In Vitro Propagation Of Sugarcane In Varieties Co86032 And Co8005.

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Abstract : Sugarcane is an important cash crop. Although there are several protocols available for sugarcane propagation. There is need of a variety and explant specific protocol adaptable commercially for Sugarcane tissue culture. We have standardised a protocol for rapid in vitro propagation of Sugarcane in varieties Co86032 and Co8005.Use of BAP (1mg/lit)was found most effective for both shoot induction and multiplication of well established cultures of sugarcane. Further it was found that use of NAA(5.0mg/lit) and activated charcoal along with half strength M.S. media is suitable for root formation. There was 92-96%success at hardening stage which indicated commercial applicability of this protocol for the propagation of both varieties mentioned above.

IndexTerms **– Sugarcane, Micropropagation, IBA, BAP**

I. INTRODUCTION

Sugarcane (*Saccharum officinarum*) is a monocotyledonous perennial crop which belongs to the grass family Poaceae. The natural variation present in sugarcane genome as it is evolved as a hetero hexapolyploid results in variation although it is propagated through vegetative mode.

The new varieties of Sugarcane are developed to harness maximum yield in the agro climatic conditions and soil types prevalent in a particular area. Two Sugarcane varieties used in this research are widely used in the western and Central parts of India in particular the Sugarcane growing regions across Maharashtra. Co86032 is used for several years across India by Sugarcane growers.Co8005 is now becoming an important variety in the region because of the yield and tiller numbers along with other favourable characters present in other Sugarcane varieties.

In vitro propagated plants are more useful than traditional method of using sets because it not only saves cropping material and time but also provide true to type ,uniform and disease free planting material. The constrains of cost and lack of awareness of using tissue cultured plants can be mitigated by developing protocols for rapid ,cost effective protocols adaptable by tissue culture industry.

We have developed a rapid method for mass propagation of Sugarcane in the verities Co86032 and Co8005.

II. MATERIAL AND METHOD

The shoots of 8-9 month old Sugarcane plants grown in the experimental fields were used as mother plants for Sugarcane propagation. The original plants of these varieties were obtained from Sugarcane Breeding Institute, Coimbtoor.

Murashige and Skoog media(M.S.)was used with 3% Sucrose and 0.8% Agar Agar. The media was autoclaved and apical meristems were inoculated aseptically on Shoot induction media after surface sterilisation. The Hormones used were BAP, Kinetin ,BA and Zeatin of which BAP has given satisfactory results and it was comparatively less costly .Hence BAP was used in final experiments where BAP was added in 0.1mg/lit, 0.5mg/lit,1.0mg/lit.,1.5mg/lit and 2.0mg/lit.

Similarly, the experiments were conducted for assessing the best hormonal concentration required for shoot multiplication using BAP.

Use of 4.0mg/lit and 5.0mg/lit NAA alone showed delayed response for root formation. Addition of 1% activated charcoal and use of 5.0 mg/lit NAA was conducted which gave rapid in vitro rooting.

The observations were recorded in standard format. The experiments were repeated twice in the laboratory scale and then a pilot experiment was conducted in the commercial scale which has given satisfactory results.

III. RESULTS AND DISCUSSION

3.1 Shoot induction in Sugarcane varieties Co 86032and Co8005

Among the different combinations of harmones studied BAP(6-Benzyl-aminopurine) was found most suitable. Hence, 0.1mg/lit,0.5mg/lit,1.0mg/lit,1.5mg/lit and 2.0mg/lit. BAP was used along with MS media. As shown bellow, MS media supplemented with 0.1mg/l BAP and 0.5mg/l BAP gave quick response to shoot induction with good length and number of shoot per plants after 4 weeks.

