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Effect of different Combinations of Plant Growth Regulators on Sugarcane Shoot Multiplication

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ABSTRACT: For the production of disease-free plant on large scale plant tissue culture technique is the best for utilization *in vitro*. The current experiment used the different plant growth regulators for the development of sugarcane plantlets using plant tissue culture technique. About 86% of the world's total sugar needs are satisfied by sugarcane. India is the world's second-largest producer of sugar. Sugarcane is traditionally replicated vegetatively, utilising setts as seed rather than real seeds. Low rates of multiplication and disease susceptibility are typically the outcome of this method of sugarcane propagation. Techniques for growing plant tissues provide a reliable and effective answer to this problem. The production of pathogen-free plants and a significant improvement in plant quality are both being explained by tissue culture. Auxins are used for roots, whereas cytokinin is used for shoot initiation and multiplication. In the current study, Murashig and Skoog (MS) medium were employed. Different sixteen combinations (15+Control) of modified MS media were used for *in-vitro* shoot multiplication in both sugarcane genotypes (Cos 13235 and Co 15023). The study showed that the combination of sterilant in sequence of labolene (2%) for 10 min, Bavistin (0.1%) for 5 min, HgCl2 (0.1%) for 90 seconds and Ethanol (70%) for 1 min was found to be best for surface sterilization. Minimum growth of explants was observed in media which is devoid of any growth regulator. It is concluded that both the genotypes have shown higher growth and shoot regeneration in T11 containing (0.5 BAP + 0.5 Kinetin mg/L). Some challenges in the present study are 1. Ensuring pathogen-free and disease-free plant production through tissue culture technique on a large scale requires meticulous and consistent sterilization procedures to prevent contamination. 2. Finding the optimal combination of growth regulators in the culture medium for shoot multiplication can be challenging, as it involves extensive experimentation and precise control of hormonal concentrations. 3. Achieving consistent and efficient growth and shoot regeneration across different sugarcane genotypes can be difficult due to variations in their inherent characteristics and responses to the tissue culture conditions.

Keywords: Sugarcane, Plant growth regulators, Culture medium, BAP and Kinetin.

INTRODUCTION

The world's primary source of sugar, sugarcane (*Saccharum officinarum* L., family Gramineae), was first cultivated in South East Asia and Western India, mostly in tropical and subtropical regions of the world it is now prominently grown as a commercial crop in nearly 108 countries. Sugarcane probably originated in North India and New Guinea. A tropical perennial grass known as sugarcane is harvested for its sucrose content. Brazil is the largest producer of sugarcane in the world, largely because of favourable edaphoclimatic conditions and advancements in technology in the productive sector. Next to Brazil, India is the highest user and producer of sugar in the world. Around the world, 1.9 billion tonnes of sugarcane are produced in an area of 26.26 million hectares (FAOSTAT, 2019; Thibane *et al*., 2023). It includes conventional cane and sugar sweeteners and accounts for 17% of the world's cane sugar production (28.31 million tonnes) (http://www.Agricoop.nic.in). Sugarcane meets about 86% of the total world sugar requirements (OECD-FAO, 2019). According to reports, Uttar Pradesh will provide the most, accounting for 38.61% of the total sugarcane production in the 2020–21 fiscal year (ICAR-sugarcane report and molasses production, 2019). The next two states that produce the most sugarcane are Maharashtra and Karnataka. Traditionally, sugarcane is multiplied vegetatively using setts as seed instead of actual seeds. This method

of sugarcane propagation frequently results in low rates of multiplication and 3 disease susceptibility. If multiplied using traditional seed multiplication techniques, it often takes more than 10 years to produce enough seed material of a new variety of sugarcane for planting in a large region (Sengar *et al*., 2011; Sharma *et al*., 2015). Techniques for cultivating plant tissues offer a productive and trustworthy solution to this challenge. Micropropagation is one of the most significant contributions of plant tissue culture for industrial plant propagation. It is a novel plant tissue culture method that uses controlled nutritional artificial media termed MS media and environmental conditions *in-vitro*to propagate selected plant species true to type by aseptic shoot tip/meristem culture (Sauvaire and Galzy 1978). Therefore, the present investigation was performed to get the best combinations of media and different growth regulators. In this paper the analysis of effect of different growth regulators were evaluated.

