EFFECT OF DIFFERENT HORMONES ON EARLY, MID AND LATE MATURING SUGARCANE *(Saccharum officinarum* L.) GENOTYPES FOR CALLUS AND SOMATIC EMBRYOGENESIS

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ABSTRACT

In the present study three sugarcane clones NIA1198-P18 (early maturing, normal yield and high sucrose sugarcane clone), NIA86-223 (late maturing, higher yield and low sucrose sugarcane clone) and Larkana-2003 (mid maturing, low yield and low sucrose) were used for callus induction and its regeneration. MS medium were modified by the use of different growth regulators (2,4-D, dicamba, IAA and BAP). Maximum callus induction / proliferation was recorded in MS medium containing 2mg and 4mg 2,4-D in all clones, while BAP 4 mg/l with 4mg/l 2,4-D has an adverse effect on callus proliferation. Five different media were used for regeneration of plantlets from callus. Sequential observations were taken after one week, 15days and 30 days of shoot induction. Varietal differences were observed in regeneration potential among three clones used. Combination of BAP (5mg/l) with kinetin (2 mg/l) and IBA (2mg/l) was found to be good for regeneration specifically in clone NIA1198-P18. Among the four different media formulated for roots induction, NAA (1mg/l) with IBA (1mg/l) and 3% sucrose produced maximum roots. Clone NIA-1198-P18 respond best among all clones in root induction media. The present investigation has been undertaken to determine the regeneration rate of different clones under different auxin and cytokinins concentrations.

Keywords: *Saccharum officinarum,* Tissue culture, Callogenesis, Somatic embryogenesis, Auxin. Callus

INTRODUCTION

Sugarcane (*Saccharum officinarum*) has a paramount importance over the cash crops of Pakistan. The country is the world's fifth largest producer of sugar cane in terms of acreage, and the 15th largest producer of sugar (Anon, 2003; Khan *et al*., 2009). In Pakistan, sugarcane is grown on around a million hectares and provides raw material for 84 sugar mills (Hashmi, 1995) .The sugar industry is the country's second largest agro-industry after textiles. In addition to sugar, sugarcane is employed in the production of a number of other products such as alcohol used by the pharmaceutical sector, ethanol for fuel, bagasse in paper, chip board manufacturing and press mud used as a rich source of organic matter and nutrients for crop production. There are more reasons for low yields of sugarcane and low sucrose recovery from the early, mid and late maturing sugarcane crop. About two-thirds of the sugarcane area remains under ratoon crops, which reduces output, particularly in the northern areas where frost is prevalent in winter. Among the several possible reasons, the most important one is non-availability of disease free elite stock for seeding (Ali *et al.,*2008). Commercially, sugarcane is propagated from stem cutting with each cutting or set having two or three buds. 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).

Tissue culture is now widely used in sugarcane improvement programmers. Somatic embryogenesis in cell and callus cultures has become the choice for high volume propagation systems and setting up such a large pathogen free delivery for multiplying new sugarcane varieties. Callus can be

initiated from any sugarcane tissue like shoot and root apical meristem, young root, leaves and node tissue immature florescence and pith parenchyma. (Khan *et al*., 1999). Present study was conducted to streamline tissue culture methodology for early, mid and late maturing sugarcane clones.

MATERIALS AND METHODS

Leaf primodia of three sugarcane clones (NIA1198-P18, NIA86-223 and LARKANA-2003) were cultured on eight different callus induction media (Table 2) and were kept at 25± 2˚ C in dark for first three weeks and the somatic embryos were induced by exposing callus to 16/8 hrs light/dark conditions at25± 2˚ C. The somatic embryos were proliferated on the same medium as described in Table 2. The regeneration potential of somatic embryos of all clones was evaluated on five different medium (Table 3). Four different media were used for the root induction (Table 4). The sucrose added in the media as carbon source and media was sterilized at 121°C and 15 lbs psi pressure for 20 min. Medium pH was adjusted to 5.6 to 5.8. Ten explants per replication were used. Time taken to induce callus, somatic embryoids and plantlet regeneration in specific hormonal supplementation was recorded (in days) on daily basis inspection. The data were analyzed using software statistic 8.1.

