J. Agric. Food. Tech., **4(3)8-11, 2014**

ISSN 2090 – 424X
Journal of Agriculture and Food Technology
www.textroad.com

© 2014, TextRoad Publication

Callus Induction in Sugarcane

Faria Soomro, Shabana Lochi, Rehana Lochi, Fazeela Lochi, Tabassum Sanam Ansari, Paras Ansari, Uzma Afreen Ansari, Arman Ali Rajper

Faculty of Crop Production Sindh Agriculture University Tandojam, Sindh, Pakistan

ABSTRACT

This study was performed as joint venture among tissue culture laboratory Plant Breeding and Genetics division at Nuclear Institute of Agriculture Tandojam. Department of Biotechnology, Sindh Agriculture University Tandojam. This study performed on three varieties of sugarcane. Results shows that the two types of callus were observed, a) compact, yellowish white, dry nodular and embryogenic which is capable of regenerating plants and b) whitish globular, smooth non-compact, non-embryogenic and non regenerable.

KEY WORDS: *Callus induction, sugarcane Sindh*

INTRODUCTION

Sugarcane (*Sacchrumofficinarum*) is one of the worlds important crop plant and is cultivated tropical and subtropical areas in more than 80 countries around the globe. Sugarcane is a tall plant of recurrent nature and is a member of the genus S*accharum*, tribe Andropogane and family Gramineae. The cultivated sugarcane varieties are the result of interspecific hybridization involving *Saccharumofficinarum, S. barberry, S. sinense*and the wild species S. *spontaneum*and S. *robustum*. It is thought that S. officinarum was originally selected by humans in Papua New Guinea, perhaps from S. *robustum*germplasm. Because of its multispecies origin, sugarcane is thought to have one of the most complex plant genomes carrying variable chromosome numbers (generally 2n =80-120 whereas x=10). Most of the commercial sugarcane varieties now in use allover the world are descendant of interspecfic hybrids within the genus *Saccharum* (Arcenaux, 1967). Sugarcane was one of the first "cash crops" of early colonial America. Countries that produce the largest amounts of sugarcane are Brazil, Cuba, Kazakhstan, Mexico, India and Australia.

In the world, Pakistan ranks 5th in cultivated area and 15th in can yield (Anonymous, 1998) thus there is a big gap between ranking in cultivated area and can yield. An extensive breeding work and management practices and required to narrow down this big gap. Although a good crop is harvested in Pakistan, it does not flower and produce seed (Fuzz) under normal planting conditions in the country. Non- sporadic flowering, natural vibal fertile seed production has ever been a problem of Pakistan.

The tissue culture techniques can play an important role in this regard. Initial attempts to regenerate plants through in vitro techniques were made on sugarcane by Nickell (1964) and Heinz and Mee (1969). Callus culture of sugarcane has been successfully established using shoot apices, young leaves and young inflorescence as ex plant. Callus is now been educed in a large number of sugarcane species indicating that, this phenomenon is not limiting. The plants, which are regenerated from the callus, are not true-to- type for their chromosomal aberration. Many new characters have been identified in these plants. If any character is found to be superior to mother plant, than the somaclone with this phenotype can be realized as a new variety. The fantasting future of callus culture is that one can alter one or few characters of the questioned genotypes, keeping the rest of genomes intact. It is easy to create somaclonal variations in the tissues of sugarcane by in vitro callus culture. Somaclonal variation cane be used for breeding new cultivars as (i) the variant cane occur for agronomics traits (ii) variation occur at high frequency (iii) some variants may be novel and have not been obtained by conventional breeding (iv) in some cases, in vitro selection may allow isolation of genotypes tolerant to biotic and abiotic stresses.

MATERIALS AND METHODS

The research was carried out as joint venture among tissue culture laboratory Plant Breeding and Genetics division at Nuclear Institute of Agriculture Tandojam. Department of Biotechnology, Sindh Agriculture University Tandojam.

Varieties/ Material

Three sugarcane varieties viz, NIA-98, BL-4 70 and NIA-2004werecomparedfor callusing and plant regeneration potential.

Corresponding Author: Shabana Lochi, Faculty of Crop Production Sindh Agriculture universityTandojam, Sindh, Pakistan

Laboratory Facilities

Well-equipped tissue culture laboratory at Nuclear Institute of Agriculture was work with following facilities:

Culture room:

Must be air-conditioned, contamination free, having laminar air flow cabinets with different scalpels, scissors, forceps and a fire facility.

