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Genetic and non genetic factors affecting callus induction and regeneration in sugarcane

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Abstract: *Four species of Saccharum (S.officinarum, S.barberi, S.sinense, S.robustum), one intergeneric hybrid (Saccharum x Zea) and five sugarcane varieties (Co 86032, Co 99004, Co 94012, Co 1148, CoJ 64) were tested for their response to callus induction and regeneration. Young leaf explants of each plant were inoculated in callus medium having different gelling agents like agar, agarose, phytagel, clarigel and bactoagar. It was found that different gelling agents responded differently for callus induction. In another experiment they were inoculated on MS medium containing different concentrations of 2,4-D (0, 1.0, 2.0, 3.0, 4.0 mg/l) for callus induction. Maximum of 95% callus induction was observed in the variety Co 99004 and Saccharum x Zea showed the lowest callus induction (40%). The actively growing calli were transferred to MS medium supplemented with different concentrations of 6- Benzyl Amino Purine (BAP), kinetin and combination of BAP and kinetin for shoot regeneration. Most of the genotypes showed maximum shoot regeneration in MS medium supplemented with a combination of BAP and kinetin (0.5 mg/l each). S. officinarum and Co 86032 showed maximum number of regenerated shoots in medium supplemented with 0.5 mg/l BAP + 0.5 mg/l kinetin and 1.0 mg/l kinetin respectively. Though the induction and shoot regeneration was satisfactory in all the clones, differences could be observed for these characters between the genotypes and media combinations. From the study it was concluded that the somatic embryogenesis and organogenesis in sugarcane is significantly influenced by genetic and non genetic factors like genotypes, gelling agents and auxins and cytokinins in the culture media.*

INTRODUCTION

In sugarcane breeding programmes, increased disease resistance and high sucrose content are the main areas targeted. In recent years, biotechnological techniques such as tissue culture and gene transfer systems have been used for the improvement of sugarcane. While considering the improvement programme through *in vitro* culture, it is necessary to develop efficient procedures to increase the regeneration rate of plantlets through the production of more friable calli.

Callus is an unorganized mass of plant cells and its formation is controlled by growth regulating substances present in the medium (Shah *et al*., 2003). The specific concentration of plant regulators, needed to induce callus formation varies from species to species and can even depend on the source of explant (Charriere *et al*., 1999). Furthermore, genotype is one of the most important factors for callus induction (Sarrafi *et al*., 1996; Punia and Bohorova, 1992).

The major area of tissue culture in sugarcane improvement is the production of somaclones from callus cultures of commercially important varieties to rectify their specific defects. The highly polyploid nature of sugarcane coupled with chromosome numerical variation in different cells of the same tissue is an added advantage in sugarcane for creating somaclonal variation for a wide spectrum of characters. Research on sugarcane tissue and cell culture was started in Hawaii in 1961. Later several somaclones were developed through tissue culture with improved productivity and eliminating certain minor defects like spines, leaf drying and disease susceptibility. This approach was utilized to rectify specific defects of the widely adapted sugarcane varieties which were rejected at the final stage of selection. Production and utilization of somaclonal variants from selected species and interspecific and intergeneric hybrids of sugarcane have a great scope in the improvement programmes. Extensive application of this method in sugarcane improvement is awaiting the standardization of optimum culture conditions of these genotypes to maximize the regeneration efficiency.

The present study was carried out to determine the effects of different gelling agents like agar, agarose, phytagel, clarigel and bactoagar and also different concentrations of 2,4-D on callus induction and different BAP and kinetin concentrations on shoot regeneration in different species and hybrids of sugarcane. Using more diverse genotypes (species and hybrids) in one tissue culture study may be useful,

showing similarities and/or differences in factors such as gelling agents, concentrations of growth regulators in the medium. Thus the same steps may be used for callus production or plant regeneration in other tissue culture and transformation programmes.

