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Review

Sugarcane seed production- Indian Scenario

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ABSTRACT

India is the largest producer of sugar in the world. In terms of sugarcane production, India and Brazil are almost equally placed. In Brazil, out of the total cane available for crushing, 45% goes for sugar production and 55% for the production of ethanol directly from sugarcane juice. This gives the sugar industry in Brazil an additional flexibility to adjust its sugar production keeping in view the sugar price in the international market as nearly 40% of the sugar output is exported. The annual projected growth rate in the area under sugarcane at 1.5% per annum has doubled during the last five years. This is because it is considered to be an assured cash crop with good returns to the farmers vis-a-vis other competing crops. A supply of good quality low cost seed can be an effective means of improving production; even in the absence of new and better varieties, adequate supplies of reliable seed of existing varieties would have a favorable effect. In addition the effect of plant breeding on production will depend on the efficiency of seed distribution: only if the improved varieties reach the farmer through a reliable seed distribution channel will be able to use them effectively to produce more.

Key Words: *Micro-propagation, Seed production, Sugar, Sugarcane, Tissue culture*

INTRODUCTION

Sugarcane is one of the most efficient converters of solar energy into sugars and other renewable forms of energy. Presently it has emerged as a multi-purpose commercial crop providing not only sugar but also a series of products such as paper, ethanol and other alcohol derived chemicals, animal feed, antibiotics, particle board, bio-fertilizer and raw material for generating electricity. Globally sugar consumption has been increasing at of 2.25 per cent per annum. Energy from ethanol has emerged as a key product from the sugarcane industry globally. With ever increasing oil prices, more and more countries are encouraging ethanol production form sugarcane as an environment-friendly fuel. 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. 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 while in India it is around 66.95 t/ha. In India most of the sugarcane farmers 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 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 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

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facilities, comprehensive disease and pest management packages and regular development of improved varieties are the necessary. Keeping these factors in view we would like to discuss seed production scenario in India including in vitro seed production of sugarcane.

Indian Scenario: Agricultural productivity in country has increased, largely due to development of good high yielding varieties. The full potential of a variety can be explored only if equal importance be given to the production of good quality seed. Majority of the newly released sugarcane varieties with exception of a few have not moved to the farmer's field. There is a wide gap between the time of release of a variety and its spread. The time lag is between 10-12 years. It is also likely that during this period, the varieties may accumulate cryptic genetic differences, physical mixtures and pathogens causing seed borne diseases. "Healthy seed production is a process designated to secure, maintain and make available to the public, seeds and vegetative propagating materials of superior crop plant varieties so grown and distributed as to ensure desirable levels of genetic purity, physical condition, quality and health. A supply of good quality low cost seed can be an effective means of improving production, even in the absence of new and better varieties, adequate supplies of reliable". The effect of plant breeding on production will depend on the efficiency of seed distribution, only if the improved varieties reach the farmer through a reliable seed distribution channel will be able to use them effectively to produce more. Hence, non-availability of quality seed material is one of the major problems faced by farmers in developing countries including in India. 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.

Seed Distribution System: There is number of factors which affect the efficiency of a seed distribution. These are the variety evaluation system, varietal maintenance, availability of new and better varieties and the control of seed production. An independent authority, i.e. one that is completely independent of the parties concerned, should carry evaluation. The latter may be plant breeders, sugar factories, seed producers. Independence is necessary to ensure proper evaluation. Stability is another characteristics desired by the farmers, because a stable variety will perform in accordance with expectations based on the results of tests of the variety. To ensure the stability of accepted varieties a suitable varietal maintenance system is essential. The major concern in vegetatively propagated crops is prevention of virus infection. The essential feature in any method for maintenance is that no genetic changes occur during maintenance or in other words that the stability of the variety is assured. Countries where breeding work is entirely in the hands of a few government institutes, regulations controlling variety release are important for the protection of the farmer but regulations can also have an adverse effect, for example excessive bureaucracy can delay in the introduction of the new varieties. Unreasonable requirements may not only delay the introduction of a good variety but can even result in its rejection. The purpose of the control systems used in the seed industry is to guarantee that the seed a farmer buys belongs to the variety he has ordered and is of good quality. This involves control of the identity and purity of the variety and recognition of seed borne diseases.

