from bottles, washed properly under running water to remove the slimy medium attached with the roots and excess roots are trimmed before transfer. A mixture of sieved sterilized soil and sand in the ratio of 2:1 should be used for transplanting. Plantlets should be immediately watered after transplanting in trays or pots and shifted to a misting chamber. With the sprouting of the first true leaf on 6th day, the misting is replaced by manual watering. If necessary, preventive measures for pest control should be applied. The hardening process takes about 20-30 days.

The hardened plant is transplanted in the field in trenches at a distance of 45 cm or 60 cm within row and 90 cm between rows. This may vary with genotype. Immediately after transplanting, irrigation should be given. Intercultural operations in crop raised through tissue culture are similar to conventional method. The seed from this crop can be multiplied further for one generation using STP (1:40) technique & thereafter, can be given for commercial cultivation.

(f) Merits of Micropropogation

1. Quick multiplication
   (1 shoot apex : several thousand plants)
2. Disease-free material
3. True-to-type plants
4. Easier transport
5. Low gestation period for exploiting new varieties
6. Rejuvenation of old varieties
7. Germplasm storage
8. Micropropagated plants are more vigorous, give higher cane yield and sucrose %. The quality of seed produced by this technique can be maintained for 3- 5 years with proper monitoring.

(g) Scope

1. Sugarcane is a vegetatively propagated crop and normally requires 7-8 years or even more, for a newly developed variety to spread at large scale. During this period, deterioration of various yield and quality characteristics is inevitable prior to commercial use on account of systemic infections during vegetative multiplication. Tissue culture method (micro-propagation) is the only alternative approach for fast multiplication of a variety in its original form.
2. Micro-propagation is very effective in rejuvenating/reviving the well adapted promising local cultivars facing gradual decline or degenerating in yield and vigour by freeing them from diseases due to accumulation of viruses and other systemic pathogens during prolonged vegetative cultivation. Unfortunately, MHAT (Moist Hot Air Treatment) is not effective against mosaic virus. The meristem culture is the only method to remove the SCMV (Sugarcane mosaic virus) as the meristematic tissue remains free from virus disease.
3. Considering the above advantages, micro-propagation has an important role to play in the 'Seed Production Chain' in sugarcane.

MICROPROPAGATION : A TISSUE CULTURE TECHNIQUE IN SUGARCANE

सत्यमेव जयते

Sponsored by :

Government of India

Ministry of Agriculture

(Department of Agriculture & Cooperation)

Directorate of Sugarcane Development

8th Floor, Hall No. 3, Kendriya Bhawan, Aliganj, Lucknow - 226 024 (U.P.)

भाकृअनुप ICAR

Developed, Designed and Published by Director

Indian Institute of Sugarcane Research

Rae Bareli Road, Dilkusha, Lucknow-226002

MICROPROPAGATION : A TISSUE CULTURE TECHNIQUE IN SUGARCANE

The production of quality seed through micropropagation technique is well recognized now. The sustained high production of sugar per unit area depends primarily on continuous supply of adequate quantity of good quality seed cane, which has to be genetically pure, free from diseases, pests and with no nutritional disorders.This can only be achieved by applying the tissue culture techniques. Since the plants are free from infections, so the original vigour of the newly bred variety is maintained**.** Sugarcane is a vegetatively propagated crop and is cultivated through stem cuttings using 3-budded 'setts'. Diseases like red rot, leaf scald, ratoon stunting, grassy shoot and mosaic are carried to succeeding crops through infected seeds. Thus, heavy financial losses occur annually on account of reduction in cane yield and sucrose recovery. In this context, use of healthy seed of recommended varieties through hot air treatment / and shoot tip culture technique becomes exceedingly important. The conventional mode of seed multiplication has a multiplication rate of 1: 8-10. As a result, a new variety takes 7-8 years to saturate the command area. A much faster multiplication rate(1:30-40) is achieved by using the IISR developed space transplanting (STP) technique. Micropropagation offers a thousand-fold rate of multiplication and is, therefore, the quickest available method in sugarcane.

In a nutshell, micro-propagation in sugarcane provides a rapid technique of providing healthy seed of new varieties and rejuvenates old run-down varieties. The technology is not only economically viable but profitable as well.

The Indian Institute of Sugarcane Research, Lucknow is routinely providing trainings in sugarcane micro-propagation to sugar factory personnel,students, entrepreneurs, etc.

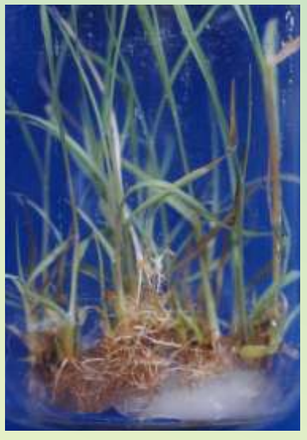
The micro propagation technique involves the following steps:

(a) Collection of explant and sterilization : Actively growing tops (shoots) are collected from 3-4 months old crop. Tops with the growing apices are cut approximately 10 cm long. Outer sheaths are removed by wiping the sheath with rectified spirit. The shoots are then washed with soap water for about 2-3 minutes followed by several changes of water. The plant segment is then thoroughly rinsed in 70% ethanol for 1 minute. Disinfection is done by treating with chlorine water or sodium hypo-chloride solution for 10-15 mts.. The smell of chlorine is removed by 3-4 washings of sterile water under aseptic condition.

(b) Initial explant culture : The isolation of shoot apex meristem is done under laminar flow by carefully removing the outer whorls of the developing leaves. The apical dome along with surrounding leaf primordia is excised with the help of sterile sharp blade. The explant is then placed aseptically on modified Murashige and Skoog medium for initial explant culture over filter paper bridge or cotton swab.

(c) Multiplication : The elongated explants are transferred to the multiplication medium that forms 2 to 5 shoots in first multiplication cycle of about 45 days. The proliferation in the second (first sub-culture) cycle occurs at the highest rate, 5 to 9 fold, which gradually declines in subsequent cycles, 3 to 5 fold in the last 7th cycle. Shoot tip or meristem culture produces normal plants up to 7 cycles of multiplication. After 7 cycles, a green mass, sometimes, starts to appear at the base of the formed shoots, which produces abnormal shoots. Therefore, it is recommended not to go beyond 7th sub-cycles of sub-culturing. The number of resulting shoots under favourable conditions may produce 36,000 to 75,600 plants, depending on the genotype in a period of four and a half months. The basal Murashige & Skoog medium (1962) along with suitable concentration of auxin and cytokinin is used for multiplication.

(d) Rooting : Rooting of plants is achieved by transferring the individual or group of plants in rooting medium. A special rooting medium has been developed for inducing root formation. Root initiation is visible in a week in many genotypes and three weeks in all the genotypes and the rooted plantlet is then ready to transfer to potting mixture for hardening.



(e) Transfer to pots / Field (Hardening / Acclimatization) : The plants are taken out from vessels in a cool and shaded area. One variety should be taken at a time and processed at the earliest. The plant-containing vessel is first inspected. If there are signs of root rotting or leaf rotting, the damaged plants with the container are to be discarded. Planting of tissue culture plants should be done in cool hours i.e. morning or afternoon. The rooted plants are taken out