Pak. J. Biotechnol. Vol. 1 (2) 17-22 (2004) ISSN. No. 1812-1837

EFFECTS OF DIFFERENT PHYTOHORMONES ON SUGARCANE (SACCHARUM SPP.) REGENERATION

Imtiaz Ahmed Khan[+], Abdullah. Khatri, Saboohi Raza, Ghulam Shah Nizamani,Muhammad Aquil Siddiqui, Nighat Seema, [*]Muhammad Umar Dahot and Mazhar H. Naqvi

Agricultural Biotechnology Division, Nuclear Institute of Agriculture, Tando Jam and [*]Institute of Biotechnology, University of Sindh, Jamshoro, Pakistan
[+]E-mail: niatjam@hyd.paknet.com.pk

ABSTRACT:

Three sugarcane clones, AEC82-223, Ghulabi-95 and AEC86-328 were investigated for callus induction and regeneration under different concentrations (2mg/L and 4mg/L) of various auxins i.e. 2,4-dichlorophenoxyacetic acid (2,4-D), indol acetic acid (IAA), 3,6-dichloro 2-methoxy benzoic acid (Dicamba) and picloram. An efficient callus induction was observed on the medium containing picloram followed by 2,4-D but high callus proliferation was achieved with picloram. Non-regenerable callus was produced when calli were transferred for proliferation on Dicamba. The maximum calli induction, its proliferation and plantlets regeneration were recorded in clone AEC82-223, while the minimum in AEC86-328. The maximum chlorophyll mutation frequency was noted in clone AEC86-328 and minimum in AEC82-223. Among auxin especially 2,4-D induces more genetic variability as compared to other auxins used in this work.

INTRODUCTION

Sugarcane (*Saccharum* spp. hybrid) is one of the most important cash crops of Pakistan. Average cane yield and sugar recovery in Pakistan are the lowest among the sugarcane growing countries of the world (Anonymous, 2002). Because of non- or sporadic flowering, natural viable fertile seed production has ever been a problem in Pakistan. Hence, alternative methods such as *in vitro* culture techniques and induced mutations are being employed to create the new genetic variability for the selection of desired clones/genotypes of sugarcane (Khatri *et al* 2002).

Realization of the full potential of somatic cell genetics in higher plants is predicted on the ability to induce desired development state (Orton, 1979). Callus has now been induced in a large number of sugarcane species indicating that, this phenomenon is not limiting (Narayanvaswamy, 1977). The fascinating feature of callus culture is that one can alter one or few character (s) of the questioned genotype, keeping the rest of the genome intact. Ahloowalia (1995) reported that the development of desired genotypes is only possible through somaclonal variation or through *in-vitro* mutagenesis in case of vegetatively propagated sugarcane plants. The ability to regenerate the plantlets from callus tissue of *Saccharum* species was first demonstrated by Heinz and Mee (1969). Liu and Chen (1976, 1978, 1984) have reported significant variations in somaclones in the important agronomic characters such as cane yield and its components, sugar contents and some morphological traits.

The objective of the present work was to assess the effects of different auxins on callus induction and plant

differentiation in different genotypes of *Saccharum* spp (hybrid) under tissue culture conditions.

MATERIALS AND METHODS

Three sugarcane clones, AEC82-223, AEC86-328 and Ghulabi-95 were used for tissue culture studies. Ten explants containing leaf primordia were taken from each genotype, sterilized by standard procedure (Siddiqui *et al*., 1988) and cultured on modified MS medium (Murashige and Skoog, 1962) containing 2 mg/L and 4mg/L, 2,4-D, dicamba, picloram and indole acetic acid for callusing. Media was solidified with 0.8% Difco bacto agar. Commercial sugar was used instead of Analar grade sucrose as carbon source in the medium.

After five weeks of explantation, the calli were weighed and cultured on shoot induction medium (MS +2 mg/L IBA + 2 mg/L IAA + 2 mg/L kinetin). The regenerated shoots were scored for chlorophyll mutations. When the plantlets attained 7-8 cm height, these were subjected to rooting by culturing on different media viz.; i) MS medium, ii) ½ MS medium, iii) MS medium + 1mg/L IBA + 3% sugar, iv) MS +1mg/L IBA+ 4% sugar, v) MS +1mg/L IBA + 5% sugar, vi) MS + 1 mg/L IBA + 6% sugar, vii) MS + 1 mg/L IBA + 7% sugar and viii) MS + 1 mg/L IBA + 8% sugar. All these operations were carried out under aseptic conditions and cultures were incubated at 28 ± 2^{o}C with 16 hours photoperiod. Rooted plantlets were acclimatized and transplanted to field.

