Pak. J. Agri. Sci.
26 (3) 202, 1989

IN VITRO PRODUCTION OF PLANTS FROM SUGARCANE TISSUE

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Callus masses were developed from pith parenchyma tissue and cut edges of the leaves of sugarcane on modified MS medium supplemented with 1-5 mg 1 of 2, 4-D. Response to callus initiation was different in various varieties and 2, 4-D concentrations. Callus differentiated to produce numerous shoots on modified MS medium adapted with kinetin and casein hydrolysate. Shoots on transfer to medium devoid of growth regulators induced rooting. Plantlets so formed (I800 in number) were grown in pots in the glasshouse and later transferred to the field. Structures comparable to embryoes were observed in callus cultures grown on low concentration of 2,4-D. Further incubation of these cultures on the same medium or medium without growth regulators, produced rooted plantlets. Morphological variants were observed in clones derived from the callus of each variety.

INTRODUCTION

Breeding work in sugarcane is confined to Iocali tic') of specific environmental conditions favouring flower production, synchronization to make crosses and get viable seed. Genetic variability resulting from sexual crossing and gene segregation mayor may not provide sufficient room to permit greater chances of improvement in important characters of the crop. The primary responsibility of the plant breeder is to find ways for enlarging magni tude of genetic var iabilit y in a particular population. In this quest ti ssue culture techniques offer such opportuni ties to the Plant Breeders.

Nickell (1964) established first sugarcane cui tures from mature internodal parenchyma tissue. Later on Heinz and Mee (1969) reported callus formation on parenchyma tissue of shoot apices and leaves of Saccharum spp, on basal medium containing coconut water and 2, 4-D. Withdrawal of 2, 4-D from the medium induced organ differentiation. Larkin (1982) described protocol for effective regeneration even after 30 months of caUus proliferation. Use of different growth regulators in culture medium was reported by Bhansali and Kishan (1982) for callus proli fera tion in sugarcane. In similar studies Zeng ~ al. (1983) reported 100% callus induction in ex plants from 1-20 leaves from the growing point. Among ca1Jus derivatives morphological variants for tiller number, stem epidermal pattern, stalk diameter, leaf angle and erectness from parental clone were observed (Heinz and Mee, 1971; Sreeni vasan and Sreenivasan, 1984). The area where tissue culture techniques can be of use for the improvement of sugar cane have been indicated by various workers (Nickell, 1977; Heinz et al., 1977; Liu, 1981, and Liu ~al., 1972).

Studies reported here, were initiated on sugarcane to monitor techniques for caUus induction, plant regeneration and to observe soma clonal variation induced by callus,.

MATERIALS AND METHODS

Plant material for this study was taken from Vigorously growing plants of three sugarcane varieties *Col-54,* BL-4 and L-116 from the fields of Sugar cane Research Institute, Fai salabad, Explants were excised from apical stem portions and from roUed young leaves. Young sub-apical 5-6 internodes of the cane stick were excised unroUing the leaf sheaths carefuUy to obtain apical stem explants, Leaf explants 1-2 cm in length, were obtained from 2nd-5th innermost roll of young leaves. Excision of explants was completed under aseptic condition with sterilized *tools.* Explants, immediately after excision, were transferred to test tubes (25 x 150 mm) contalnirg 10 ml agar medium for caUus induction; while 100 ml capacity Jam Jars containing 20 ml medium were used for shoot differentiation from callus. Shoots regenerated from callus were transferred either singly or in groups to rooting medium for root induction. Murashige and Skoog (962) basal medium was modified supplementing its mineral

salts with myo-inositol 100 mg (1, thi!lfline HCl 10 mg C!, nicotinic acid and pyridoxin HCl lrng 1 • Sucrose was added at the rate of 2 96 and the medium was jelled with 0.8 or 0.9 96 Agar-A Mast U.K. This medium was used' in all the studies except where variations specified.

