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INFLUENCE OF PHYTOHORMONE ON THE ORGANOGENESIS OF SUGARCANE

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Abstract

Three sugarcane *(Saccharum* sp. Hybrid) varieties viz., AEC82-1026, NIA-98 and BL4 were used in this study in order to compare their response to different concentration of phytohormones (Dicamba 2,4D and Picloram). Best callus induction was observed using dicamba fallowed by 2,4-D whereas best proliferation was achieved with dicamba. The maximum callus formation, and plantlets regeneration were recorded in AEC82-1026 and minimum in BL4.The growth regulators dicamba and 2,4D induced more genetic variability as compared to picloram. After regeneration, the plantlets were transferred on eight different rooting medium and best rooting was observed on media containing 2mg/l IBA with 4% sugar. The AEC82-1026 produced maximum number of roots secondary roots followed by NIA 98. The well rooted plantlets were transferred to the green house and than into the field for evaluation.

Introduction

Sugarcane (*Saccharum* sp. Hybrid), belongs to the family Poaceae, is a tropical grass of high polyploidy (2n=80-270) (Heinz & Mee., 1969). Sugarcane accounts for approximately 70% of the world's sugar and is an economically important cash crop in tropical and sub-tropical region in 70 countries spread over five continents (Chatenet *et al*., 2001). Properties such as an efficient photosynthesis and efficient biomass production make this an excellent target for industrial processing and a valuable alternative for animal feeding and the production of by- products. It is also a prime candidate as a fuel crop (production of ethanol) because of its efficient biomass production (Gallo-Meagher *et al*., 2000). The average yield of sugarcane in Pakistan is 48.9t ha^{-1} as compared with the world which is around 65.5t ha^{-1}, while India and Egypt are getting around 60t and 121t ha^{-1}, respectively (Anon., 2009). Thus there is big gap between cultivated area and cane yield. An extensive breeding work and management practices are required to narrow down this big gap. The genetic complexity, non- or sporadic flowering and the production of non-viable seeds under natural conditions render traditional breeding laborious (Ingelbrecht *et al*., 1999**;** Khan *et al*., 2004). Under such circumstance *In-vitro* culture technique offers unique opportunity for the exploitation of genetic variability and rapid isolation of clones with desired characteristics in sugarcane (Heinz & Mee, 1969; Khan *et al*., 1999). Somaclonal variation in sugarcane was first demonstrated by (Heinz & Mee, 1969). Larkin & Scowcroft (1981) coined the term somaclonal variation to describe the occurrence of genetic variance derived from *In vitro* procedures. Liu & Chen, (1976, 1978 and 1984) have reported significant variation in somaclones for characters such as cane yield and its components, sugar contents and some morphological traits. Krishnamurthi & Tlaskal, (1974) and Larkin & Scowcroft (1981) have developed improved sugarcane clones through *In-vitro* culture technique endowed with high cane yield, resistance to disease and tolerance to stress. Callus culture of sugarcane has been successfully established using shoot apices, young leaves and young inflorescences as explant. Callus has now been induced in a large number of sugarcane species indicating that this phenomenon is not limiting (Narayanaswamy, 1997).

However, in Pakistan very few studies were carried out on callus induction from shoot tip which is a base for genetic studies in sugarcane for developing varieties resistant to various diseases and insect pests. In the present study we have optimized conditions for callus induction in sugarcane. This optimized protocol will help in establishing efficient system for the genetic transformation in callus of the important sugarcane varieties for resistance to various stresses.

Material and Methods

Eight month-old field-grown sugarcane plants of NIA-98, AEC82-1026 and BL4 were used for tissue culture studies. Ten explants containing leaf primordia were taken from each genotype, sterilized by standard procedure (Siddiqui *et al*., 1994; Khan *et al*., 2009) and cultured on modified MS medium (Murashige & Skoog, 1962) containing 1-3 mg/l, 2,4-D, dicamba and picloram for callusing designated as G1 (1mg/l 2,4-D), G2 (2mg/l 2,4-D), G3 (3mg/l 2,4-D), G4 (1mg/l Dicamba),G5 (2mg/l Dicamba),G6 (3mg/l Dicamba), G7 (1mg/l Picloram),G8 (2mg/l Picloram) and G9(3mg/l Picloram). Medium was solidified with 0.4% gerlite. Commercial sugar was used as carbon source. After four weeks of explantation, the callus was weighted and one gram of callus was cultured on regeneration medium containing different concentrations IAA, IBA and kinetin. The appearances of green shoot from callus were counted for calculating the shoot organogenesis. The regenerated shoots were scored for chlorophyll mutations. When the plantlets attained 7-8 cm height, these were subjected to rooting. Rooting media contains different concentrations of IBA and sucrose. All these operations were carried out under aseptic condition and cultures were incubated at 28 ± 2°C with 16 hours photoperiod. The *In vitro* grown plantlets were then transferred to the net house for the hardening purpose.