RESULTS AND DISCUSSION

Callus induction: Based on morphological appearance, two types of calli were observed: (i) type A-yellowish white, compact, dry and nodular (Fig 1) and (ii) type B- whitish globular, non-compact and wet(Fig. 2). Such type of calli have also been reported by Khan *et al.*, 1998 and Khatri *et al.*, 2002. Best callus induction and proliferation was observed on medium containing 4mg/L 2,4-D and 4mg/L picloram. Siddiqui et al., 1988 and Begum et al., 1996, have reported similar results. Picloram in 2mg/L also yielded good callus. AEC82-223 yielded the maximum callus followed by Ghulabi-95 while AEC86-328 produced the minimum (Table 1). No callus induction was observed in indole acetic acid medium. Dicamba yielded very few calli in AEC82-223 and Ghulabi-95 but on second transfer the calli were turned into non-regenerable callus. Similar trend was observed in callus proliferation on sub-culture. Callus weight reduced in Ghulabi-95, because of high percentage of type B callus. According to Orton (1979), the type B callus of *Hordeum vulgare* has twice intrinsic growth rate as compared to type A, but in our study, it was observed that type B callus of sugarcane did not exhibit the same attributes, rather its growth substantially decreased with similar results vclones AEC86-328 yielded type A callus on dicamba, but on subculture it got converted into type B. Aging of the medium affected morphological status of callus in AEC86-328, whereas calli of AEC82-223 and Ghulabi-95 were converted into somatic embryos.

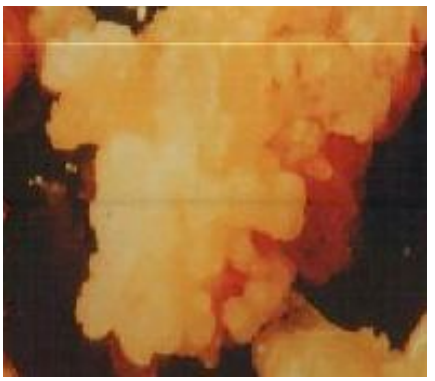

Fig 1.Type A callus

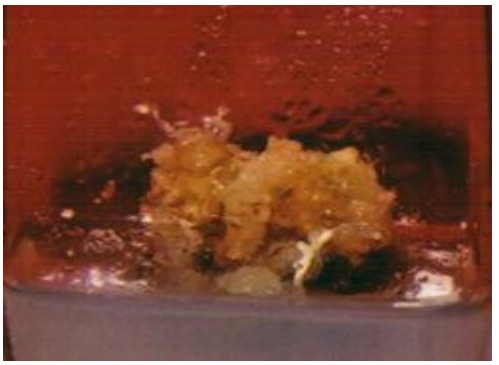

Fig 2. Type B callus

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. It was also observed that callus of different auxins showed different regeneration potential (Table 2). AEC82-223, yielded maximum plantlets on callus derived from picloram followed by 2, 4-D and minimum plantlet regeneration was recorded on callus derived from dicamba. Similar trend of regeneration was observed in all the genotypes. Whereas, minimum plantlets was produced by AEC86-328 in all the calli as compared to other genotypes. Callus induction, proliferation and regeneration potential in sugarcane exhibited synchrony to each other. However, regeneration was low as compared to its callus production in Ghulabi-95. This might possibly be due to the conversion of regenerable callus type A to non-regenerable callus type B on sub-culturing of callus (Orton, 1979).

Regeneration of albino and viridis plantlets exhibited the appearance of chlorophyll mutations in *in-vitro* plantlets (Fig. 3 & 4). According to Zubko and Day, (1998), chlorophyll deficiency or albinism, is a standard marker in plant cytoplasmic genetics. Its stability is consistent with mutations in the plastid genome because nuclear mutations induce plastid ribosome deficiency. A chlorophyll deficient phenotype can also result of recessive mutations such as *iojap* in maize (Han *et al*., 1992) and *albostrians* in barley (Bradbeer *et al*. 1979). Maximum numbers of chlorophyll mutants were observed in callus derived from 2,4-D. and minimum number of chlorophyll mutants was recorded in calli of dicamba. The highest percentage of chlorophyll mutants was recorded in AEC86-328 and the lowest in AEC82-223 (Table 2). The presence of chlorophyll deficient plantlets confirmed the induction of genetic variability (Shepard *et al*., 1980 and Evan and Sharp, 1986). Present study revealed that the calli derived from 2,4-D produces more genetic variability as compared to the calli of dicamba. Plants obtained through *in vitro* cultures show high phenotypic variability, which was due to true genetic changes (Orton, 1980). Chaleff and Keil (1982), reported that some phenotypic variability was the result of physiological changes during *in vitro* conditions; hence such plantlets normally revert to their parent type in field conditions.

