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EFFECT OF SUCROSE AND GROWTH REGULATORS ON THE MICROPROPAGATION OF SUGARCANE CLONES

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Abstract

The rapid multiplication of early, mid and late maturing sugarcane clones were obtained under different concentrations of commercial sugar and plant growth regulators in this study. Late maturing sugarcane clone AEC82-223 did not produce any auxillary shoot at 6% commercial sugar concentration. In contrast multiplication rate was higher in early and mid maturing sugarcane clones NIA-2004, BL4 and NIA-98, respectively. Apical meristems were cultured on modified MS medium containing different concentrations of auxins and cytokinins. An optimal multiplication was observed on M4 (1.0 mg/1 IAA + 1.0 mg/1 BAP + 0.1mg/l Kinetin) and M5 (0.l mg/1 kinetin + 1.5mg/l BAP + 1.5mg/l IAA). Maximum numbers of shoot were observed in BL4 followed by NIA-2004 when 1.0 mg/l of BAP and IAA were applied with low concentration of kinetin (0.1 mg/l). Shoot elongation and multiplication was improved on media devoid of BAP (MS+ 2 mg/1 kinetin + 2 mg/1 IBA + 2 mg/1 IAA). Best rooting was observed on media containing MS+ 1 mg/1 IBA + 6% sucrose.

Introduction

Sugarcane is the second largest cash crop of Pakistan (Naz, 2003) with the country's sugar industries solely dependent on the fate of this crop. Although Pakistan is the fifth largest sugarcane growing country in terms of production area, its per hectare yield is the lowest among all sugarcane producing countries (Anon., 2003; Khan *et al*., 2004). Uneconomical yield and lower sugar recovery cause very high production cost which makes Pakistan the least competitive in domestic and international sugar markets (Khan *et al*., 2005).

There are many causes of low yield, one of which is the lack of a rapid seed multiplication procedure. Once a desired clone is identified, it usually takes 6-7 years to produce sufficient quality of improved seed material. This long duration causes a major bottleneck in breeding programmes (Siddiqui *et al*., 1994). Another important reason for low yield in sugarcane is its susceptibility to attacks by pathogens such as fungi, virus, bacteria and mycoplasma which cause up to 70% in yields reduction (Xue & Chen., 1994; Oropez *et al*., 1995; Bhavan & Gautam, 2002). For instance, sugarcane mosaic virus (SCMV) is found in almost all the cultivars grown in the sub-continent (Naz, 2003). A significant part of the yield (39-40%) is lost each year due to SCMV (Malik & Munir, 1990). As sugarcane is mostly propagated by vegetative means, once a plant becomes infected by a pathogen it can easily transfer the pathogen from one generation to another. For this reason, sugarcane seed (seed cane) production through micropropagation is a suitable and effective method for rapid propagation in comparison to conventional methods.

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Initial attempts to regenerate plants through *In vitro* techniques were conducted on sugarcane by Nickell (1964) and Heinz & Mee (1969). Protocols for *In vitro* plant regeneration of sugarcane through callus culture, axillary bud and shoot tip culture have been developed by many authors (Lee 1986, 1987; Hu & Wang 1983; Hendre *et al*., 1983; Nagai, 1987; Milton & Alien 1995; Baksha *et al.,* 2002). One of the major obstacles to the *In vitro* micropropagation of plants is the genotype / media interaction and rooting of the plantlet. Sugarcane is a highly heterozygous, polyploid and aneuploid crop (Jannoo *et al*., 1999) and as a consequence the frequency of shoot differentiation from apical shoots in most sugarcane varieties varies greatly in number (Siddiqui *et. al.*, 1994). Mulleegadoo & Dookun (1999) examined the effect of explant and genotype on growth of sugarcane under *In vitro* conditions. Although considerable advancement in tissue culture systems for micropropagation has been achieved, there is little information regarding how stalk sucrose concentration influences *In vitro* propagation of the plant. All commercial clones are divided into 3 groups on the basis of stalk sucrose concentration (Javed *et al*., 2000). The present investigation has been undertaken to determine the propagation rate of different genotypes under different auxin and sugar concentrations. Commercial sugar was used rather than AnalaR grade sucrose, making this technique economically viable and technically feasible.