Characteristics of Good Quality Seed: Good quality seed is a pre requisite to substantially increase agriculture production. Healthy seed production helps in keeping pedigree records, to make available source of genetically pure seed propagating materials for general distribution. Healthy seeds insure the standard such as; high yield, uniform maturity, disease / insect resistance, genetic purity, physical purity, freedom from seed borne diseases, high germination, synergistic effect with other inputs, farmers faith, better quality of production etc.

Spread of Quality Seed and Productivity: The sugarcane area in the country could be broadly classified as-subtropical and tropical belts. The productivity in terms of sugarcane per unit area is very poor in subtropical belt compared to the tropical belt. Critical analysis of factors that restrict yield has revealed that there is a wide gap between the time of release of a variety and its spread. The time lag is generally between 10-12 years. Lack of appropriate quality seed production is suspected to be behind the backlog of improvement varieties awaiting development. It is also likely that during this period, the genotype may accumulate cryptic genetic differences and plant pathogens causing seed borne disease. Commercial crop of sugarcane is propagated vegetatively. The advantage lies in the fact that the crop thus raised is true to the type. However, a serious drawback, this practice carries, is concomitant propagation of many of the serious diseases through the propagating seed cane. The pathogens within the seed-pieces find an easy avenue into the young plant which in turn serves as a source of inoculum for secondary infection in adjoining healthy plants. Thus, in every crop, the inoculum potential keeps on building up, which reflects in increasing incidence of diseases like, red rot, smut, wilt, grassy shoot, ratoon stunting, leaf scald, mosaic, etc. These diseases exercise deleterious effect on the yields of cane as well as sugar. Within a few years, a stage is reached when a variety, as such, is said to have gone bad or 'deteriorated' in a zone. It is then replaced with a new variety and the story repeats itself.

Sugarcane Seed Production: Sugarcane used for seed production 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. In addition to it, 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 give 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 and known as "setts" or "seed canes" are used as seed. In some cases, buds scooped out of the cane with a bud chipping machine are used for raising the seed nursery. For raising a healthy crop, setts should be harvested from 7 to 10 months old crop which is totally free of diseases and pests and with 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.

Seed Production 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. 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.

Heat Treatment: Sugarcane setts may host number of diseases such as sugarcane smut, red rot, grassy shoot, ratoon stunting, sugarcane mosaic and yellow leaf. 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 proportions 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.

Sugarcane Seed Production System in India: To overcome these problems, it is necessary to organize seed production in sugarcane on systematic lines to safe guard genetic architecture of the variety on one hand and to prevent its degeneration due to diseases and other counter productive forces on the other. While conventional technique are known to help maintain varietal stability and promote intrinsic productive potential on a continuing basis, the volume of seed material required in a crop like sugarcane is rather extremely high. Added to that is low multiplication rate (1:10). These are some of the limitations, which come in the way of any systematic seed production programme on a commercial scale. The answer to these problems is the "three tier seed production programme" In India, developmental agencies have been practicing a system of seed supply from approved nursery, which has been better than a commercially well-grown crop. The consciousness and realization of growing a 'seed crop' different from commercial crop has been restricted to a few progressive growers. However, for production of quality seed on scientific lines, the idea of a three-tier seed programme have to produce first Breeder's seed followed by Foundation seed and finally Certified seed.

Primary or Breeder's seed Production: The breeder's seed, by convention, has to be produced by the original breeder, who has bred this variety either by him or by the station from where it has been released. This is the best way of maintaining genetic purity of the planting material over a longer period. By this way, it would also be possible to maintain the prototype of the variety without loosing its original identity. In case, a variety has spread to a larger area and the research station originally bred and released this variety would not be in a position to cope up with the demand of the breeder's seed due to sheer quantity so demanded, a sponsored breeder could be designated in such States where this variety has been adopted irrespective of its State or Station of origin. The sponsored breeder will, however, arrange to produce sufficient quantity of breeder's seed from the basic material. The sponsored breeder will be in constant touch with the original breeder/station in order to obtain the guidance of the latter to maintain the genetic purity and prototype characteristics of the variety. The breeder's seed so produced should conform to the highest standard of purity, approaching 100 per cent. There should not be any compromise on this point. The breeder's seed is raised from nucleus seed after giving heat treatment for elimination of sett-borne infections of diseases, if any. The seed material to be used for raising breeder's seed is obtained from nucleus seed nurseries. The breeder's seed is raised at the farms of research stations under the supervision of the scientists. Breeder's seed being the very basis of seed programme, naturally, calls for utmost attention. Primary or Breeder seed production is done in scientifically supervised farms of research stations, state seed farms or research and development 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.