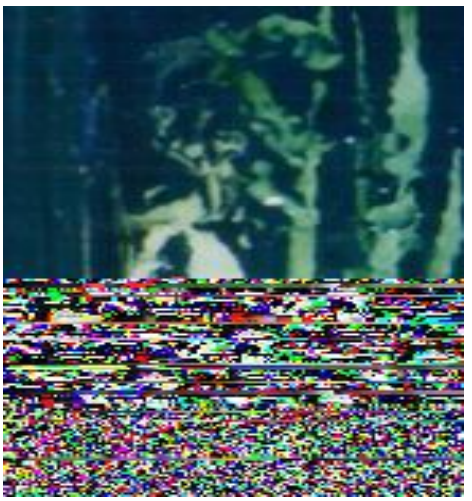

Fig 3. Chlorophyll mutant (Albino)

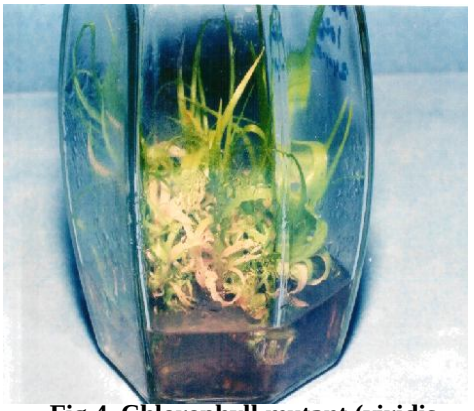

Fig 4. Chlorophyll mutant (viridis

Rooting: The roots were developed from the nodal primordial region when the plantlets are well developed (Khan *et al.*, 1998). Eight different media were used for the optimisation of the root induction rate (Table 3). Root induction was observed in the regeneration medium when plant hormones were exhausted, but more vigorous root development was achieved, when the plantlets were cultured on the root induction medium supplemented with 1mg/L IBA + 6% sucrose (Khatri *et al*., 2002). The plantlets with well developed shoots and roots were transferred to jiffy pots having sterilized perlite. After acclimatization the plantlets were first transferred to the earthen pots for hardening and afterwards in the field. These plantlets are being evaluated for desired agronomic traits (Fig. 5).

Fig – 5: *In vitro* regenerated sugarcane

plantlets in the field for evaluation

Among the four auxins, picloram and 2,4-D showed best results for callusing. Maximum chlorophyll mutant frequency was observed when 2,4-D was applied in 4mg/l concentration (31.8%) in AEC86-328 (Table 2). This showed that 2,4-D induces more genetic variability as compared to other auxins under study.

Table 1. Effect of auxins concentration on callogenesis of different genotypes/clones

Auxins Conc./ Genotypes	AEC82-223		Ghulabi-95		AEC86-328	
	Callus (g)	Proliferation of callus (g)	Callus (g)	Proliferation of callus (g)	Callus (g)	Proliferation of callus (g)
2mg/l 2,4-D	1.56	1.83	1.32	1.20	0.93	0.34
4mg/l 2,4-D	3.56	2.83	3.32	2.24	1.93	0.80
2mg/l Picloram	3.68	2.90	3.10	2.25	1.55	0.56
4mg/l Picloram	4.68	3.61	3.98	2.98	1.85	0.88
2mg/l Dicamba	0.78	0.53	0.70	0.25	0.24	0.35
4mg/l Dicamba	0.94	2.2	0.88	0.37	0.58	0.86
2mg/l IAA	--	--	--	--	--	--
4mg/l IAA	--	--	--	--	--	--

2,4-D= 2,4 dichlorophenoxy acetic acid, IAA= Indol acetic acid

Table 2. Response of genotype on regeneration and frequency of chlorophyll mutants