MATERIAL AND METHODS

Experimental site. In the present investigation, all the laboratory and field experiments were conducted carefully in a systematic manner under *in-vitro* conditions at Sugarcane Tissue Culture Laboratory, College of Biotechnology and molecular work at PG Laboratory, Department of Agriculture Biotechnology, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut, India, during 2020- 2021 and 2021-2022.

Plant materials

Source of plant material. The planting material of two sugarcane genotypes namely Cos 13235 and Co 15023 were used in the present research work collected from field grown sugarcane plants maintained in the Sri Ram Sugarcane Farm and Sardar Vallabhbhai Patel University of Agriculture and Technology, Modipuram, Meerut respectively.

Media preparation

Preparation of stock solution of media and growth regulators. The formulation of modified MS (Murashige and Skoog 1962) basal medium supplemented with various growth regulators was tested for *in-vitro* clonal propagation of sugarcane, as it is the most widely used media for tissue culture work. Stock solutions of nutrients used in the culture medium were prepared in advance for convenience and accuracy. Once dissolved, the final volume was made up with autoclaved distilled water in an appropriate volumetric flask and poured in bottles followed by storage at 4°C in refrigerator till use. At the time of media preparation, they were brought to room temperature and mixed proportionately to get desired media.

Preparation of culture media from stocks. The preparation of 1 L MS medium as used in the present study for *in-vitro* propagation. All the stock components of the medium were mixed in an appropriate quantity. The final volume of the solution was made by the addition of autoclaved double distilled water. The pH of the medium was then adjusted to 6.0 with 1 N NaOH or 1 N HCl. The agar (0.8 g/L) was added and the medium was heated in a microwave till boiling to melt agar. The medium was then poured in presterilized culture vessels while exposing to flame to prevent contamination.

Sterilization of explant and Preparation of explant. Healthy sugarcane tops were collected and the explants were thoroughly washed in running tap water for 25-30 min. After that, the tops were washed in labolene (2.0%) solution by continuous shaking for 10-15 min, and then washing with tap water for 4-5 times to remove the traces of detergent after that treat the explants with Bavistin (0.1%) for 5-10 min and place under running tap water to remove excess Bavistin on explants now wash the explants after each chemical treatment, tops were rinsed for 3-4 times with autoclaved distilled water. The sterilization with $HgCl_2$ and 70% Ethanol was given under aseptic conditions in laminar air flow hood. The standardized treatment of sterilant was then used for further sterilization of explants.

***In-vitro* aseptic establishment of shoot-tip explants on media.** Establishment of *in-vitro* cultures of two sugarcane genotypes was carried out by removing the outer layers of the sterilized explants under aseptic conditions in laminar air flow chamber. About 2.0-4.0 cm long shoot tip explants containing apical and axillary meristem were carefully excised from the sterilized tops and immediately inoculated onto full strength MS media with different combinations of hormones (Table 1), containing 100 mg/L myo-inositol and 30 g/L (w/) sucrose combinations solidified by 0.8% (w/v) bacteriological grade agar. The inoculated cultures were incubated in culture room providing the standard culture conditions of temperature (25+2°C) for 16 hours at light intensity of 3000-4000 lux provided by cool fluorescent tube lights and humidity (approx. 60%).

Culture Conditions. All the cultures were kept in growth chambers at 25±2°C under 16 hr illuminations (followed by 8 hr dark period) of cool white fluorescent tubes with a light intensity of 4000 lux, the relative humidity is maintained at 90-95% in the chamber.

Optimization of culture media for shoot multiplication in both sugarcane genotypes. Healthy and contamination free *in-vitro* raised sugarcane plantlets of both genotypes were used in order to study the effect of different levels of cytokinin's (BAP and Kinetin) alone or in combination on shoot multiplication

Growth hormones. Different concentrations of cytokinin's (BAP and Kinetin) used for *in-vitro* shoot multiplication A total of sixteen combinations (15+Control) of modified MS media as shown in (Table 1) were used for *in-vitro* shoot multiplication in both genotypes. The modified MS basal medium devoid of BAP and Kinetin was used as control. About 1-2 cm of healthy and contamination free plantlets were inoculated on the media in Jam bottles (each Jam bottle containing single shoot). The cultures were incubated in a culture room under above mentioned standard culture conditions. Comparative studies were made on various aspects of *in-vitro* shoot multiplication of both sugarcane genotypes.

Table 1: Different culture media used for *in-vitro* shoot multiplication in sugarcane genotypes.

