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Establishment the Synchronization System for Mass Clonal Propagation of Sugarcane (*Saccharumofficinarum L*) through Shoot tip Culture

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Abstract*—The aim of present investigation was to examine the efficiency of three different sugarcane (Saccharumofficinarum L.) verities viz. NIA 1026-P7, NIA-87 and SG NIA-2476.A total of 20 explants (apical meristems) shoot tip were inoculated on MS Basal media along with various concentrations of 2, 4-D, BAP, Kinetin and IAA. The results of different verities showed that initial growth, number of micro shoots and shoot length was obtained in variety NIA-1026-P7, on MS medium supplemented with low concentration of 2, 4Dichlorophenoxyacetic acid (2, 4-D) along with highest concentration of Benzyl aminopurine (BAP) and Kinetin. The variety SG NIA 2476 expressed modestly and was not sufficient as compared with other varieties. The established plantlets were transferred on the MS half strength rooting medium along with various concentrations of IBA and IAA. The results of different varieties showed that maximum number of micro roots and roots length were established in variety NIA 87, on the half strength MS medium plus 1.50 mg/l IBA, 3.0 mg/l IAA and 25 g/l of sugar.*

***Keywords*— Auxin, Cytokinin, In-vitro, Plant Growth hormones, Sugarcane.**

I. INTRODUCTION

Sugarcane (*Saccharumofficinarum,* L.) is the agro-industrial cash crop of Pakistan belongs to the *poaceae* family and cultivated in tropical and subtropical area of the world. Due to high economic importance in Pakistan mostly contributed share of country; where accounts about 70% of worlds total sugar production [1, 2]. The production of sugarcane described a very promising picture and reached to historical high of 73.6 million tons showing with increase of 12.4 % over the production of 65.5 million tons during 2015-16 and comfortably exceeded target of 67.5 million tons by a considerable margin of 9.0 % [3]. Pakistan occupies 5thposition in respect to area under sugarcane crop and almost 15thposition in cane production but ranks far below in sugar production [4]. Pakistan average yield of sugarcane is 48.9 tonnes per hector as compared with globally which is nearby 65.5 tonnes per hector, however Egypt and India are getting around 60 tonnes and 121 tonnes per hector individually [5]. Inefficient yield and lower sugar recovery cause high production cost that's why Pakistan the minimum aggressive in domestic and global sugar markets [6]. Generally conventional propagation of sugarcane multiplied vegetatively by stem cuttings which suffered from low propagation rates, expensive labour, time consuming and potential transmission of pathogens through seed cane from generation to generation [7]. It is still a problem due to unfavourable climatic conditions. Thus, lack of viable fuzz production makes it difficult to improve sugarcane through conventional breeding in Pakistan [8]. Therefore, now a day's most widely used techniques plant tissue culture like a Micro propagation and *in vitro* 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 [9]. Plant tissue culture is currently a powerful tool that plays a major role in rapid multiplication of disease free planting material of newly improved varieties through in vitro technique on continuous year rounded basis [10]. Shoot tip culture is relatively simple *in vitro* method for rapid propagation of selected sugarcane materials and the clean or pathogen free plant materials [11]. As a result of

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the regeneration of plants through tissue culture technique could be a viable option for improving the quality and productivity of sugarcane. So far, a lot of reports have been published in the tissue culture of sugarcane from different countries [12, 13].

The aim of present investigation to determine the efficiency of three different sugarcane varieties were tested their performance under synchronization system for regeneration of shoots and roots.

II. MATERIAL AND METHOD

2.1. Collection of explants/ Growth media preparation

The present experiment was done at Plant tissue Culture Laboratory, Plant Breeding Genetics Division, Nuclear Institute of Agriculture (NIA) Tando Jam. Three sugarcane` varieties Viz. NIA-1026-P7, NIA-87 and SG-NIA-2476 were tested in this experiment. Fresh, healthy and young apical meristems were collected from Experimental Farm, Nuclear Institute of Agriculture (NIA) Tando Jam.The Murashige and Skoog [14]medium containing with different concentration of 2, 4-D, BAP, IAA and Kinetin for shoot induction, for induction of rooting plantlets were transferred half strength MS basal media added various concentrations of IBA and IAA.

