

## Factors To Be Considered For Successful Sugarcane Micro Propagation For Quality Seed Production

Akula Dinesh<sup>1\*</sup>, G. Rakesh<sup>1</sup>, M. Sai charan<sup>2</sup>, A. Krishna chaithanya<sup>3</sup>, G. Narender<sup>4</sup>

<sup>1</sup> Scientist, Plant Breeding, Regional Sugarcane and Rice Research Station, Rudrur-503188

<sup>2</sup> Scientist, Entomology, Regional Sugarcane and Rice Research Station, Rudrur-503188

<sup>3</sup> Scientist, Soil Science, Regional Sugarcane and Rice Research Station, Rudrur-503188

<sup>4</sup> Scientist, Agronomy, Regional Sugarcane and Rice Research Station, Rudrur-503188

Corresponding author: Dine.a32@gmail.com

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### Abstract

The production of sugarcane in India's tropical and subtropical regions has been transformed by governmental decisions and the nobilization of the crop. The farming community has been cultivating clones like Coc 671, Co 86032, Co 205, Co 419, Co 99004, Co 210, Co 213, Co 419, and Co 11015 because of their high yields, superior sugar recovery and adaptability. This has increased demand for high-quality seed. Sugarcane's low seed multiplication rate (1:8 to 1:10) and infestation by seed-borne pests and diseases such as shoot borers, red rot, sugarcane mosaic virus, and grassy stunt result in a significant yield loss in both the current crop and the succeeding crop when used as source seed material. However, implementing scientific cane seed production and seed certification through an authenticated certification agency will support a high seed replacement rate. The problem of low seed multiplication rate and disease free material production can be addressed by using new age technologies such as micro propagation followed by deploying standard seed production chain via breeder seed, foundation seed, and certified seed. Micropropagation via meristem aids in the rejuvenation of old deteriorated clones as well as the rapid dispersal of newly developed clones, which is not possible with the traditional seed multiplication system. The current article focuses on the factors to consider in successful invitromicropropagation as well as the issues encountered during the process and agencies involved in certification of quality tissue culture seedling production.

**Keyword:** Micro propagation, Sugarcane, seed production,

Sugarcane is an important industrial crop owing to humungous production of sugar, jaggery, fibre, fertilizer, ethanol etc., and one of the main sources for earning foreign exchange in India (PIB, 2022). Sugarcane is cultivated in an area of 58.83 lakh ha with production of 494.22 million tonne having productivity of 84.01 tonne/ha and the major growing states include Uttar Pradesh, Maharashtra followed by Karnataka in India (Fig.1) (E&S, DAC, New Delhi, 2023).