Auxins concentration	AEC82-223		Ghulabi-95		AEC86-328	
	GP	CM	GP	CM	GP	CM
2mg/l 2,4-D	96	5	90	4	53	9
4mg/l 2,4-D	112	16	107	14	66	21
2mg/l Picloram	138	4	120	3	84	5
4mg/l Picloram	159	8	150	8	88	10
2mg/l Dicamba	36	2	34	2	22	3
4mg/l Dicamba	54	4	41	6	31	5

GP= Green plants CM= Chlorophyll mutants

Table 3. Effect of medium composition on root induction of sugarcane

Medium	Root induction
MS medium	-
½ MS medium	-
MS + 1 mg/I IBA + 3% sugar	+
MS + 1 mg/I IBA + 4% sugar	+
MS + 1 mg/I IBA + 5% sugar	+
MS + 1 mg/I IBA + 6% sugar	++++
MS + 1 mg/I IBA + 7% sugar	+++
MS + 1 mg/I IBA + 8% sugar	+++

-, No root, +, weak root, +++, good rooting, ++++, excellent rooting

REFERENCES

Ahloowalia, B.S. *In vitro* mutagenesis for the improvement of vegetatively propagated plants. In: Extended Synopsis FAO/IAEA Int. Symp. on the use of induced Mutation and Molecular Techniques for Crop Improvement, IAEA-SM 340/203 (1995).

Anonymous, Agricultural Statistics of Pakistan, 2000-01. Govt. Pakistan pp. 27-28 and 106 (2002).

Begum, S., M.A. Samad and M.A.Q Shaikh. Induction of mutations in sugarcane callus. Bangladesh J. Nucl. Agric. 12: 91-94 (1996).

Bradbeer,J.W., Y.E. Atkinson, T. Borner and R. Hagemann. Cytoplasmic synthesis of plastid polypeptide may be controlled by plastid synthesized RNA. Nature 279: 816-817 (1979).

Chaleff, R.S. and R.L. Keil. Origins of variability among cultured cells and regenerated plants of *N. tabacum* In: Earle ED and Demarly Y (ed) Variability in Plants Regenerated from Tissue Culture, (pp. 175-187). Praeger Publication (1982).

Evans, D.A. and W.R. Sharp. Somaclonal and gametoclonal variation. In: Evans DA, Sharp WR & Ammirato PV (ed) Hand Book of Plant Cell Culture, Vol 4 (pp. 99-132). Macmillan Publishing Co. New York (1986).

Han,C.D.,E.H.Coe and R.A.Martienssen. Molecular cloning & characterization of iojap (ij), a pattern striping gene of maize. EMBO J. 11: 4037-4046 (1992).

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

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

Khatri, A., I.A. Khan, M.A. Javed, M.A. Siddiqui, M.K.R. Khan, M.H. Khanzada, N.A. Dahar and R. Khan. Studies on callusing and regeneration potential of indigenous and exotic sugarcane clones. Asian J. Plant Sci. 1(1): 41-43 (2002).

Liu,M.C. and W.H.Chen. Tissue and cell culture as aids to sugarcane breeding I. Creation of genetic variation through cell culture. Euphytica 25: 393-403 (1976).

Liu, M.C. and W.H. Chen. Tissue and cell culture as aids to sugarcane breeding II. Performance and yield potential of callus derived clones. Euphytica 27: 272-282 (1978).

Liu,M.C. and W.H.Chen. Tissue and cell culture as aids to sugarcane breeding III. High sucrose and vigorously growing cell clone 71-489. Euphytica 31: 77 (1984).

Murashige, T and F. Skoog. A revised medium for rapid growth and bioassays with tabacco tissue culture. Physiol. Planta. 15: 473-497 (1962).

Narayanaswamy, S. Regeneration of plants from tissue culture. In: Reinert J & Bajaj YPS (ed). Plant Cell Tissue and Organ Culture (pp. 179-206) Springer Berlin (1977).

Orton, T.J. A quantitative analysis of growh and regeneration from tissue cultures of *Hordeum vulgare, H. tubatum* and their intraspecific hybrid. Environ. Exp. Bot. 19: 319-333 (1979).

Orton, T.J. Chromosomal variability in tissue cultures and regenerated plants of *Hordeum*. Theor. Appl. Genet. 101-112 (1980).

Shepard, J.F., D. Bidiney and E. Shahin. Potato protoplast in crop improvement. Science 208: 17-24 (1980).