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TRANSFORMING SUGARCANE SEED SYSTEMS THROUGH MICROPROPAGATION: A REVIEW OF PROGRESS AND FUTURE STRATEGIES

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ABSTRACT

Sugarcane mobilization and policy decisions have transformed cane cultivation in the tropical and subtropical regions of India. Varieties such as Co C 671, Co 86032, Co 205, Co 419, Co 99004, Co 210, Co 213, Co 419, and Co 11015 have become popular among farmers due to their high yield, better sugar recovery and adaptability to various climatic conditions, increasing the demand for quality seed supply. However, sugarcane being a clonally propagated crop with a low seed multiplication rate (1:8 to 1:10) and susceptible to set-borne pests and diseases such as shoot borers, red rot, sugarcane mosaic virus and grassy stunt resulted in significant yield losses in both cropping season and subsequent crops when used as seed material. This issue can be addressed using technologies like micropropagation of meristems followed by a standardized seed production process that includes breeder seed, foundation seed and certified seed. Despite these measures, the growth of sugarcane micropropagation as an industry has not progressed as rapidly as that of crops like banana, pineapple, teak and ornamental plants, due to various challenges. This review focuses on the progress of regeneration and hardening protocols for popular varieties, the challenges faced in the process, solutions to address these issues and strategies for rapid sugarcane seed production and multiplication.

Keywords **:** Micro propagation, Microbial contamination, Quality seed, Tissue culture, Virus indexing.

Introduction

Sugarcane is one of the important industrial crops due to its contribution for sugar, jaggery, fiber, fertilizer, and ethanol, making it a key source of foreign exchange for India. In the past, sugarcane farming in India was limited to the *Saccharum officinarum* species, which had low yields and were vulnerable to pests and diseases. However, following the discovery of sugarcane seed germination and crossing behavior, efforts to enhance sugarcane through a crossing program were intensified. This initiative, led by pioneering scientists Venkataraman and Barber, aimed to create sugarcane varieties with higher sucrose, cane yield, improved resistance to major pests and diseases, and greater adaptability referred as nobilization. The nobilization process involves crossing *Saccharum spontaneum*, which has genes for biotic and abiotic stress resistance, with *Saccharum officinarum*, known for its high sucrose content and yield potential. Improved sugarcane varieties such as Co 205, Co 210, Co 213, Co-0238, CoJ-8, Co-89003, Co 0238, Co 419, Co 86032, and Co 99004 were developed and widely adopted by farmers across India due to their high productivity and sugar recovery rates. Sugarcane is grown over an area of 58.83 lakh hectares, producing 494.22 million tonnes with a productivity rate of 84 tonnes per hectare, primarily in states like Uttar Pradesh, Maharashtra, and Karnataka (Directorate of Economics and Statistics, 2023). Typically, developing improved sugarcane varieties takes about 10-12 years, and the process of supplying seeds for large areas is prolonged due to a

low seed multiplication rate of 1:8 to 1:10, which poses a significant challenge for varietal adaptation. Additionally, the seed replacement ratio for sugarcane is very low across various states in India, largely due to the lack of quality seed and a standardized seed production system (Trivedi and Gunasekaran, 2013). Since sugarcane is clonally propagated, pests and diseases will be passed on to subsequent generations when used as seed material. Traditionally, sugarcane farmers reuse seed from previous harvests, which often does not meet seed standards and is poorly managed, leading to minor genetic changes, developmental variations, and lethal mutations that negatively affect yield due to seed deterioration (Mall, 2018). Fringe attention given to the health of sugarcane seeds, as there is no distinction between seed crops and commercial crops. The lack of effective seed programs and the use of low-quality seed, combined with poor management practices, have contributed to the degeneration of many varieties. It is emphasized that the wide disparities between potential yield and actual yield realized within narrow regions is due to use of poor quality and non-availability of certified seed to the most of the farmers. Unfortunately, the system of scientific seed production i.e., breeder seed, foundation seed and certified seed production is not being followed in India in most of the sugarcane cultivating areas. Above all the yield potential of a clone is maintained only when the planting material is replaced every 4 years. This necessitates the importance of good quality seed material in sugarcane for realizing the actual yield potential. The institutes like Sugarcane Breeding Institute, Tamil Nadu, Vasanth Dada Sugar Institute, Pune and Indian Institute of Sugarcane Research, Lucknow few state agricultural universities and private industries are engaged in supply of pure true to type high quality truth fully labeled seedling material developed through conventional and modern seed multiplication methods.