2.2. Surface Sterilization

The excised young and mature shoot tip removed leaf sheath on the surface of explants and cut into 1 to 1.5 cm long segments and washed in running tap water for five minutes, surface disinfected using 70 % ethanol for 40 second and then immersed in 10 % sodium hypochlorite solution of commercial laundry bleach (5.25% NaOCl). The pH of medium was adjusted at 5.7-5.8 before autoclaving and media was autoclaved at 121 °C for 20 minutes.

2.3. Tissue culturing

After that plant samples were brought under the laminar airflow cabinet and washed thrice with sterilized distilled water. The soft segment of apical meristem then cut into small disc and placed on MS basal medium supplemented with different concentration of plant growth hormones.

2.4. Culture maintenances

After successfully explants were inoculated in the culture bottles then these culture bottles were transferred in the incubator for one week, after one week it is again transferred in the growth room forUniform culture conditions were maintained as 16-hour photoperiod at 25±2 ^{o}C for growth temperature.

2.5. Obtained data and phenotypic analysis

The experiments were laid out in completely randomized design (CRD) with three replications. The phenotypic data were collected, days taken shoot initiation, number of micro shoots, shoot length (cm), number of micro roots and root length (cm).

2.6. Statistical Analysis

The experimental data were recorded and subjected to factorial design of analysis of variance (ANOVA) under linear models of statistics to observe statistical differences among different traits of wheat using computer program, Student Edition of Statistix (SWX), Version 8.1 (Copyright, Analytical Software-USA). Further least significant difference (LSD) test was also applied to test the level of significance among different combination means 15, 16].

III. RESULTS AND DICUSSION

3.1 Hormonal Concentration Regulates the Micro-Shoot Initiation

The results of different varieties showed that days taken to shoots initiation (40.91) was observed in variety NIA-1026-P7 and later days taken to shoots initiation (50.83) was recorded in variety SG-NIA-2476 (Figure 1).The results of different varieties indicated that maximum number of micro shoots were recorded (40.75) in variety NIA-1026-P7 and minimum number of micro shoots were observed (28.41) in variety SG-NIA-2476 (Figure 2).The results of varieties indicated that highest shoots length were recorded (6.22 cm) in variety NIA-1026-P7 and lowest shoots length were recorded (5.58 cm) in variety SG-NIA-2476 (Figure 3).The results of different varieties indicated maximum number of roots were recorded (14.58, 11.17 and 10.16) in varieties NIA-1026-P7, NIA-87 and SG-NIA-2476 separately (Figure 4).The results of different varieties showed that days taken to shoots initiation concurred with [17] publicised a protocol for stimulation and organogenesis shoots proliferation, found early days to shoots initiation for tip extension were observed 15 and 20 days with the concentration MS media containing 3 % sugar combined with BAP and Kin, 1.50 mg L^{-1} GA_3 3.0 mg L^{-1} resulted about 100 % explants showing shooting. However the result number of shoots which corresponded with [18] developed the method for callus and regeneration was set up under *in vitro* culture developing immature shoot tip as explants, maximum shoot enlistment was observed in MS medium added with 2.50 mg L^{-1} 2, 4-D, 2.0 mg L^{-1}BAP and 0.5 mg L^{-1} NAA.The results equally comparable with [19] publicised that variety B4906 gave the highest (16.88) shoots with 5.94 cm average shoot length in MS medium added with 1.5 mg L^{-1}BAP and 0.4 mg L^{-1} NAA while, Pr1013 delivered highest 11.70 shoots explants^{-1} with 4.48 cm shoot length on MS media sustained by 2.0 mg L^{-1} BAP and 0.5 mg L^{-1} NAA [17].

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3.2 Rooting of established shoots inhibited by the interaction of Auxin-Verities

The results of different varieties indicated that highest roots length were observed (5.90 cm) in variety NIA-87 and lowest roots length were recorded (4.66 cm) in variety SG-NIA-2476 (Figure 5). Among the combinations, IBA and NAA indicated best response with professed establishing MS½medium added with 2.50 mgl^{-1} NAA showed best response and the highest number of roots per micro shoots were 13.9 obtained within 8-10days with average root length 4.3 cm for the variety Co-91017. The stronger root growth development was impacted by IBA at the concentration of 1.0 mg $L^{-1}$with the maximum number of 41 roots $plant^{-1}$ and poor quality rooting response was observed at 0.1-0.5 mg L^{-1}IBA combined with 0.5-2.0 mg L^{-1} BAP. The concurred with[19] observed the rooting response at concentration of 2.50 mg L^{-1} IBA and it exhibits 16 numbers of roots at the length of 1.1 cm, whereas 0.5 mg L^{-1} NAA + 2.50 mg L^{-1} IBA indicated rooting response with 11.3 with a number of roots and 3.7 cm root length.

