Administration

has established five laboratories around the country to provide seed from micropropagated plants for the sugar industry (Barredo, 2006). With effective delivery of production technologies through the Mill District Development Councils, sugarcane production has increased by 19.33 per cent from 21.67 mt in 1998 to 25.87 mt in 2004.

In Pakistan, micropropagated sugarcane seedlings of nine varieties are being produced and marketed by AgriBiotech since 2001. The company grows micropropagated seedlings with the help of contract farmers and supplies seed as cuttings for commercial cultivation. Sugarcane micropropagation is also being commercially utilized in China, the exact area covered with micropropagated seed is, however, not readily available.

4.2 THE WAY AHEAD

Between 2005 and 2015, total world trade in sugar is predicted to increase by 3 per cent with increasing imports in Asia being made by China, Japan and South Korea (Koo and Taylor, 2006). Exports are predicted to increase from Australia and Thailand due mainly to increase in sugar prices driven by higher sugar consumption as also substantial diversion of sugarcane for ethanol production. Hence, there is reason for enhancing production of the crop in Asia-Pacific countries despite high sugarcane production during 2006-2007 and the consequent depression in sugar prices (FAO, 2007). With limited land available for sugarcane area expansion, production increase must be substantially based on improving productivity through development of improved varieties, better seed quality and better crop management practices. Micropropagation provides means of producing uniform high quality, disease free seed at a substantially faster rate than the conventional seed production system. However, a number of issues would need to be addressed to render the technology more useful and widely acceptable.

Somaclonal variations in tissue culture raised sugarcane plants have been reported by some workers (Rani and Raina, 2000; Zucchi *et al.*, 2002). Since such variations could lead to instability in crop growth and yield, the recommended micropropagation protocols should be thoroughly tested for production of uniform and true to type plantlets. Following these protocols very strictly during large-scale micropropagation is also necessary for ensuring desirable field growth and propagation.

Attempts to promote excessive multiplication and prolonged culture cycles often lead to plants with aberrant morphology. These epigenetic changes caused due to culture environment and hormonal imbalances generally express by producing plants with profuse tillering, thin canes, short internodes, narrow and short leaves, germination of buds at the nodes throughout the length of the cane and grass-like clumps. A quality control mechanism should be in place to ensure that proper micropropagation procedures are followed. For this purpose, development of step-wise guidelines for micropropagation-based plant production, and practical training of the staff are very helpful.

For efficient transfer of micropropagation technology and its acceptance by the sugarcane farmers, it is essential to set up the micropropagation facilities as an integral component of

sugar industry. The cane development personnel of the sugar mills must be trained to handle the entire process of three-tier seed production chain. The basic cultures being supplied for seed production should be true to type, of desired uniformity and disease indexed to ensure that the plantlets are free of diseases and pests.

Sugarcane varieties reach the release stage generally after 14 or 15 years from the time they are developed from true seed, a time frame during which the stock is likely to get infected with diseases and pests. If disease-free cultures are available at the time of release, totally clean seed of the new variety can be made available for distribution to the farmers. In countries where a large number of sugar mills are in operation, it is desirable to constitute zone-wise networks of sugarcane micropropagation facilities so that multiplication of new varieties can be done as per the requirements of the mills of a particular zone. The hardening facilities should also be established zones-wise to facilitate ready availability of seedlings for the primary seed plots established in each zone.

The price of micropropagated seedlings is often too high for direct field planting. The technology detailed in this report mitigates this problem by following the micropropagation cycle with two cycles of conventional seed multiplication, which results in significant reduction in per unit seed production cost. Additional cost reduction can be achieved by adopting low-cost alternatives in the tissue culture facility (Anon, 2004). Replacing expensive culture vessels with household jars and other glassware, use of commonly used sugar in place of expensive sucrose and alternatives to gelling agents can substantially reduce the cost of plantlet production. Such low-cost technologies are reported to have been successfully employed in Cuba for micropropagation of sugarcane (Ahloowalia, 2004). Ordinary village houses are converted into tissue culture facilities employing local labor and using low-cost media and containers. Natural sunlight is utilized to provide light for growing cultures.