Sugarcane, a crop that is propagated vegetative, facilitates the accumulation and dissemination of pathogens to new regions. Historical epidemics associated with red rot, smut, wilt, grassy shoot, ratoon stunting, yellow leaf, early shoot borer, and top shoot borer have demonstrated that the use of infected seed can significantly contribute to the emergence and propagation of these diseases. Though certain pests and diseases can be managed through effective agronomic practices and preventive measures, others, such as the sugarcane yellow leaf virus (SCYLV) and grassy shoot, remain uncontrollable. The primary strategy to address these issues, involves the development of resistant varieties and the delivery of virus-free seed material to farmers. Research indicates that the sugarcane mosaic virus can lead to yield reductions ranging from 10% to 50%, contingent upon the pathogenicity of the strain, environmental conditions, the plant's disease response, and the crop's growth stage (Lockhart and Cronje, 2000; Agnihotri, 1996). Canes infected with SCYLV, when utilized as seed material, exhibit low germination rates and reduced seedling vigor. Although thermotherapy, specifically hot water treatment, has been shown to improve the germination and vigor of virus-infected single-node setts, it does not eradicate the virus from the seedlings, which subsequently affects crop performance (Varma *et al*., 2022). Furthermore, the commercial application of this method is not feasible due to its resource-intensive nature and the economic burden it imposes on producers. Given the various challenges associated with the production and supply of quality seed material, tissue culture technology (TCT) through micropropagation of meristem culture, along with the implementation of a standardized seed production chain, emerges as the most effective approach. Micropropagation has been acknowledged as the optimal method for generating large quantities of virus-free seedlings (Jalaja *et al*., 2008). For which the process of micropropagation encompasses several key activities, including: 1) explant collection, 2) explant sterilization, 3) shoot induction, 4) shoot multiplication, 5) root induction, and 6) acclimatization (Fig. 1).

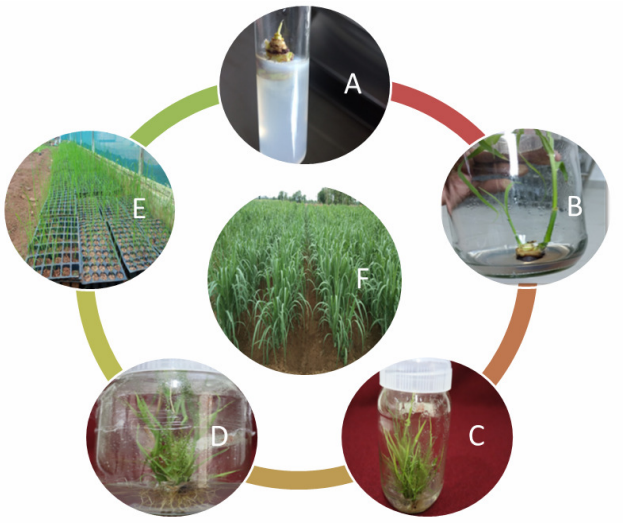

Fig. 1: Stages in sugarcane micropropagation through shoot tip. A, Shoot tip with apical meristem, B, Shoot Inducation, C, Shoot multiplication D, Root formation E, Acclimatization stage F, Field establishment of seedling

Generally, it is recommended that explants should be collected from crops that are 4 to 6 months old, specifically targeting the actively growing shoot tips. At this stage, the growing apices should be excised to a

length of approximately 10 cm (tops). The outer sheaths should be removed sterilized with rectified spirit. Subsequently, the tops should be rinsed in soapy water for two to three minutes, followed by multiple rinses with water. The plant material is then thoroughly sterilized by immersing in 70% ethanol for one minute. Disinfection is further achieved through treatment with chlorine water or a sodium hypochlorite solution for 10 to 15 minutes, followed by three to four washes with sterile water in an aseptic environment to eliminate microorganisms. The shoot apex meristem is isolated by carefully removing the outer whorls in the laminar hood. The explant is then aseptically placed on Murashige and Skoog media supplemented with growth hormones (auxins and cytokinins). To mitigate phenolic leakage from the explant, the test tubes are kept in a dark environment for three to four days (Sarwar and Siddiqui, 2004). Approximately 10 days post-inoculation, the apical bud begins to sprout, subsequently developing into a stem and leaves. The elongated explants are then transferred to a multiplication medium containing varying concentrations of auxins and cytokinins to promote the formation of multiple shoots, thereby completing the first cycle. During this phase, the number of culture vessels increases rapidly (Jalaja *et al*., 2008). This sub culturing can be done upto seven cycles without inducing variations (somaclones) in the seedlings, which often exhibit high mortality rates during the acclimatization phase. Rooting of the plants is achieved by transferring individual seedlings into a rooting medium characterized by high auxin concentrations and low cytokinin levels, which facilitates root development. Seedlings exhibiting robust root and shoot system should then be transitioned to a hardening phase. The survival rate of tissue-cultured seedlings is contingent upon the quality of the potting soil and the surrounding environment. It is advisable to plant tissue-cultured plants during cooler hours with high humidity levels around the seedlings to enhance their establishment.

