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Advances In Tissue Culture: An Innovative Approach For Single Bud Propagation In Sugarcane

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Abstract: Sugarcane is a member of grass family Poaceae, previously it was classified as Saccharum Officinarum and now it is designated as Saccharum Spp.Sugarcane (Saccharum Officinarum L.) is monocotyledon crop plant that mostly propagate through conventional methods (bud chip method , sugarcane initiating technology SSI, Cane node technology).However conventional propagation lacks rapid multiplication. Micropropagation is best alternative of conventional methods . Procedure in Micropropagation is broadly classified as collection of explant,sterilization of explant, inoculation, explant culture, multiplication,rooting , hardening. The variety was cultured on MS medium supplemented with different concentrations of growth regulators on shoot initiation, multiplication,rooting and acclimatisation stages , for initiation stage the best performance was observed on MS medium supplemented 1.0mg/l of BAP . On the other hand multiplication stage was best on MD media enriched with 2.0 mg/l BAP+ 1.0 mg/ l NAA , with regard to root induction, the best rooting response achieved best on ½ MS media enriched with 2.0 mg/l NAA +0.5 mg /l BAP. Healthy seeds are a prerequisite for improving the productivity of sugarcane crop .The best way to ensure the production of disease free sugarcane seeds via a technique called `Meristem Culture ' (Tissue culture). According to a study about 80% of seed material can be saved by planting single bud setts over the conventional 3 bud setts planting.

Key words: single bud propagation, Micropropagation, bud chip method, MS medium, Tissue culture.

Introduction: Plant tissue culture is collection of techniques used to maintain or grow plant cells,tissues or organs under sterile conditions on a nutrient culture medium of known composition. Plant tissue culture is widely used to produce clones of a plant in a method known as micropropagation.Several technique have been developed for rapid multiplication of seed cane which can be categorised in two groups

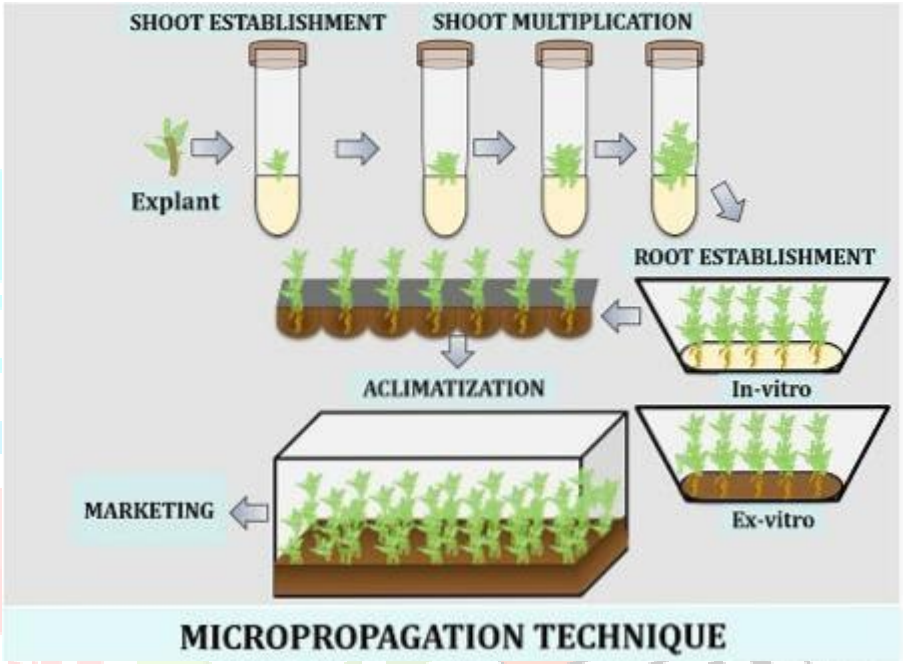
(a) Biotechnological and

(b) Conventional or traditional method of sett plantation .