Secondary or Foundation Seed production: This is the second stage of seed production chain. It is produced from the breeder seed under the supervision of the breeder (original/sponsored breeder). Here, apart from maintaining varietal purity through rigorous rouging and so on, the material has to be free from the known seed borne diseases and pests. During the crop growth period, the strayed diseased plants in the foundation seed plots need to be scrupulously uprooted, in addition to off-types, if any. Thus, the purity and the healthiness of the foundation seed are ensured.

The requirement of the foundation seed of a variety is rather extremely high depending upon the acreage under that variety in the jurisdiction of a particular research center station. The facilities of the research station may or may not permit such a large-scale operation to produce adequate quantities of foundation seed. Under these circumstances, the facilities of Govt. seed farms and the neighbouring sugar factory farms would be utilized. However, the responsibility to maintain purity and healthiness of the crop and to produce adequate quantity of foundation seed squarely rest with the breeder/station/concerned Cane Development Agency of the State. Setts from primary seed or breeder 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. The crop is harvested at 7 to 10 month age and setts are used for planting commercial seed nurseries. The foundation seed is grown at the government seed multiplication, sugar factory or progressive grower's farm. The field in which heat treated seed cane is to be planted, should be easily approachable and having a fairly rich soil and assured water supply. The field should be well drained with no history of waterlogging. Inspection of foundation seed nursery A minimum of three inspections shall be made as under:

Stage I-The first inspection shall be made at 45-60 days after planting in order to verify isolation and detection to volunteer plants, designated diseases and pests and other relevant factors. The permissible tolerance limits for red rot and smut are 0% and 0.01% respectively. If the smut infection is found with in 0.01% limit, the affected plants are rouged out. In case it is more than that, the field is liable to be rejected.

Stage II-The second inspection shall be made after 120-130 days after planting to verify off-types, designated diseases and pests and other relevant factors. In case the incidence of smut and leaf scald is within 0.01% and grassy shoot disease within 0.05% level, the diseased clumps should be rouged out carefully. Should it exceed this limit, the field may be rejected.

Stage III-The final inspection of seed nursery is done 15 days prior to the anticipated date of harvest to verify the age of cane, off types, designated diseases and pests and other relevant factors. At this stage, no incidence of red rot, smut, GSD or leaf scald should be there; otherwise the field should be rejected. The minimum tolerance limit for wilt shall be 0.01%. If the incidence of wilt is within at his limit, the affected

plant shall be rouged out. During the examination, if the scale insect is detected on more than 5 per cent of the stalks on their lower one-third portions, the field will not be selected for seed purpose. It is important that the personnel responsible for foundation seed plots be thoroughly conversant with the epidemiology of the diseases and capable of taking control measures, as demanded by situation.

Commercial or Certified Seed Production: The foundation seed, so produced, would be handed over to the State Department of Agriculture/State Cane Development Department/Sugar Factories, as the case may be, depending upon the recipient agency to be named by the State Cane Development authority for organizing certified seed production. The certified seed, so produced, will be distributed to the farmers and sugar factories for growing commercial crop. The seed so produced is expected to result in a pure, healthy and vigorous crop with an assurance of at least 10 to 15%, if not more, incremental yield over the average yields obtained in the respective regions. The advantages of the quality seed are found not only in the plant crop, the one that receives the certified seed as a planting material, but also in the following ratoon crop (crops). The crop raised in the foundation seed plots serves as seed for raising certified seed nurseries. No heat treatment is required at this stage. Once again, selection of fields, facilities therein and cooperation of the farmers who own it are essential. Surveillance and measures against secondary infection of the diseases are imperative. The inspection of the seed plots will be carried out as per schedule given for foundation seed. 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. 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.

Agronomy of Seed Crop

Seed Material: Immature internodes may form the seed material for a seed crop. Top portions from flowered canes should be avoided. The seed material should be high in nitrogen and reducing sugars. The buds should not be covered with too many dry scales or desiccated. One hundred per cent varietal purity should also be ensured.

Prophylactic Plant Protection Measures: The setts intended for planting should be pretreated with an appropriate and officially recommended fungicide. As preventive measures against termites and shoot borers, gamma HCH 20 EC at the rate of 1.0 kg a.i. per ha should be applied over the setts in furrows at planting.

Time of planting: Time of planting should be so adopted that the seed crop is ready for harvest at 6-8 months and 8-10 months in tropics and sub-tropics, respectively.