While micropropagation technology offers the advantage of producing high-quality seedlings in short period, it remains insufficient to supply humongous agricultural community in India. Furthermore, the seedlings that originate from tissue culture are referred to as nucleus seeds which are known for their exceptional quality and are sold to farmers at a considerably high cost which is not amicable to poor farmers. This pricing structure presents a barrier to the widespread adoption of tissue culture technology. To mitigate this challenge and enhance the acceptance of tissue culture seedlings among farmers, it is essential to provide planting materials at a more affordable cost. The seed production system is integral to this endeavor, as it necessitates the rigorous implementation of a structured process that encompasses breeder seed production, followed by foundation seed and certified seed production multiple. Adhering to this systematic approach not only enhances the availability of seed materials but also ensures that high-quality seedlings are accessible at lower prices.

Despite the numerous benefits associated with tissue culture technology, several significant challenges persist in the generation of quality seed materials, which will be addressed in this review. These challenges include:

1. Standardization of efficient micropropagation protocols
2. Microbial contamination
3. Phenol exudation
4. Vitrification
5. Somaclonal variations

1. Standardization of efficient micropropagation protocol:

As previously mentioned, the principal stages of micropropagation in sugarcane include shoot induction, shoot multiplication, and root formation. These stages are influenced by various factors, including the age, endogenous levels of hormones, size of the explant, and position of the explant, as well as the seasonal conditions of the plant during its growth period along with any externally supplemented hormonal combinations and their concentrations, which plays a critical role in this process. In a study, shoot tips collected at three distinct ages viz., 103 days, 115 days, and 145 days post-planting were inoculated onto media enriched with equal proportions of hormones, revealing a differential response of explants and underscoring the significance of the source plant's age (Houllou and Souz, 2015). Notably, even when the age of the explant is consistent, variations in response may arise due to differing levels of cytokinin present in the buds, with younger tissues exhibiting higher cytokinin concentrations than older ones. Research on the response of apical and axillary meristems MS medium indicated that the frequency of shoot initiation was greater in apical meristems compared to axillary meristems (Khan *et al*., 2013). Apical meristems are widely regarded as the most effective source of explants for sugarcane micropropagation due to their high establishment frequency (Sughra *et al*., 2014; Adilakshmi *et al*., 2014; Jahangir *et al*., 2014). Furthermore, the size of the explant significantly

influences its response to the medium; apical meristems measuring 4 mm have demonstrated a high establishment rate. However, this finding is not universally applicable across all laboratories, as Jahangir *et al* (2014) successfully regenerated apical meristems at a length of 7 mm for disease-free and rapid mass production. Generally, apical meristems within the size range of 4-8 mm have been identified as optimal for sugarcane micropropagation, exhibiting efficient survival rates.

Apart from the effect of size and position of explant the surface sterilization also has profound impact on explant regeneration. The over sterilization of explant cause browning slow response to the media death and improper sterilization causes microbial contamination of explant (Moutia and dookun, 1999). The highest sugarcane expalnt survival rate, minimal contamination, and reduced necrosis were achieved when explant treated sequentially with 0.1% Bavistin for 10 minutes, followed by 0.1% $HgCl_2$ for 5 minutes, 6% NaOCl for 10 minutes, and 70% ethanol for 1 minute (Tiwari *et al*., 2012). In our lab the shoot tops are initially surface sterilized with 2 % carbendizm and 1 % streptomycin for 10 min fallowed by treatment with 4 % NaOCl for 10 min in laminar airflow and final treatment with 70 % ethanol for 5 to 10 sec shoot tops are thoroughly washed with sterile water for 3 times for 2-3 min to remove the debris dead microbes and to avoid side effects of the chemical (data not published). To avoid the contamination lab should be properly cleaned with sterilizing agents and fumigated every fort night with potassium dichromate and formaldehyde to kill the air suspended micro-organisms.

