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VIRUS FREE PLANTLETS PRODUCTION OF SUGAR (SACCHARUM OFFICINARUM (L)) THROUGH INVITRO MICROPROPAGATION OF SHOOT TIP CULTURE

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ABSTRACT
Standardization of protocol for virus-free sugarcane production were investigated through shoot tip culture of sugarcane was isolated from grown sugarcane tip of explants and cultured on modified Murashige and Skoog (M&S 1962) medium containing auxin (2, 4-D) and cytokinins (KN, BA, BAP) was used to cultivate shoot tip of sugarcane (L) in vitro. Concentration of sucrose was at 3% W/V level, the PH media was adjusted to 5.6 prior to the addition of agar 8% W/V. young shoot tip were cut into thin smaller pieces of 1.0 to 1.5cm length-and inoculated to the media (various levels of hormones and in different combinations), then incubated at 27 ± 2^0C under light intensity of 2000 – 3000 Lux in the culture room. Treatments 2, 4 – D and BA at levels of 0.8mg/L and 0.25mg/L respectively with GA_3 (0.1mg/L) produce optimum multiplication. After having DAS-ELISA test the invitro grown plantlets were being used for massive micro propagation. Visual evaluation of the morphological trials of the shoot tip culture. Derived plants showed normal and free from various diseases. Gross yield was obtained in shoot tip derived plants over their source plants.
Key Words: *shoot tip, virus free, plantlets production, sugarcane, invitro micropropagation, culture.*

INTRODUCTION
The tissue culture techniques have become a powerful tool for studying and solved food insecurity of the nation. Tissue culture can increase the propagation potential by 20 – 35 times (Geijskes et al (2003)). And Syman et al (2006). In addition plants can be disease-indexes (Synman *et al* (2007)). And healthy material multiplied in half the time compared to the conventional vegetative route. Research on in vitro culture of sugarcane began in the 1960s and several protocols have been documented. These include plantlet regeneration via somatic embryos from callus, shoot tip and immature leaf sections, or directly from apical shoot material (Lee, (1987) and Bourg, (1989). During the last thirty years, micropropagation and other in vitro techniques have become more widely used in commercial horticulture and agriculture for the mass propagation of crop plants Sherrington, (1984) and Dodds (1991). Sugarcane (*saccharum afficinarum L.*) is an important industrial cash crop of Nigeria. It is an economically important, clonally propagated crop that accounts for more than 60% of the of rapid multiplication has been a serious problem in sugarcane breeding (Ali and Afghan,

2001). Poor or inadequate suitable multiplication procedure and contamination by systematic diseases are the serious problem of sugarcane in the open field Lal and Singh (1994). In vitro multiplication of sugarcane has received considerable research attention because of its economic important as a cash crop. Micropropagation 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 Feldmenn et al, 1994, Lal and Krishna, 1994 and Lorenzo *et al*, 2001. Consequently, Barba, et al, (1978) reported that within nine (9) months callus culture of apical meristem produce planting material from a single spindle which was sufficient to plant a hectare of land. Sauvaire and Glozy, (1978) used auxiliary buds for micropropagation of sugarcane. Lee, (1987) and Heinz *et al*, (1977) also reported shoot tip culture for mass propagation of sugarcane. The use of biotechnology – based methods for virus-free mass micropropagation of improved sugarcane varieties as demonstrated in the above standardized protocol is one of the strategies that will help to solve the constraint of diseases as in the conventional methods.

MATERIALS AND METHOD

Explants Source: Healthy young shoot tips were collected by removing the leaf sheath from field grown plants of sugarcane (*saccharum afficinarum L*) maintained Botanical Garden of University of Maiduguri and brought to the laboratory.

Size of Explants: The young shoot tip was cut into thin smaller pieces of 1.0 to 1.5cm length using sharp scarpel.

SURFACE STERILIZATION OF EXPLANT

The explants were washed thoroughly under running tap water for 25 – 30 minutes followed by Bavistin 0.2% for 10 minutes and then washed with sterile distilled water and transferred to laminar air flow cabinet. The young shoot tip explants were treated with 70% alcohol for 30 second to one minute, followed by another treatment in 0.1% mercuric chloride ($HgCl_2$) for another five minutes. Finally, the young shoot tip was washed thoroughly 3 to 5 times with sterile distilled water before the inoculation into sterilized nutrient agar media pre-packed in culture tubes. All the above experiment was conducted under aseptic condition in laminar air flow cabinet.

Culture Medium: The young shoot tip explants were inoculated on to sterilized MS medium (Murashige and Skoog's 1962) supplemented with different concentrated and combination of plant hormone.