Spacing: To facilitate inspection, row to row spacing should not be less than 90 cm. Spaced transplanting technique may be adopted for faster multiplication of cane especially when the quantity of planting material is a limiting factor.

Heat Treatment of Seed Cane: Heat therapy is known to kill the sett-borne pathogens by

denaturation of proteins and impairing vital functions at a temperature not lethal to sugarcane. Its application in production of healthy seed cane has become the most essential. The systems of heat treatment of seed cane can broadly be classified into two groups-the water and the air system. In the former case, the water is the medium of heat transfer, while in the latter; it is either dry air or humidified air, which is also referred to as moist hot air or aerated steam. Humidified air is a better source of heat transfer than the dry air and does not cause desiccation of sugarcane setts and, therefore, sprouting of buds is not adversely affected. The heat therapy systems, which are currently used on a large scale, are mentioned below:

Moist hot air treatment (MHAT): Full-length canes or cuttings of desired lengths are subjected to MHAT at 54ºC for 2-3 hours. Maintenance of a high humidity (more than 95%) inside the treatment chamber is essential to avoid desiccation of buds. MHAT has shown its effectiveness against many seed-piece transmissible diseases of sugarcane, such as, grassy shoot, ratoon stunting, smut, red rot and leaf scald.

Hot water treatment (HWT): Cane cuttings are treated either at 52ºC for 20-30 minutes (short HWT) or 50ºC for 2-3 hrs (long HWT). The long HWT is preferred over short HWT because it gives better sprouting of buds as well as disease control. HWT has been found effective against a number of sett-borne diseases of sugarcane *viz* grassy shoot, ratoon stunting and smut.

Aerated steam treatment (AST): In this system, cut canes are placed in trays inside the unit. Steam and air are mixed together in the ratio of 1:4 and, at a fixed temperature the aerated steam is passed inside the treatment chamber. AST has shown its effectiveness against grassy shoot disease at 50ºC for 1 hr.

Rapid Multiplication of Seed Cane

Spaced Transplanting (STP) Technique: STP based on single but sett will lend scope for reducing the seed rate at least to one third of the present rate of 6-7 tonnes per hectare. The system involves planting of single bud setts. Alternatively, a nursery may be raised from single bud setts and then the settlings are transplanted in the field. This has many advantages. First, it releases atleast 2/3 of the material, which is required for seed purposes, for commercial crushing as a result of reduction in seed rate. Secondly, nursery technique enables better management of the settlings at early stages and selection of healthy and vigorous settlings from the nursery for transplanting in the field to raise a seed crop. Thirdly, the transplanted settlings would have a better chance of establishment without much scope for gaps in the field which are a normal feature in the fields planted with cane setts. Fourthly, settlings so transplanted being healthy and vigorous, will not only have quick establishment, but also maintain the initial vigour in their subsequent stages of growth and consequently, produce a uniform crop with significantly better yields. Finally, the crop occupies the field for a proportionately shorter period since the first one and a half to two months in the beginning are spent in the nursery. As a result, the efforts and the costs involved in the management of the field crop in the first two months are considerably reduced, which will definitely add to the credit of the system.

Tissue Culture: The second alternative technique to solve the chronic problem of low multiplication of seed is the clonal propagation of sugarcane through tissue culture technique. This system, by virtue of its character to multiply the planting material rapidly without impairing the genetic purity, has established its merit in several of the clonally propagated horticultural species, especially in orchids and some plantation crops. The experiments, so far conducted, have shown the feasibility of this system to work satisfactorily with sugarcane also. Multiplication rate of sugarcane clones through this technique is

extremely high in a relatively shorter time and the survivability and the field establishment capacity of the tissue cultured seedlings are also fairly high. However, it is yet to be tested as a tool for large scale seed multiplication and its economics have to be worked out.

Micropropagation as Mode of 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. When disease-free material is used as the source of explants or the explants are heat-treated to eliminate diseases, the resultant micropropagated plants are disease free and healthy. 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

Meristem Tissue 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).

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 1).

Success Story in 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.

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.Presently, a number of sugar factories in Tamil Nadu meet their seed

requirements from micropropagated plants. 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 yields 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 twobudded setts obtained from conventionally raised material (Lakshmanan 2006). Multiratooning 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. 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).

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. 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.

Future Plan and Scope: In future 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. 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 in near future and the consequent depression in sugar prices. 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. 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 the 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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