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In-Vitro Conservation of Sugarcane Germplasm

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Abstract

Lack of in vitro multiplication procedure has long been a serious problem in sugarcane breeding programs. This problem has been solved to maximum extent by *In Vitro* propagation. In the present study, 6 genotypes of sugarcane (SC 06, SC 04, SC 25, SC 11, SC 13 and SC 30) was acquired & excised for apical and sub apical parts (eyes/ lateral buds). The explants were surface sterilized with Clorox (70% +100%, 20 & 10 min). Results showed that highest survival % was achieved with apical buds (66%) as compared to lateral buds (33%). In addition, effect of different concentrations of rooting media (NAA, 5% Sucrose and 6% Sucrose) and shooting media (GA3, 0.1mg/, Kinetin, 0.1mg/L and BAP 0.1mg/L, Kinetin 0.1mg/L,GA_3 0.1mg/L, NAA 0.1mg/L) was studied. Synergistic response was observed on the growth of plant with hormone consortia. Significant differences were observed in No. of roots, shoot length @ No. of shoots at all tested treatments. Highest No. of roots recorded for SC 30 at with 6% Sucrose. Highest No. of shoots was recorded for SC 04 with GA_3 @ 0.1mg/L and highest shoot length for SC 06 at 0.1mg/ L of Kinetin.

Keywords: Growth regulators, *in vitro* preservation, *saccharum officinarum*, sugarcane

Introduction

Conservation of plant genetic resources via tissue culture received immense attention from researchers in the last three decades. Storage of shoot tips or meristem derived explants under slow rate of growth has a significant use in the international germplasm resources units. This procedure makes germplasm available at any times for international distribution. Several types of plant materials have been used for in vitro preservation of clonally propagated crops. Meristem derived explants such as shoot tips and buds were mostly suggested for their genetic and generative stability (Baksha *et al.*, 2002).

Sexually propagated plants may be stored as seeds and are not in danger if compared with vegetatively propagated plants. Clonally propagated plants are usually maintained in botanical gardens. Some of those plants are usually preserved by continuous multiplication of tubers, roots, cuttings or bulbs. Such a procedure is laborious and exposes plants to pests and environmental stresses. Tissue culture has proved to be a useful tool for storage of vegetatively propagated commercial crops like potato, palm, forest trees, fruit trees and other species.

Sugarcane is a member of the genus Saccharum from family Gramineae. It is a high valued cash crop and exclusive source of 75% world sugar production (Lakshmanan *et al.*, 2006). This crop provides many by-products for bio-factory such as alcohol, butanol, acetic acid, animal feed and paper besides, sugar and energy (Garcia *et al.*, 2007). Varieties of sugarcane are highly heterogeneous and generally multiplied vegetatively by stem cutting. Lack of suitable multiplication procedure and contamination by systemic diseases are the

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serious problem to multiply an elite genotype of sugarcane in the open field (Lal and Singh, 1994). Tissue culture of sugarcane has received considerable research attention because of its economic importance as a cash crop. Plant regeneration through tissue culture technique would be a viable alternative for improving the quality and production of sugarcane.

Material and methods

Sugarcane varieties namely SC 06, SC 04, SC 25, SC 11, SC 13 and SC 30 were acquired. Sugarcane *in Vitro* cultures were established using standard procedure (Uzma *et al.*, 2012). Surface sterilization was done using 70% and 100% Clorox (Commercial bleach comprising 5.25% v/v sodium hypochlorite; an active agent) or 20 and 10 min of time duration under aseptic conditions. After culture establishment, the effect of different concentrations of rooting media (NAA, 5% Sucrose and 6% Sucrose) and shooting media (GA3, 0.1mg/L, Kinetin, 0.1mg/L and BAP 0.1mg/L, Kinetin 0.1mg/L,GA3 0.1mg/L, NAA 0.1mg/L) on rooting and shooting respectively, was studied. The cultures were incubated at 25˚C temperature and 16hr light period. Data for number of leaves, number of roots was recorded after 1 month of culturing.