Results and Discussion

Callus induction: Two types of callus were observed, a) compact, yellowish white, dry nodular and embryogenic which is capable of regenerating plants and b) whitish globular, smooth non-compact, non-embryogenic and non regenerable (Fig. 2). Similar results were also reported by Khan *et al*., (1998). It was recorded that quantity and quality of callus were equally important for obtaining good regeneration. Distinction between embryogenic and non-embryogenic callus was performed on the basis of callus external aspect as reported by Gandonou *et al*., (2005). For callus induction, 9 different media were used (Table 1). Clone AEC82- 1026 yielded maximum callus on media containing 1mg/l dicamba (Fig. 1), whereas, clone NIA-98 produces maximum callus on MS + 1mg/l 2, 4-D (G4). Lowest callus induction was observed on media containing picloram in all three genotypes. High degree of embryogenic callus were observed on 2,4-D followed by dicamba and least was observed on picloram. However 2,4-D did not produce type 'b' callus in our experiment.

Table 1. Effect of different auxins concentration on callus induction.

Concentration of auxin	Initial weight of callus (g)	Weight of callus after Ist sub-culture (g)	Type of callus
		AEC82-1026	
Dicamba(1mg/lit)	2.646^{b}	6.183^{a}	Nodular Compact (Type A)
Dicamba(2mg/lit)	2.786^{ab}	5.413^{b}	Nodular Compact
Dicamba(3mg/lit)	2.346^{c}	4.556^{c}	Nodular Compact
2,4D(1mg/lit)	2.043^{d}	3.276^{d}	Nodular Compact
2,4D(2mg/lit)	1.860^{e}	2.406^{h}	Nodular Compact
2,4D(3mg/lit)	1.670^{fg}	2.440^{gh}	Nodular Compact
Piroram(1mg/lit)	1.593^{gh}	1.756^{i}	Nodular/smooth non- Compact (Type B)
Piroram(2mg/lit)	1.580^{ghi}	1.116^{j}	Nodular/smooth Compact
Piroram(3mg/lit)	1.440^{ijk}	1.110^{j}	Nodular/smooth Compact
		NIA98	
Dicamba(1mg/lit)	1.906^{de}	2.506^{fgh}	Nodular Compact (Type A)
Dicamba(2mg/lit)	1.843^{e}	2.763^{fg}	Nodular Compact
Dicamba(3mg/lit)	1.880^{e}	3.103^{de}	Nodular Compact
2,4D(1mg/lit)	2.656^{b}	3.263^{d}	Nodular Compact
2,4D(2mg/lit)	2.870^{a}	2.790^{ef}	Nodular Compact
2,4D(3mg/lit)	2.783^{ab}	2.536^{fgh}	Nodular Compact
Piroram(1mg/lit)	1.383^{jk}	1.056^{jk}	Nodular/smooth non- Compact(Type B)
Piroram(2mg/lit)	1.480^{hij}	1.106^{j}	Nodular/smooth Compact
Piroram(3mg/lit)	1.766^{ef}	1.026^{jk}	Nodular/smooth Compact
		BL4	
Dicamba(1mg/lit)	2.373^{c}	1.750^{i}	Nodular Compact / smooth non compact (type B callus)
Dicamba(2mg/lit)	1.843^{e}	1.836^{i}	Nodular Compact
Dicamba(3mg/lit)	2.370^{c}	1.150^{j}	Nodular Compact
2,4D(1mg/lit)	1.623^{g}	2.020^{i}	Nodular Compact
2,4D(2mg/lit)	1.533^{ghi}	2.626^{fgh}	Nodular Compact
2,4D(3mg/lit)	2.470^{c}	1.830^{i}	Nodular/ compact
Piroram(1mg/lit)	1.233^{l}	1.130^{j}	Nodular/ smooth non- Compact(Type B)
Piroram(2mg/lit)	1.443^{ijk}	0.890^{jk}	Nodular/smooth Compact
Piroram(3mg/lit)	1.323^{kl}	0.753^{k}	Nodular/smooth Compact
SE	**0.0700**	**0.1667**	
LSD (5%)	**0.1404**	**0.3345**	
LSD (1%)	**0.1871**	**0.4457**	