The establishment of shoot cultures is the most crucial and important step in vitro micropropagation and which intern depends on factors like hormonal concentration and the genotype. Hormonal combination of auxin and cytokines determine the fate of the meristem inoculated It is well known that the higher the concentration of auxin to cytokines promotes the root formation and vice versa (Jalaja *et al.,* 2008). The rate at which the shoot tip or meristem sheds new leaf or new bud depends on the concentration and proportion of hormones (Biradar *et al.,* 2009; Godheja *et al.,* 2014). The importance of the protocol standardization will be discussed by taking example of two prominent varieties ie Co 86032 and CoC 671 According to a study conducted by Godheja *et al* (2014) the researchers found that MS medium supplemented with 1 mg/lit of BAP and 0.3 mg/lit of BAP yielded the best outcomes for shoot induction and multiplication for Co 86032 clone. Another study by Biradar *et al* (2009) showed that MS medium supplemented with 20 mg/lit of BAP resulted in successful establishment and high shoot multiplication in CoC 671 clone. According to Mehta *et al* (2020) the most effective medium for inducing shoot growth in both Co 86032 and CoC 671 clones was MS medium with 1 mg/l of BAP, 0.75mg/l of IBA, 0.5 mg/l of GA3 and in terms of shoot multiplication for Co 86032 optimal outcomes were achieved using liquid MS media with 2.0 mg/l BAP, 1 mg/l Kinetin, 0.5 mg/l of GA3, 0.5 ml/l of NAA along with 10 % coconut water and 2 % sucrose. Similarly, for CoC 671 the best results were obtained using liquid MS medium containing 1 mg/l of BAP, 0.5 mg/l of Kinetin, 0.5 mg/l of GA3, 0.25 mg/l of NAA along with 15% coconut water and 3% sucrose demonstrating the differences in varietal responses. In another study, the sugarcane variety Co86032 exhibited the highest shoot formation on MS medium supplemented with 0.2 mg/l BAP and 0.1 mg/l kinetin, whereas the clone CoC671 showed maximum shoot development on MS medium containing 0.5 mg/l kinetin, along with 3% sucrose and 10% coconut water (Sawant and Meti, 2016). Similarly, high shoot multiplication in Co86032 was recorded on MS medium with 0.2 mg/l BAP and 0.1 mg/l kinetin, while CoC671 produced the greatest number of shoots on MS medium supplemented with 0.2 mg/l kinetin and 0.2 mg/l BAP. Amit *et al* (2018) reported 1 mg/l of BAP was effective for shoot induction and multiplication of Co 86032. It can be concluded from the above results that micropropagation protocol of one's laboratory may not work for all due to prevailing crop cultivation practice and environmental effect on endogenous hormone levels. It would be advisable to standardized one's own protocol. Efficient shoot multiplication should be fallowed with sound root induction programme for better hardening. High root induction was noticed in CoC 671 inoculated in ½ strength MS medium with 2 mg/l of NAA (Biradar *et al.,* 2009). Similarly in another study, maximum rooting was reported in ½ strength MS medium supplemented with 5 mg/l NAA for Co 86032 and CoC 671 (Mehta *et al.,* 2020; Amit *et al.,* 2018; Sawant and Meti, 2016). It is evident, that the above results on root induction protocol will vary from lab to lab based the type combination and concentration of the hormone. The ultimate success of the micropropataion lies in efficient hardening of the seedling. The success of the hardening/acclimatization will inturn depends on the hardening environment (relative humidity, potting mixture, temperature, and also hormonal balance of the seedling). Hence, it has become mandatory for standardizing the hardening environment which gives more surviving plants. Many

laboratories worked on this aspect to draw highest immortal seedlings. The relative humidity and temperature can be regulated in polyhouses fitted with misting unit or fogger units and their automation gives good results. Varied proportion of potting mixture (clay, sand, FYM, red soil, cocopeat, as sole material or combinations) was used to get high establishment. Our lab uses potting mixture containing red soil sand and cocopeat in the ratio of 1:1:1 for high establishment rate in Co86032. This hardening process generally takes place for 10-15 days for appearance of new leaf shortly after the first leaf appeared and the seedlings increased in size the plant was transferred to secondary hardening outside the greenhouse to adapt it to the harsh environment and achieve high seedling development when transplanted into open ground. Salokhe (2021) used soil, vermiculite and vermicompost in varied composition for better hardening of sugarcane seedling. Highest survival percent was observed in potting mixture of soil: FYM: sand (1: ½: ½) compared with soil: FYM: sand (1:1:1) (Gadkari and Yamagar, 2015)