Table 3.1: Results of shoot induction in MS media with different concentrations of BAP

Variety	Media	BAP (mg/lit)	No. of explants cultured	Days of shoot formation	No. of shoots per explant	Shoot length (cm)
Co86032	MS+BAP	0.1	3	10	15	3
Co8005	MS+BAP	0.1	3	10	14	2.18
Co86032	MS+BAP	0.5	3	15	10	2.84
Co8005	MS+BAP	0.5	3	15	12	1.92
Co86032	MS+BAP	1.0	3	15	11	2.08
Co8005	MS+BAP	1.0	3	15	11	2.04
Co86032	MS+BAP	1.5	3	15	10	2.48
Co8005	MS+BAP	1.5	3	15	11	2.38
Co86032	MS+BAP	2.0	3	15	9	2.04
Co8005	MS+BAP	2.0	3	15	10	2.08

(0.1mg/l BAP) (0.5mg/l BAP) (1mg/l BAP) (1.5mg/l BAP) (2mg/l BAP)

Figure 3.1.Shoot multiplication in Sugarcane variety Co86032

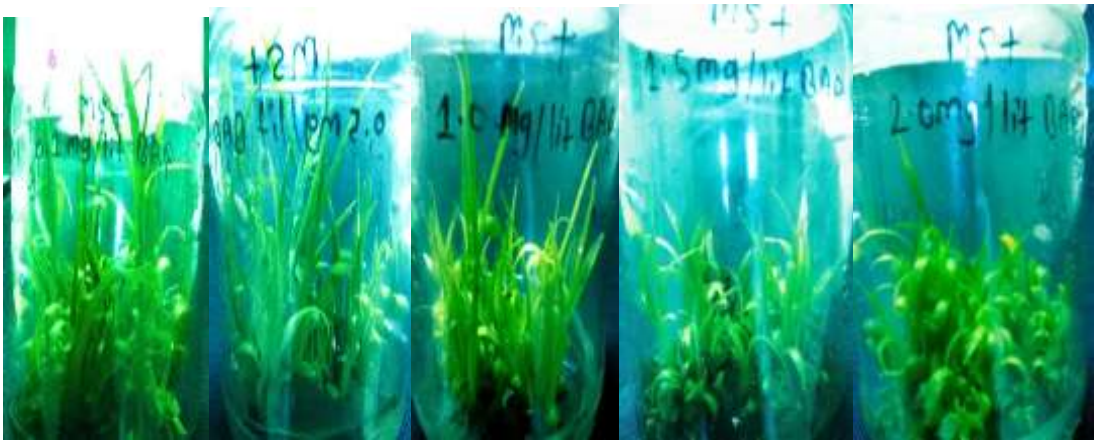
3.2 Multiple shoot formation in Sugarcane varieties Co 86032and Co8005

After 4-5 weeks of shoot growth, actively growing shoots were transferred to fresh medium for further growth and proliferation. The results were observed until formation of a dense mass of shoots (25-30days) in each culture.

Table 3.2: Multiple shoot formation in MS media with different concentrations of BAP

Variety	Media	Concentration (mg/lit)	No. of explants cultured	Days of shoot formation	No. of multiple shoots	Shoot length(cm)
Co86032	MS+BAP	0.1	3	12	27	1.52
Co86032	MS+BAP	0.1	3	12	23	
Co8005	MS+BAP	0.1	3	12	20	1.48
Co8005	MS+BAP	0.1	3	12	25	
Co86032	MS+BAP	0.5	3	12	26	1.61
Co86032	MS+BAP	0.5	3	12	17	
Co8005	MS+BAP	0.5	3	12	20	1.34
Co8005	MS+BAP	0.5	3	12	11	
Co86032	MS+BAP	1.0	3	12	18	1.40
Co86032	MS+BAP	1.0	3	12	11	
Co8005	MS+BAP	1.0	3	12	12	1.5
Co8005	MS+BAP	1.0	3	12	20	
Co86032	MS+BAP	1.5	3	12	11	1.40
Co86032	MS+BAP	1.5	3	12	12	
Co8005	MS+BAP	1.5	3	12	17	1.3
Co8005	MS+BAP	1.5	3	12	15	
Co86032	MS+BAP	2.0	3	17	12	1.18
Co86032	MS+BAP	2.0	3	12	11	
Co8005	MS+BAP	2.0	3	12	12	1.4