Micropropagation based on bioreactor technology can help in reducing production costs by saving on energy, space and labor requirements. However, use of disease-free explants and maintenance of aseptic cultures is essential for success of bioreactor-based micropropagation. Further, care needs to be taken in developing countries so that the adoption of labor-saving technologies does not lead to loss of job opportunities, particularly in the rural sector. Hence, adoption of cost-saving approaches that do not adversely affect the quality of planting material as well as employment opportunities would be ideal for developing countries.

Producing good quality, disease-free sugarcane seed through micropropagation is now successful in Australia, India, Pakistan, and the Philippines. Efforts are being made in Bangladesh, Indonesia, Thailand and Sri Lanka to introduce the technology for rapid propagation of new varieties and for seed production. As detailed earlier, the Philippine Sugar Industry has moved a step ahead in disseminating the technology throughout the country. A similar system may be adopted with suitable modifications by other countries of the region to accelerate the adoption of technology and delivery of the benefits to farmers.

The sugar industry needs to provide the required support by establishing micropropagation facilities, adopting appropriate technology and popularizing it.

It is hoped that the above-suggested refinements will accelerate the pace of integrating micropropagation in the formal sugarcane seed production system. Availability of quality planting material in adequate quantities will substantially contribute to increasing sugarcane productivity and farmers' incomes. APCoAB will contribute to these efforts by disseminating information and promoting adoption of appropriate, environmentally safe biotechnologies that benefit farmers and other stakeholders. This will be done through publication of status reports and success stories, and promoting regional networking of research and development programs, and public-private partnerships.

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Annexure-I

SEED CANE STANDARDS FOR SUGARCANE IN INDIA

(As approved by the Government of India)

Age of the seed cane crop at harvest for seed purpose shall be 6 to 8 months and 8 to 10 months for the sowing in tropics and subtropics, respectively. Seed cane material should be undamaged and reasonably clean. Each node of seed cane shall bear one sound bud. The number of nodes without sound buds shall not exceed 5 per cent (by number) of the total number of buds per seed cane. The number of buds which have swollen up or have projected beyond one centimeter from the rind surface, shall not exceed 5 per cent (by number) of total number of buds.

1. Application and Amplification of General Seed Cane Certification Standards

The general Seed Cane Certification Standards are basic and together with the following specific standards constitute the standards for certification of sugarcane seed cane.

The certified classes will be produced from seed canes and/or mericlones whose sources and identity may be assured and approved by the certification agency.

2. Land Requirements

A. A seed crop of sugarcane shall not be eligible for certification if planted on land on which sugarcane was grown in the previous season.

B. Land/seed crop shall be kept free from sugarcane residues and drainage from other sugarcane fields.

3. Heat Treatments

Foundation stage shall be raised from heat-treated seed cane.

4. Field Inspection

A minimum of three inspections shall be made as under:

Stage-I. The first inspection shall be made at 45 to 60 days after planting in order to verify isolation and detect volunteer plants, designated diseases and pests and other relevant factors.

Stage-II. The second inspection shall be made at 120-130 days after planting to verify off-types, designated diseases and pests and other relevant factors.

Stage-III. The third inspection shall be made 15 days prior to the harvesting of seed canes to verify the age of cane, off-types, designated diseases and pests and other relevant factors.

5. Field Standards

A. *General Requirements*

Isolation: The sugarcane seed production fields shall be isolated from other fields with a minimum distance of 5 m to avoid mechanical mixture of other varieties.

B. *Specific Requirements*

S. No.	Factors	Stage of field Inspection	Maximum permissible limit (%)	
			Foundation seed	Certified seed
1	**Off-type**	I, II, III	None	None
2	**Plants affected by designated diseases**			
	Red rot (*Glomerella tucumanensis* Speg. Arx & Muller)	I, II, III	None	None
	Smut (*Ustilago scitaminea* Sydow)	I II III	0.02* 0.01* None	0.10* 0.10* None
	Grassy shoot (Mycoplasma-like organism)	II III	0.05* None	0.05* None
	Wilt (*Cephalosporium sacchari* Butler)	III	0.01*	0.01*
	Leaf scald *Xanthomonas albilineans* (Ashby) Dowson	II III	0.01* None	0.05* None
3	**Plants affected by designated insect pests**			
	Top borer (*Scirpophaga excerptalis* walker)	II, III	5.0	5.0
	Internode borer (*Chilo sacchariphagus indicus* Kapur)	III	10.0# None**	20.0 None**
	Stalk borer (*Chilo auricilius* Dudgeon)	III	20.0+ None**	20.0 None**
	Plassey borer (*Chilo tumidicostalis* Hampson), Gurudaspur borer (*Acigona steniellus* Hampson), scale insect (*Melanaspis glomerata* Green), mealybug	III	5.0 None**	5.0 None**

* Subject to immediate rouging of the whole clump
** In area where the presence of the pest has not been recorded.
# Around 10% affected buds.
+ Around 0.5% affected buds.