IV. CONCULSION

It was concluded that days to micro shoots initiation, number of micro shoots, can be obtained on MS + 2.0 mg L^{-1} 2, 4-D + 4.0 mg L^{-1} BAP + 20 g L^{-1} sugar in variety NIA-1026-P7 (Figure 6-7). while the shoots length (cm) can be achieved on MS + 1.0 mg L^{-1} 2, 4-D + 4.0 mg L^{-1} IAA + 2.0 mg L^{-1} Kin + 20 g L^{-1} sugar in variety NIA-1026-P7, however the number of micro roots and roots length (cm) can be obtained on MS½ + 3.0 mg L^{-1} IBA + 1.50 mg L^{-1} IAA + 30 g L^{-1} sugar in variety NIA-87.

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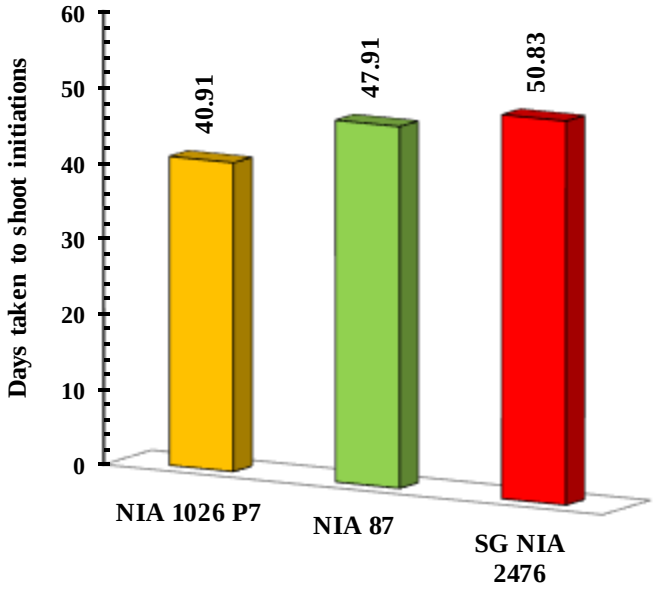

Fig.1: Invitro regeneration of sugarcane through shoot tip culture. *The treatment means were compared using Least Significant Difference (LSD) at 5% level of Probability. NIA 1026 P7, NIA87, SG NIA 2476 represents the different verities of sugarcane. Data are the average of three different biological replications.*

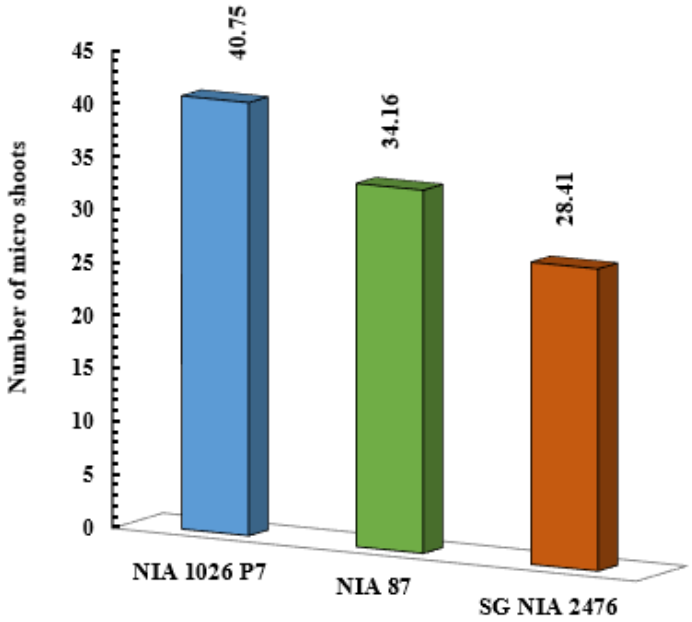

Fig.2: Encountering the number of micro shoots. The treatment means were compared using Least Significant Difference (LSD) at 5% level of Probability. NIA 1026 P7, NIA87, SG NIA 2476 represents the different verities of sugarcane. Data are the average of three different biological replications.