Co8005	MS+BAP	2.0	3	12	14	

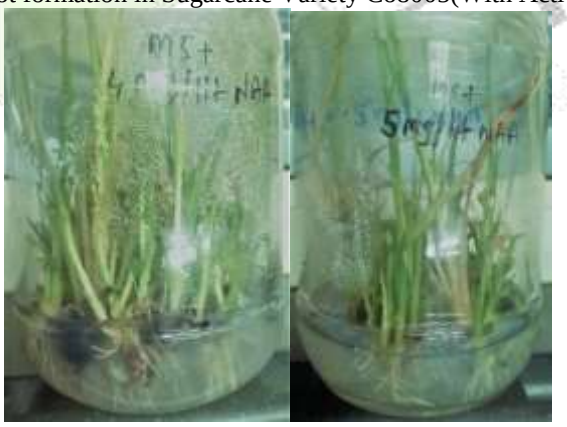


(0.1mg/l BAP) (0.5mg/l BAP) (1mg/l BAP) (1.5mg/l BAP) (2mg/l BAP)

Figure 3.2.Shoot multiplication in Sugarcane variety Co8005

3.3 Root formation in Sugarcane varieties Co 86032and Co8005 *In vitro* generated clumps of Sugarcane were transferred on rooting media which consisted half strength M.S.alongwith 4mg/lit and 5mg/lit NAA.Rooting was observed after 3-4 weeks .Use of activated charcoal hastened the rooting procedure and rooting was observed in two weeks.

Figure 3.3. a)Root formation in Sugarcane Variety Co8005(With Activated Charcoal)



MS+4mg/L NAA MS+5mg NAA

Figure 3.3. b) Root formation in Sugarcane Variety Co86032(Without Activated Charcoal)

3.4 Hardening of *in vitro* cultured sugarcane plants variety Co 86032 and Co8005

All the plants showed 94-98 % response to hardening process. Primary and secondary hardening was conducted in the mist chamber and green house respectively.

a) Co 86032 b) Co8005

Figure 3.4Primary hardening of sugarcane variety a)Co 86032and b)Co8005

3.5 Genetic fidelity analysis and virus indexing

The genetic fidelity was analysed using RAPD and SSR markers and there was no variation within the in vitro grown plants and mother culture .

The virus indexing was conducted for commercial propagation by providing samples to the government recognised laboratories. The plants were free from disease and viruses.

The results indicated possibility of using the same protocol for commercial propagation of Sugarcane in the varieties Co86032and Co8005.M.S.media along with 1mg/lit BAP for shoot induction and multiplication and use of half strength M.S. with activated charcoal and 5mg/lit NAA was satisfactory for root formation.

Similar studies have been conducted by different researchers to standardise the protocols for sugarcane multiplication and shoot induction using various combinations of media and hormones .The results presented here are in concurrence with the methods adapted by earlier workers. But, there are very few reports of correlating the results obtained in laboratory level experiments with those at the commercial level. We have successfully tested the protocols developed in laboratory scale experiments to the commercial scale. The successful adaptation of the results obtained in laboratory level experiments for commercial tissue culture propagation reiterate success of this protocol for Sugarcane propagation in the two varieties.

IV.ACKNOWLEDGEMENT

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REFERENCES

[1] Ali, A., Naz, S. and Iqbal, J.,2008, An efficient protocol for large scale production of sugarcane through micro propagation. *Pakistan journal of botany*.40 (1): 139-149.

[2] Ali, A.,Naz, S.andIqbal, J. ,2007, Effect of different explants and media compositions for efficient somatic embryogenesis in sugarcane (*saccaharum officinarum*). *Pakistan journal of botany*. 39(6): 1961-1977.