Medium was enriched with different doses of 2, 4.-0 (Table-1) for callus induction. Use of coconut water (l0 % v/v) in the medium was also studied both for calogenesis and prganogenesis. Medium !prtified with casein hydrolysate (4.00 mg I) and Kinetin (I mg I) was used for shoot regeneration. To induce rooting modified MS medium was adopted at full or half concentration without addition of any growth regulator and organic supplement. Before autoclaving the medium at 121°C 15 lbs psi its pH was adjusted at 5.7 with O.lN NaOHor O.lN HCI.

Cultures for callogenesis .were kept in dark for first two weeks in incubator running at 28°C and thereafter transferred to an environment controlled chamber kept at 28°C ± 2°C under continuous cool white fluorescent light with an intensity of about 2500 lux. Callus maintenance and proliferation cultures were incubated either in light or in dark but for differentiation of both shoot and root in ll~ht only. Callus was maintained on medium containing 3 mg I of 2, 4.-0 and subculturing was carried out regularly after every 4-5 weeks on fresh medium.

A part of the callus at each subculturing was carried to differentiation medium and plantlets were regenerated continuously. Rooted plantlets were transferred to pots, carrying sterilized manure-soil-mixture, initially in glasshouse and later to the field for further studies.

RESUL TS AND DISCUSSION

Callus masses developed from the exposed parenchyma cells of the excised internodes and from the cut edges of the leaf explants on modified MS medium containing 2, 4-0. Callus proliferation started from the cut edges of the tissue and covered the whole surface of the explants (Fig.vI). In general callus initiated within two weeks after explantation. Results of these studies are given in Table 1.

Table 1. Response of different explant sources and concentration of 2,4-D in callogenesis of Sorghum

Concentration of 2,4-D	Varieties					
	[illegible]		CO-[illegible]		[illegible]	
	Leaf	Stem	Leaf	Stem	Leaf	Stem
1 mg	+	+	+++	+++	+	+
2 mg	++	++	++++	+++	++	++
3 mg	++	+++	+++	++	+++	++

+ Some callus   +++ Good callus

++ Moderate callus   ++++ Excellent callus

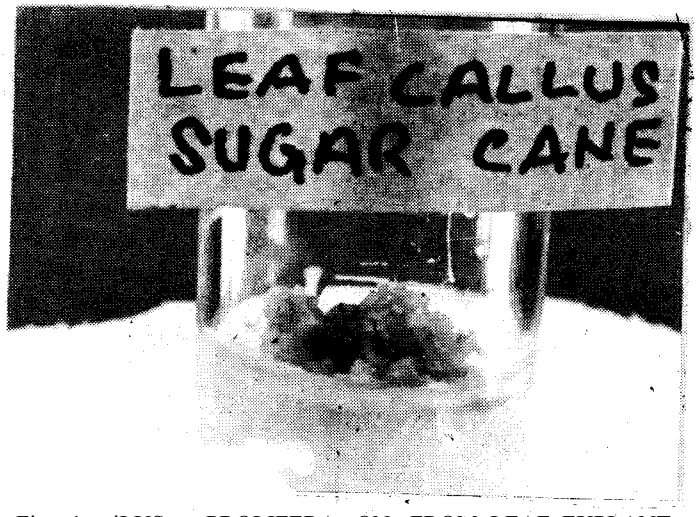
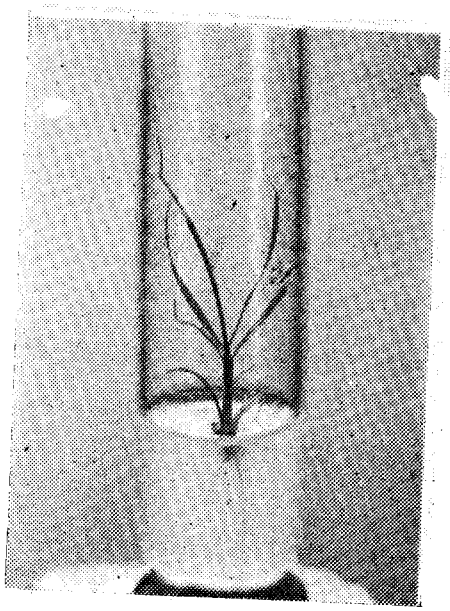


