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PRODUCTION OF DISEASE FREE QUALITY SUGARCANE PLANTING MATERIALTHROUGH MICROPROPAGATION

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ABSTRACT

Sugarcane is an important commercial crop in India. Sugarcane yields are deteriorating day by day because of lack of good quality seed.Inadequate availability of quality seed of new sugarcane varieties and poor seed replacement rate adversely affect the realization of potential cane yield of varieties. Availability of disease and pest-free, true to type planting material is an important prerequisite for achieving the desired yield improvementin sugarcane. Micropropogation is not only a popular mean of clonal propagation but also the most viable and successful method for the production of pathogen free seed material. The micropropagation technique can be used for large scale production of newlyreleasedsugarcane varieties in order to speed up the sugarcane breeding process and for rejuvenation of outstanding old varieties.The advantages of using micropropaged sugarcane's planting material have been realized by the sugarcane growers and the sugar industry. This technology helps farmers for enhancing their crop productivity in a sustainable manner.

Key Words: Sugarcane, Biotechnology, Micropropagation, Planting Material

I. INTRODUCTION

Sugarcane is a perennial crop and one of the few crops which stores its carbohydrate reserves as sucrose. Its economic value lies in the stalks and the sugar they contain after crushing. The main product of Sugarcane is sugar, however, there are many byproducts of sugarcane industry like bagasse, molasses, press mud and green top, which are used by various industries.

Sugarcane is an important commercial crop of India and having largest area under sugarcane cultivation. India is second in the world for sugarcane production. The sugar industry is the second largest agro-based industry, next only to textiles, in the country. By-products of sugarindustries are also playing an important role in the national economy.Sugarcane provides raw material for the second largest agro-based industry after textile. About 527 working sugar factories are there in India.Sugarcane is vegetatively propagated for commercial cultivation and requires huge quantity of seed. Different kindsof planting materials viz., cane setts; settlings and bud chips are used for raising sugarcane crop. Stem cuttings orsections of the stalks called "setts"or seed pieces propagate sugarcane. Each set contains one or more buds. Cuttings are taken from the selected canes. The normal practice in Sugarcane growing States of the country is to use commercial crop of sugarcane for seed purposes.

The accounting of different classes of sugarcane seed i.e. breeder, foundation and certified are not being maintained by the different sugarcane growing States, therefore the exact quantum of sugarcane certified seed

distributed by different agencies inmajor sugarcane growing state could not be assessed and resulted in failure of assessment of SRR in sugarcane (DIRECTORATE OF SUGARCANE DEVELOPMENT, 2013).

1.1 Problems of Sugarcane Production

Sugarcane yields are deteriorating day by day because of lack of good quality seed. Recovery of sugar is also come down because good quality canes are not available.Inadequate availability of quality seed of new sugarcane varieties and poor seed replacement rate adversely affect the realization of potential cane yield of varieties. Seed replacement with fresh commercial seed is done only after 4 years (Sundara, 2000). Diseases are one of the major constraints in the profitable cultivation of sugarcane.

Sugarcane is vegetatively propagated and it favors accumulation of pathogens of most of the diseases. Hence along with seed canes disease causing pathogens are also introduced into new areas.Slow accumulation of different pathogens over a period of time makes minor diseases into major one. Several epidemics due to red rot, smut, wilt, grassy shoot, ratoon stunting, yellow leaf and leaf scald occurred in the past indicated that disease infected seed can played significantly in their creation and further spread. Affected planting material poses a major problem in propagation and exchange of germplasm, and eventually in breeding and distribution of superior genotypes.

In order to achieve the demand for sugar in 2020, sugarcane production has to be increased. For increasing sugarcane production availability good quality seed material of high yielding varieties is very essential. High yielding sugarcane varieties are developed and released by various sugarcane research stations in India.But seed of these varieties is not available for large scale cultivation.

1.2 Solution to the Problems

Some of the methods applied for elimination of viruses in sugarcane cropfor developing good quality seedare thermotherapy and meristem-tip culture technology.Apical meristem culture was used by Coleman (1970) and Hendre *et al.* (1975) to obtain sugarcane mosaic virus free plants. Hendre *et al.* (1983) standardized an apical meristem culture technique for rapid multiplication of mosaic virus-free plants of variety Co 740. Heat therapy has been used to successfully eliminate many viruses from a variety of plant species and is the proven approach to eliminate the pathogen from seed canes but there are some disadvantages of this therapy.Techniques involving thermotherapy or tissue culture and frequently a combination of both have been successfully used to eradicate viruses from infected plants (Walkey, 1980).When disease-free material is used as the source of explant or the explants are heat-treated to eliminate diseases, the resultant micropropagated plants are disease free and healthy (Jalaja *et al*., 2008).