Sr. No.	Treatment	Media composition
1.	T_o	M S Basal
2.	T_1	M S Basal + 0.5 BAP mg/L + 30g Sucrose
3.	T_2	M S Basal + 1.0 BAP mg/L + 30g Sucrose
4.	T_3	M S Basal + 1.5 BAP mg/L + 30g Sucrose
5.	T_4	M S Basal + 2.0 BAP mg/L + 30g Sucrose
6.	T_5	M S Basal + 2.5 BAP mg/L + 30g Sucrose
7.	T_6	M S Basal + 0.5 Kinetin mg/L + 30g Sucrose
8.	T_7	M S Basal + 1.0 Kinetin mg/L + 30g Sucrose
9.	T_8	M S Basal + 1.5 Kinetin mg/L + 30g Sucrose
10.	T_9	M S Basal + 2.0 Kinetin mg/L + 30g Sucrose
11.	T_{10}	M S Basal + 2.5 Kinetin mg/L + 30g Sucrose
12.	T_{11}	M S Basal + 0.5 BAP mg/L + 0.5 Kinetin mg/L + 30g Sucrose
13.	T_{12}	M S Basal + 0.5 BAP mg/L + 1.0 Kinetin mg/L + 30g Sucrose
14.	T_{13}	M S Basal + 0.5 BAP mg/L + 1.5 Kinetin mg/L + 30g Sucrose
15.	T_{14}	M S Basal + 0.5 BAP mg/L + 2.0 Kinetin mg/L + 30g Sucrose
16.	T_{15}	M S Basal + 0.5 BAP mg/L + 2.5 Kinetin mg/L + 30g Sucrose

Observations. Effect of different treatment on shoot multiplication on sugarcane genotypes after 8 weeks was recorded by calculating number of shoots obtained, observing shoot growth and frequency of shoot regeneration.

RESULTS AND DISCUSSION

Effect of different growth regulators on shoot multiplication and root proliferation in both sugarcane genotypes. Two separate sugarcane types, Cos 13235 and Co 15023, were studied in a series of tests to find the best combination and concentration of plant growth regulators for promoting shoot multiplication. Daily data collection included factors like the frequency (%) and quantity of shoots per explant. The established shoots were moved to growth media and cultured in a growth chamber with a variety of doses of plant growth regulators.

Table 2: Effect of BAP and Kinetin alone or in combination on *in-vitro* shoot multiplication of sugarcane genotype Cos 13235 after 8 weeks(Mean± SE).

Sr. No.	Media code	Frequency of shoot regeneration(%)	No. of Shoots/explant	Shoot growth
1.	T_O	44±0.76	2.2±0.04	Poor
2.	T_1	88±1.51	14.2±0.43	Good
3.	T_2	79±1.37	12.2±0.19	Good
4.	T_3	74±1.26	10.9±0.19	Moderate
5.	T_4	63±1.07	8.3±0.14	Moderate
6.	T_5	52±0.88	5.8±0.10	Poor
7.	T_6	72±1.23	11.9±0.19	Good
8.	T_7	66±1.13	9.4±0.16	Moderate
9.	T_8	54±0.92	7.8±0.14	Moderate
10.	T_9	51±1.50	6.4±0.11	Moderate
11.	T_{10}	48±0.81	4.5±0.08	Poor
12.	T_{11}	81±1.39	17.3±0.32	Good
13.	T_{12}	74±1.30	14±0.26	Good
14.	T_{13}	71±1.25	15.2±0.25	Moderate
15.	T_{14}	72±1.26	12.9±0.24	Moderate
16.	T_{15}	67±1.18	8.2±0.12	Moderate
	C.D.	4.47	0.71	
	SE(m)	1.17	0.12	
	SE(d)	1.66	0.25	
	C.V.	1.15	0.43	

The results, presented in Table 2 and 3, demonstrated that both genotypes exhibited high rates of shoot regeneration frequency and number of shoots. Genotype Co 15023 displayed the highest shoot regeneration frequency (88.16±1.08%), followed by Cos 13235 (81±1.39%), using the T11 hormonal combination. Similarly, for the number of shoots per explant, Co 15023 had the highest count (19.12±0.24), followed by Cos 13235 (17.3±0.32) after 8 weeks of inoculation (Table 2 and 3) using the T11 combination. In contrast, the culture media without any hormone supplementation showed significantly lower shoot regeneration frequency for both Co 15023 (41.06±2.51) and Cos 13235 (44±0.76), as well as a lower number of shoots per explant for Co 15023 (2.31±0.14) and Cos 13235 (2.2±0.04) in both genotypes (Fig. 1 and 2).