2. Microbial contamination

The major challenge faced by the scientific community in the realm of tissue culture experimentation is the inability to effectively manage contamination. This issue is particularly pronounced in the micropropagation of sugarcane, where contamination not only hinders the establishment of meristems but also adversely affects shoot multiplication and rooting processes (Thorat *et al*., 2016; Kidus and Teka, 2020). Various microorganisms, including bacteria, fungi, viruses, mycoplasmas, and yeasts, can readily proliferate in the culture medium due to the conducive pH, temperature, and elevated sucrose concentrations, rendering contamination a persistent problem (Permadi *et al*., 2023). Such contamination may arise from inadequate cleaning of glassware, improper handling within laminar airflow hoods, insufficient sterilization protocols for explants, deviations from established standard operating procedures during machine use, and a failure to maintain hygienic laboratory conditions. Over the years, researchers have proposed various strategies to mitigate contamination; however, it remains one of the most significant obstacles in plant tissue culture like adhering to standard operating procedures in laboratory and incorporating antibiotics into the culture media. Several antibiotics, including Gentamicin, Chloramphenicol, Ciprofloxacin, Tetracycline, Vancomycin, Streptomycin and Kanamycin, have demonstrated efficacy in controlling contamination at varying concentrations (Wakil and Mbah, 2012). For example, antibiotics are particularly useful for managing endogenous bacterial populations that are challenging to eliminate through surface sterilization. While antibiotics can effectively reduce contamination, their higher concentrations may exhibit phytotoxic effects on explants, thereby diminishing proliferation rates (Fang and hue *et al*., 2012, Liang *et al*., 2019). Furthermore, the incorporation of antibiotics into tissue culture protocols can escalate production costs and necessitate their use in every subculture until the acclimatization phase is reached (Kaur *et al*., 2008).

3. Phenol exudation

Plant phenolic compounds exhibit significant antioxidant properties, primarily released in response to abiotic factors such as pH, salinity, chemical stress, and fluctuations in light intensity and temperature, thereby protecting cells by scavenging reactive oxygen species (ROS) (Abbas *et al*., 2014; Varela *et al*., 2016). In sugarcane, the phenolic compounds identified include tricine, apigenin, luteolin, as well as caffeic, chlorogenic, coumaric, and ferulic acids (Duarte *et al*., 2011). During micropropagation, the accumulation of phenols may occur due to several factors, including excessive sterilization of explants, injuries to shoot tips during processing, and alterations in environmental conditions (temperature and light intensity), as well as changes in the pH and mineral composition of the growth medium, which can lead to cellular imbalances. The phenolic compounds released from the meristem undergo oxidation under high light intensity with disruption of cellular ion homeostasis leading to protein and lipid oxidation, and destabilizes DNA and RNA culminating in programmed cell death at the base of the meristem resulting in the formation of a black-brown melanin substance that impedes nutrient transport into the meristem, ultimately causing mortality, retarded growth, and a reduced multiplication rate (Zhao *et al*., 2021). The issue of browning due to phenolic leakage can be mitigated through various strategies, such as pre-treating explants with antioxidants like ascorbic acid, citric acid, and activated carbon, or by incorporating these substances into the growth medium (Ishaq and Ehirim, 2011; Jalaja *et al*., 2008; Khan *et al*., 2013). Other methods, including frequent transfers of explants at short intervals and placing explants in a dark chamber for 7-10 days, may also reduce phenolic release by alleviating stress associated with high light intensity.

4. Vitrification

Vitrification, a term synonymous with glassiness, translucency, and vitrescence is a physiological

disorder frequently observed in tissue cultures, specifically characterized by hyperhydricity. This condition manifests through an accumulation of excess fluid on leaf surfaces and within intercellular spaces, accompanied by deficiencies in chlorophyll A and B, hypolignification of the cell wall, and a reduction in leaf thickness. Affected plants exhibit drooping leaves with spongy mesophyll and palisade cells, as well as a weakened epicuticular layer on leaf surfaces, distorted cellular membranes, disrupted stomatal functions, and decreased mechanical strength in vitro plants (Casanova *et al*., 2008; Sen *et al*., 2013). Several factors contribute to the vitrification process, including the type of gelling agent, the combination and concentration of hormones, the presence of organic and inorganic compounds in the nutrient medium, water potential, growth room temperature, light intensity, and the characteristics of the culture container (Polivanova and Bedarev, 2022). It is well-documented that micropropagation in liquid culture significantly increases the incidence of hyperhydricity (Ascough and Fennell, 2004). For instance, Gelrite-inoculated MS media demonstrates a higher chance of vitrification compared to agar-enriched media. Furthermore, elevated concentrations of cytokinins in the media is associated with increased hyperhydricity (Tsay *et al*., 2006; Ivanova and Van, 2008). The type and concentration of sugar utilized in the media also play a critical role in this process, as carbon sources such as fructose and higher concentrations of sucrose can induce hyperhydricity (Bouza *et al*., 1992; Xiao *et al*., 2003). The factors like fluctuating temperatures, high relative humidity, and elevated concentrations of CO_2 and ethylene within culture vessels, may further exacerbate tissue hyperhydricity (Gribble, 1999). However, the incidence of vitrification can be mitigated by incorporating mannitol or sorbitol, utilizing low concentrations of cytokinins in the media, and maintaining optimal laboratory conditions (relative humidity of 65%, temperature of 24 ± 2 °C, and a photoperiod of 16 hours of light followed by 8 hours of darkness with standardized light intensity), as well as employing solid media (Thomas *et al*., 2020).