RESULTS AND DISCUSSION

Callus induction was observed within three-weeks after plating of leaf sheath on all modified MS media. Two type of calli were produced (1) embryogenic and (2) non embyogenic. Where, good regeneration potential was observed in embryogenic callus. The best callus induction was observed on MS medium supplemented with 2,4-D from leaf explant of all clone. The application of 2,4-D produced Whitish, compact callus, soft friable nodular callus in clone NIA1198-P18 whereas, whitish, soft compact and friable embrogenic callus was produced in clone NIA86-223. Larkana-2003 produced whitish, compact, yellow color, less compact and nodular callus. The maximum callus induction and somatic embryogenesis was observed on MS medium contains 2, 4-D 2mg/l in clone NIA1198-P18 followed by MS medium contains 4mg/l of 2, 4-D whereas the lowest callus induction was observed MS medium containing 4mg 2,4-D + 4mg BAP in all clones. The maximum callus proliferation was observed in MS medium containing 2 mg/l 2, 4-D and 4mg/l 2,4-D in clone NIA1198-P18 (1.26g), followed by NIA86-223 (1.23g) in MS medium containing 2mg dicamba and minimum callus proliferation was observed in MS Medium augmented with 4mg 2,4-D + 4mg BAP NIA86-223 clone (0.59g).

The initial shoot induction was observed within one week after plating the callus on regeneration media (Table 3).The maximum number of plantlets was observed in MS medium supplemented with 5mg BAP+2mg/l Kinetin + 2mg/l IBA (21.66) and followed by MS medium supplemented with 2mg/l IAA+ 2mg/l Kinetin +2mg/l IBA (20.6). The minimum plantlets were observed in MS medium augmented with 2mg/l GA_3+2mg/l Kinetin (9.36). After 15 days of plating, regeneration from embryogenic calli was observed in almost all clones, however the discrepancies was observed in number of regenerated plantlets. NIA1198-P18 produced the maximum number of plantlets (93.00) and followed by Larkana-2003 (87.00), while minimum plantlets were observed NIA86-223 (47.33). After a month maximum number of plantlet was observed on MS medium with 5mgBAP+ 2mg Kinetin +2mg IBA in clone NIA1198-P18 (107.00).

Four different media were used for root induction (Table 4). During the first fifteen days of root induction the effects of the growth regulators were statistically non significant. However after a month maximum roots were produced in half strength MS medium augmented with 1mg/l NAA with 1mg/l IBA with 3% sucrose in Clone NIA-1198-P18(10.0) and followed by half strength MS medium supplemented with 1mg/l IBA with 4% sucrose in NIA86-223(9.66) .The plantlets with well developed

shoots and roots were transferred to the jiffy pots. After acclimatization, plantlets were first transferred to the earthen pots for hardening and then to soil.

The present work, to investigate the effect of different growth regulators with different concentrations reveals that among all the growth regulators tested, 2,4-D proved to be the best growth regulator for callus induction. The embryogenic type A callus and non-embryogenic type B callus was observed on the basis of callus external morphology.) similar results were reported by compact and dry nodular and embryogenic callus forms. Somatic embryogenesis is efficient for the production of large number of plants within a short period (Arencibia, 1998); (Yasmin *et al*., 2011; Zahra *et al,* 2010; Athar *et al.,* 2009; Badawy, *et al.,* 2008; and Gandonou *et al.,* 2005.

In this study callus under different growth regulators showed different callus regeneration potential All these studies concluded that regeneration potential of callus was specific and genotype dependent phenomenon and at the same time it parallel with the hormonal concentration and combinations(Kaur *et al.,* (2001). It was also observed that callus derived from different auxins showed different regeneration potential.

The regeneration started with the appearance of green dots on one week on regeneration medium the regeneration in these clones was observed. Five different media were used in plantlets regeneration. Sequential observations were taken after 30 and 45 days of shoot induction. Varietals differences were observed in the regeneration potential among three varieties used as combination of BAP with kinetin and IBA was found to be suitable for NIA1198-P18 in contrast with MS medium containing GA3, where minimum regeneration was observed. Whereas combination of IAA, Kiniten and IBA medium were found to be suitable for NIA1198-P18 and NIA86-223 regeneration. The regeneration of the plants from leaf tissues of sugarcane in *vitro* can occur through organogenesis, (Larkin, 1982; Chen *et al.,* 1988); as well as embryogenesis (Falco *et al.,* 1996); reported by both organogenesis and somatic embryogenesis (Taylor *et al*.,1992); depending upon the culture conditions

The effect of different concentrations of NAA, IBA and sugar for root induction was also non significant. Aamir *et al*., (2007); Safdar *et al.* (2010); Alain *et al.,* (2002); observed similar results in their studies. Khan *et al*., 1998 reported that roots grow from the nodal primordia only when the plantlets are well developed.