Fig. 1 ~iLUS PROLIFERA nON FROM LEAF EXPLANT



.Fig.:3 SUGARCANE PLANTLET RE GENERATED FROM CALLUS

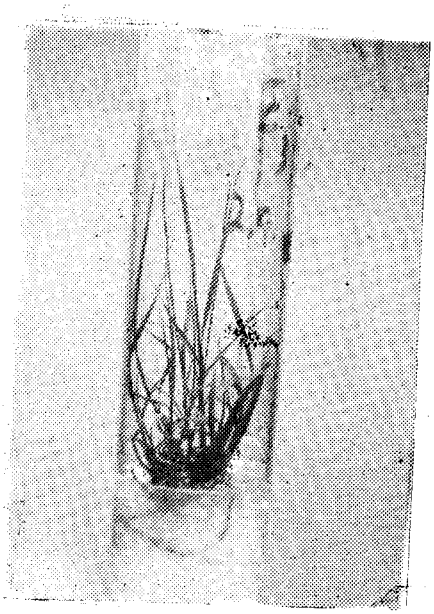


Fig. 2 SHOOT REGENERA nON FROM CALLUS ON DIFFER-ENTIA TION¥EOIUM

Varietal response to different concentrations of 2, 4-D is evident from Table- 1. Variety Col-54 responded to lower concentrations of 2, 4-D and the best callus masses were obtained on 3 mg/l concentration, while the other two varieties BL-4 and L-116 produced good callus at higher concentration i.e. 5 mg/l. Variety Col-54 provided more callus on both the tissue sources as compared to other varieties. The callus colour varied with variety. Shining golden yellow callus was obseved in variety Col-54 in contrast to dusty yellow callus of BL-4 and reddish green in L-116. The results of the present study support the findings of Liu et al., (1972) who reported varietal difference in ability to callogenesis and variation in callus colour of different clones.

Differences in callus induction to explant source i.e, leaf or stem tissue were not prominent as the quantity of callus produced from both the tissues of varieties BL-4 and Col-54 were the same. The behaviour of the tissue source in variety L-116 was somewhat different. Liu et al. (1972) indicated different responses of various tissues to callusproduction.

Use of coconut water 25 % v/v in the medium had no obvious effect in improving early callus initiation and proliferation, therefore, it was not used in later studies. Many workers like Heinz and Mee, (1969) and Liu, (1981) reported use of coconut water in the medium. Need for coconut water was not felt at any stage of this study. Positive results obtained in these studies without coconut milk in medium were probably due to the change in vitamin concentrations or due to the behaviour of genotype. These findings are similar to those of Zeng et al., (1983) who reported production of plant lets in MS medium without the use of coconut water.

CALLUS CHARACTERS AND BEHAVIOUR

Callus cultures maintained in dark produced shining golden yellow colour, while callus incubated in light was granular, comparatively slow in proliferation and of dull golden colour with greenish regions. The quantity of callus produced in cultures incubated in light was comparatively less as compared to cultures incubated in dark and was comparatively better in regeneration

capacity. Callus, maintained in dark, was slow and shy in regeneration.

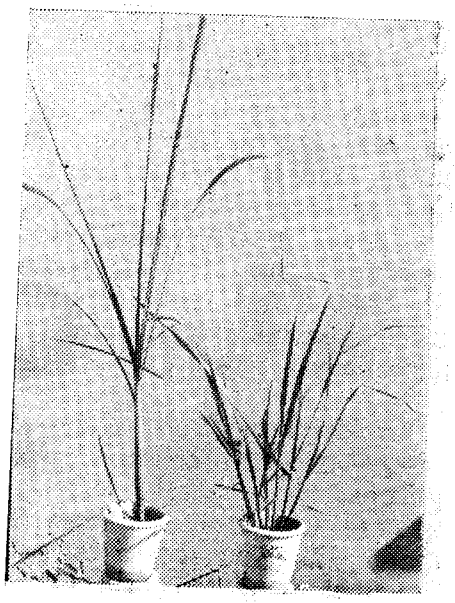
At each sub-culturing Callus pieces were transferred to regeneration. medium (modified MS medium + 400 mg C casein hydrolysate and 1 mg 1 Kinetin) for organ differentiation. Granular callus was better in regeneration capacity as compared to soaked homogenious one. Upper surface of callus incubated in dark, on transfer to light for differentiation, turned pink brown in the beginning but later on green nodular areas developed out of it which differentiated into shoots. The change in callus colour on transfer from darkness to light may be due to physical effect of light or due to some chemical changes. Liu *et al.* (1972) reported colour differences in various clones and genotypes of sugarcane in such studies.

