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In vitro shoot proliferation in commercial cultivars of sugarcane (*Saccharum officinarum* L.)

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Abstract: A study was carried out with the objective to standardize the media supplements for shoot proliferation of Co 86032 and Co 7805. The result revealed that MS medium fortified with Kinetin 3.0 mg/l and 1.0 mg/l BAP were found to be optimum for early shoot initiation, Kinetin 2.5 mg/l and 1.0 mg/l significantly produced maximum number of shoot (5.10) in sugarcane cv. Co 86032. MS medium fortified with Kinetin 2.0 mg/l and 1.0 mg/l BAP was found to be most suitable for early shoot initiation. Kinetin 2.0 mg/l and 1.0 mg/l BAP significantly enhanced available days for shoot proliferation (10.00), Kinetin 2.5 mg/l and 1.0 mg/l BAP significantly increased number of shoots (4.32) in cv.7805.Hence the protocol is useful for shoot proliferation, rapid *in vitro* propagation and production of large quantity of quality planting material of this sugarcane cultivars.

Key words: Bio regulators, Explants, *In vitro, Saccharum officinarum,* Shoots

Introduction

Plant tissue culture of sugarcane has received considerable research attention because of its economic importance as a cash crop. Sugarcane (*Saccharum officinarum* L.) is an important agricultural cash crop in tropical and subtropical region of the world and it is the major source of sugar with respect to export product in many developing countries that accounts for more than 60% of the world's sugar production Guimarces and Sobrala (1998); Behera *et al. (*2009). In India, agriculture is not an agri-business, but way of life. Sugarcane, an agro-industrial crop, is an important integral component of the agriculture. It assumes the important position in the economy by contributing, nearly 1.9% of National GDP. Sugarcane is cultivated in over 4 million hectares spread over a wide range of agro-ecological situations, both in tropical and sub-tropical regions. Sugar sustains a second largest production in organized agro-industry. Presently, the total production of sugarcane is around 300 million tonnes (Shahi *et al.,* 2002). Sugarcane is the production of natural pharmaceutical compounds (Menendez *et al.,* 1994). Further, agricultural and industrial by-products of the sugar production process are extensively employed for animal nutrition, food processing, paper manufacturing and fuel (Ali *et al.,* 2010). This crop is traditionally propagated by means of setts, which is the major bottleneck for perpetuation. Transportation of huge quantities of setts from one place to another is quite cumbersome and also the cut ends are infected by various pathogens. Besides, the conventional method of propagation is slow process with lower multiplication rate. Under such predicaments, it is essential to find out and implement technological interventions for the same. *In vitro* cloning is a tool for obtaining true to type and disease free quality planting materials in shorter period of time. Once its protocol developed for a particular crop variety can be used for bulk production of planting materials. *In vitro* multiplication of sugarcane has received considerable research attention because of its economic importance as a cash crop. Micro propagation is currently the only realistic means of achieving rapid, large-scale production of disease-free quality planting material as seed canes of newly developed varieties in order to speed up the breeding and commercialization process in Sugarcane (Behera *et al.,* 2009). In view of the above facts, research will be carried out to develop and standardize an economically viable method for production of disease free quality planting material through *in vitro* cloning and meristem culture in large scale in shortest possible time to standardize of media supplement for shoot proliferation of commercial cultivars of sugarcane Co86032 and Co7805.

Materials and Methods

The investigation was carried out at the biotechnology-cum- Tissue Culture Center, OUAT, Bhubaneswar. Healthy and disease free plants varieties of Co86032 and Co7805 were taken from the mother Sugarcane blocks for conducting *in vitro* research in the laboratory Biotechnology-cum-Commercial tissue Culture