Due to high pollen sterility, unviable seed production and scarce facility of artificial hybridization promotes non conventional methods like mutation and in vitro culture or in combination for obtaining genetic variability in sugarcane.

Biotechnological method consist of micropropagation come under tissue culture technique(Salokhe,2021) while conventional methods chiefly include bud chip method,cane node technology (developed by ICAR- IISR), Special transplanting technique (STP), Sustainable sugarcane initiating (SSI)technology.

Fig 1: Micropropagation technique chart



Biotechnological method: Micropropagation

It has many synonyms: tissue culture,mass propagation,in-vitro culture,cloning.Briefly we can define micropropagation as the art and science of multiplying plants in vitro.The complete phenomenon of micropropagation is broadly classified into three stages : Establishment, Proliferation,Rooting and hardening.One of the most pricise definition of micropropagation is clonal micropropagation in vitro.Micropropagation (Dinesh et.al,2015) is the best methodology available for rapid multiplication and production of quality seed free of any disease and pest which ensures maximum production or varieties(Urges et.al.,2021). Though this technique from one shoot apex about 180000 plants can be produced within a year which can cover about 14 hactares of nursery area.

History: In 1902, Gottlieb Haberlandt proposed his views on plant tissue culture in the German Academy of Science for which he is recognised as the father of plant tissue culture. The first known plant tissue culture medium used for growing roots was developed by White in 1939 and the callus culture medium was developed by Gautheret. In 1962, Murashige and Skoog developed another plant tissue culture medium that is most accepted today.Micropropagation methods were used in 1960 by George Morel for the production of orchid plants at a commercial level. It can also refer to use of a tissue culture technique for clonal propagation of plants.

Protocol: The ventilation unit should be equipped with a high efficiency particulate air (HEPA) filter.A 0.3 micro metre HEPA filter of 99.97-99.99% efficiency works well.In a sterile transfer room large number of cultures are being utilized and screened for further processing. Depending upon the nature of explant and objectives of in vitro studies there are different types of media that are available in the market. Compositional analysis of growth medium assists in the optimization and selection of the suitable medium for the desirable explants.Media and their preparation require a lot of precautionary measures (pH control,PPT of salts, autoclaving of sucrose and synthetic hormones etc. to avoid contamination and other problems.

Multiple shoots were observed on Murashige and Skoog's medium (MS medium) supplemented with BAP 0.2 mg/l and kinetin 0.1 mg/l. Best rooting was observed on MS media supplemented with IAA 1 mg/l and NAA 1 mg/l (table1).Soil mixture containing (soil+vermiculite+sand in 4:1:1 proportion by volume). 2 ft distance between 2 plants and distance between 2 rows was found as an appropriate method for planting tissue

Fig 2 : autoclave operation in biotechnology lab.

During plant tissue culture sucrose act as fuel source or energy source (carbon source).The most preferred carbon energy source is sucrose at a concentration of 20-60 g /litre. During autoclaving the medium sucrose is hydrolyzed to glucose and fructose which are then used by the plant material for their growth.

Table1. Media composition

Ingredients	Shoot apex medium (mg/l) (whites basal)	Multiplication medium (mg/l) (modified MS)	Rooting medium (mg/l) (whites medium)
Ammonium nitrate	-	1640	-
Potassium nitrate	-	1900	-
Calcium chloride	-	440	-
Magnesium	720	-	720
sulphate	200	-	200
Sodium sulphate	65	-	65
Potassium chloride	80	-	80
Potassium nitrite	300	-	300
Calcium nitrite	16.5	-	16.5
Sodium dihydrogen	-	170	-
orthophosphate	1.5	6.2	1.5
Potassium	-	0.025	-
dihydrogen	-	0.25	-
orthophosphate	-	0.25	-
Boric acid	0.75	-	0.75
Cobalt chloride	3	-	3
Sodium molybdate	7	22.5	7
Cupric sulphate	36.7	36.7	36.7
Potassium iodide	100	100	-
Zinc sulphate	1.07	1.07	-
Manganese sulphate	0.5	0.5	-
Ferric EDTA	1	-	-
Meso inositol	2	2	3
Kinetin	-	0.5	0.5
Gibberlic acid	-	0.5	0.1
Indole Butyric acid	-	0.1	0.1
Glycine	-	0.5	1
Nicotinic acid	-	0.25	-
Pyridoxine	-	-	1
Thiamine	-	-	1
Naphthalene acetic	100 ml	100 ml	-
acid	20 g	20 g	20 g
6 Benzyl amino	5..8	5.8	7.0
purine			
Calcium			
pantithenate			
Riboflavin			
Coconut water			
Sucrose			
pH			

