Asian Journal of Plant Sciences

ISSN 1682-3974

science alert

ANSInet
an open access publisher
http://ansinet.com

Asian Journal of Plant Sciences
Volume 1 Number 1, 41-43, 2002

Studies on Callusing and Regeneration Potential of Indigenous and Exotic Sugarcane Clones

Abdullah Khatri, Imtiaz Ahmed Khan, Muhammad Aslam Javed, Muhammad Aquil Siddiqui, Muhammad Kashif Riaz Khan, Muhammad Hussain Khanzada, Nazir Ahmed Dahar and Raziullah Khan
Plant Genetics Division, Nuclear Institute of Agriculture, Tandojam, Pakistan

Abstract: Eleven sugarcane clones were compared for callusing and regeneration potential. All these clones showed varied response to the traits under study. However, the highest callus formation and plantlets regeneration were recorded in clone AEC81-8415, while the lowest in L116. The maximum chlorophyll mutation frequency was noted in clone AEC82-1026 and minimum in AEC81-0819.

Key words: Sugarcane (*Saccharum* spp. hybrid), clones, callus, plantlets regeneration

Introduction

Sugarcane (*Saacharum* 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, 2000). Enhancement of sugarcane productivity mainly depends on the combination of the potential genes. Natural viable seed production has ever been a problem in Pakistan because of non- or sporadic flowering. Arrangements for hybridization under artificial conditions are scarce and meager. Hence, alternative methods such as *in vitro* culture techniques and induced mutations are needed to be used to create the new genetic variability for the selection of desired clones/genotypes of sugarcane.

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 (Narayanaswamy, 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 regeneration potential of exotic as well as local sugarcane cultivars.

Materials and Methods

Eleven sugarcane clones comprising of 9 local (AEC80-4725, AEC81-0819, AEC80-2046, AEC80-3029, AEC82-1026, AEC81-8415, AEC80-5108, L116 and BL4) and 2 exotic (CP68-1067 and CP67-412) 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) + 2 mg/1 2,4-D, 3 mg/1 2,4 -D and 4 mg/1 2,4-D with pH 5.8 and solidified with 0.8% Disco bactor agar for callusing. Commercial sugar instead of analar grade sucrose was used in the medium. After five weeks of explanation, the calluses 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 + 1 mg/IBA + 3%sugar, ii) MS + 1 mg/l IBA + 6% sugar, iii) MS + 1 mg/l IBA + 9% sugar, iv) MS+1 mg/l IBA + 12% sugar and v) MS + 1 mg/l IBA + 15% sugar. All these operations were carried out under aseptic conditions and cultures were incubated at 28 ± 2°C with 16 hours photoperiod. Rooted plantlets were acclimatized and transplanted to field.

Results and Discussion

Callus induction: Based on their 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 Shaheen and Mirza (1989) and Khan *et al*. (1998). Best callus induction and proliferation was observed on medium containing 3 mg/l 2,4-D. More or less same results were reported by Siddiqui *et al*. (1988) and Begum *et al*. (1996). Clone AEC81-8415 yielded the maximum callus followed by BL4 and AEC82-1026, while L116 produced the minimum (Table 1). Similar trend was observed in callus proliferation on sub-culture. Callus weight got reduced only in AEC80-4725, 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 with type A, but in this study, it was observed that type B callus of sugarcane did not exhibit the same attributes, rather its growth substantially decreased. Explant of clones AEC82-1026 and AEC80-5108 yielded type A callus, but on subculture it got converted into type B. Aging of the medium affected morphological status of callus in all clones, except AEC81-8415 and BL4 in which calli were converted into somatic embryos.

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 variety dependent phenomenon (Table 1). Clone AEC81-8415, yielded maximum plantlets followed by BL4. The minimum plantlets were produced by L116. Callus induction/proliferation and regeneration potential in sugarcane exhibited in synchrony to each other.

However, regeneration was low as compared to its callus production in AEC82-1026 and AEC80-5108. 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). The highest percentage of chlorophyll mutants were recorded in AEC82-1026 and the least in AEC81-0819 (Table 1). The presence of chlorophyll deficient plantlets confirmed the induction of genetic variability (Shepard *et al*., 1980 and Evans and Sharp, 1986). Plants obtained through *in vitro* cultures gave phenotypic variability which was due to true genetic changes (Orton, 1980). Chaleff and Keil (1982) reported that some phenotypic variability was the result of

Table 1: Callus induction, proliferation, regeneration and chlorophyll mutants in indigenous and exotic clones of sugarcane

Clone/variety	Callus (gm)	Proliferation of callus (gm)	Plantlets reg--enerated(Nos.)	Chlorophyll mutants (CM) in regenerated plantlets (Nos.)				
				Albino	Viridis	Others	Total	CM(%)
AEC80-4725	1.9	1.3	78	1	1	0	2	2.5
AEC81-0819	0.4	1.0	162	0	3	0	3	1.8
CP68-1067 (E)	0.7	1.0	124	5	0	0	5	4.03
CP67-412 (E)	0.7	1.0	32	1	0	0	1	3.12
AEC80-2046	1.8	2.0	203	20	0	0	20	9.85
AEC80-3029	2.0	2.0	169	7	2	0	9	5.32
AEC82-1026	2.3	3.8	210	35	10	4	49	23.33
AEC81-8415	2.8	4.3	333	8	5	1	14	4.20
AEC80-5108	2.0	3.5	166	25	0	0	25	15.06
L116	0.3	0.7	23	3	0	1	4	17.34
BL4	2.6	4.0	302	7	3	0	10	3.31