Differences among varieties in their ability to regenerate shoots were prominant. Variety Col-54 was very efficient in differentiation to produce shoot primodia, followed by L-116; but BL-4 was shy in regeneration. Similar variation in differentiation ability in *Saccharum* spps. had been reported by Liu *et al.* (1972). Exclusion of casein hydrolysate from the medium reduced the shoot regeneration to a marked degree and very few shoot primodia appeared in callus which later developed into shoots. A systematic procedure for mass production of *in vitro* plantlets was monitored. Transfer of actively proliferation callus pieces to differentiation medium in test tubes/jam jars during callus subculturing produced numerous shoots (Fig. 2).

Root development in variety Col-54 usually started after the shoot growth had advanced in regeneration medium, possibly, the shoots had produced enough auxin to stimulate root initiation. Other two varieties viz. L-116 and BL-4 produced roots only when shoots were transferred to full or half concentration of modified MS medium (Fig. 3). Variety Col-54, in general, gave better response to *in vitro* plant regeneration. Shoots in groups were better in stimulating *in vitro* root development than solitary shoots. It appears that greater quantity of auxin was produced by many shoots to condition the medium for stimulation and induction of rooting as suggested by Liu *et al.* (1972). Regenera tion of sugarcane plants from callus had also been reported by many workers (Bansali and Kishan, 1982; Liu *et al.* (1972); Larkin (1982) etc.

Fig. j PLAN'TLETS GROWING IN POTSAN SOIL MEDIUM

Fig. 4 EMBROIDS PRODUCING SHOOTS AND ROOTS SIMUL-TANEOUSLY



t-ig. 6 MOTHER PLANT AND SOMA CLONAL VARIA TION P,OR NUMBER. QF TILLERS

EMBRYOGENESIS

Embryogenesis studies were conducted only in variety Col-54. taBus cultures of this variety grown on medium containing I mg'-. of 2, 4-0, incubated over 6 weeks produced structures comparable to embryoes which produced roots and shoots simultaneously (Fig. 4). This organised callus also produced roots and shoots simultaneously on medium containing low concentration of 2, 4-0 (below 0.5 mg I^{-1}) or without growth regulator. Regeneration of plantlets from organized callus appeared to be due to embryogenesis. The development of somatic embryoes on medium containing 2, 4-0 indicated that the growth regulator had probably been depleted from the medium to the extent that it did not influence the formation of embryoes.

Plantlets with well developed root system produced in this study were transferred in sterilized manure-soil mixture in glasshouse (Fig.-5) and thereafter in the field during sugarcane planting season. Over 1800 plants were produced dur ing the study in all the three varieties.

Genetic differences to in vitro methods were exhibited clearly in this study. Variety Col-54 had great response to these methods as compared to L-116 and BL-4. Morphological variants for leaf angle, leaf shape and number of tillers were observed among callus derivatives (Fig. 6). These variations suggested that genetic changes had accomplished through somatic means Le. soma clonal variation. These studies are to be reported separately. In similar studies, Heinz and Mee (1971) pointed out that callus cells maintained in vitro over long. period were usually unstable cytologically and gave rise, after regeneration, to plants which were characterized by genetic variability.

Tissue culture techniques, therefore, play very important role in creating genetic variability, particularly when the opportunities for sexual hybridization are limited. It also permits the use of mutagenic treatments at cellular level. In Pakistan where very few localities suitable for sugarcane breeding exist, the application of this technique in adjunct to conventional methods can give new dimentions to sugarcane improvement.

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