5. Somaclonal variation

The belief that micropropagation consistently yields true-to-type plants that are genetically identical to the mother plant is a misconception. Scowcroft, in his extensive research on tissue culture, noted that "clonal uniformity is an exception rather than the rule." This observation has been corroborated by various researchers worldwide (Bairu *et al*., 2011; Rizvi *et al*., 2012). The genetic variation rendered from somatic cells is termed as somaclonal variation (Karp, 1995). The underlying causes of somaclonal variation may include factors such as culture growth conditions, the concentration and combination of chemicals and hormones, and light intensity (Joyce *et al*., 2003). Frequent subculturing in micropropagation can elevate the levels of reactive oxygen species within the cells, potentially leading to chromosomal breakage, deletions, gene mutations, chromosomal and gene rearrangements, and the activation of transposable elements, all of which may contribute to somaclonal variation (Czene and Harms-Ringdahl, 1995; Krishna *et al*., 2016). The occurrence of somaclones in micropropagules is generally considered undesirable for quality seed production, while, it can serve as a valuable resource for crop improvement. Notably, sugarcane varieties such as 'Phule Savitri' and 'VSI 434' have been developed through somaclonal variation and have gained significant popularity. Commercially, micropropagation techniques utilizing meristem, shoot tips, and leaf discs are widely employed, with meristem and shoot tip cultures yielding a higher proportion of genetically identical, true-to-type seedlings (Jalaja *et al*., 2008; Adilakshmi *et al*., 2014; Sughra *et al*., 2014; Jahangir *et al*., 2014). While, micropropagation via indirect organogenesis involves the use of leaf discs measuring 5-10 mm in size, which are cultured on a callus induction medium enriched with 2,4-Dichlorophenoxyacetic acid (2,4-D) to facilitate callus formation. During this process, fully developed leaves undergo dedifferentiation, resulting in a mass of unorganized cells known as callus, which possesses the ability to proliferate and differentiate into a complete new plant. When the callus is subsequently transferred to a shoot induction medium, it develops into complete plant form characterized by a higher multiplication rate and exhibiting subtle variations (Doule *et al*., 2008; Kumar *et al*., 2012). These somaclonal variations can be identified at morphological, biochemical, and molecular levels. Morphological variations may encompass alterations in stalk diameter, sucrose content, leaf length, fiber percentage, the number of millable canes, leaf sheath color, plant architecture, internode length, the number of internodes, leaf color, and the presence of spines on the leaf blade (Praveen *et al*., 2019; Memon *et al*., 2023). However, the morphological assessment of somaclonal variations is often labor-intensive and sensitive to environmental conditions. Analyzing chromosomal morphology, pairing, and chromosome number can aid in differentiating somaclones from their parental material (Birchler, 2013). DNA-based markers are increasingly favored by researchers for the precise detection of somaclones, as they are environmentally stable, available in large quantities,

amenable to automation, and facilitate ease of evaluation. Techniques such as Inter-retrotransposon Amplified Polymorphism (IRAP), Inter-Simple Sequence Repeats (ISSRs), SSR-SSCP markers, and Random Amplified Polymorphic DNA (RAPD) markers were employed to assess the genetic fidelity of tissue culture-derived seedlings (Tawar *et al*., 2008; Srivastava *et al*., 2015). In the context of sugarcane micropropagation, the National Certification System for Tissue Culture Raised Plants (NCS-TCP) permits a maximum of seven subcultures to achieve genetically identical seedlings. Notably, Brazilian researchers utilized 1 mm meristems from two clones, RB943365 and RB92579, and reported no variations in seedlings even after 15 subcultures, as determined by ISSR markers.