Initial callus weight was taken after 30 days of explantation (Table 1) and one gram callus of each genotype was transferred on fresh media for proliferation. Best proliferation of callus was observed on medium containing 1mg/l Dicamba and 1mg/l 2,4-D (Fig. 3). Minimum proliferation of callus was observed on 3mg/l Picloram. Maximum proliferation of callus was observed in clone AEC82-1026 followed by NIA98 and minimum callus proliferation was recorded in BL4. Callus weight reduced in BL4 because of high percentage of type-B callus. Similar result was reported by Khatri *et al*., (2002).

Regeneration: Regeneration started with the appearance of green dots on callus within a week on regeneration medium (Fig. 4a). Clone AEC82-1026, yielded maximum plantlets on callus derived from dicamba followed by 2,4-D and minimum plantlet regeneration was recorded on callus derived from picloram (Table 2). Similar trend of regeneration was observed in NIA-98, whereas, minimum plantlet regeneration was observed in BL4.

The effect of different growth regulators on shoot elongation of *In vitro* grown sugarcane was evaluated in this study. Maximum shoot elongation was observed on callus derived from dicamba fallowed by 2,4-D and minimum shoot length was recorded on callus derived from picloram (Fig. 4c). Fitch & Moore (1993) showed that the total number of regenerated shoots decreased with time in the long-term culture of embryogenic sugarcane calli, while the number of pale green plants increased after five months of culture. Callus cultures capable of regenerating normal plants and showed a gradual decrease in their cell re differentiation potentiality from the 7th subculture.

The regeneration of albino, virids and other plantlets exhibited the appearance of chlorophyll mutation in *In-vitro* plantlets (Fig. 4b). Maximum numbers of chlorophyll mutants were observed in callus derived from dicamba and minimum was recorded in calli derived from picloram. The highest percentage of chlorophyll mutant was recorded in AEC82-1026 and the lowest in BL4. The presence of chlorophyll deficient plantlets confirmed the induction of genetic variability (Shepard *et al*., 1980).

Root induction: The effect of different concentrations of IBA and sugar against root number was significant. Clone AEC82-1026 produced maximum numbers of primary and secondary roots on 2mg/l IBA with 4% sugar fallowed by NIA98 (Fig. 5a). Minimum root number was achieved on 4mg/l IBA with 6% sugar in BL4 (Table 3). Khan *et al*., (1998), reported that roots grow from the nodal primordia only when the plantlets are well developed. *In vitro* regenerated plantlets were transferred to small pots containing mixture of soil and sand (2:1) for hardening (Fig. 5b).

Table 2. Effect of different phytohormone concentration on plant regeneration.