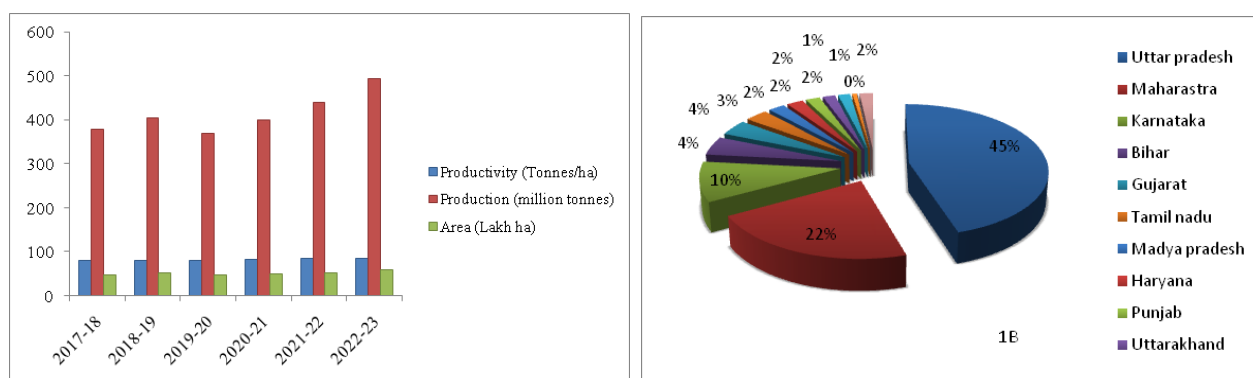


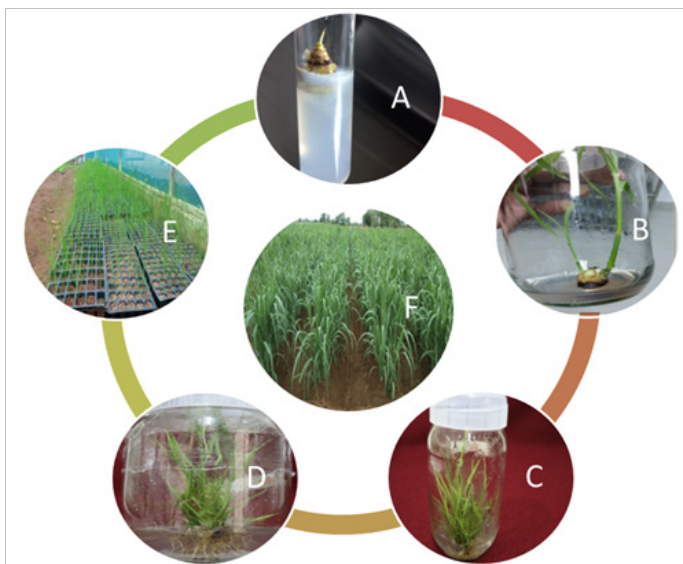
Fig. 1: 1A, The sugarcane production trends in India from 2017-2018 to 2022-23. 1B, Major sugarcane growing area in India (Average from 2017-18 to 2021-22).

In general, the development of improved cane takes nearly 10-12 years with the entire test done. Unlike other crops, the seed supply of newly released varieties for large area takes more number of years due to exceptionally low seed multiplication rate 1:8 to 1:10 which is regarded as major obstacle in varietal adaptation. Apart from this, the seed replacement ratio of sugarcane is almost negligible various in various sugarcane-growing states in India due to non availability of quality seed material and standard seed production system (Trivedi and Gunasekaran 2013). Further instance, sugarcane being clonally propagated crop, the pest and disease inculcated during the cultivation will be transmitted to the next generation when used as seed material. Traditionally, over the years sugarcane growers reuse seed material from the previous crop harvest which was not in accordance with the seed standards and minimally managed field causing minor genetic changes, developmental variation and lethal mutation impacting the yield levels due to seed deterioration (Mall et al., 2018). Least care is given to the seed health of sugarcane, as there is no difference between seed crop and commercial crop. It is estimated that sugarcane mosaic virus can cause yield reduction of 10 to 50 % depending upon the pathogenicity of strain, environment, and disease response of the plant and also on the stage of the crop (main crop or ratoon) (Lockhart and Cronje, 2000). In view of several constraints encountered with the supply of quality seed material, the tissue culture technology (TCT) through micropropagation of meristem culture and adopting standard seed production chain would be best method.

Micropropagation has been recognized as the best method for multiplying lakhs of seedling which are free from virus (Jalaja et al., 2008). Efficient micropropagation protocol can yield 2 lakh plants in six months from a single shoot tip (Anita et al., 2000). Jalaja et al. (2008) showed that theoretically it is possible to generate 1.8 lakhs genetically identical, true to type, virus free seedling material from single apical meristem in one year. Micropropagation as a whole involves various activities like 1. Collection of explant 2. Sterilization of explant 3. Shoot induction 4. Shoot multiplication 5. Root induction 6. Acclimatization.