Environmental Condition: The PH of the medium was adjusted to 5.7 prior to gelling with Agar and before auclaving for 15 minutes at 1 ib/in^2 at 121^{0}C. All the cultures were incubated in a growth room with a 16h photoperiod and maintained at 27±2^{0}C, with artificial illumination of 2000 – 3000 Lux by placing them at 25 – 30cm blow fluorescent light.

Acclimatization and Transfer of Plantlets to Sail: Sugarcane plantlets with well-developed roots were removed from the culture medium. Washed the roots gently under running tap water and were transferred aseptically to plastic trays containing autoclaved garden soil, farmyard manure, and coconut husk – for hardening. The harden plantlets in the plastic trays were covered with porous polyethylene sheets for maintaining humidity and were kept under shade in a net house for subsequently, they were irrigated with 1/8 MS basal salt solution devoid sucrose and inositol every 4 days for two weeks. After 30 days the plantlets were transplanted into the soil in field condition.

Virus Indexing: Confirmation of virus elimination in shoot tip derive plant sample were conducted by DAS-ELISA test: prior to mass micropropagation of shoot tip derive plantlets. Field performance of shoot tip derived plantlets: on the field performance of shoot tip derived plants shows, morphological characters of shoot tip derived plants were found normal varietal stability was also seen.

Results and Discussion

Primary establishment of shoot tip: Apical shoot tips were used as explants for meristem isolation. It was collected from field grown plant were quickly rinsed in 70% alcohol for 1-2 minutes in a sterilized Erlenmeyer flask. Among the different time period used for sterilization five minutes was found healthy and free of contamination. As shown in Table 2, some of the treatment induced the formation of either plantlets or callus while other did not induce any plantlets or callus formation totally. Early appearance of roots and shoots was observed in CO – 997 followed by CB – 53/98, followed by C – 1001, followed by CO – 62175 and C – 957 serially (Table 2) When the hormones supplements were considered low levels of 2, 4 – D, BA with GA_3 produced plantlets. The best hormones combination among the treatments (table 1) to produce plantlets was 0.8mg/L 2, 4 – D, 0.25mg/L BA and 0.1mg/L GA_3. The second best performance was found on MS medium supplemented with BAP (2.0mg/L) + IBA (1.0mg/L). Shoot tips were found excellent starting material for the micropropagation of sugarcane. Anita et al, (2000) also used shoot tip as Table 1 below show hormones in different concentrations and combinations in the modified M&S medium

CONCENTRATION OF GROWTH REGULATORS (Mg/L)			
Treatment	**2, 4 - D**	**BA**	**GA_3**
1	0.5	0.10	0.1
2	1.0	0.25	0.1
3	1.5	0.40	0.1
4	2.0	1.00	0.1
5	2.0	1.00	0.1
6	2.5	1.50	0.1
7	1.0	0.10	0.2
8	1.0	0.20	0.2
9	1.5	0.40	0.2

10	2.5	1.50	0.2
11	0.5	0.10	0.1
12	1.0	0.20	0.1
13	1.5	0.30	0.1
14	2.0	0.60	0.1
15	2.5	1.30	0.1

2,4 – D: 2, 4 Dichclorophenoxy acetic acid
BA: Benzyl adenine
GA_3: Gibberellic acid
KN: Kinetic
BAP: 6 – Benzyl amino amino purine

Table 2 Growth of Storage Shoot Tips Culture of 5 Sugarcane Cultuivars

Treatment	**C – 1001**	**C – 957**	**CB – 53/98**	**CO - 62175**	**CO - 997**
1	+++	+	+	-	+
2	+	+++	++	+	++
3	++	*	+++	**	*
4	**	**	*	++	**
5	*	+	**	+++	+
6	+++	-	+	*	-
7	-	-	-	-	-
8	-	*	-	+	+
9	*	*	*	**	++
10	*	-	*	-	+++
11	+	-	-	+	*
12	-	-	+	-	-
13	-	**	***	**	++
14	**	+	++	+	+
15	***	***	*	***	+++

+++: Best plantlets formation
++: Good plantlets formation
+: Poor Plantlets formation
***: Best callus formation
**: Good callus formation
*: Poor callus formation
–: No callus and plantlets formation

Table 3: Days of shoots and Roots from shoot tips of five (5) sugarcane cultivars

Varieties	**Formation of shoot**	**Formation of Root**	**Well formed Plantlets**
C – 1001	33	42	85
C – 957	32	41	82
CB – 53/98	26	38	70
CO – 62175	32	40	78
CO – 997	31	40	76

As explants source for mass micropropagation of sugarcane crop, which supported strongly our choice of explants selection. Shoot tip culture is a unique technique to free from various pathogens including vituses, viroides, mycoplasma, bacteria and fungi Walkey, D. G. A, (1978).