Micropropagation chiefly consist of four major steps:

(a) Collection of explant and sterilization : shoots(Purnamaningsih.et.al,2021)are collected from 3-4 months old crop and selection of apices of 10 cm long ,outer sheaths are removed by rectified spirit. Sample thoroughly rinsed in 70% ethanol for 1 minute. Disinfection is done by treating with chlorine water or sodium hypochloride solution for 10-15 minutes. The smell of chlorine is removed by 3-4 washing of sterile water under aseptic condition.

(b) Initial explant culture:

Apex meristem put in laminar flow where explant(Purnamaningsih et.al.,2021) placed aseptically on MS medium.

The basal MS medium along with suitable conc.of auxin and cytokinin is used for multiplication (Patel et.al.,2018)

(C) Multiplication:

2 to 5 shoots in 1st multiplication cycle(Baday .et.al.,2020)of about 45 days.It is advised not to go beyond 7th cycles of sub-culturing because after 7th cycle,a green mass start to appear at base of formed shoot. The number of resulting shoots under favourable conditions may produce 36000 to 75000 plants depending on the genotypes.

(d)Rooting :

It achieved by transferring the individual or group of plants in rooting medium , root initiation is visible in many genotypes in 1 to 3 weeks . During tissue culture(Hendre et.al.,1983)adventitious roots or shoots can be induced by transferring the callus to medium containing different ratios of auxin and cytokinin.