Table: 1 Response of different varieties callus growth and morphology effect

NIA1198-P18	++++	Whitish, compact callus, soft frible nodular callus	Embryogenic (type callus)
NIA-223	++	Soft compact and frible embrogenic callus	Non embryogenic Embryogenic (type callus)
Larkana-2003	+++	Yellowish-white, compact callus	Embryogenic (type callus)

Table:2 Response of different varieties to different media concentration for callus induction and callus proliferation

Media	Callus Induction			Callus Prolification		
	NIA1198-P18	NIA86-223	Larkana-2003	NIA1198-P18	NIA86-223	Larkana-2003
2,4-D 2 mg/l + MS	0.98[a]	0.92[ab]	0.89[ab]	1.26[a]	1.20[bc]	1.20[bc]
2,4-D 4 mg/l + MS	0.92[ab]	0.90[ab]	0.76[d]	1.26[a]	1.16[cd]	1.13[cd]
Dicamba 2 mg/l + MS	0.85[bc]	0.79[cd]	0.63[f]	1.23[b]	1.23[bc]	1.13[cd]
Dicamba 4 mg/l + MS	0.72[de]	0.7[2de]	0.63[f]	1.00[e]	1.03[ef]	1.10[de]
Dicamba 2 mg/l + IAA 2mg/l	0.65[ef]	0.53[g]	0.53[g]	0.88[j]	0.96[gh]	0.94[hi]
Dicamba 4 mg/l+ BAP 4mg/l	0.45[g]	0.42[hi]	0.43[h]	0.77[lm]	0.82[jk]	0.84[jk]
2,4-D 2 mg/l + IAA 2mg/l	0.30[kl]	0.33[jk]	0.35[l]	0.77[l]	0.73[mn]	0.80[kl]
2,4-D 4mg/l +BAP 4 mg/l	0.21[lm]	0.21[lm]	0.22[lm]	0.59[p]	0.62[op]	0.67[no]

DMR test (0.05): Different letters show significant differences at P≤0.05

Table 3: Response of different varieties to different media concentration for shoot number

Media	NIA1198-P18	NIA86-223	Larkana-2003	NIA1198-P18	NIA86-223	Larkana-2003	NIA1198-P18	NIA86-223	Larkana-2003
	15 after shoot induction			30 days after shoot induction			45 days after shoot induction		
BAP5mg/l+Kint2 mg+IBA2mg/l	21.66[a]	14.00[de]	16.33[c]	93.00[a]	74.00[d]	87.00[ab]	107.00[a]	89.33[bc]	90.67[bc]
IAA2mg/l+Kint 2mg/l+ IBA 2mg/l	20.66[ab]	13.00[ef]	16.66[c]	80.33[c]	54.33[gf]	82.00[bc]	93.00[b]	90.67[bc]	76.33[de]
BAP 4mg/l+Kint3mg/l	16.66[c]	12.33[e]	15.00[cd]	81.33[bc]	62.00[e]	74.00[d]	85.33[c]	79.00[d]	63.6[f]
IAA 1.5mg/l +Kint .5mg/l	19.00[b]	10.66[gh]	11.33[fh]	62.33[e]	72.66[d]	59.00[ef]	72.33[e]	71.67[e]	62.33[f]
GA3 2mg/l + Kint 2mg/l)	11.66[fg]	9.36[i]	10.33[hi]	48.66[gh]	47.33[H]	48.33[gh]	64.67[f]	52.00[g]	53.67[g]

DMR test (0.05): Different letters show significant differences at P≤0.05

Table 4: Response of different varieties to different media concentration for shoot number