Centre, Odisha, Bhubaneswar. The terminal shoots of two varieties of sugarcane were taken for the experiment from the pre established shoot initials in the laboratory. The chemicals used for the present study were analytical reagents of excel R grade of Titan Biotech Ltd., Merck(India), qualigen fine Chemicals, and Himedia Laboratories Ltd. (India). Auxin, cytokinin, myoinositol and fe-EDTA were supplied by Sigma (USA) and Agar from Ranbaxy Laboratory Limited. The glassware used for culture work comprised of 250 ml, 500 ml and 1000 ml conical flasks, culture bottles, test tubes, jars, pipettes, measuring cylinder (100 ml, 500m1). The glassware was properly cleaned with teepol detergent, washed under running tap water and left to dry. The glassware was then steam sterilized in an autoclave at pressure of 15 psi pressure at 121°C for 20 minutes. For the preparation of MS culture medium (Murashige and Skoog, 1962) required quantities of macronutrients, micronutrients, Fe-EDTA, vitamins and plant bio regulators were taken from the stock solution and required quantity of sucrose dissolved in water was added fresh to the medium. Then volume was made up to 1 liter with distilled water. The pH of the solution was adjusted to 5.7$\pm$ 0.1 using 0.1N NaOH or 0.1 N HCL. Agar (0.6% w/v) was added to the medium boiled and poured to the culture bottles and capped. Capped culture bottles containing culture medium were autoclaved for the 20 minutes at 121^{0}C and 15 Psi pressure. The autoclaved medium was kept in a laminar air flow bench for cooling. All the glassware were dipped in detergent solution for overnight and washed under running tap water. They were rinsed with distilled water and then dried in an oven for 2hrs at 150^{0}C. Forceps, petridishes and scaples were thoroughly cleaned with isopropanol or rapped with paper and kept in a clean sterilized in autoclave at 15 psi and 121^{0}C for 20 minutes. The working chamber of laminar air flow cabinet was wiped with isopropanol . Filtered air (80-100 cft/min) to ensure that particles do not settle in working area was blown for 5minutes. The sterilized materials to be used (except living tissue/ explant) were kept made the chamber and exposed to UV light for 30 minutes. While working, filtered air was continuously passed the laminar air flow cabinet. The culture were kept at 25$\pm$2^0 C in an air conditioned room with a 16 hours light period (3000-3200 lux) supplied by fluorescent tubes and 80% relatively humidity (Sahoo *et al.,* 2015). The micro shoots were inoculated in MS medium alone (Control), in combination with Kinetin (1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0 mg/l) and all the concentration of Kinetin with BAP (1.0 mg/l). Three replication per treatment and 10 cultures bottles per replication were used during the culturing for 14 days each for micro shoots production, shoot proliferation. Three culture/ bottles, three culture bottles per replication were marked for observation in each treatment i.e. Days to shoot initiation, days available for shoot proliferation, number of shoots, length of the shoot and colour of the leaf. The data recorded from the experiments were analyzed following the method of Gomez and Gomez (1984) using one way Analysis of variance (ANOVA) in Completely Randomized Design (CRD). The treatment effects were tested by F-test at 5% level of significance. The critical difference at 5% level was calculated for comparing the treatment means.

Results and Discussion

The results of the experiment on impact of plant bioregulators on shoot proliferation of sugarcane cv. Co86032 presented in table1 revealed that MS medium fortified with Kinetin 3.0mg/l and BAP 1.0 mg/l advanced the days for shoot initiation (5.20). In case of shoot proliferation MS medium supplemented with Kinetin 3.0 mg/l and BAP 1.0mg/l significantly enhanced the maximum available days for shoot proliferation and the data stood at par with the treatment T_{12} (Kinetin 2.5mg/l and BAP 1.0 mg/l),T_1 (Kinetin 2.0mg/l and BAP 1.0 mg/l),T_{14}(Kinetin 3.5mg/l and BAP 1.0 mg/l), T_1 (MS medium),T_2 (Kinetin 1.0mg/l).From the perusal of the data it was evident that the treatment T_{12} (Kinetin 2.5mg/l and BAP 1.0mg/l) significantly produced maximum number of shoots (5.10) which stood at par with T_{11} (Kinetin 2.0mg/l and BAP 1.0mg/l) and T_{13} (Kinetin 3.0mg/ l and BAP 1.0mg/l). Significantly longer shoots (2.25cm) was recorded in control i.e.; T_1(MS medium) followed by T_{13} (Kinetin 3.0mg/l and BAP 1.0mg/l), T_2 (Kinetin 1.0mg/1) and T_{12} (Kinetin 2.5mg/l and BAP 1.0mg/l). Colour of the plants as well as leaves remained green in all the treatments. Considering all the above mentioned characters the treatment T_{13} (Kinetin 3.0mg/l and BAP 1.0mg/l), T_{12} (Kinetin 2.5mg/l and BAP 1.0mg/l) and T_{11} (Kinetin 2.0mg/l and BAP 1.0mg/l) advanced the days for shoot initiation and also increased more number of multiple shoots/explant. However the treatment T_1 (MS medium) increased the length of the shoot in the cultivar Co86032. MS medium fortified with mediocre concentrations of Kinetin and lower concentration of BAP advanced the shoot initiation in Sugarcane cultivar Co86032. The treatment also enhanced number of shoots for the explant. However length of the shoot was maximum in MS medium only. Increase in concentration of Kinetin decreased the number of shoot/explant and also delayed the shoot initiation process. MS medium fortified with various concentration of Kinetin had not shown any spectacular result. Role of MS was tremendous as far as length of the shoot was concerned. This was in agreement with the work done by Dash (2004); Dinesh *et al.* (2015).