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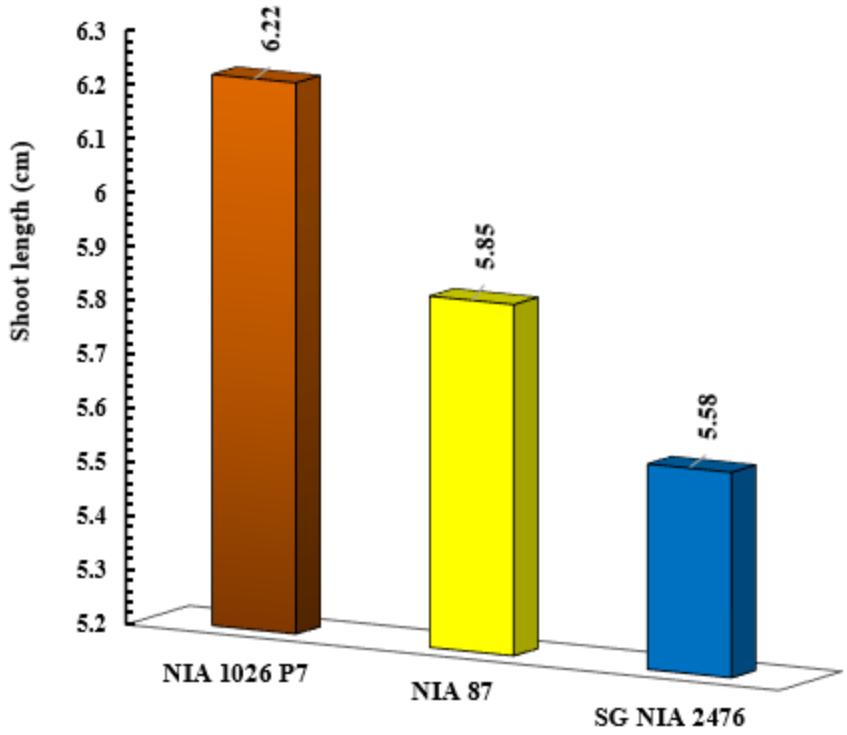

Fig.3: Shoots length modulated by different varieties of sugarcane. The treatment means were compared using Least Significant Difference (LSD) at 5% level of Probability. NIA 1026 P7, NIA87, SG NIA 2476 represents the different verities of sugarcane. Data are the average of three different biological replications.