Concentration of auxin	Plantlets	Shoot length (cm)	chlorophyll mutant
	AEC82-1026		
Dicamba(1mg/lit)	77.66^{a}	13.60^{a}	11.20^{a}
Dicamba(2mg/lit)	74.33^{ab}	12.50^{b}	10.56^{b}
Dicamba(3mg/lit)	75.00^{ab}	12.40^{b}	9.50^{e}
2,4D(1mg/lit)	71.66^{bc}	11.20^{cdef}	10.40^{bc}
2,4D(2mg/lit)	68.33^{cd}	10.53^{fgh}	9.56^{de}
2,4D(3mg/lit)	64.33^{de}	10.70^{efgh}	8.56^{f}
Piroram(1mg/lit)	50.33^{fg}	10.43^{gh}	8.60^{f}
Piroram(2mg/lit)	45.66^{gh}	10.40^{h}	8.53^{f}
Piroram(3mg/lit)	43.66^{hi}	10.46^{gh}	7.46^{g}
	NIA98		
Dicamba(1mg/lit)	67.66^{cd}	12.46^{b}	11.26^{a}
Dicamba(2mg/lit)	64.33^{de}	12.43^{b}	11.53^{a}
Dicamba(3mg/lit)	65.00^{de}	12.56^{b}	10.00^{cd}
2,4D(1mg/lit)	61.66^{e}	13.33^{a}	9.76^{de}
2,4D(2mg/lit)	55.00^{f}	13.40^{a}	9.50^{e}
2,4D(3mg/lit)	54.33^{f}	13.46^{a}	8.63^{f}
Piroram(1mg/lit)	42.66^{hi}	11.13^{cdefg}	7.53^{g}
Piroram(2mg/lit)	40.00^{ij}	11.63^{c}	7.53^{g}
Piroram(3mg/lit)	35.66^{jk}	11.53^{cd}	7.33^{g}
	BL4		
Dicamba(1mg/lit)	54.33^{f}	11.40^{cde}	9.80^{de}
Dicamba(2mg/lit)	45.66^{gh}	10.83^{defgh}	8.50^{f}
Dicamba(3mg/lit)	43.33^{hi}	11.00^{cdefgh}	7.50^{g}
2,4D(1mg/lit)	44.33^{hi}	11.23^{cdef}	7.53^{g}
2,4D(2mg/lit)	35.00^{k}	11.20^{cdef}	7.30^{g}
2,4D(3mg/lit)	31.00^{kl}	10.93^{cdefgh}	7.56^{g}
Piroram(1mg/lit)	28.00^{lm}	11.23^{cdef}	6.73^{h}
Piroram(2mg/lit)	27.33^{lm}	11.26^{cde}	5.06^{i}
Piroram(3mg/lit)	24.66^{m}	11.06^{cdefgh}	4.26^{j}
SE	**2.4597**	**0.3588**	**0.2441**
LSD (5%)	**4.9358**	**0.7200**	**0.4899**
LSD (1%)	**6.5767**	**0.9594**	**0.6528**

Table 3. Effect of different concentration of IBA and sugar on root induction.

Concentration of phytohormones	Number of primary roots	Number of secondary root
	AEC82-1026	
2mg IBA+4%Sug	10.40^{a}	22.30^{a}
2mg IBA+5%Sug	9.56^{c}	21.10^{b}
2mg IBA+6%Sug	8.76^{e}	19.53^{c}
3mg IBA+4%Sug	8.36^{f}	19.16^{d}
3mg IBA+5%Sug	7.66^{h}	18.66^{e}
3mg IBA+6%Sug	7.26^{i}	18.06^{f}
4mg IBA+4%Sug	6.66^{j}	17.60^{gh}
4mg IBA+5%Sug	6.26^{k}	17.06^{i}
4mg IBA+6%Sug	5.83^{m}	16.73^{j}
	NIA98	
2mg IBA+4%Sug	9.76^{b}	18.53^{e}
2mg IBA+5%Sug	9.16^{d}	18.06^{f}
2mg IBA+6%Sug	8.46^{f}	17.73^{g}
3mg IBA+4%Sug	7.80^{h}	17.33^{hi}
3mg IBA+5%Sug	7.30^{i}	15.26^{m}
3mg IBA+6%Sug	6.76^{j}	16.73^{j}
4mg IBA+4%Sug	6.23^{kl}	16.13^{k}
4mg IBA+5%Sug	5.60^{n}	15.73^{l}
4mg IBA+6%Sug	5.26^{o}	14.86^{n}
	BL4	
2mg IBA+4%Sug	9.03^{d}	17.80^{fg}
2mg IBA+5%Sug	8.53^{f}	17.43^{h}
2mg IBA+6%Sug	8.06^{g}	16.76^{j}
3mg IBA+4%Sug	7.30^{i}	16.36^{k}
3mg IBA+5%Sug	6.63^{j}	15.70^{l}
3mg IBA+6%Sug	6.06^{l}	15.16^{m}
4mg IBA+4%Sug	5.76^{mn}	14.70^{n}
4mg IBA+5%Sug	5.26^{o}	14.06^{o}
4mg IBA+6%Sug	4.76^{p}	13.83^{o}
S.E	**0.0962**	**0.1449**
LSD (5%)	**0.1930**	**0.2908**
LSD (1%)	**0.2571**	**0.3875**
CV	**1.60**	**1.04**