Materials and Methods

Four sugarcane clones (*Saccharum* spp. hybrid) were selected on the basis of their maturity viz., NIA-2004, BL4 (early maturing), NIA-98 (mid maturing) and AEC82-223 (late maturing) were used in this study. Maturity parameter was set on the basis of their sucrose concentration in cane stalk (Khan *et al.,* 2005). Ten explants containing apical meristems were taken from each genotype, sterilized by a standard procedure (Siddiqui *et al.,* 1994) and cultured on modified MS medium (Murashige & Skoog, 1962) supplemented with different concentrations of growth regulators (Table 1&2). Two different concentrations of commercial sugar (4% and 6%) were used in order to study the effect of sucrose on initial multiplication. The explants were kept in the dark for 15, 20, 25, and 30 days to check the effect of darkness on phenol production in the explant. Data on shoot initiation were recorded and shootlets were then subcultured on shoot elongation and multiplication media Sl) MS+ 2 mg/1 kinetin + 2 mg/1 IBA + 2 mg/1 IAA and (S2) MS+ 4.5 mg/1 BAP having two different sugar concentration i.e., 4 and 6%. Plantlets of different height (3, 4, 5, 6, 7 cm) were subjected to rooting by transferring them on three different media i) MS + 1 mg/I IBA + 6% sugar, ii) MS + 1 mg/I NAA + 6% sugar and iii) MS + 1 mg/I IBA+1mg/l NAA + 6% sugar. All these operations were carried out under aseptic conditions and cultures were incubated at 28 ± 2°C with a 16 hours photoperiod. Media was solidified with 0.2% gelrite. Commercial sugar was used instead of AnalaR grade sucrose as a carbon source in the medium. Rooted plantlets were acclimatized and transplanted to the field. The mean and standard deviations were computed from each treatment. Data was analysed by using Duncan Multiple range test.

Results and Discussions

Significant effect of different combination of Kinetin, BAP and IAA were observed. Apical meristems elongated a few days after incubation (Fig 1a). It was observed that shoot initiation in most of the clones started 15 days after explantation. Excessive phenol

Table 1. Effect of different concentration of Kinetin, BAP and IAA 4% sucrose concentration on shoot regeneration of different sugarcane varieties after one month of incubation.

Medium (mg/1) with 4% sucrose	Average number of shoots observed after one month of incubation			
MS+ Kin+BAP+IAA	**NIA-98**	**NIA-2004**	**BL4**	**AEC82-223**
Ml = 0.1 + 0.1 + 0.0	4.25 ± 0.50b	7.50 ± 0.57b	4.75 ± 0.50b	6.75 ± 0.50b
M2 = 0.1 + 0.5 + 0.0	3.50 ± 0.57c	3.00 ± 0.81c	4.00 ± 0.81b	5.25 ± 0.50c
M3 = 0.1 + 1.0 + 0.0	4.25 ± 0.52b	3.50 ± 0.57c	4.00 ± 0.81b	4.00 ± 0.81d
M4 = 0.1 + 1.0 + 1.0	7.75 ± 0.51a	9.25 ± 0.50a	8.25 ± 0.95a	11.50 ± 0.57a
M5 = 0.1 + 1.5 + 1.0	4.75 ± 0.49b	7.50 ± 0.57b	7.25 ± 0.95a	3.75 ± 0.95d

DMR Test: Means denoted by similar letter showed non significant difference among the treatments

Table 2. Effect of different concentration of Kinetin, BAP and IAA at 6% sucrose concentration on shoot regeneration of different sugarcane varieties after one month of incubation.

Medium (mg/1) with 6% sucrose	Average number of shoots observed after one month of incubation			
MS+ Kin+BAP+IAA	**NIA-98**	**NIA-2004**	**BL4**	**AEC82-223**
Ml = 0.1 + 0.5 + 0.0	6.75 ± 0.50c	8.50 ± 0.57c	8.25 ± 0.50d	-
M2 = 0.1 + 1.0 + 0.0	6.50 ± 0.57c	5.25 ± 0.50d	6.00 ± 0.81e	-
M3 = 0.1 + 1.5 + 0.0	9.25 ± 0.95a	10.00 ± 0.81b	9.50 ± 0.57c	-
M4 = 0.1 + 1.0 + 1.0	7.50 ± 0.57bc	11.75 ± 0.50a	10.50 ± 0.57b	-
M5 = 0.1 + 1.5 + 1.0	8.25 ± 0.95a	12.00 ± 0.81a	11.75 ± 0.95a	-