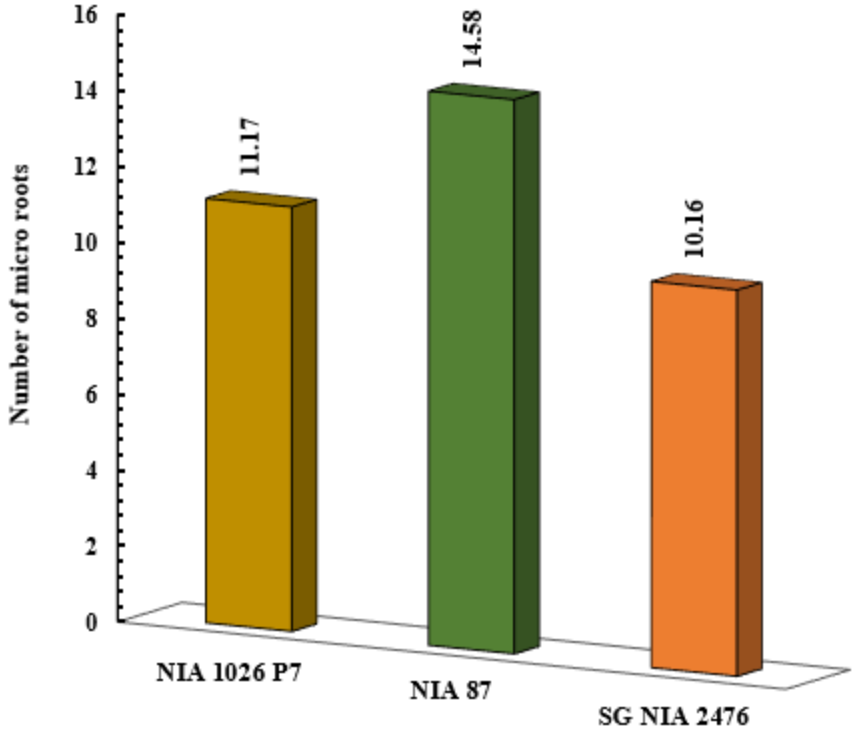

Fig.4: Micro roots maximization in different varieties of sugarcane. The treatment means were compared using Least Significant Difference (LSD) at 5% level of Probability. NIA 1026 P7, NIA87, SG NIA 2476 represents the different verities of sugarcane. Data are the average of three different biological replications.

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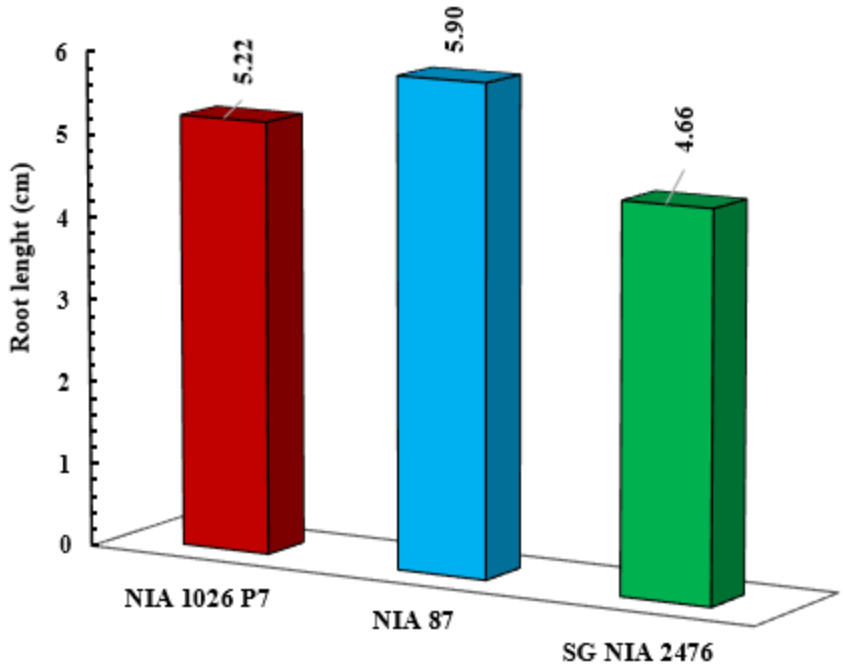

Fig.5: Slight inhibition of root length. The treatment means were compared using Least Significant Difference (LSD) at 5% level of Probability. NIA 1026 P7, NIA87, SG NIA 2476 represents the different verities of sugarcane. Data are the average of three different biological replications.