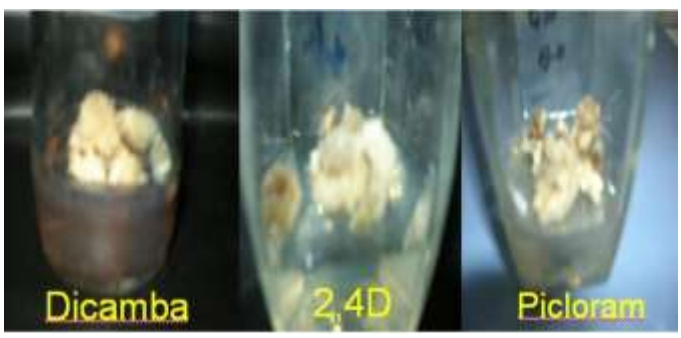

Fig. 1. Effect of different phytohormone on callus induction in sugarcane.

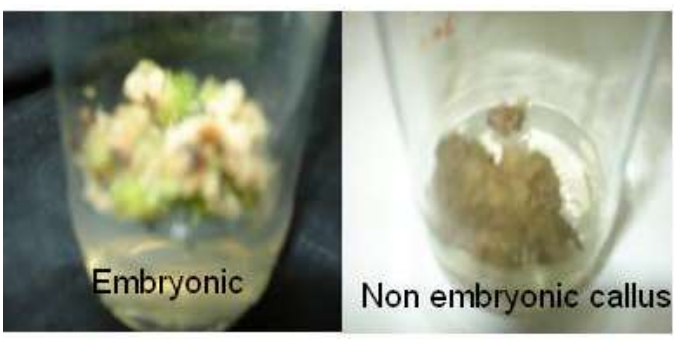

Fig. 2. Production of embryogenic and non embryogenic callus under the influence of different phytohormone concentration in sugarcane.

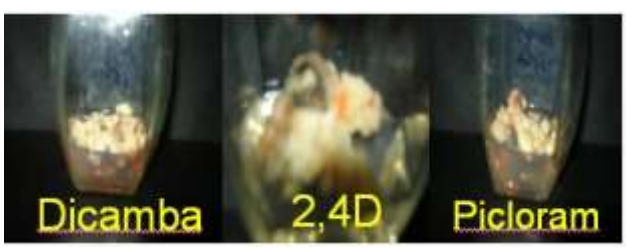

Fig 3. Effect of different phytohormone on callus proliferation in sugarcane.

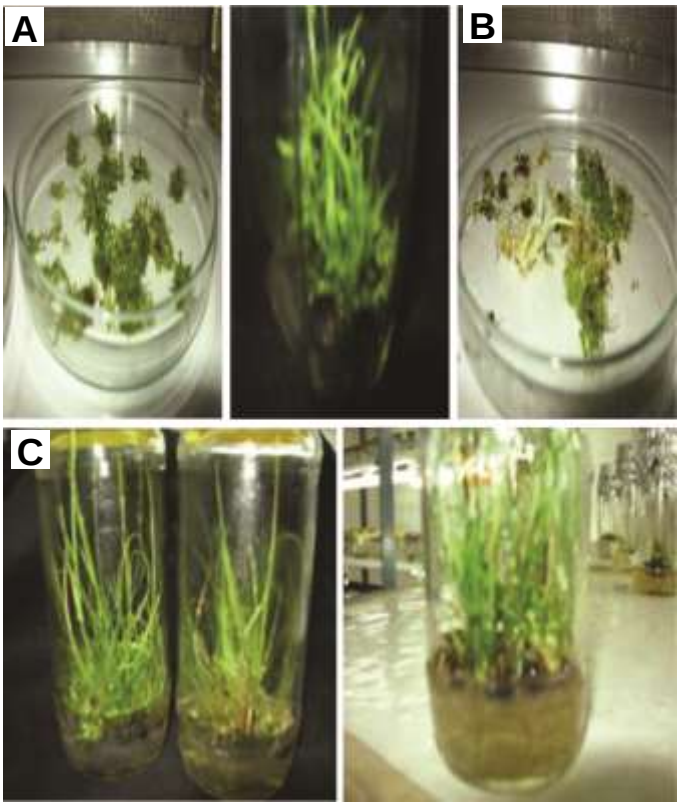

Fig 4. A. Shoot regeneration, B. Chlorophyll mutant, C. Shoot elongation under the influence of different phytohormone concentration.

Fig. 5. A. Rooted plantlets, B. plantlets in the earthen pots.

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