Note:

1. All off-types and diseased plants shall be rouged out along with roots and destroyed.
2. Maximum permissible limits for the stripping of dry foliage shall be 2.0 per cent.
3. The crop should not have more than 10 per cent lodged canes.
4. Seed canes should not have nodal roots. In water-logged areas relaxation may be given up to a maximum of 5 per cent.
5. Moisture in seed cane should not be less than 65 per cent on wet weight basis.
6. Germinability of buds should not be less than 85 per cent.
7. Physical purity of seed should be 98 per cent.
8. Genetic purity of seed should be 100 per cent.

Annexure-II

ORGANIZATION OF SUGARCANE MICROPROPAGATION LABORATORY

The ideal location of sugarcane micropropagation laboratory being established by a sugar mill is within the mill premises. There are a number of advantages of such a location: proximity of planting fields thus avoiding the need for long-distance transportation of seedlings; availability of uninterrupted power supply from the mill; and availability of labor from the mill for various laboratory and field operations. However, care should be taken to locate the laboratory in an area free of dust and ash. The production capacity of the laboratory should be adjusted to the available area for sugarcane cultivation in the mill zone and the crushing capacity of the mill. It is also a good idea to establish one micropropagation facility to meet the requirements of two to three adjoining sugar mills.

The tissue culture laboratory should ideally have six interconnected rooms: 1) general laboratory, 2) media and plant material preparation room, 3) inoculation room, 4) culture storage room, 5) washing room, and 6) autoclave room.

1. General Laboratory

This laboratory should have major equipments like hot air ovens, refrigerators, hot plate, distillation units, deionizing units, and working tables for initial cleaning, trimming and surface sterilization of the plant materials before they are taken to the preparation room. A laboratory sink should be installed in one corner for all cleaning work.

2. Media and Plant Material Preparation Room

This room is used for making stock solutions and media, preparation of plant material, dispensing the media in culture vessels and storing the latter before being taken to autoclave room for sterilization. The room should be air-conditioned, free of dust and provided with washable flooring and walls. The infrastructure required for this room includes: (i) laboratory tables with laminated tops, (ii) refrigerators and deep freezers for storing fine chemicals and stock solutions, (iii) racks for storing chemicals and culture vessels, (iv) filter sterilization units and vacuum pump, (v) automatic media dispenser, (vi) cabinets for storing prepared media, and (vii) trolleys for transporting vessels with media to the autoclave room.

3. Inoculation Room

This room should have two compartments, the first to be used for storing media containing culture vessels, instruments and other articles used for culture inoculation and the second for carrying out the inoculation. The latter should be equipped with laminar flow chambers for providing sterile air across the working area, and trolleys for transporting culture vessels and other items. The inoculation room must remain dust free and should be fumigated regularly to maintain microbe-free environment. Installing high efficiency particulate air (HEPA) filters with pressure module is also recommended to create sterile atmosphere in the inoculation room.

4. Culture Room

This room is used for growing cultures under controlled conditions of temperature, humidity and photoperiod. The required photoperiod for sugarcane micropropagation is eight hours dark and sixteen hours light. The room temperature is maintained at 26°C with the help of air-conditioners. The relative humidity is maintained at above 50 per cent. Fluorescent lamps fitted with timers for adjusting light and dark phases provide light intensity of about 2500 lux. The cultures should be inspected daily and contaminated flasks, if any, removed immediately without opening the lids. Maintaining a dust free, clean environment and restricting access to visitors are essential to minimize contamination.

5. Washing Room

The washing room should be located away from inoculation room and culture room. It should be provided with workbenches, large washing sinks, running water, water heating and deionizing units, and ovens to dry the washed laboratory wares. Sufficient number of plastic buckets or tubs to presoak the laboratory wares should be provided. A number of well-covered racks to ensure protection from dust must be available to dry the washed glassware. Laboratory detergents are used for washing glassware under running tap water followed by a final rinse in deionized water. Automatic washing and drying machines have, however, replaced manual washing in many laboratories.