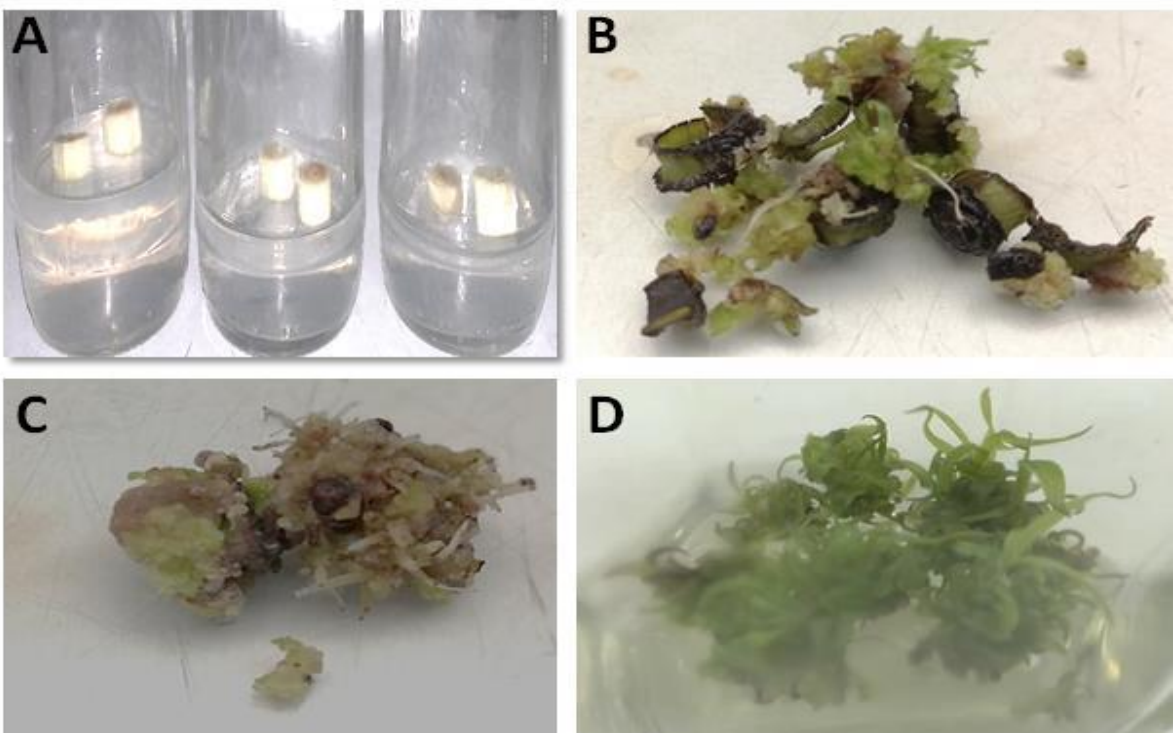

Fig.6: Mass-clonal proliferation of sugarcane on invitro condition. (A)Disc inoculated in the culture bottles(B)Growth starting from small segment of meristic tissue (C) Differentiate the tissue in the micro shoots(D)Establishment of seedling. Experiments performed thrice as different biological replications. Data so obtained at two days interval on each replications. Regenerated shoots were then transferred to rooting media.

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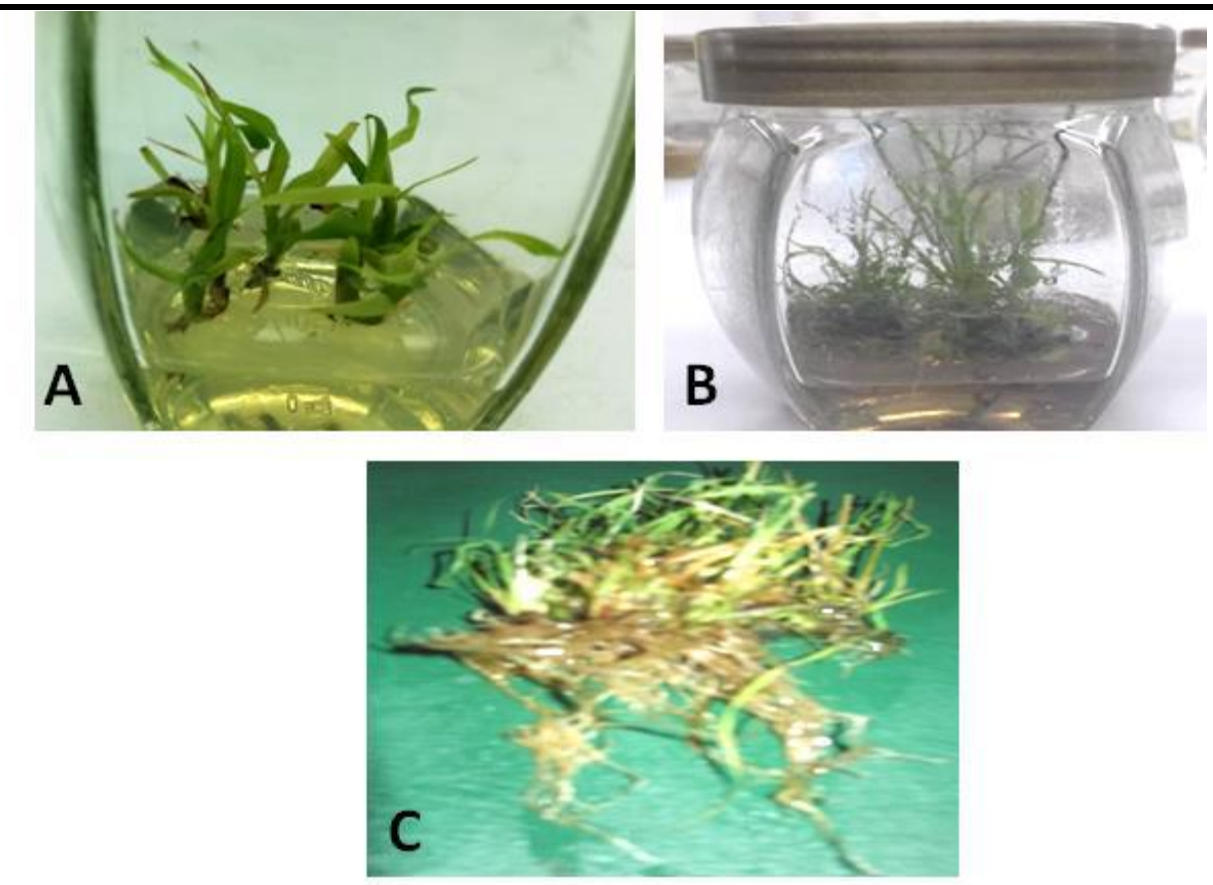