Quality assurance of tissue culture derived seedlings

Many high yielding sugarcane varieties are being released every year and notified under seed act 1966. But the notified varieties of sugarcane are facing difficulties in entering to seed production chain due to absence of seed certification by the state certification agency due to the bulkiness and non-storability of seed cane. In India, it's interesting that till 2000, there was no specific seed certification standards for cane seed production. Under the chairmanship of Dr. Kishan Singh former Director of IISR, Lucknow, a committee was constituted in 1978 to establish the sugarcane seed standards. After series of discussions the field and seed standards for sugarcane planting material were approved by the Technical Committee of Central Seed Certification Board in October 2001 and later notified by the Central Seed Certification Board. In 2006, the Department of Biotechnology (DBT) of the Government of India instituted the National Certification System for Tissue Culture Raised Plants (NCS-TCP) to ensure the quality of tissue culture seedlings, particularly concerning genetic uniformity and virus indexing. Through a structured certification process, the NCS-TCP has played a pivotal role in enhancing the capacity of tissue culture enterprises to produce high-quality planting material and to broaden their market presence. The NCS-TCP has different sectors working on NCS-TCP Management Cell (NMC), Referral Centers (RCs) and Accredited Test Laboratories (ATLs). Based on the quality is seed material, the ATL issues certification labels with barcodes. The National Certification System for Tissue Culture Raised Plants (NCS-TCP) has given accreditation to 78 commercial tissue culture production facilities in India (Fig 2).These Accredited Test Laboratorys can certify the genetic fidelity testing, viral indexing and batch certification of tissue culture seedlings from phytoplasma, sugarcane bacilliform virus, sugarcane mosaic virus, and sugarcane yellow leaf virus. As per NCS-TCP the minimum standards for growing the sugarcane tissue culture seedling depicted in Fig. 3.

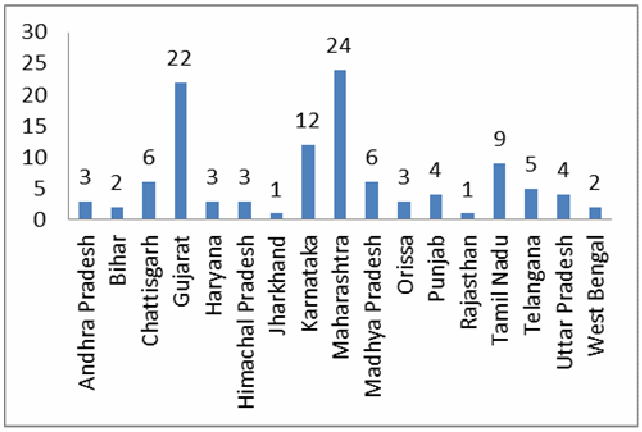

Fig. 2: State wise number of registered labs under NCS-TCP

The Tissue culture derived seedling should be routed through standard seed production to mass multiple seed material and to reach the every corner of the village. Sugarcane seed production involves four generation systems of seed multiplication namely nucleus seed, breeder seed, foundation seed and certified seed (Fig. 4). In sugarcane, three tier seed production system is fallowed which includes primary nursery (breeder seed), secondary nursery (foundation seed), and commercial nursery (certified seed). The canes produced from the field grown micropropagated plants are regarded as primary seed which is used for raising the primary nursery. The canes are cut into two-budded or three budded setts from the primary nursery to raise the secondary (foundation seed) nursery and the seed from latter is used to raise commercial seed plots (certified seed). The seed harvested will be supplied to the farmers for commercial planting. It is essential to maintain genetic and physical purity in seed production plots by taking up the standard seed certification programme. The foundation and certified cane seed production system should support the features listed in Table-1 and Table-2 in order to supply the good seed material

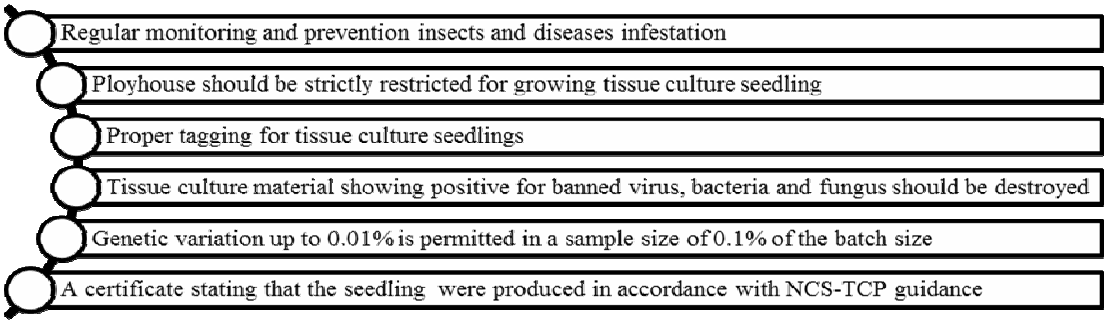

Fig. 3: Minimum Quality Standards for growing of plants inside greenhouses/polyhouses