DMR Test: Means denoted by similar letter showed non significant difference among the treatments

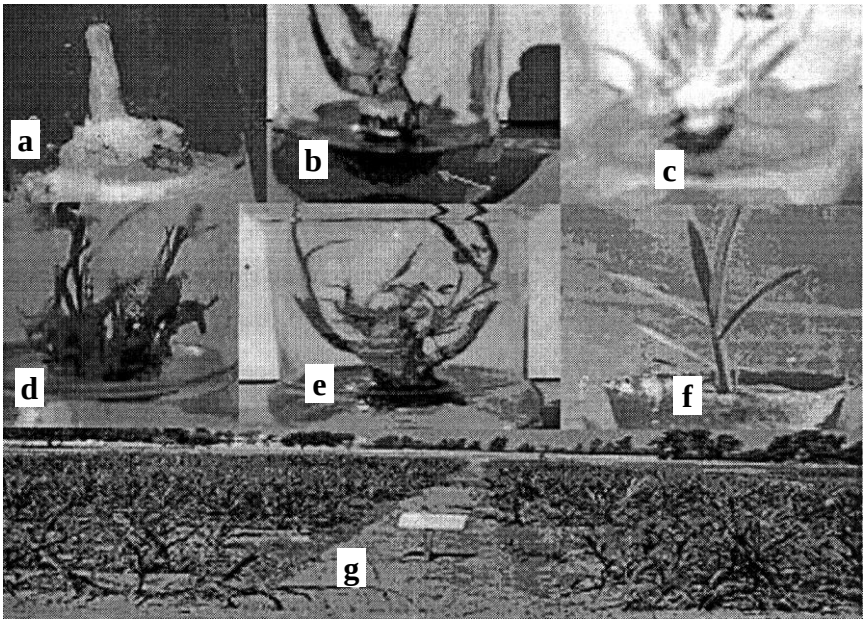

Fig. 1. a- Explant of sugarcane; b- Excessives phenol production due to no dark treatment; c- Shoot initiation; d & e- Shoot multiplication; f- Sugarcane lanlfet in jiffy pot; g- Plantlets in the field for evaluation.

production was observed in cases when explants were not kept in darkness (Fig. 1b), with 20 days of darkness treatment showing good result. When 1.0 mg/l of BAP and IAA were applied alongwith 0.1mg/l kinetin at a 4% sugar concentration, better shoot regeneration was observed in all clones when compared to other hormonal combinations (Table 1, Fig. 1c). Clone AEC82-223, which is a late maturing clonal line, showed a variable response according to sugar concentrations. It showed significantly higher shoot multiplication at 4% sugar concentration. In contrast, clones NIA-2004 and BL4, which are early maturing sugarcane lines, and NIA-98, mid maturing sugarcane line, all exhibited better multiplication at 6% sugar concentration. These results showed that in addition to hormonal concentration, sugar plays a vital role in sugarcane shoot multiplication under *In*-vitro conditions (Table 1 & 2). Chengalrayan & Meagher (2001) reported that auxins and cytokinin concentration influence shoot regeneration. Siddiqui *et al*., (1994), reported the positive effect of lower concentrations of BAP and Kin on shoot proliferation of sugarcane. Sorory & Hosien (2000) also reported that the use of 6% sucrose concentration enhanced shoot regeneration in sugarcane.

Higher shoot multiplication was observed in media containing kinetin, IBA and IAA (Table 3, Fig. 1d & 1e). Clone NIA-98 showed a higher shoot multiplication on medium containing high concentration of BAP (4.5mg/l) at both sugar concentrations (Table 3 & 4). Statistically non-significant differences were observed between NIA-2004, BL4 and AEC82-223. However, maximum shoot multiplication was observed in clone AEC82-223 and the lowest in NIA-98 at 4% sugar concentration. At 6% sugar concentration maximum shoot multiplication was observed in NIA-2004. The result suggests that shoot multiplication in sugarcane is dependent on the genotype/media interaction and sugar concentration also play significant role in shoot multiplication. Serving as a carbohydrate source, it also regulates the osmolarity of the culture media and plays a role during morphogenesis (Sopory, 1979). Nagai (1988) reported that high concentration of BAP suppressed the shoot proliferation in sugarcane. Geetha & Padmanadhan (2001) reported that the combination of BAP with Kin gave the maximum response in most varieties. In our study we found that addition of IAA along with IBA and Kin enhances the production of *In vitro* sugarcane plantlets. Rajesh *et al*., (1994) studied *In vitro* clonal propagation of sugarcane with modified MS media supplemented with IAA, BAP and Kin with 0.5mg/l of each for optimal growth. Pawar *et al*., (2002) also obtained similar results with low concentration of IAA + BAP + Kin for mass multiplication of sugarcane.