6. Autoclave Room

It is desirable to keep the autoclaves in a separate room near the media preparation and dispensing room. There should be one or more horizontal autoclaves for steam sterilization of culture vessels with media. Another horizontal or vertical autoclave may be installed exclusively for autoclaving the culture vessels with contaminated cultures before taking them to the washing facility.

A list of laboratory consumables required in the micropropagation laboratory is given below:

Borosilicate culture tubes (25 mm x 150 mm)	For initiating shoot tip cultures
Erlenmeyer flasks (250 ml)/ Culture jars/bottles	For culturing shoots
Glassware/plastic wares like conical flasks, volumetric flasks, beakers, measuring cylinders, graduated pipettes, pasture pipettes, test tubes, Petri dishes, reagent bottles	For various laboratory activities
Stainless steel forceps, scissors, scalpels, scalpel blade holders, sterile surgical blades, syringes, needles, Petri dishes, watch glasses, magnifiers	For explant preparation and aseptic operations inside the laminar flow chamber
Filter paper (Whatman No. 1), autoclave indicator tapes, polypropylene closure caps, cotton plugs, tissue paper, parafilm, aluminum foils, glass rods, blotting paper, rubber bands, autoclavable bags	For general purpose laboratory use
Laboratory coats, protective hoods, goggles, slippers, latex gloves	For the use of the laboratory workers
Soap, absorbent cotton, bandage cloth, brown paper, sieves	For laboratory use
Polythene bags, earthen pots, plastic pots, tags and labels	For nursery operations
Glass marking pens, note books, data sheets, measuring scales, eraser, tapes of different colors	For recording data, labeling
First aid box, fire extinguishers	For laboratory safety

SOME DO'S AND DON'TS

Sterilization of Glassware and Other Equipment

Do's

- While cutting canes and preparing setts, the knives should be disinfected by dipping them in any commercial disinfectant solution.
- Both new and used culture vessels should be autoclaved before washing with detergent.
- Contaminated culture vessels should be autoclaved without opening the lid. Autoclaving should be done for 1hr at 121°C at 15 psi.
- Washing of glass culture vessels should be done carefully to avoid deep scratches.

Don'ts

- Never open the lid of infected containers to save uninfected plants.
- Glass vessels with deep scratches should not be used for culture.
- Do not use vessels with deposits for culture.
- Do not dry glassware in open space where dust settles.

Media Preparation

Do's

- Autoclaving of the media should be done for 20 minutes at 121°C at 15 psi. The autoclaved medium should be kept aside for 2-3 days before inoculation so that microbial contamination, if any, is detected before the medium is put to use.
- While autoclaving the culture medium it is better to fix indicator tapes at different places on the vessel.
- The autoclaved vessels with medium should be stored in a clean, dust-free place.
- pH of the medium should be adjusted carefully by adding acid/alkali drop by drop.
- Sucrose is added to the medium after adjusting the pH.

Don'ts

- Do not dump the glassware in the autoclave. Leave ample space for steam circulation.

Collection of Material

Do's

- The sugarcane variety to be multiplied through micropropagation should be obtained from a reliable source.
- Explants should be collected only from a vigorously growing, healthy crop raised through conventional stem cuttings.
- The explant source nursery should be planted in a staggered manner to ensure continuous supply of sugarcane tops of specified age as per the requirements and the capacity of micropropagation laboratory.

Don'ts

- Do not collect explants from a crop derived from tissue culture multiplied plants or from ratoon crops.
- Do not collect tops during or soon after rains as chances of contamination will be high.

Sterilization of the Material

Do's

- The shoot apex should be quickly excised and transferred to the filter paper support on the medium to avoid excessive browning of the explant.
- As and when the shoot apex medium gets discolored, change to fresh medium to avoid cessation of growth.

Don'ts

- During preparation of shoot apices for culture, do not exert too much pressure while removing the tender leaf sheaths.
- To avoid undesirable tissue softening, the material should not be left for long in sterilizing solution.