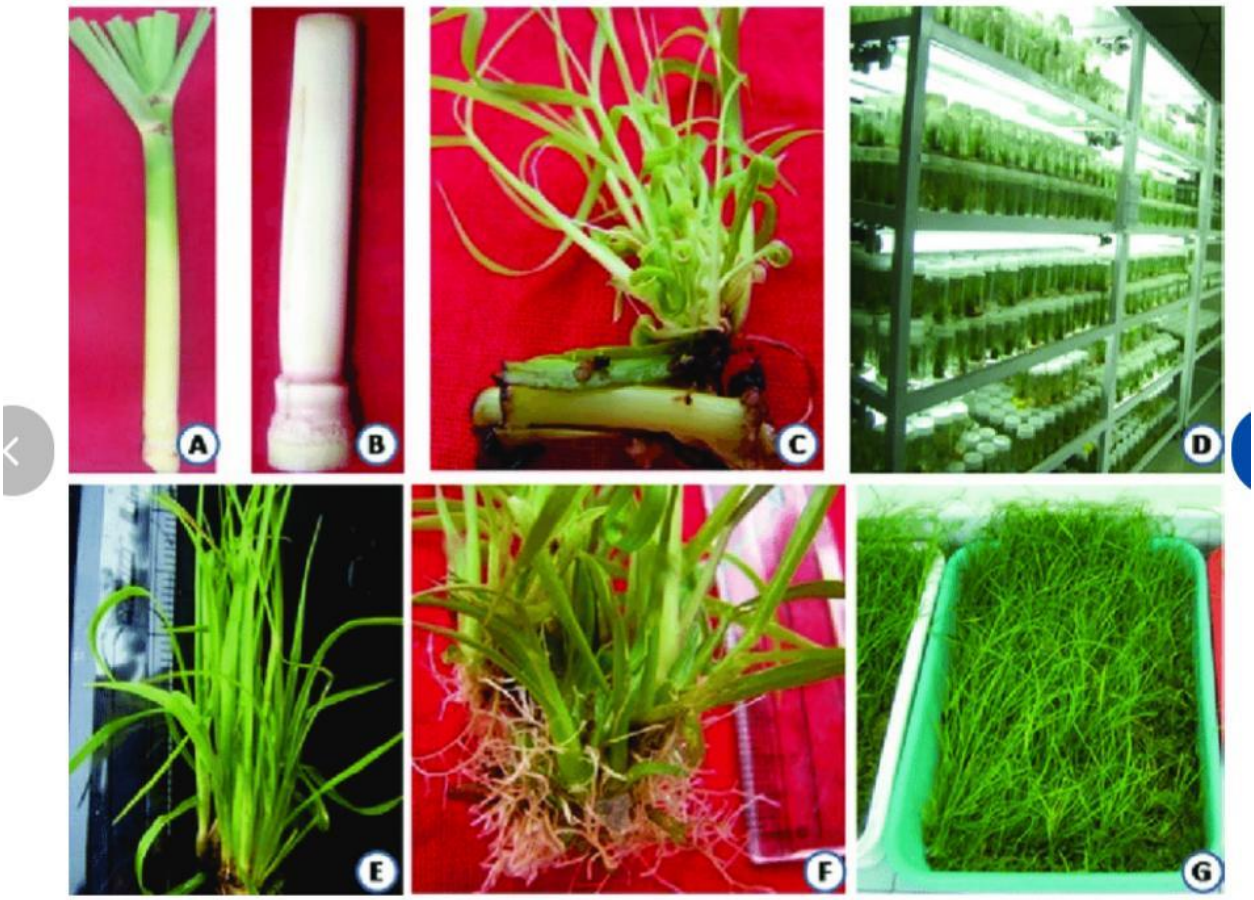
(e) Hardening: It means transfer of rooting plantelets in field or pots which is also known as acclimatisation. Damaged plants should be eliminated. A mixture of sterilized sieved soil and sand in the ratio of 2:1 should be used for transplanting. The hardening process takes about 20-30 days. The hardened plant is transferred in the field in trenches at a distance of 45 cm or 60 cm within row and 90 cm between rows . Intercultural practices in crop raised through tissue culture are similar to conventional method.

Merit: There are certain merits of micropropagation(Sawant et.al.,2014) that's why it attained a certain degree of popularity in field of single bud propagation through tissue culture or biotechnological technique. Merits of micropropagation are: quick multiplication(Hendre et.al.,1983), disease free material ,true to type plants, easier transport, rejuvenation of old varieties,germplasm storage,micropropagated plants are more vigorous, give higher cane yield and sucrose %. The quality of seed produced by this technique can be maintained for 3 to 4 years.

fig 3: Growth of plantelets under sterile and aspetic conditions

Plant tissue culture lab should have proper sanitation and hygiene maintenance environment,so that no any chances of contamination remain left.

fig 4 : Different steps of micropropagatio in sugarcane



Micropropagation of sugarcane-laboratory. (a) Actively growing top. (b) Spindle. (c) Establishment of shoot cultures in vitro. (d) Rapid shoot multiplication through subculturing. (e) Rate of shoot multiplication per cycle. (f) Root formation in shoots. (g) Hardening of plantlets ex vitro

Conventional methods of sugarcane propagation:

Material required: single bud setts,sett cutting machine, solution of malathiyan and bavistin 2%,gunny bags,cuvettes,polytrays, micro irrigation system,NPK ratio (19:19:19), specially designed racks.

Method: 1: Single budded nodes obtained with the help of sett cutting machine.