It is preferable to collect the explant from 4-6 month old crop. The actively growing tops (shoots) are harvested at this stage. Cut the growing apices of the tops to a length of about 10 cm. By washing the sheath with rectified spirit, outer sheaths can be removed. The shoots are then washed for two to three minutes in soapy water, followed by many water changes. After that, the plant piece is thoroughly rinsed in 70% ethanol for one minute to sterilize the surface. Treatment with chlorine water or a solution of sodium hypochlorite is used to disinfect for 10 to 15 minutes. Three to four washings with sterile water in an aseptic environment will get rid of micro organism. By carefully removing the outer whorls of the developing leaves, the shoot apex meristem is isolated in a laminar flow environment. Following that, the explant is positioned aseptically on Murashige and Skoog media that has been treated with growth hormones (auxin and cytokines). To lessen phenol leaking from the explant, the test tubes are kept in a dark room for three to four days. The apical bud sprouts approximately 10 days after inoculation and later develops into a stem and leaf. The elongated explants are added to a medium for multiplication that contains various ratios of auxins and cytokines to create a number of shoots that finish the first cycle. In this stage, the number of bottles will expand rapidly. micropropagation can be carried out for up to 7 cycles without causing variation in seedlings and the formation of abnormal seedlings with high mortality in the acclimation phase. Rooting of plants occurs by transferring individual or multiple plants into a rooting medium. A special rooting medium with high auxin concentration and low cytokine content supports the root development. The seedlings with good roots and shoots must be moved for hardening (fig.2). The survival percentage of the tissue culture seedling depends on the potting soil and environment. Planting tissue culture plants should be done in cool hours, with high humidity around the seedling to ensure better establishment.

Fig2: Stages in sugarcane micropropagation through shoot tip. A, Shoot tip with apical meristem, B, Shoot Induction, C, Shoot multiplication D, Root formation E, Acclimatization stage F, Field establishment of seedling



Although micropropagation technology provides high quality seedlings in a shorter time, it is not enough to meet the needs of the humane agriculture group living 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. This aspect poses a challenge to the widespread adoption

of tissue culture technology. To address this issue and promote the acceptance of tissue culture seedlings among farmers, it is crucial to provide planting materials at an affordable price. The seed production system plays a vital role in achieving this objective, as it involves the strict implementation of a structured process that includes breeder seed production followed by foundation seed and certified seed. Adhering to this system not only increases the availability of seed material but also ensures the accessibility of high-quality seedling materials at a lower price (Sawant et al., 2014).

Despite several advantages associated with the tissue culture technology, pain staking hurdles still exist in generating the good seed material and the important factors which decide the success of sugarcane Micropropagation are:

1. Standardization of efficient micro propagation protocol
2. Microbial contamination
3. Phenol exudation
4. Vitrification
5. Somaclonal variations
6. Non availability of skilled personal
7. Maintenance of lab environment
8. Hardening protocol
9. High initial cost for lab establishment

#### Quality assurance of tissue culture derived seedlings:

As like any other crop, several 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 were no authorized 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. The National Certification System for Tissue Culture Raised Plants (NCS-TCP) was established in 2006 by the Department of Biotechnology (DBT), Government of India, to guarantee the quality of tissue culture seedlings with regard to genetic uniformity and virus indexing. Through a certification process, NCS-TCP has been

crucial in helping tissue culture enterprises develop their capacity for producing high-quality planting material and expanding their market reach. The NCS-TCP is a one-of-a-kind system that is distinctive, dynamic, and complete. It has different sectors working on NCS-TCP Management Cell (NMC), Referral Centers (RCs) and Accredited Test Laboratories (ATLs). Under this quality management system, tissue culture manufacturing facilities are recognized based on their adherence to technical requirements, infrastructure, a set of practices, and documentation/recordkeeping.

### Conclusion:

Sugarcane is a wonder crop in tropical and sub-tropical India for its multipurpose uses and pivotal role in foreign exchange. Exceptionally low seed multiplication rate and vegetative propagation hinders faster seed supply of newly developed varieties making the variety degenerated by the time it enters the farmers field. Further instance, sugarcane being clonally propagated crop, the pest and disease-like shoot borers, red rot, smut, viruses inculcate during the cultivation will be transmitted to the next generation when used as seed material. Thermo therapy coupled with sett treatment using fungicide has shown to reduce the inoculum load of fungal spore but not viruses paving way to enhanced germination. These viruses grow with the plant and cause significant yield loss. The only viable method for completely eliminating the virus for the seed material is through micropropagation of shoot tip meristem. The efficient micropropagation depends on large number of factors like age of the explant, source, standardized regeneration protocol and also hardening procedure. With growing demand for tissue culture seedlings, to ensure the quality of the material the government of India under department of biotechnology initiated National Certification System for Tissue Culture Raised Plants (NCS-TCP) to monitor the genetic fidelity and virus indexing of the material supplied.

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