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IN-VITRO CULTURE STUDIES IN SUGARCANE

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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 NIA-98 while the lowest in CP67-412 followed by SPSG-26. The maximum chlorophyll mutation frequency was noted in clone AEC82-1026 and minimum in AEC81-0819.

INTRODUCTION: Sugarcane (Saacharum Spp. hybrid) is one of he 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 (Anon., 2002). Improvement of sugarcane mainly depends on the combination of the potential genes. Natural viable fertile seed production has ever been a problem in Pakistan because of non-or sporadic flowering. Arrangements of hybridization under artificial conditions are scare 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 predicated 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. Ahlowalia (1995) reported that the development of desired genotype is only possible through somaclonal variation of thorough 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 variation 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 sugarcane clones under tissue culture conditions.

MATERIAL AND METHODS:

Eleven sugarcane clones viz. Thatta-10, AEC71-2011, AEC82-223, AEC82-1026, NIA-98, BF-129, SPSG-26 and BL4 were used for tissue culture studies. Ten explants containing leaf premordia were taken from each genotype, sterilized by standard procedure (Siddiqui et al., 1988) and cultured on Modified MS medium (Murashige and Skoog, 1962) + 2mg/ 1 2,4-D, 3mg/1 2,4-D and 4mg/1 2,4-D with pH 5.8 and solidified with 0.8% Difco bacto agar for callusing. Commercial sugar instead of Analar grade sucrose was used in the medium.

After five weeks of explantation, the calluses were weighed and cultured on shoot induction medium (MS +2mg/I IBA + 2mg/I IAA + 2mg/I kinetin). The regenerated shoots were scored for chlorophyll mutations. When the plantlets attained 7-8cm height, these were subjected to rooting by culturing I=on different media viz,; i) MS medium ii) ½ MS medium iii) ½ MS medium + 6% sugar iv) MS + 6% sugar v) MS + 9% sugar vi) MS + 1mg/ I IBA + 3% sugar vii) MS + 1mg/I IBA + 6% sugar and viii) MS + 1mg/I IBA + 9% sugar. All these operations were carried our under aseptic conditions and cultures were incubated at 28 + 2°C vi) with 16 hours photoperiod. Rooted plantlets were acclimatized and transplanted to field.

RESULT AND DISCUSSION:

Callus induction: Based on their morphological appearance, two types of calli were observed: (I) type A-yellowish white, compact, dry and nodular and (ii) type B-whitish globular, non-compact and wet 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 2mg/I 2,4-D. More or less same results were reported by Siddiqui et. al., 1988 and Begum et. al., 1996. NIA-98 yielded the maximum callus followed by BL4 and AEC82-1026, while SPSG-26 produced the minimum (Table-1). Similar trend was observed in callus proliferation on sub-culture. Callus weight got reduced only in Thatta callus of Hordeun 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 and similar results were reported by Khatri et al., 2002. Explant of clones AEC82-1026 and BF-129 yeilded 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 NIA-98 and BL4 in which calli were converted in to 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 genotype dependent phenomenon (Table-1). NIA-98, CP67-412. 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 BF-129. This might possibly be due to the conversion of regenerable callus type A to non-regenrable callus type on sub-culturing of callus (Orton, 1979).

Regeneration of albino and viridis plantlets exhibited the appearance of chlorophyll mutations in *in-vitro* plantlets. The highest percentage of chlorophyII mutants were recorded in AEC82-1026 and the least in AEC81-0819 (Table-1). The presence of chlorophyII deficient plantlets confirmed the induction of genetic variability (Shepard et al., 1980 and Evan and Sharp, 1986). Plants obtained through in-vitro cultures gave phenotype 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.

Rooting: Roots grow from the normal primordial when plantlets are well developed (Khan et al., 1998). Eight different media were used for root induction (Table-2). 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/I IBA + 6% sucrose. Khatri et al., (2002) observed that use of IBA with 6% sucrose in growth medium induced vigorous root development. 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 afterward in the field. These plantlets are being evaluated for desired agronomic traits.

Table1: Callus induction, proliferation, regeneration and chlorophyll mutants in indigenous and exotic clones of sugarcane.

Clone/variety	Callus (gms)	Proliferation Ofcallus (gms)	Plantlets regenerated (Nos.)	Chlorophyll mutants (CM) regenerated plantlets (nos.)				in
				Albino	Virids	Others	Total	CM(%)
Thatta-10	0.9	0.7	58	1	1	0	2	3.4
AEC81-0819	0.4	1.0	162	0	3	0	3	1.8
CP68-1067	0.7	1.0	124	5	0	0	5	4.03
CP67-412	0.7	1.0	32	1	0	0	1	3.12
AEC71-2011	1.3	1.6	103	15	1	0	16	15.53
AEC82-223	1.4	2.0	196	6	1	1	8	4.08
AEC82-1026	2.3	3.8	210	35	10	4	49	23.33
NIA-98	2.8	4.3	333	8	5	1	14	4.20
BF-129	2.0	3.5	166	25	0	0	25	15.06
SPSG 26	0.4	0.8	37	2	0	1	3	8.11
BL4	2.6	4.0	302	7	3	0	10	3.31

Table-2. Effect on medium composition on rot induction of sugarcane

Medium	Root induction
MS Medium	-
½ MS Medium	-
½ MS Medium + 6% sugar	+
MS + 6% sugar	++
MS + 9% sugar	+
MS + 1mg/I IBA + 3% sugar	+
MS + 1mg/I IBA + 6% sugar	+++
MS + 1mg/I IBA + 9% sugar	++

-, No root, +, week root, ++, good rooting, +++, excellent rooting variation. In: Hand Book of Plant Cell Culture Vol. **4**. Techniques

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Studies are recommended with increasing the duration of the priming treatments and more detailed study of the culture throughout its productive cycle. Keywords. Salinity Stress, MicropropagationÂ ex vitro. saline stress. The difference in results may have oc-curred due to the use of different methodologies and varieties of sugarcane, since [12] used plants of the variety RB98710, micropropagated in a temporary im-mersion bioreactor and the present work made use of traditional methodology, based on semi-solid culture medium. The temporary immersion bioreactors make use a liquid nutrient medium, allowing the renewal of air and nutrients during cultivation, resulting in higher plant growth and multiplication, when compared to semi-solid medium cultiva-tion [24].
@inproceedings{Khatri2004INVITROCS, title={IN-VITRO CULTURE STUDIES IN SUGARCANE}, author={Abdullah Khatri and Nighat Seema}, year={2004} }. Abdullah Khatri, Nighat Seema. 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 NIA-98 while the lowest in CP67-412 followed by SPSG-26. The maximum chlorophyll mutation frequency was noted in clone AEC82-1026 and minimum in AEC81-0819. View PDF. Save to Library.