These results were similar to the findings of some earlier researchers (Khaskheli *et al*., 2019; Pathak *et al*., 2009; Qazi *et al*., 2019; Bhalerao and Kumar 2019; Singh and Gupta 2019; Yadav *et al*., 2019; Amente and Feyissa 2021).

Table 3: Effect of BAP and Kinetin alone or in combination on *in-vitro* shoot multiplication of sugarcane genotype Co 15023 after 8 weeks (Mean±SE).

Sr. No.	Media code	Frequency of shoot regeneration %	No. of Shoots/explant	Shoot growth
1.	To	41.06±2.51	2.31±0.14	Poor
2.	T1	78.59±2.48	14.65±0.43	Good
3.	T2	77.78±2.34	11.96±0.33	Good
4.	T3	73.31±1.31	11.23±0.19	Moderate
5.	T4	73.44±1.83	8.610.19	Moderate
6.	T5	65.06±1.20	4.81±0.11	Moderate
7.	T6	78.30±1.76	13.12±0.29	Good
8.	T7	74.68±1.65	10.93±0.24	Good
9.	T8	63.41±1.65	8.31±0.22	Moderate
10.	T9	61.91±1.41	7.31±0.17	Moderate
11.	T10	58.72±0.82	5.05±0.07	Moderate
12.	T11	88.16±1.08	19.12±0.24	Good
13.	T12	80.00±4.39	16.90±0.93	Good
14.	T13	69.43±2.28	14.70±0.49	Moderate
15	T14	67.77±1.82	12.33±0.33	Moderate
16	T15	64.98±1.64	10.78±0.28	Moderate
	C.D.	5.904	1.00	
	SE(m)	2.049	0.35	
	SE(d)	2.898	0.49	
	C.V.	0.19	0.40	

Out of all the hormonal combinations it is observed that there is low growth rate when a single hormone (BAP or Kinetin) is used in the MS media and when used in combinations (BAP + Kinetin) there is higher growth **(**Table 2 and 3) From this, it is concluded that both thegenotypes have shown higher growth in T11 containing (0.5 BAP + 0.5 Kinetin mg/L) which is given in the (Table 2 and 3).

The results which were observed in this study indicate that the addition of at least a single cytokinin (BAP or Kinetin) to the MS basal medium proved highly effective as remarkably high-frequency shoot establishment and number of shoots in both the genotypes. It is also observed that both the hormones BAP and Kinetin when used in combination have shown green, healthy plants with vigorous growth.

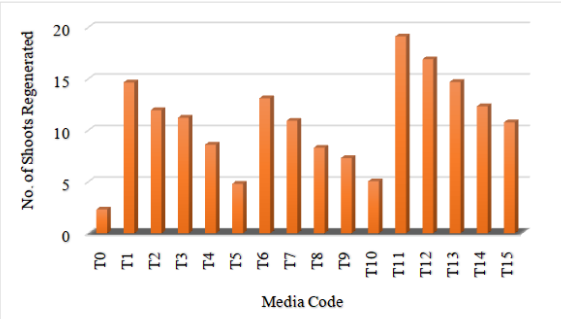

Fig. 1. Effect of different hormonal combinations on number of shoots regenerated of sugarcane genotype Co 15023 after 8 weeks.

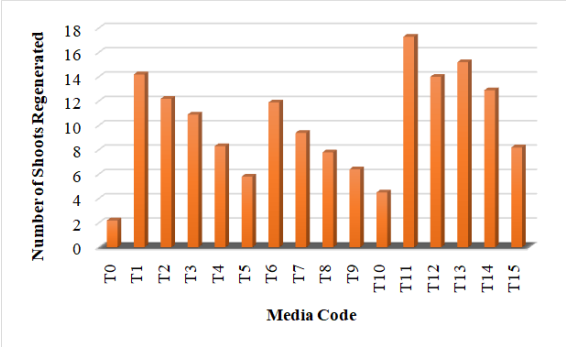

Fig. 2. Effect of different hormonal combinations on number of shoots regenerated of sugarcane genotype Cos 13235 after 8 weeks.