Confirmation of virus elimination in shoot tip derive plant sample by DAS-ELISA Test: before mass micropropagation shoot tip derive plantlets were tested for virus detection by DAS-ELISA technique: virus free plantlets were used for mass-propagation. Field performance of shoot tip derived plantlets. The on field performance of shoot tip derived plantlets shows, morphological character of shoot tip derived plants were found normal. Through the use of tissue culture technique it may be easy to obtain diseases free plants. The protocol used in the present study can be used for mass micropropagation of sugarcane.

CONCLUSION

In conclusion, visual observation of the plants shows that, no symptoms of viral disease were noticed sugarcane yield of shoot tip derived plant (cane) was more than tat of derived plants. Since good plant health is major advantage in the development of elite mother (stock material) production virus free/disease free micropropagation offers many advantage over conventional method of plants (sugarcane).

RECOMMENDATION

In recommendation, sugarcane produce through shoot tip micropropagation can be easily exploited on a large scale, generating millions of plants in few days, and these may become a profitable multibillion rupees industry in near future, if this technique is employed as a means of disease free/virus free sugarcane production in the study area.

REFERENCES

1) Geijiskes R.J, Wang L, Lakshmannan P, Mckeon M.G, Berding N, Jackson J.A and Smith G.R, (2003). Smartsett^{TM} Seedling: tissue cultured seed plants for the Australian sugar industry. Sugarcane international. May/June. PP 13 – 17.
2) Synman S.J, Meyer G.M, Richards J.M, Haricharan N, Ramgareeb S and Huckett B.I, (2006). Refining the application of direct embryogenesis in sugarcane: effect of the

developmental phase of leaf disc explants and the timing of DNA transfer on transformation efficiency. Plant cell Rep 25: 1016-1023.

3) Synman S.J, Van Antwerpen T, Ramdeen V, Meyer G.M, Richards J.M and Rutherford R.S, (2007). Micropropagation by Direct Somatic embryogenesis: is disease eliminating also a possibility? Proc Int Soc sugarcane Technol 26:943 – 946.
4) Lee TSG (1987). Micropropagation of sugarcane (*saccharum Spp*) Plant cell TISS Org cult 10:47 – 55.
5) Bourg D, (1989) and Grisham M.P, Efficiency of in vitro propagation of sugarcane plants by direct regeneration from leaf tissue culture. Handbook and directory of commercial laboratories. Exegenetics Ltd, Basingstoke, Hants, England PP. 444 – 447.
7) Dodds J.H, (1991). In vitro resources. Book, published by chapman and Hall, London.
8) Guimarces C.T, Sobral W.S, (1998). The *Saccharum* complex: relation to other andropogneae. Plant Breed Re. 16:269 – 288.
9) Ali. K, Afghan S, (2001). Rapid multiplication of sugarcane through micropropagation technique. Park. Sugar J, 16 (6): 11 – 14.
10) Feldmenn, P., Sapotile, J. Gredoire, P and Rott, P. 1994. Micropropagation of sugarcane. In: Teisson C, ed. In vitro culture of tropical plants. France: CIRAD: 15 – 17.
11) Lal, N and Krishna, R. 1994. Sugarcane and its problems: Tissue culture for pure and diseases free seed production in sugarcane. Indian sugar, 44: 847 – 848.
12) Lorenzo, K.C., Ojeda, E., Espinosa A and Borroto, C. 2001. Field performance of temporary immersion bioreactor derived sugarcane plant Y.S. in vitro cell Dev. Bio I, plant, 37: 803 – 806.
13) Barba R.C, Zamora A.B, Malion A.K, Linga C.K, (1978) sugarcane tissue culture research. Proc. Int. Soc. Sugarcane Technol. 16: 1843 – 1863.
14) Sauvaire D, Glozy R (1978). Multiplication vegetative deccanne a sacre (*saccharum Sp*) par bouturage in vitro Cr Acad Sci. paris, seri D., PP. 467 – 470.
15) Lee TSG (1987). Micropropagation of sugarcane (*saccharum SPP*) plant cell tissue Org. Cutt. 10: 47 – 56
16) Anita, P.m Jian, R.K, Schrawat, A.R and Punia, A. 2000. Efficient and cost-effective micropropagation of two early maturing varieties of *sugarcane saccharum Spp*) Indian sugar, 50: 611- 618.
17) Walkey, D.G.A, 1987 in vitro methods for virus elimination. In T.P.S Thorpe (ed) Frontiers in plant tissue culture. University Calgary Press. Calgary, Canada, PP. 245 – 254.