Roots grow from the nodal primordial when the plantlets are well developed (Khan *et al*., 1998). Rooting was highly influenced by the different types and concentrations of auxin used (Table 5). Appropriate amounts of auxin in the rooting medium are crucial for root induction. Among three auxins concentrations, IBA at 1 mg/l produced the highest percentage of rooting. These results confirms the previous findings of Khatri *et al*., (2002), whereas, Lal & Singh (1994) who reported that the most efficient auxin for root initiation was NAA. Shenk & Hildebrandt (1972) have also reported a requirement of high concentration of auxin for rooting in sugarcane. A More vigorous root development was achieved when the plantlets were separated, the leaves trimmed and plantlets cultured on a root induction MS medium containing lmg/1 IBA and 6% sucrose (Fig. 5). It was also observed that plantlets of less than 5 cm height did not produce good roots. Plantlets with well developed shoots and roots were transferred to jiffy pots containing sterilized perlite (Fig. 6). After acclimatization, plantlets were initially transferred to earthen pots for hardening and subsequently to the field (Fig 7). These plantlets are currently being evaluated for desired agronomic traits.

Table 3. Effect of phytohormone on shoot multiplication of different sugarcane varieties after one month of incubation.

Medium (mg/1) with 4% sucrose	Average number of shoots multiplied after one month			
MS+Kin+BAP+IBA+IAA	**NIA-98**	**NIA-2004**	**BL4**	**AEC82-223**
MS1-2.0+ 0.0+2.0+2.0	7.50 ±0.57b	10.50 ±0.57a	10.0 ±0.81a	11.00 ±0.81a
MS2-0.0 + 4.5 + 0.0 + 0.0	8.25 ±0.95b	9.50 ±0.57a	8.25 ±0.95b	8.50 ±0.57b

DMR Test: Means denoted by similar letter showed non significant difference among the treatments

Table 4. Effect of phytohormone on shoot multiplication of different sugarcane varieties after one month of incubation.

Medium (mg/1) with 6% sucrose	Average number of shoots multiplied after one month			
MS+Kin+BAP+IBA+IAA	**NIA-98**	**NIA-2004**	**BL4**	**AEC82-223**
MS1-2.0+ 0.0+2.0+2.0	6.75 ±0.95b	10.75 ±0.96a	10.50±0.58a	-
MS2-0.0 + 4.5 + 0.0 + 0.0	8.25 ±0.95a	9.50 ±0.58b	8.75 ±0.50b	-

DMR Test: Means denoted by similar letter showed non significant difference among the treatments

Table 5. Effect of phytohormone on root induction of different sugarcane varieties after one month of incubation.

Medium (mg/1) with 6% sucrose	Average number of shoots multiplied after one month				
MS +IBA +NAA	**NIA-98**	**NIA-2004**	**BL4**	**AEC82-223**	**Thatta-10**
MS + 1.0 + 0.0	+++	+++	+++	+++	+++
MS + 0.0 + 1.0	+	+	+	+	+
MS + 1.0 + 1.0	-	-	-	-	-

- , No rooting, +, week rooting, +++, Profuse rooting

The results of the experiments with different concentrations of sucrose showed that the rates of sugarcane micro shoots obtained from micropropagule were greatly influenced by the concentration of sugar in the medium. Of the two concentrations tested, 4% commercial sugar appeared to be optimum for shoot regeneration and the same can be used for multiplication, whereas 6% commercial sugar was recommended for rooting. The present study would suggest an efficient and easy to handle protocol for micropropagation of sugarcane. This protocol provides a successful technique that can be used for rapid propagation. The application of this protocol can help minimize the variety assessment period and contribute to the rapid propagation of high yielding sugarcane cultivars.

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