Media preparation:

Room must have the required chemicals i.e. Chemicals, absolute alcohol, distilled water, pH meter with buffers for the calibration, solutions for pH adjustments. Growth regulators and growth promoters i-e 2,4-D (2,4-dichlorophenoxy acetic acid), Dicamba (dichloro-2-methoxy benzolic acid), IAA (indole acetic acid), Kinetin, IBA (indolebutaric acid). Electronic balances should be there and hot plate with stirrer as well.

Incubation room: should have controlled conditions of temperature, light, humidity.

Autoclave:

An autoclave is basically a large-size but sophisticated pressure cooker, and is used for the sterilization of the medium, glassware and instrument**.**

Glass house:

Must be with all the controlled conditions to grow sensitive plantlets.

Laminar air flow chamber:

The laminar air flow chambers provide clean filtered air that allows cultured to be handled under contamination –free environment .Several types of laminar flow chambers are sold on the market and are available in different size .The laminar air flow cabinets are located in the culture transfer area . Some large –sized laboratories have sterile rooms in addition to laminar flow cabinets.

Preparation of MS (Murashige and Skoog) Basal Medium

basal medium:

MS basal medium was first discovered by Murashige and Skoog in 1962. It is high salt based mixture, which is commercially available in the market in powder form, but for this research purpose it was prepared according to the composition of the salts. (Murashige, and Skoog. 1962).

Composition of plant tissue culture media

Ingredient	Chemical composition	Weight in mg/lit
MICRO NUTRIENTS		
Manganese sulphate	MnSO4 .4H2 O	22.2
ZineSulphate	ZnSO4 . 7H2 O	8.2
Boric acid	H3BO3	6.2
Potassium lodide	Kl	0.83
Sodium Molybdate	Na2 MoO4 . 2H2 O	0.025
Copper sulphate	CuSO4 . 5H2O	0.025
Cobalt chloride	CoCl2 . 6H2O	0.025
IRON		
Iron Sulphate	FeSO4 . 5H2O	27.8
Sodium EDTA (Sodium Ethylene diamine tetra acetic acid)	Na2 ETDA.2H2O	37.26
VITAMINS		
Pyridoxol Hydrochloride	C8Hl2 ClNO3	0.5
Thiamine HCl	C12H8N4OSCl2	0.5
Glycine	NH2 CH2.COOH	2.0
Nicotinic	C6H5NO2	0.5
Myo-inosital		0.1 gm/1
MACRO NUTRIENTS		
Potassium nitrate	KNO3	1.09 gm/1
Ammonium nitrate	NH4 NO3	1.65 gm/1
Calcium chloride	CaCl2 .2H20	0.44 gm/1
Potassium phosphate	KH2 PO4	0.17 gm/1
Magnessiumsulphate	MgSO4 . 7H20	0.37 gm/1
Sugar	C12H22O11	**25.00 gm/1**
Casein		**2.00 gm/1**
AGAR		**8.00 gm/1**
Ph		**5.7**

(Murashige and Skoog, 1962)

MEDIA
The media can be defined as matter comprising of macro and micro nutrient, growth regulators, sugar and solidifying agent which facilitate the *in vitro* growth of organism or plant material.

Explants source
Explants source was used from the top portion of the plant and 6-7 month age of plant. Leaf explants were taken from innermost 1-5 leaves and were (2-3 mm) in length.

Sterilization of the explants and culture
The top portion of sugarcane plant was taken. Unnecessary portion of the cane top was removed and the remaining was first washed with distilled water and then sterilized with 70% ethonal for 1 minutes and 10% sodium hypochloride solution for 20 minutes. After sterilization the explant material was washed with double distilled water 2-3 times to remove any traces of disinfectant under aseptic conditions in laminar air flow cabinet. These sterilized leaves were too removed and only the innermost leaves, which are supposed to be the infection free, were cut into (2-3 mm) long pieces. These pieces were cultured aseptically into the bottles of the media.

Media used for callogenesis
MS + 4 mg/lit 2,4-D
MS + 4 mg/lit Dicamba

Five cultures of each explant were inoculated at each of the media. Each culture bottle was kept under controlled conditions at 28 ± 2 ^{0}C for 16-8 hours photo period at the light intensity of 2500-3000 lux.

Subculturing of the callus produced
The callus produced was sub cultured twice at an interval of 15 days to increase the amount of callus. It was done on the same medium at which callus was produced initially.