In case of sugarcane cv. Co7805 from the result revealed that the treatment T_{11} (Kinetin 2.0 mg/l and BAP1.0 mg/l) was found to be most suitable one for early shoot initiation requiring 4 days for shoot initiation (Table 2). It remained at par with the treatments T_7 (Kinetin 3.5mg/l), T_6 (Kinetin 3.0mg/l), T_2 (Kinetin 1.0mg/l). Regarding the days to shoot proliferation, MS medium fortified with Kinetin 2.0mg/l and BAP 1.0mg/l Significantly enhanced the days for shoot proliferation remaining at par with the treatment T_7 (Kinetin 3.5mg/l), T6 (Kinetin 3.0mg/l), T_2 (Kinetin 1.0mg/l) and T_9(Kinetin 1.0mg/l and BAP 1.0mg/l). From the perusal of the data from the table 2 it was evident that MS medium fortified with Kinetin 2.5mg/l and BAP 1.0mg/l Significantly enhanced the number of shoot (4.32) followed by the treatment T_2 (Kinetin 1.0 mg/l) followed by T_{11}(Kinetin 2.0mg/l and BAP 1.0mg/l) and T_8(Kinetin 4.0mg/l). Significantly longer shoot (3.78cm) were recorded in T_8 (Kinetin 4.0mg/l) followed by T_7(Kinetin 3.5mg/l) and T_6 (Kinetin 3.0mg/l). It was interesting to note that the treatment such as T_4(Kinetin 2.0mg/l), T_5(Kinetin 2.5mg/ l) and T_{15}(Kinetin 4.0 mg/l and BAP 1.0 mg/l) albino colour shoots were regenerated in the variety Co7805.Considering above

Table-1: Impact of plant bioregulators on shoot proliferation of Sugarcane cv. Co86032

	Treatments	Days to shoot initiation	Days available for shoot proliferation	Number of shoots	Length of the shoot	Colour of the leaf
T_1	MS	6.53	7.47	2.27	2.25	Green
T_2	MS+1.0 mg/l Kn	6.90	7.10	3.24	1.69	Green
T_3	MS+1.5 mg/l Kn	9.50	5.50	0.71	0.00	Green
T_4	MS+2.0 mg/l Kn	12.07	1.93	0.71	0.50	Green
T_5	MS+2.5 mg/l Kn	12.33	1.67	0.71	0.50	Green
T_6	MS+3.0 mg/l Kn	9.68	4.32	0.71	0.50	Green
T_7	MS+3.5 mg/l Kn	9.40	6.60	2.00	0.50	Green
T_8	MS+4.0 mg/l Kn	7.23	6.77	2.52	0.70	Green
T_9	MS+1.0 mg/l Kn+ 1.0 BAP mg/l	7.10	6.90	2.97	1.00	Green
T_{10}	MS+1.5 mg/l Kn+ 1.0 BAP mg/l	6.77	6.90	4.31	1.20	Green
T_{11}	MS+2.0 mg/l Kn+ 1.0 BAP mg/l	6.00	8.00	5.00	1.40	Green
T_{12}	MS+2.5 mg/l Kn+ 1.0 BAP mg/l	5.67	8.33	5.10	1.47	Green
T_{13}	MS+3.0 mg/l Kn+ 1.0 BAP mg/l	5.20	8.60	5.00	1.83	Green
T_{14}	MS+3.5 mg/l Kn+ 1.0 BAP mg/l	6.87	7.13	1.40	1.11	Green
T_{15}	MS+4.0 mg/l Kn+ 1.0 BAP mg/l	7.33	6.67	1.31	0.82	Green
	SE(M)±	0.28	0.48	0.04	0.03	
	CD (P=0.05)	0.81	1.35	0.13	0.10	

Table-2: Impact of plant bioregulators on shoot proliferation of Sugarcane cv. Co7805