Major emphasis should be given on micropropagation (meristem tip culture technology) for rapid clonal multiplication of newly released varieties so that seed of new varieties will be available to the farmers in short span of time.We can develop good quality, disease free and healthy seed through micropropagation of sugarcane.Meristem-tip culture can be successfully used for producing virus free plantlets. This technique involves the use of apical dome or shoot tip with a few leaf primordia of the size less than 1 mm in length as the explant.

II. SUGARCANE MICROPROPAGATION

Micropropagation is the first major and widely accepted practical application of plant biotechnology. It is a key tool of plant biotechnology that has been extensively exploited to meet the growing demands for elite planting material in the current century. Sugarcane micropropagationinvolves the use of small explants (meristems) which are cultured on a nutrient medium under sterile conditions. Using the appropriate growth medium and growing conditions explants can be induced to rapidly produce multiple shoots, and, with the addition of suitable hormones produce new roots.

Sugarcane micropropogationis the practice of rapidly multiplying stock plant material to produce a large number of progeny plants under aseptic conditions using modern plant tissue culture methods. This is a simple method because of the ease of multiplication, saves cost of producing planting material.The micropropagation technique can be used for rapid multiplication of newly developed sugarcane varieties and for rejuvenation of old deteriorated varieties (Lat et.al, 2015). Development of tissue culture technology for rapid multiplication of disease-free planting material has greatly facilitated mass production of quality seed in sugarcane (Jalaja et.al. 2008). Micropropagatedsugarcane plants are used as breeder's seed in seed multiplication system and seed obtained from microprogated plants are used as foundation seed (Nerkar 2006, Tawar, 2006)

2.1 Advantages of Application of Micropropagation in Sugarcane

Application of micropropagation in sugarcane has the following advantages:

- Rapid multiplication of elite varieties: Micropropagation facilitates production of large numberof plantlets in short span of time, thus helping in rapid introduction and dissemination of new varieties.
- Limited mother stock requirement: The rapid multiplication technology ensures that limited numbers of mother plants are required for raising large number of progeny plants.
- Uniform product:Through micropropagation we can develop genetically andphenotypically uniform progeny plants.
- Agronomic advantages: Micropropagated plants exhibit uniform growth and maturity. So we can do harvesting at one time.
- Season independent production: We can develop micropropagated plantlets as per need in all seasons.
- Disease free planting material using tissue culture: it is possible to develop planting material which is free from diseases and pests.
- High returns:Increase in yield up to 20 to 25% in the commercial crop due to higher number of millable canes. Since the micropropagation based progeny is genotypically and phenotypically similar to the mother plant, which is often a superior selection, the yield and returns are expectedly higher.
- Uniform cane maturity in the commercial crop raised from tissue cultured derived certified seed.
- Higher germination (95 to 98%) of the setts produced from the tissue cultured (TC) plantlets.

2.2 Different Stages of Sugarcane Micropropagation

1. Selection of mother plants and establishment of mother block nursery.
2. Selection of superior initial planting material(Explants)and preparation for inoculation.