It is observed that similar results were reported by several researchers in their findings about the effect of cytokinins for enhancing shoot multiplication of sugarcane they are (Ajadi *et al*., 2018; Rahman *et al*., 2020) both genotypes showed decreased shoot growth parameters *viz.,* frequency of shoot regeneration (%) and number of shoots per explant with increase in concentration of BAP and Kinetin and at higher concentrations decline in growth rate of plants is observed. These results were similar with (Kumar *et al*., 2020; Azu *et al*., 2022). The least multiplication rate was observed in control where there is no presence of hormones in the basal MS media. Thus, BAP and Kinetin had major influence on *in-vitro* shoot multiplication in both the sugarcane genotypes. This result was similar in conformity with earlier researchers

of sugarcane crop (Khaskheli *et al*., 2019; Qazi *et al*., 2019; Bhalerao and Kumar 2019; Yadav *et al*., 2019; Amente and Feyissa 2021; Kumar *et al*., 2020; and Azu *et al*., 2022) who obtained higher *in-vitro* shoot multiplication at o.5 mg/L concentration of both the cytokinin BAP and Kinetin.

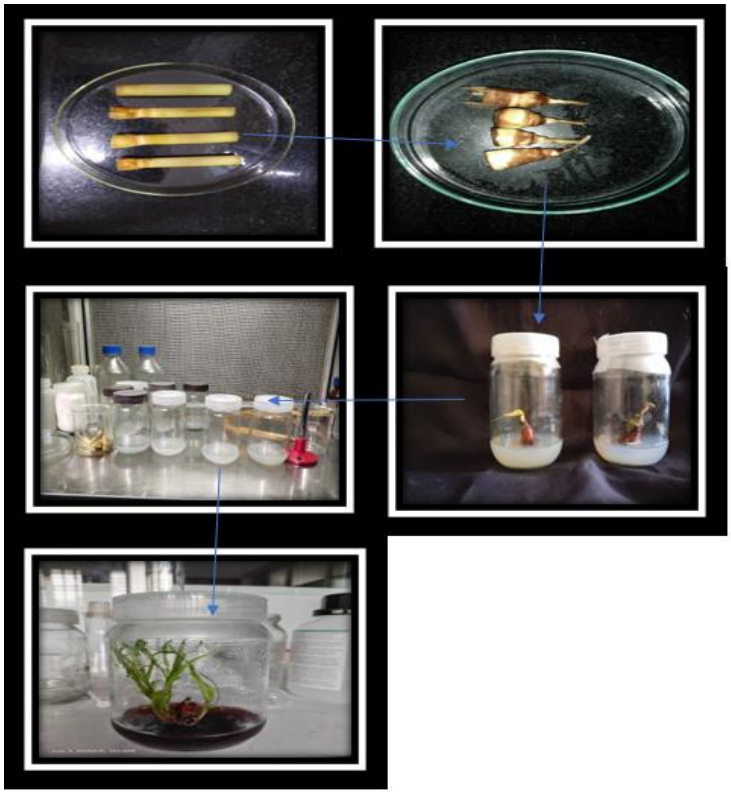

Fig. 3. Steps involved in *an in-vitro* micropropagation of sugarcane genotypes.

CONCLUSIONS

A well-standardized protocol for *in-vitro* shoot tip culture of sugarcane genotypes Cos 13235 and Co 15023 is done by treating with different hormonal combinations. The best growth was observed in the MS medium supplemented with a hormonal concentration of 0.5 mg/L BAP + 0.5 mg/L Kinetin in both of the sugarcane genotypes Cos 13235 and Co 15023. On the basis of results obtained in this study, it can be concluded that the developed protocol for shoot tip culture can be used for *in-vitro* shoot tip culture of sugarcane in commercial level, it also shows relative advantage such as taking short duration for shoot multiplication, disease free culture plants.

FUTURE SCOPE

The future scope of sugarcane micropropagation using different hormonal combinations holds great potential for enhancing the efficiency, speed, and quality of sugarcane propagation. The development of novel hormone combinations and culture protocols can help achieve higher multiplication rates in sugarcane micropropagation. This would result in the production of a greater number of plantlets from a single explant, allowing for larger-scale propagation and increased supply of quality planting material. By starting with virus-free explants and implementing appropriate protocols, the production of virus-free sugarcane plants can be achieved, thereby reducing the spread of diseases and ensuring healthier crops. With improved protocols and optimized hormonal combinations, sugarcane micropropagation can potentially lead to cost reductions in production and labour, as well as increased overall productivity. This can positively impact the economics of sugarcane cultivation and provide benefits to farmers and the sugarcane industry as a whole. It is important to note that research, optimization, and development of these techniques will require rigorous scientific investigations, field trials, and collaboration between researchers, agricultural institutions, and industry stakeholders.

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Conflict of Interest. None.

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