[3] Barba, R. and Nickell, L.G. ,1969. Nutrition and organ differentiation in tissue cultures of sugarcane, a monocotyledon. *Planta.* 89(3):299-302.

[4] Cheema, K. and Hussain, M. ,2004, Micro propagation of sugarcane through apical bud and axillary bud. *International journal of agriculture &biology.* 12(2): 1560- 8530.

[5] Dinesh, P., Thirunavukkarasu, P., Saraniya, A.R. and Ramanathan, T. ,2015, *In vitro* studies of sugarcane variety Co 91017 through micro propagation of shoot tip culture. *Adv Plant Agric Res.* 2(6): 70-71.

[6] Etienne, E.,Teisson, C., Alvard, D., Lartaud, M., Berthouly, M., Georget, F. and EscalonaM.,1999,Temporary immersion for plant tissue culture. *Plant biotechnology and in vitrobiology*.36:629-632.

[7] Geijskes, R.J., Wang, L., Elliott, A., Grof, C.P., Barding, N. and Smith, G.R. ,2006, Developmental and hormonal regulation of direct shoot organogenesis and somatic embryogenesis in sugarcane (*saccharum spp*. interspecific hybrids) leaf culture. *Plant cell report.*25 (10):1007-15.

[8] Hoy, J.W., Bischoff, K.P. and Milligan, S.B. ,2003, Effect of tissue culture explants source on sugarcane yield components.*Euphytica.*129 (2): 237–240.

[9] Kaur, A. andSandhu,J. S. ,2014, High throughput *in vitro* micro propagation of sugarcane (*saccharum officinarum l.*) from spindle leaf roll segments: cost analysis for agri-business industry. *Plant cell tissue organ cult*.12 (3): 339–350.

[10] Khan, M. R. and Rashid, H. ,2003, Studies on the rapid clonal propagation of *Saccharum officinarum. Pakistan journal of biological sciences*.6: 1876- 1879.

[11] Khan, R. H., Chaudhary M. F., Chaudhry, Z., Siddiqui S. and Zia M. ,2009, Effect of cytokines on shoot multiplication in three elite sugarcane varieties. *Pak. j. bot*. 41(4): 1651-1658.

[12] Khan, S., Rashid, H., Chaudhary, F.and chaudhry, Z. ,2008, Rapid micro propagation of three elite sugarcane (*saccharum officinarum* l.) varieties by shoot tip culture. *African journal of Biotechnology*.7 (13):2174-2180.

[13] Savant, R.A. and Meti, N.T. ,2016, Cost effective techniques for *in vitro* propagation of two varieties of sugarcane. *int.j. curr. Res. biosci*. *Plantboil*. 3(5);65-72.

[14] Sawant, R. A., Tawar, P. N., Meti, N.T. and Ranjekarp. K. ,2014, Role of sugarcane micro propagation for production of quality seed.*int. J. rec. biotech*. 2 (4): 34- 41.

[15] Sheng, T., Lee, G. ,1987,Micro propagation of sugarcane (*saccharum spp*.).*Plant cell tissue organ cult*.10: 47–55.

[16] Sood, N., Gupta, P. K., Srivastava, R.K. and Gosal, S.S. ,2006, Comparative studies on field performance of micro propagated and conventionally propagated sugarcane plants. *plant tissue cult. &Biotech*.16 (1): 25-29.

[17] Wekesa, R., Onguso, J., Nyende, and B.and Wamocho, L. S. ,2015, Sugarcane in vitro culture technology: opportunities for Kenya's sugar industry. *African Journal of Biotechnology*.14 (47): 3170-3178.

[18] Yadav, S., Ahmad, A.and Rastogi, J. ,2012, Tissue culture strategies in sugarcane (*saccharum officinarum* l.).*International journal of pharma and Bio sciences*. 3 (2):426-441.