2.Nodes with sound buds are filled in gunny bags and dipped in solution of malathiyan and bavistin 2%

3. These treated nodes in gunny bags are kept under shade for six days to allow the buds to sprout and root initiation.

4. Water curing every day in morning and evening times

5. Workers are filling the cuvettes with root media.

6. Sprouting nodes are transferred to the cuvettes or holes of polytrays.

7. Unsprouted/ unrooted nodes are rejected in the second grading.

8. Transfer of polytrays in nursery farm.

9. Microirrigation system in nursery farm.

10. Sprouting nodes are coming outside.

11. N:P:K is 19:19:19 solution is sprayed on the growing seedlings at 10 days interval for vigorous growth.

12. Polytrays which are half filled with the rooting media ,followed by complete filling, pressing/compacting with fingers.

13. Workers are doing in grading for ready to issue the farmers.

14. Seedlings are ready in our nursery farm to issue the farmers.

15. Ready to issue the disease free sugarcane seedlings.

16. Healthy plants are ready to issue for farmers.

17. Sugarcane seedlings are transferred from nursery farm to sugarcane growers by tracks which are specially manufactured.

Merit: Single bud propagation completely eliminates the impact of the apical dominance, therefore emergence of neat, healthy and sturdy tillers .

It promotes uniform growth of sugarcane by improving luminous energy and fertilizers. Effort come under the sustainable sugarcane initiative (SSI) which aim to focus on using less seeds with wider seed spacing and better water and nutrient management to increase the cane yield significantly.

It has been observed that due to saving in seed cane material, the highest net return can be obtained with setting raised from single bud setts. According to study about 80% of seed material can be saved by planting single bud setts over the conventional 3 bud setts planting . The food reserves and moisture content in the single bud setts deplete at a faster rate compared to 2 or 3 bud setts, therefore sufficient soil moisture and nutrient are pre-requisite for viability of single bud setts as planting material.In view of the benefits of using single bud setts for sugarcane planting ,extensive field experiments were carried out at ICAR- IISR, Lucknow and its validation was also done at the farmers field in different sugar factory zones of Uttar Pradesh.

Mission: To fulfill the requirement of sugarcane with economical plantation. To revolutionize the field of sugarcane production through research and development with integrated viable technologies. To be a vibrant force in the sugarcane based industries and facilitate sustainable prosperity for all its participant.

Vision: The culmination of an enterprises endorsed with technological reference for sugarcane based industries.

A dedicated approach to always deliver high yield and disease free sugarcane.

Update: Development of protocol for tissue culture of sugarcane and development of agronomy for tissue cultured plants was essential to make use of tissue culture(Baday et.al.,2020) plantelets in three tier system of sugarcane seed production. The nutritional requirements for in vitro propagation protocol of sugarcane is highly dependent on the genotype and explant used .In vitro propagation that enables the rapid and large scale production of disease free planting material as being exercised with different crops is a prerequisite .

Result indicated that 15 days interval is the best optimum period of sub-culturing to enhance the rate of multiplication and produce more number of elongated and healthy shoots in a seed multiplication program through in vitro micropropagation .

Recent significant advances in technique apply directly to sugarcane so we can proudly say in vitro culture technique proved to be a tool for the development of new genetic architecture in vegetatively propagated (sugarcane) crop .

Future prospects: sugar is becoming an essential commodity to fulfill the requirement of ever increasing demand of population especially in contrast with South Asian and African continent. Our chief goal will be maximize sugarcane production in less amount of seed sett. Biotechnological route in form of micropropagation assured guarantee to fulfill this goal at rapid pace in comparison with conventional methods. Construct of tissue culture laboratory should be mandatory in every sugarcane industry unit to encourage rapid multiplication of single bud propagation.

Conclusion:

Sugarcane tissue culture research has profited from independent approach, technique that are employed by different laboratories around the world,a more integrated view point of progress being made by an increasingly greater number of investigators is timely.

Single bud propagation technique broadly categories as micropropagation and conventional method respectively. Micropropagation is best methodology available for rapid multiplication and production of quality seed of disease frees. MD media supplemented with IAA and NAA,BAP and kinetin are important ingredients of routine tissue culture.Single budded nodes obtained with the help of sett cutting machine in conventional method.

There will be need to strengthen R & D sector to promote micropropagation activities for single bud propagation in sugarcane.

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