Media for regeneration / organogenesis
MS + 3 mg/lit IBA + 3 mg/lit lAA + 3 mg/lit Kinetin.

Culture for regeneration
After the first 4 weeks of explantation, the calli, were weighed and cultured on regeneration medium for proliferation of callus. After the 40 days intervals the calli were again weighed and sub cultured on same regeneration medium. The regenerated shoots were scored for chlorophyll mutations.

RESULTS AND DISCUSSION

Results and discussion
This stud was performed on three sugarcane varieties viz, NIA-98, BL-4 70 and NIA-2004 were compared for callusing and plant regeneration potential. Following results were observed.

Two types of callus were observed, a) compact, yellowish white, dry nodular and embryogenic which is capable of regenerating plants and b) whitish globular, smooth non-compact, non-embryogenic and non regenerable. Similar results were also reported by Khan *et al.* (1998).For callus induction, two different media were used 2,4-D and dicamba. Callus weight was taken after 30 days of culturing transferred the callus on same medium for proliferation. Best callus induction was observed on medium containing 2,4-D and followed by Dicomba .The maximum callus was observed in NIA 98 and BL4 –P70 The present research is also supported by Rehman *et al.* (2002).

Concentration of auxins	Varieties	Explants wt/ bottle	One month after callus induction
2,4-D 4mg/l	NIA-98	0.45	+++
2,4-D 4mg/l	NIA-2004	0.40	+
2,4-D 4mg/l	BL4-P70	0.36	++
Dicomba 4mg/l	NIA-98	0.41	++
Dicomba 4mg/l	NIA-2004	0.30	-
Dicomba 4mg/l	**BL4-P70**	**0.28**	+

Effect of regeneration
Regeneration started with the appearance of green dots on callus within a week on regeneration medium and generally produced normal stem and leaves. Regeneration potential was specific and a genotype dependent

phenomenon. Similar results was reported by Karim *et al*. (2002). The variety NIA-98 produced maximum number of plantlets followed by BL4-P70 and minimum plantlet regeneration was recorded from NIA-2004.

Regeneration media	Varieties	Regeneration of plantlets
2mg/l IAA+2mg/lIBA +2mg/l Kint	NIA-98	+++
2mg/l IAA+ 2mg/l IBA +2mg/l Kint	NIA-2004	+
2mg/l IAA+ 2mg/l IBA +2mg/l Kint	BL4-P70	++

REFERENCES

Anonymous. 1998. Guide to efficient plant nutrition management. Land and Water Development Division. Food and Agric Orgof the United Nation Rome. Pp-19.

Arcenaux, G. 1967.Cultivated sugarcane of the world and their botanical derivation Proc. Cong. Int. Soc. sugarcane Techno.(Puerto Rico) 12:844-854

Bhojwani and Razdan. 1992. Plant tissue culture theory and practice Elsever publication.

Evans, D.A, Sharp, W.R, Ammirato, P.V. 1986. *Handbook of Plant Cell culture,* 419-456. Macmillan Publ. Co., New York.

Heinz, D.J. and Mee, G.W.P. 1969. Plant differentiation from callus tissue of *Saccharum* species. Crop Sci., 9: 346-348.

Karim, M.Z., M.N. Amin, M.A. Hossain, S. Islam, F. Hossain and R. Alam. 2002. Micropropagation of two sugarcane (*Saccharumofficinarum*L.) varieties from callus culture. *Online J. of Biol. Sci*., 2(10): 682-685.

Khan, I. A., Khatri, A., Ahmad, S.M., Siddiqui, S.H., Nizamani, G.S., Khanzada, M.H., Dahar, N.A. and Khan, R. 1998. In vitro mutagenesis in sugarcane. Pak. J.Bot., 30(2): 253-261.

McCoy, T and Phillips, RL. (1982). Can J Genet Cytol24:559-566

Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant*, 15: 473-497.

Nickell, L.G. 1964. Tissue and cell cultures of sugarcane: Another research tool. Hawaii. Plant. Rec., 57: 223-229.

Rehman ,S.,M. T.H.Shahid, M.Hussain,M. K. Tanvir, and M.A.Javed.2002.Genotypic effects on callogenesis and organogenesis in sugarcane. Pakistan Sugar Journal, 17:6,13-2.

Skirvin, R.M., Janick (1976). Natural and induced variation in tissue culture. Euphytica27:241-266.