Fig.7: Mass-clonal production and multiplication of sugarcane. (A) Plantlets ready for establishing further height (B) Height of seedling (C) Established maximum micro roots and its length Experiments performed thrice as different biological replications. Data so obtained at two days interval on each replications. Rooted seedlings ready for acclimatization

Supplementary Tables

Table S.1. Regeneration of sugarcane on controlled condition.

	Days taken to shoot initiations		
Varieties	NIA-1026-P7	NIA-87	SG-NIA-2476
Mean	40.91 c	47.91 b	50.83 a
	Number of micro shoots		
Varieties	NIA-1026-P7	NIA-87	SG-NIA-2476
Mean	40.75 a	34.16 b	28.41 c
	Shoot length		
Varieties	NIA-1026-P7	NIA-87	SG-NIA-2476
Mean	6.22 a	5.85 b	5.58 b
	Number of micro roots		
Varieties	NIA-1026-P7	NIA-87	SG-NIA-2476
Mean	11.17 b	14.58 a	10.16 b
	Root length		
Varieties	NIA-1026-P7	NIA-87	SG-NIA-2476
Mean	5.22 b	5.90 a	4.66 c

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Table S.2 Composition of MS basal medium (Murashige and Skoog, 1962)

INGREDIENTS OF MS MEDIUM			
Serial No	**Macro Nutrients**		
	Ingredients	**Chemical composition**	**Weight mg l^{-1}**
1	Potassium nitrate	KNO_3	23.5 g l^{-1}
2	Ammonium nitrate	NH_4NO_3	20.5 g l^{-1}
3	Calcium chloride	$CaCL_2$ $2H_2O$	5.5 g l^{-1}
4	Potassium diphosphate	KH_2PO_4	2.5 g l^{-1}
5	Magnesium sulphate	$MgSO_4$ $7H_2O$	4.5 g l^{-1}
6	Potassium nitrate	KNO_3	23.5 g l^{-1}
	Micro Nutrients		
7	Boric Acid	H_3BO_3	0.31
8	Manganesesulphate	$MgSo_4$ $4H_2O$	1.15
9	Zinc sulphate	$ZnSo_4$ $7H_2O$	0.430
10	Ferrous sulphate	Fe SO_4 $7H_2O$	1.390
11	Sodium molybdate	Na_2 M_oO_4 $2H_2O$	0.12
12	Copper sulphate	$CuSO_4$ 5 H_2O	1.25
13	Cobalt chloride	$CoSo_3$ 6 H_2O	1.25
	Iron source		
14	Iron Sulphate	$FeSo_4$ 7 H_2O	27.8
15	Sodium EDTA	Na_2 EDTA	1.865
	Vitamins		
16	Thiamine HCL	$C_{12}H_8N_8$ $OSCL_2$	2.0 mg l^{-1}
17	Myo-inosital		200 mg l^{-1}
	Other		
18	Sugar	$C_{12}H_{22}O_{11}$	20 g l^{-1}
19	Agar		7.5 g l^{-1}
20	pH		5.8