MATERIALS AND METHODS

Shoots from 4-6 months crops of sugarcane species (*S. officinarum*, *S. barberi* and *S. sinense*, *S. robustum*) and intergeneric hybrids (*Saccharum* x *Zea*) and five varieties of sugarcane (Co 86032, Co 99004, Co 94012, Co 1148, CoJ 64) were collected from germplasm collection and experimental fields of Sugarcane Breeding Institute, Coimbatore. The outer leaves were removed and surface of shoots wiped with alcohol. Two or three outer whorls were removed and the apical portion of the stem (10cm) was cut and taken into laminar air flow chamber. The developing leaves encircling the growing points were dissected out and bits of about 0.5 cm x 0.5 cm were cut with the help of sterile forceps and scalpel. The excised explants (10/ culture bottle) were immediately inoculated on MS medium (Murashige and Skoog, 1962) supplemented with different gelling agents and various concentrations of 2,4-D (Table 1). Three replications of each treatment were done and incubated in dark at 25±1ºC temperature. Subculturing was done at 15-20 days intervals. Observations were made on callus induction time, callus weight, callus induction percentage, callus morphology and embryogenic callus production.

RESULTS AND DISCUSSION

Time taken for callus induction in each clone was recorded in general without considering the gelling agent effect. Normally callus induction takes place within two weeks. In this experiment all the species and hybrids have produced callus within two weeks. While comparing, the species took long time to produce calli than hybrids.

While considering the role of gelling agent in callus induction, the species showed good callus growth in clarigel where as the intergeneric hybrids responded well in all the callus induction media irrespective of gelling agents. The varieties showed high callus induction percentage than the species and intergeneric hybrids. From this experiment it is proved that callus induction is genotype dependant and the role of gelling agents is minor in callus induction (Fig.1).

Callus induction time was found to be different in different genotypes. Most of the varieties showed callus initiation within 12-14 days. Callus initiation in *S. sinense* was observed within 10 days. *S. barberi* took 32 days to produce callus and the intergeneric hybrid, *Saccharum* x *Zea* took the maximum time (36 days) for callus induction. Data presented in Table 2 indicate clear genotypic variation in time taken for callus initiation in clones. Similar observations have also been made in *Sorghum* (Patil *et al*., 1998; Baskaran *et al*., 2005). It was also observed that use of lower concentration of 2,4-D generally required more days to initiate callus formation from explants. A minimum period of 10-14 days was required to initiate callus in medium supplemented with 3.0 and 4.0 mg/l 2,4-D.

Average callus weight of clones was recorded. Among the species, *S. officinarum* showed 1.26g, *S. barberi* 1.13g and *S. sinense* 1.70g callus weight. The intergeneric hybrid, *Saccharum x Zea* showed the lowest callus weight of 0.83g. Among the varieties Co 99004 produced the maximum (2.81g) and Co 1148 produced the minimum (1.03g) callus weight. It is reported that the varieties produced more callus than the species and the intergeneric hybrid and callus weight is dependent on the genotype of the explants used (Gandonou *et al*., 2005). Co 99004, which produced the highest callus weight showed the highest callus induction percentage (95%) also. A positive relationship between callus weight and callus induction percentage was observed in the case of Co 99004. (Melahat *et al*., 2001). Callus growth was influenced by 2,4-D concentration used. High callus weight was recorded in medium containing 3.0 mg/l or 4.0 mg/l 2,4-D and callus weight was less in medium with lower concentration of 2,4-D.

The actively growing calli of a set of species and hybrids were transferred to MS medium supplemented with different concentrations of BAP and kinetin. The line diagram (Fig. 2) indicates that regeneration is affected by the concentration of growth regulators. *S. officinarum* showed the maximum regeneration (100%) in both S4 and S5 media. *S. barberi* produced the maximum regeneration in S4 and S5. *S. sinense* showed 90% results in S2, S3 and S5 media. *Saccharum* x *Zea* showed 80%results in S2 and S5 media. Co 86032, Co 99004 and Co 94012 produced the maximum shoot regeneration in S5 medium.