	Treatments	Days to shoot initiation	Days available for shoot proliferation	Number of shoots	Length of the shoot	Colour of the leaf
T_1	MS	7.30	6.70	3.28	0.78	Green
T_2	MS+1.0 mg/l Kn	4.33	9.67	3.63	1.68	Green
T_3	MS+1.5 mg/l Kn	5.40	8.67	3.00	2.50	Green
T_4	MS+2.0 mg/l Kn	6.30	8.33	3.00	2.58	Albino
T_5	MS+2.5 mg/l Kn	5.20	9.10	2.72	2.62	Albino
T_6	MS+3.0 mg/l Kn	4.33	9.67	2.00	2.78	Green
T_7	MS+3.5 mg/l Kn	4.20	9.80	3.53	3.10	Green
T_8	MS+4.0 mg/l Kn	5.33	8.67	3.58	3.78	Green
T_9	MS+1.0 mg/l Kn+ 1.0 BAP mg/l	4.67	9.33	3.33	2.59	Green
T_{10}	MS+1.5 mg/l Kn+ 1.0 BAP mg/l	6.30	8.03	3.50	2.50	Green
T_{11}	MS+2.0 mg/l Kn+ 1.0 BAP mg/l	4.00	10.00	3.62	2.50	Green
T_{12}	MS+2.5 mg/l Kn+ 1.0 BAP mg/l	5.00	8.00	4.32	1.81	Green
T_{13}	MS+3.0 mg/l Kn+ 1.0 BAP mg/l	7.20	8.33	1.34	1.97	Green
T_{14}	MS+3.5 mg/l Kn+ 1.0 BAP mg/l	10.20	4.67	0.83	1.79	Green
T_{15}	MS+4.0 mg/l Kn+ 1.0 BAP mg/l	10.00	4.33	0.83	0.50	Albino
	SE (M)±	0.17	0.31	0.08	0.03	
	CD (P=0.05)	0.48	0.88	0.23	0.09	

mentioned characters it was concluded that the treatment T_{11}(Kinetin 2.0 mg/l and BAP 1.0 mg/l) was suitable for shoot initiation and proliferation whereas the treatment T_{12}(Kinetin 2.5 mg/l and BAP 1.0 mg/l) was found to be best for production of multiple shoots (4.32) and the treatment T_8(Kinetin 4.0 mg/l) was found to be best for production of multiple and long shoots. In cultivar Co 7805, MS medium supplemented with Kinetin at mediocre to somewhat higher, advanced the days to shoot initiation and enhanced the plant height. However MS medium with mediocre level of Kinetin and lower level of BAP increased the number of multiple shoots/explants. Higher concentration of Kinetin delayed the process of shoot initiation reduced the multiple shoot /explant and plant height.

In both the cultivars such as Co7805 and Co86032, shoot multiplication was influenced tremendously by the presence of cytokinin such as Kinetin and BAP. The ability of cytokinin to promote the growth of dicotyledons have been reported by Murashige and Skoog (1974). Cytokinin at moderate concentrations enhances shoot development; at higher levels it promotes multiple shoots through precocious axillary shoot formation (Ammirato, 1976; Godheja *et al*., 2014). Shoot tip and axillary bud culture of various species (Palai, 2001). During cell cycle the DNA synthesis and mitosis is controlled by auxin while cytokinesis in controlled by Kinetin (Jain, 2000). Cytokinin helps in cell division and influence growth through two forms, one incorporated inside tRNA and in other free states. The cytokinin occurs as constituents of tRNAs. They determine the conformation of anticodon and control the synthesis of protein and hence enzymes. The free cytokinin protect tRNAs from ribonucleases. Cytokinin also helps the plant cells to accumulate

solutes very actively and regulate transport of nitrogen during development. In the presence of cytokinin phloem carries more of nitrogen (Bhatia and Parasher, 2003). The MS media fortified with varying concentration of Kinetin and 1.0 mg/l and BAP for the two cultivars Co86032 and Co7805 gave explants exhibiting shooting. The result obtained in this experiment is at par with the finding of Toma *et al.* (2001). Cytokinins are an important regulatory factor of plant meristem activity and morphogenesis, with opposing roles in shoot and roots. Growing on cytokinin rich media can reduce apical dominance leading to more numbers (Theodore, 1964). The above results are in agreement with Chattha (2001) for sugarcane.

It was concludes that *in vitro* shoot proliferation of two commercial sugarcane varieties Co 86032 and Co 7805 has been developed. The result indicated that in shoot proliferation sugarcane was highly depends on the interaction effect of Kinetin and BAP. MS medium in combination with 3.0 mg/l Kinetin and 1.0 mg/l BAP is most suitable for shoot proliferation and shoot length in sugarcane cv. Co86032. MS medium supplemented with Kinetin 2.0 mg/l and 1.0 mg/l BAP suitable for shoot initials and shoot proliferation for sugarcane cv.Co7805. Thus the study bestowing a new vantage for the increased demand for production of quality planting materials in the current agricultural productivity scenario of the world.

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