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3. Culture medium: Success of *in vitro* culture depends largely on the choice of nutrient medium, including its chemical composition and physical form. Several media formulations have been reported for sugarcane micropropogation and most of them are modified MS media. Media based on Murashige and Skoog (1962) is used for micropropagation.For shoot multiplication MS media supplemented with BAP and Kinetin is generally used. Good rooting is obtained on MS media supplemented with IAA or NAA.
4. After autoclaving, the culture medium is stored in a clean dust free chamber for 2-3 days before use in order to check for any contamination.
5. Culture initiation by using surface sterilized apical meristems. The inoculated tubes are kept in the culture room under light (2500 lux) at 25°C.
6. Culture proliferation: first subculture is done after 40-45 days of inoculation. The cultures are first checked for contamination and then subculture is done. Illuminated Rotary Shakers are used for shaking the cultures. Temp of the culture room is maintained at 25^0c.
7. Subsequent subculture is done after 15-20 days.
8. Rooting: After 5-6 subculture cycles cultures are transferred to rooting medium containing Auxins (NAA or IAA) and keep cultures on illuminated racks.
9. Hardening: Once the plantlets are ready for shifting outside the laboratory, they are carefully acclimatized to adapt to the green house and later to least protected field conditions. During hardening, the plantlets undergo physiological adaptation to changing external factors like water, temperature, relative humidity and nutrient supply.For hardening of the tissue culture plantlets small sized green houses are preferred.In case of hardening of tissue culture plantlets special soil mixture needs to be made depending on the requirement & survival of a particular plants species.
10. Secondary hardening:After primary hardening for 4-5 weeks, plantlets are taken out of green house and kept outside about 4-5 weeks. It is essential to further harden them outside the green house in the open so that the survival under field condition is increased. Tissue cultured plantlets require special attention during the acclimatization process. During both primary and secondary hardening, the rooting media should be completely free from pathogens. Water used for irrigating the plants should be free from pests and pathogens.
11. Micropropagated plantlets are absolutely disease free & true to type when they are ready for plantation. These plantlets are used for planting breeder seed nursery in three-tier system of seed production.
12. The watering of plantlets should be withheld the day before planting in the field so that the soil in the plastic bag becomes hard and compact. This is necessary for avoiding damage to the root system of the plantlets.
13. Plot should be ready with application of green and organic manures for improving the soilfertility.Furrows are opened at a distance of three feet and recommended basal doses of chemical fertilizers are applied and mixed in the soil.
14. Keep the plantlets in the field on the ridges along with poly bags. Take the pits of the size of the plastic bags in the center of the furrow at a distance of two feet. Plant the plantlet with intact soil ball in the pits and subsequently cover it with sufficient soil.
15. Immediately after transplanting irrigation needs to be given.

16. The after care of the seed nursery planted with tissue-cultured plantlets needs special attention. All intercultural operations should be performed within time.
17. Cane obtained from tissue cultured plantlets should be harvested within ten months.
18. One eye buds obtained from tissue cultured sugarcane crop can be used as a planting material.

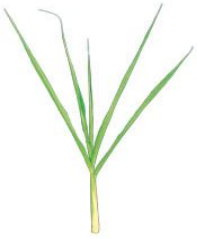
Fig 1.Sugarcane top


Fig 2.Inner portion


Fig 3. Meristem


Fig 4. Shoot Formation


Fig 5.Tillering


Fig6. Rooting


Fig7. Well rooted plants


Fig 8. Hardening in Greenhouse


Fig. 9 Hardening outside the greenhouse


Fig. 10Transplanting in Field


Fig. 11 T.C. Sugarcane

III. QUALITY CONTROL

The following aspects have been emphasized for maintaining the quality of tissue culture raised sugarcane plants (Sinha, 2006):

1. Genetic purity of source material: The genetic purity of the variety to be micropropagated should be certified by the breeder/research organization identified for the maintenance of the variety.
2. Source material: The explant should be taken from vigorously growing healthy plants raised from heat-treated setts and grown under optimum moisture and nutritional conditions. The crop raised from micropropagated seedlings should not be used as source material.
3. Accreditation of micropropagation laboratory: Micropropagation laboratory should be accredited by an appropriate authority to ensure technical competence and satisfactory infrastructure.

4. Micropropagation protocol: Micropropagation protocol should ensure only minimal genetic changes. Shoot multiplication cycles should be restricted to avoid morphological variation.
5. Seedling establishment: The seedlings should be well-established in soil mixture with good root system and with 4 to 5 green leaves at the time of supply to user agencies.
6. Disease indexing: The micropropagation-raised plants should be indexed for freedom from viruses and virus-like diseases through ELISA, and molecular methods. Standard molecular techniques may be used to assess the genetic purity of plants.
7. Seed production: The micropropagation-raised seedling should be treated as breeders' (primary) seed. This seed should be further propagated through vegetative cuttings to produce foundation (secondary) seed and then commercial seed. Inspection of the field at the breeders' seed production stage must be done to remove any off types.
8. Commercial seed: Commercial seed thus produced should be used up to four years

IV. CONCLUSION

Micropropagation is a viable and successful method for production of quality planting material. Through micropropagation we can produce plants which are geneticallyand phenotypically similar to the mother plant and it gives a much more rapid multiplication rate when compared to the other procedure of sugarcane multiplication. This technology is usefulfor rapid multiplication of newly released varieties;rejuvenation of old deteriorated varieties, production of disease free seed; easy transportation of seed material; elimination of viruses; high cane productivity and sugar yield etc.If sugarcane plantlets are micropropagated as per the norms of quality tissue culture plantlets production and if its seed is used by farmers then it helps farmers forenhancing their crop productivity in a sustainable manner.The Government of India has identified micropropagation industry as a priority area for further research, development and commercialization.

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