Co 1148 showed the weakest response to regeneration (20%) and CoJ 64 produced 90% regeneration in S5 medium. The results indicate that a combination of BAP and kinetin (0.5 mg/l each) induced more regeneration in cultures.

The number of shoots regenerated in the sugarcane species and hybrids in each treatment was counted (Table-3). In *S. officinarum*, maximum shoots (250) were regenerated in the SDM medium supplemented with a combination of BAP and kinetin (0.5 mg/l BAP + 0.5 mg/l kinetin). In Co 86032 also 250 shoots were regenerated from medium supplemented with 0.5 mg/l BAP and 100 shoots from medium containing 1.0 mg/l kinetin. Co 1148 showed only less capacity of regeneration. The highest amount of shoot (20) was obtained in medium containing 0.25 mg/l BAP and no shoots were produced in medium with 1.0 mg/l BAP. Though the regeneration of calli and shoot formation was satisfactory in almost all clones, differences could be observed for these characters between the genotypes and media combinations. Data were statistically analyzed with SPSS software and it has revealed that significant difference was obtained for callus induction and regeneration in genotypes studied and hence in sugarcane these characters are highly dependent on genotype and phytohormone.

Table 1. Different media used for callus induction

Sl. No.	Medium	2,4-D concentration	Gelling agent
1	Co	CM + 0 mg/l 2,4-D	CM+8 g/l agar.
2	C_1	CM + 1 mg/l 2,4-D	CM+4g/l agarose.
3	C_2	CM + 2 mg/l 2,4-D	CM+2.5 /l phytagel.
4	C_3	CM + 3 mg/l 2,4-D	CM+2.5 g/l clarigel
5	C_4	CM + 4 mg/l 2,4-D	CM+8 g/l bactoagar.

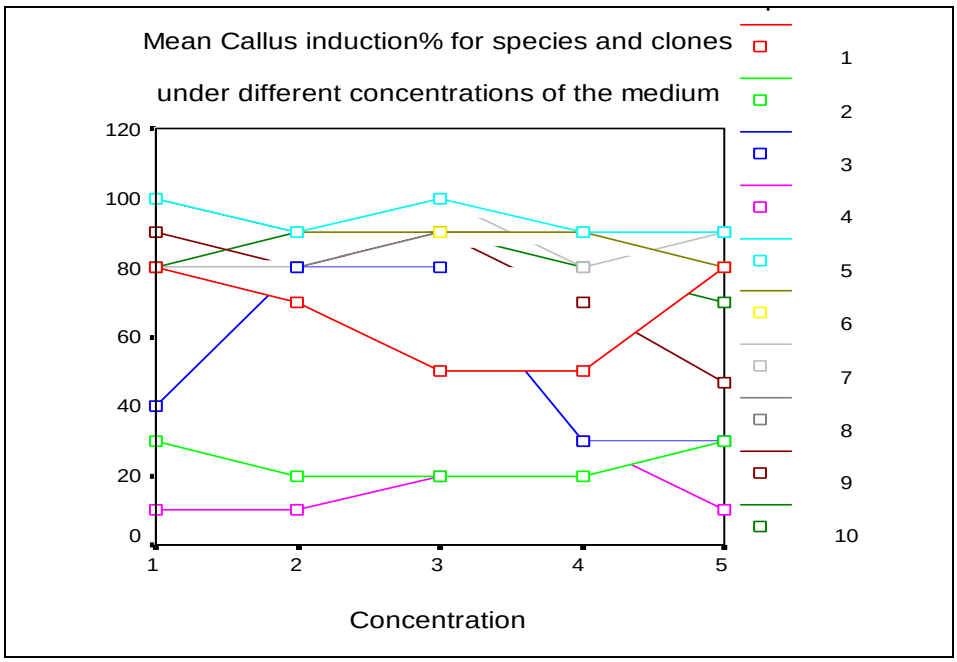
Table 2. Time taken for callus induction (in days)