Shoot Multiplication

Do's

- To ensure fast multiplication, the concentration of 6 BAP may be kept at a high level (6 mg/l) for 15 days. Subsequently transfer the developing shoots to 0.25 mg/l to avoid development of grass-like plants of poor quality.
- Ball formation due to excessive shoot multiplication and lack of elongation can be avoided by subculturing in media without 6 BAP. Adding 0.5 mg/l gibberellic acid will help in shoot elongation.

Don'ts

- Do not culture the shoots in high concentrations of 6 BAP for long periods in an attempt to achieve high multiplication rates. This is the major cause of variation observed in the field grown tissue culture raised plants.
- Avoid growing more than 25 plantlets per vessel in a bunch to obtain healthy plants.

Rooting

Do's

- Only plants with 4 to 5 leaves and sturdy bases should be carried forward to rooting.
- Only when a well developed fibrous root system is developed should the plants be transferred to soil mixture in polybags.

Don'ts

- Do not break the bases of plants at the time of separating them for rooting.

Planting in Polybags

Do's

- Plants should be washed well in running tap water to remove all traces of the medium.

Don'ts

- Excess watering should be avoided during irrigation of the polybags.

Field Planting

Do's

- Field should be kept ready well before planting with adequate application of organic manure.
- After planting, the field should be inspected at regular intervals for off types which should be removed promptly.

Don'ts

- Tissue culture seedlings should not be planted in a field where sugarcane crop has been grown in the previous year.
- Do not use ratoon crop for breeders' seed production.

Annexure III

CULTURE MEDIA USED AT DIFFERENT STAGES OF SUGARCANE MICROPROPAGATION

(for one litre)

Ingredients	Shoot Apex Medium*	Shoot Multiplication Medium**	Rooting Medium***
Ammonium nitrate	–	1640.0 mg	–
Boric acid	1.5 mg	6.2 mg	1.5 mg
6-benzyl amino purine	–	6.0 mg	–
Calcium chloride	–	440.0 mg	–
Calcium nitrate	300.0 mg	–	300.0 mg
Calcium pantothenate	–	–	1.0 mg
Cobalt chloride	–	0.025 mg	–
Coconut water	100.0 ml	100.0 ml	–
Cupric sulphate	–	0.25 mg	–
Ferric EDTA	36.7 mg	36.7 mg	36.7 mg
Gibberillic acid	0.5 mg	0.5 mg	–
Glycine	2.0 mg	2.0 mg	3.0 mg
Indole-3-butyric acid	1.0 mg	–	–
Kinetin	1.07 mg	1.07 mg	–
Magnesium sulphate	720.0 mg	370.0 mg	720.0 mg
Manganese sulphate	7.0 mg	22.5 mg	7.0 mg
Mesoinositol	100.0 mg	100.0 mg	–
α-Napthaleneacetic acid	–	0.5 mg	1.0 mg
Nicotinic acid	–	0.5 mg	0.5 mg
Potassium chloride	65.0 mg	–	65.0 mg
Potassium dihydrogen orthophosphate	–	170.0 mg	–
Potassium iodide	0.75 mg	0.83 mg	0.75 mg
Potassium nitrate	80.0 mg	1900.0 mg	80.0 mg
Pyridoxine	–	0.5 mg	0.1 mg
Riboflavin	–	–	1.0 mg
Sodium dihydrogen orthophosphate	16.5 mg	–	16.5 mg
Sodium molybdate	–	0.25 mg	–
Sodium sulphate	200.0 mg	–	200.0 mg
Sucrose	20.0 g	20.0 g	20.0 g
Thiamine	–	0.1 mg	0.1 mg
Zinc sulphate	3.0 mg	8.6 mg	3.0 mg
pH	5.8	5.8	7.0

* Based on White (1963); ** Based on Murashige and Skoog (1962); *** Based on White (1963)

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

Achieving food security for all is at the heart of FAO's efforts – to make sure people have regular access to enough high-quality food to lead active, healthy lives.

FAO's mandate is to raise levels of nutrition, improve agricultural productivity, better the lives of rural populations and contribute to the growth of the world economy.

FAO provides the kind of behind-the-scenes assistance that helps people and nations help themselves. If a community wants to increase crop yields but lacks the technical skills, we introduce simple, sustainable tools and techniques. When a country shifts from state to private land ownership, we provide the legal advice to smooth the way. When a drought pushes already vulnerable groups to the point of famine, we mobilize action. And in a complex world of competing needs, we provide a neutral meeting place and the background knowledge needed to reach consensus.