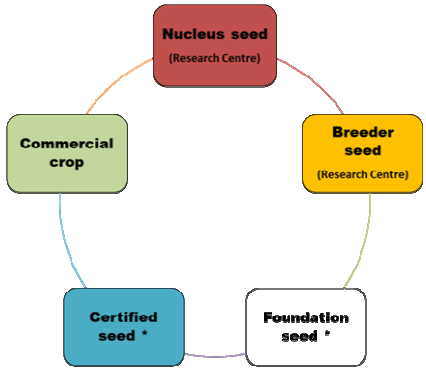

Fig. 4: Standard sugarcane seed production chain * seed production in Research Centre / State Dept. of Agriculture / State Cane Development Dept. / Sugar factories

Table 1: Features to be followed in cane seed production system

Age of the seed cane	Seed must be collected from 6 to 8 months old crop in tropics and 8 to 10 months subtropical regions Any component including the floral axis and the three internodes below the top node of a flowering cane must be excluded At the age of 12 months the lowest half of the plant may also be rejected
Appearance and physical purity	The seed should be 98% physically pure Canes that are lodged should not make up more than 10% of the crop It is not advised to de-trash a seed crop 20% is the highest allowed limit for the striping of dry foliage Aerial roots or nodal roots are not accepted in seed canes and under water logged conditions up to 5% of relaxation may be permitted
Sett moisture content	On a wet weight basis the moisture content of seed cane should not be less than 65%
Genetic Purity	There can be only one type of seed used Admixture is not allowed The seed cane should be 100% genetically pure
Germination	Buds shouldn't have less than 85% germinability
Bud quality	A seed cane must have one viable bud at each node The number of nodes without sound buds shall not exceed 5 per cent (by number) of the total number of buds in a stalk The percentage of buds that are swollen or have protruded more than 1 cm from the rind surface is not to exceed 5% (by number)
Seed Source	The certified classes will be produced from seed cane and/or mericlones whose sources and identity may be assured and approved by the certification agency

Above all to maintain the best genetic and physical purity of the seed cane minimum of three filed inspections shall be made at different stages:

Stage-I: The first inspection shall be made at 45-60 days after planting 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 Whenever the off-types and diseased plants are noticed it should be rouged out along with roots and destroyed

Table 2: Specific requirements of field inspection:

Particulars	Stage of field inspection	Maximum permissible limit %	
		Foundation seed	Certified seed
1) Off-types	I II III	None	None
2) Plants affected by designated diseases			
a) Red rot: *Glomerella tucumanensis* Speg Arx and Muller	I II III	None	None
b) Smut: *Ustilago scitaminea* Sydow	I II III	002* 001* None	010* 010* None
c) Grassy shoot: caused by MLO	II III	005* None	005* None
d) Wilt: *Cephalosporium sacchar*i Butler	III	001*	001*
Leaf scald: *Xanthomonas albilineans* (Ashby) Dowson	II III	001* None	005* None
Plants affected by designated insect pests			
a) Top borer : *Scirpophaga excerptalis* walker	II and III	50	50
b) Internode borer: *chilo sacchariphagus indicus* Kapur	III	100# None**	200 None**
c) Stalk borer : *chilo auricilius* Dudgeon	III	200+ None**	200 None**
d) Plassey borer: *chilo tumidicostalis* Hampson Gurudaspur borer: *Acigona steniellus* Hampson Scale insect: *Melanaspis glomerata* Green Mealy bug: *Sacchariphagus sacchari* Cockerell	III	50 None**	50 None**

Around 10% affected internodes * subject to immediate rouging of the whole clump
+ Around 05% affected internodes ** In areas where the presence of the pest has not been recorded

Conclusion

Sugarcane is a remarkable crop in tropical and subtropical regions of India, due to its diverse applications and significant contribution to foreign exchange. However, the non-availability of quality seed material and the exceptionally low seed multiplication rate impede the rapid supply of newly developed varieties leading to yield reduction. Additionally, since sugarcane is propagated clonally, pests and diseases such as shoot borers, red rot, smut, and viruses can be transmitted to subsequent generations when infected material is used as seed intensifying the yield loss. To address this, thermotherapy combined with sett treatment using fungicides was effective in reducing the fungal diseases but fails to eliminate viruses, which can severely impact yield as they proliferate alongside the plant. The only effective method for completely eradicating viruses from seed material is through the micropropagation of shoot tip meristems. This technology not only facilitates the production of virus-free plants but also allows for the regeneration of thousands of genetically identical seedlings in a small space. To ensure widespread availability of quality seed material, tissue culture-raised seedlings should be integrated into a three-tier seed production system under the oversight of certification agencies.

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