Media	NIA1198-P18	NIA86-223	Larkana-2003	NIA1198-P18	NIA86-223	Larkana-2003	NIA1198-P18	NIA86-223	Larkana-2003
	One week after root induction			15 days after root induction			One month after root induction		
$MS_{1/2}$ + 1 mg/I NAA + 1 mg/I IBA 3% sugar	1.27[ab]	1.27[ab]	1.28[ab]	5.16[a]	5.10[a]	5.10[a]	10.00[a]	7.13[cd]	7.33[bc]
$MS_{1/2}$ + 1 mg/I IBA + 4% sugar	1.29[ab]	1.29[a]	1.28[ab]	4.33[b]	4.43[b]	4.36[b]	8.06[b]	9.66[a]	8.10[b]
MS $_{1/2}$ + 2 mg/I IBA + 5% sugar	1.25[ab]	1.28 ab	1.26[ab]	4.03[bc]	3.66[cd]	4.00[bc]	7.13[cd]	5.53[f]	7.13[cd]
MS ½+ 2 mg/I NAA+ 6% sugar)	1.24[cd]	1.25 bc	1.29[ab]	3.26[de]	3.30[de]	3.60[cd]	6.13[ef]	7.13[cd]	6.50[de]
MS ½+ 3 mg/I NAA+ 7% sugar)	1.25[ab]	1.24[cd]	1.26[ab]	3.50[cd]	3.10[e]	3.20[de]	6.33[de]	5.30[g]	5.30[g]

DMR test (0.05): Different letters show significant differences at P≤0.05

CONCLUSION

Summarizing the main findings it is concluded that 2,4-D is more potent to callus initiation as compared to other hormonal combinations. Callus was subcultured in different media for induction of somatic embryogenesis. The present study reported the medium composition of plantlet with MS + 5mg BAP+ 2mg Kinetin +2mg IBA was found to be most favorable for regeneration of embryogenic calli in sugarcane varieties. In present study, it is observed the sugarcane clones NIA1198-P18 (early maturing, normal yield and high sucrose sugarcane clone in good response.

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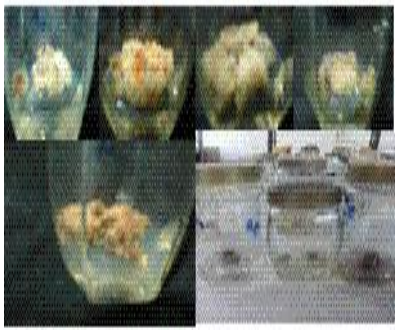

Fig.1. Types of callus developed under dark conditions in sugarcane cultivar Compact embryogenic callus with globular structures like somatic embryos after one month. Non response callus induction different media

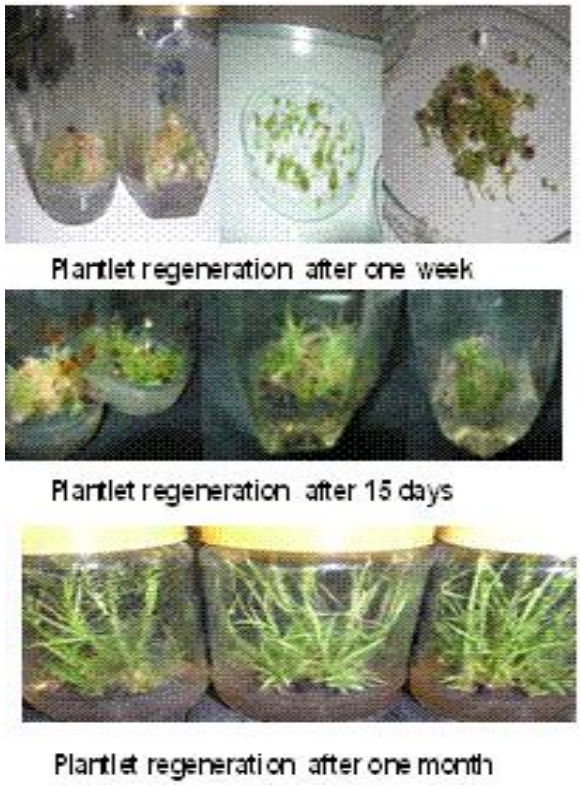

Fig.2. Regeneration plantlet in sugarcane

Fig.3 Root formation on MS medium containing. plantlets in the earthen pots plantlets with well developed shoots and root