Sl. No	Clones	Time taken(in days)
1.	*Saccharum officinarum*	12
2	*Saccharum barberi*	32
3	*Saccharum sinense*	10
4	*Saccharum robustum*	15
5	*Saccharum x Zea*	36
6	Co 86032	12
7	Co 99004	11
8	Co 94012	12
9	Co 1148	13
10	Co J64	11

Table 3. Number of shoots regenerated from sugarcane species and hybrids

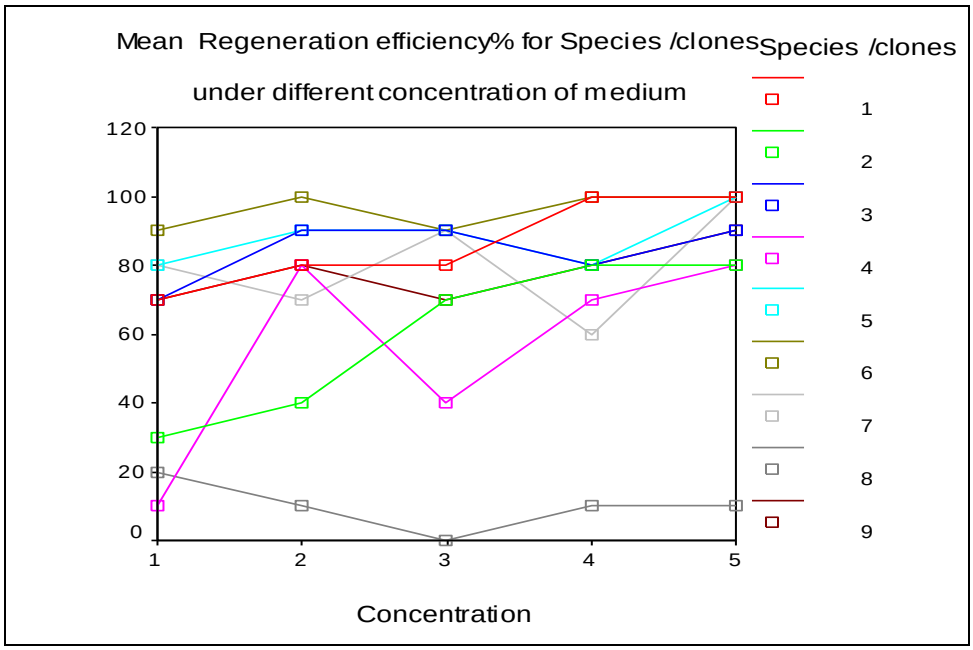
Sl. No.	Clones	No. of shoots regenerated				
		S1	S2	S3	S4	S5
1	*Saccharum officinarum*	100	200	110	160	250
2	*Saccharum barberi*	120	140	150	160	130
3	*Saccharum sinense*	110	130	120	140	150
4	*Saccharum robustum*	120	140	150	150	160
5	*Saccharum x Zea*	90	170	200	150	170
6	Co 86032	150	250	150	100	140
7	Co 99004	170	160	140	150	160
8	Co 94012	140	130	120	130	180
9	Co 1148	20	10	0	8	5
10	CoJ 64	90	80	120	140	160

Fig. 1. Line diagram showing mean callus induction percentage for clones in different gelling agents.



Clones: 1. *S. officinarum*; 2. *S. barberi*; 3. *S. sinense*; 4. *S. robustum*; 5. *Saccharum* x *Zea*; *6.* Co 86032; 7. Co 99004; 8. Co 94012; 9. Co 1148; 10. CoJ 64.

Fig. 2. Line diagram showing mean callus induction percentage for clones in different gelling agents.



Clones: 1. *S. officinarum*; 2. *S. barberi*; 3. *S. sinense*; 4. *Saccharum* x *Zea*; 5. Co 86032; 6. Co 99004; 7. Co 94012; 8. Co 1148; 9. CoJ 64

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