The activities of the Food and Agriculture Organization (FAO) comprise four main areas:

Putting information within reach. FAO serves as a knowledge network. We use the expertise of our staff – agronomists, foresters, fisheries and livestock specialists, nutritionists, social scientists, economists, statisticians and other professionals – to collect, analyse and disseminate data that aid development. A million times a month, someone visits the FAO Internet site to consult a technical document or read about our work with farmers. We also publish hundreds of newsletters, reports and books, distribute several magazines, create numerous CD-ROMS and host dozens of electronic fora.

Sharing policy expertise. FAO lends its years of experience to member countries in devising agricultural policy, supporting planning, drafting effective legislation and creating national strategies to achieve rural development and hunger alleviation goals.

Providing a meeting place for nations. On any given day, dozens of policy-makers and experts from around the globe convene at headquarters or in our field offices to forge agreements on major food and agriculture issues. As a neutral forum, FAO provides the setting where rich and poor nations can come together to build common understanding.

Bringing knowledge to the field. Our breadth of knowledge is put to the test in thousands of field projects throughout the world. FAO mobilizes and manages millions of dollars provided by industrialized countries, development banks and other sources to make sure the projects achieve their goals. FAO provides the technical know-how and in a few cases is a limited source of funds. In crisis situations, we work side-by-side with the World Food Programme and other humanitarian agencies to protect rural livelihoods and help people rebuild their lives.

ASIA-PACIFIC ASSOCIATION OF AGRICULTURAL RESEARCH INSTITUTIONS

Asia Pacific Association of Agricultural Research Institutions (APAARI), established in 1990 at the initiative of FAO, is an apolitical, neutral, non-profit forum of Agricultural Research Institutions, National Agricultural Research Systems (NARS) in the Asia-Pacific region, in the pursuit of common objectives.

The 'Mission' of APAARI is to promote the development of national agricultural research systems in the Asia-Pacific region through facilitation of intra-regional and inter-institutional, and international cooperation/partnership.

The overall objectives of APAARI are to foster agricultural research for development in the Asia-Pacific Region so as to help address the concerns of hunger, poverty, environmental degradation and sustainability of agricultural production. More specifically, the objectives are as follows:

a. Promote the exchange of scientific and technical know-how and information in agriculture;

b. Encourage the establishment of appropriate co-operative research and training programs in accordance with identified regional, bilateral or national needs and priorities;

c. Assist in prioritizing NARS/Regional needs, strengthening of research organizational and management capabilities of member institutions including information and communication technology;

d. Strengthen cross-linkages among national, regional and international research centers and organizations, including universities, through involvement in jointly planned research and training programs; and

e. Promote collaborative research among member institutions, including need based support to regional research networks.

ASIA PACIFIC CONSORTIUM ON AGRICULTURAL BIOTECHNOLOGY

The Asia Pacific Consortium on Agricultural Biotechnology (APCoAB), was established in 2003 under the umbrella of Asia-Pacific Association of Agricultural Research institutions (APAARI). APCoAB has the mission to "harness the benefits of agricultural biotechnology for human and animal welfare through the application of latest scientific technologies while safeguarding the environment for the advancement of society in the Asia-Pacific Region".

APCoAB main thrust is:

1. To serve as a neutral forum for the key partners engaged in research, development, commercialization and education/learning of agricultural biotechnology as well as environmental safety in the Asia-Pacific region.
2. To facilitate and promote the process of greater public awareness and understanding relating to important issues of IPRs, *sui generis* systems, biosafety, risk assessment, harmonization of regulatory procedures, and benefit sharing in order to address various concerns relating to adoption of agricultural biotechnology.
3. To facilitate human resource development for meaningful application of agricultural biotechnologies to enhance sustainable agricultural productivity, as well as product quality, for the welfare of both farmers and consumers.

SUCCESS STORIES AND STATUS REPORTS PUBLISHED BY APAARI AND APCoAB

- Tissue Cultural Innovations for Production of Quality Potato Seed in Asia-Pacific Region
- Bt Cotton in India – A Status Report
- Selected Success Stories on Agricultural Information System
- Commercialization of Bt corn in the Philippines: A Status Report
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