E = Exotic

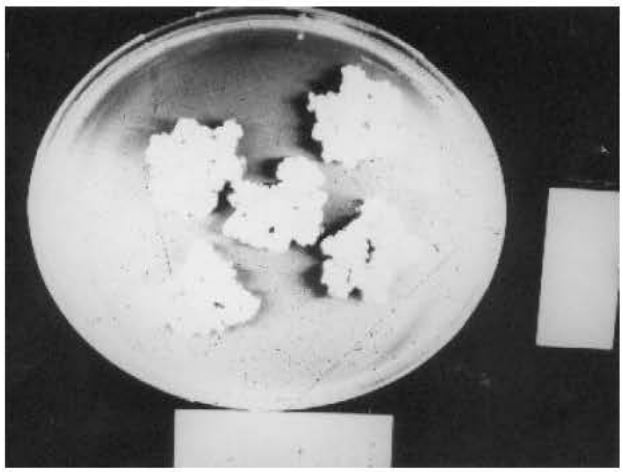

Fig. 1: Yellowish white, compact, dry and nodular type A-callus

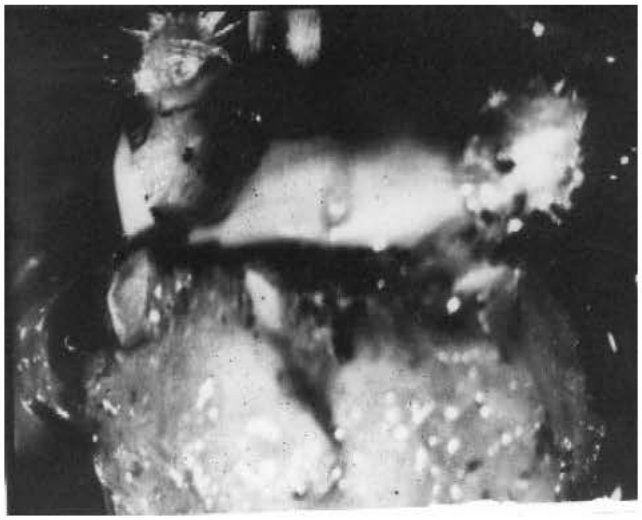

Fig. 2: Whitish globular, non- compact and wet type B-callus

physiological changes during *in vitro* conditions; hence such plantlets normally revert to their parent type in field conditions.

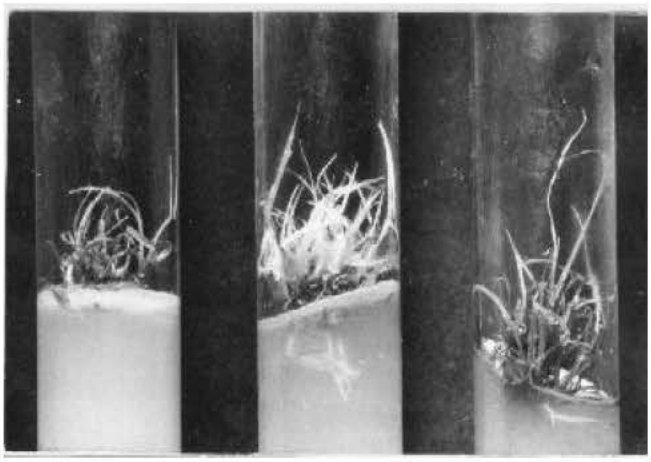

Fig. 3: Chlorophyll mutations in *in vitro* plantlets

Rooting: Root induction was observed in the regeneration medium when plant hormones (auxins) were exhausted, but more vigorous root development was achieved, when the plantlets were separated, the leaves were trimmed and plantlets were cultured on the root induction medium containing MS + 1mg/l IBA + 6% sucrose. Khan *et al*. (1998) observed that use of IBA with 6% sucrose in growth medium induced vigorous root development. The plant lets 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 afterward in the plant lets were field. These plantlets are being evaluated for desired agronomic traits. The foregoing results revealed that the indigenous clones showed better response in tissue culture as compared with exotic ones, because of good callusing and regeneration potential. Moreover, the higher frequency of phenotypic variation indicated the regeneration of variants through *in vitro* culture techniques for the development of new genotypes of sugarcane with improved agronomic characters.

References

Ahloowalia, B. S., 1995. *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.

Anonymous, 2000. Agricultural Statistics of Pakistan, 1999-2000. Govt of Pakistan, pp: 27-28 and 106.

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

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

Evans, D. A. and W. R. Sharp, 1986. Somaclonal and gametoclonal variation. In: Hand Book of Plant Cell Culture Vol. 4. Techniques and Applications. (Ed: D. A. Evans, W. R. Sharp and P. V. Ammirato) Macmillan Publishing Co. New York, pp: 99-132.

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

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

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

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

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

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

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

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

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

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

Shaheen, S. and M. S. Mirza, 1989. *In vitro* production of plants from sugarcane tissue Pak. J. Agric. Sci., 26: 302-312.

Siddiqui, S. H., A. Khatri and M. A. Javed, 1988. *In vitro* culture studies in sugarcane. In: Proc. *In vitro* Selection and Propagation of Economic Plants (Ed: I. Ilahi and K. W. Hughes) Department of Botany, University of Peshawar, Peshawar, pp: 43-51.