Results and discussion

Results showed that highest survival percentage was achieved with apical buds (66%) as compared to lateral buds (33%). %age survival of explants is generally low in case of treatment with 70% Clorox for 10 min. However, when the concentration of Clorox is increased to100%, the survival percentage of explants is elevated 2 to 4 times. In addition, effect of different concentrations of rooting media (NAA, 5% Sucrose and 6% Sucrose) and shooting media (GA3, 0.1mg/, Kinetin, 0.1mg/L and BAP 0.1mg/L, Kinetin 0.1mg/L,GA3 0.1mg/L, NAA 0.1mg/L) was studied. Significant differences were observed in number of roots, shoot length and number of shoots at all tested treatments. (Table 1). The genotype SC 30 exhibited highest number of roots on Treatment 6% Sucrose. Whereas, highest number of shoots (8) were recorded for SC 04 and maximum shoot height (9.667cm) was given by SC 30 at GA3+0.1mg/L. These results indicate that SC 30 was the best performer among all genotypes. These genotypes are now available in disease-free state at the *In Vitro* Conservation Lab, Plant Genetic Resource Institute (PGRI), NARC which can be used any time for further multiplication, characterization and research.

Table 1: Analysis of variance table for rooting

Source of variation	DF	Number of Roots	Number of shoots	Shoot length
Rep	2	6.222	8.722	0.728
Var	5	336.500**	12.388**	41.713**
Treat	2	149.733**	0.722	18.623**
Var X Treat	10	67.367**	7.677**	9.697**
Error	34	3.065	0.741	0.102
Total	53			
CV		27.64	29.25	5.27

Table 2: Effect of different rooting media on number of roots

Variety X Treatment	Number of Roots
SC 06 XNAA	4.333^{EFGH}
SC 06 X 5% Sucrose	1.000^{H}
SC 06 X 6% Sucrose	0.333^{H}
SC 04 X NAA	2.333^{GH}
SC 04 X 5% Sucrose	1.000^{H}
SC 04 X 6% Sucrose	18.333^{AB}
SC 25 X NAA	2.000^{H}
SC 25 X 5% Sucrose	1.333^{H}

SC 25 X 6% Sucrose	8.333^{CDEF}
SC 11 X NAA	2.333^{GH}
SC 11 X 5% Sucrose	10.000^{CD}
SC 11 X 6% Sucrose	7.667^{CDEFG}
SC 13 X NAA	0.667^{H}
SC 13 X 5% Sucrose	3.667^{FGH}
SC 13 X 6% Sucrose	9.667^{CDE}
SC 30 X NAA	5.333^{DEFGH}
SC 30 X 5% Sucrose	13.000^{BC}
SC 30 X 6% Sucrose	22.667^{A}

Table 3: Effect of different shooting media on number of shoots and shoots length

Variety X Treatment	Number of Shoots	Shoot Length
SC 06 X GA3, 0.1mg/L	1.3333^{D}	5.1333^{CDE}
SC 06 X Kinetin, 0.1mg/L	1.6667^{CD}	8.1667^{B}
SC 06 X BAP 0.1mg/L, Kinetin 0.1mg/L,GA3 0.1mg/L, NAA 0.1mg/L	3.6667^{BCD}	7.9667^{B}
SC 04 X GA3, 0.1mg/L	8.0000^{A}	3.8667^{FG}
SC 04 X Kinetin, 0.1mg/L	4.0000^{BC}	2.5333^{H}
SC 04 X BAP 0.1mg/L, Kinetin 0.1mg/L,GA3 0.1mg/L, NAA 0.1mg/L	2.3333^{CD}	9.9000^{A}
SC 25 X GA3, 0.1mg/L	1.6667^{CD}	4.7333^{DEFG}
SC 25 X Kinetin, 0.1mg/L	1.6667^{CD}	6.0000^{C}
SC 25 X BAP 0.1mg/L, Kinetin 0.1mg/L,GA3 0.1mg/L, NAA 0.1mg/L	2.3333^{CD}	4.8000^{DEF}
SC 11 X GA3, 0.1mg/L	2.6667^{CD}	3.7667^{G}
SC 11 X Kinetin, 0.1mg/L	5.6667^{AB}	4.0000^{FG}
SC 11 X BAP 0.1mg/L, Kinetin 0.1mg/L,GA3 0.1mg/L, NAA 0.1mg/L	3.6667^{BCD}	5.4667^{CD}
SC 13 X GA3, 0.1mg/L	3.3333^{BCD}	2.6667^{H}
SC 13 X Kinetin, 0.1mg/L	2.0000^{CD}	5.9667^{C}
SC 13 X BAP 0.1mg/L, Kinetin 0.1mg/L,GA3 0.1mg/L, NAA 0.1mg/L	2.6667^{CD}	4.2333^{EFG}
SC 30 X GA3, 0.1mg/L	2.0000^{CD}	9.9667^{A}
SC 30 X Kinetin, 0.1mg/L	2.3333^{CD}	9.9000^{A}
SC 30 X BAP 0.1mg/L, Kinetin 0.1mg/L,GA3 0.1mg/L, NAA 0.1mg/L	2.0000^{CD}	9.9667^{A}

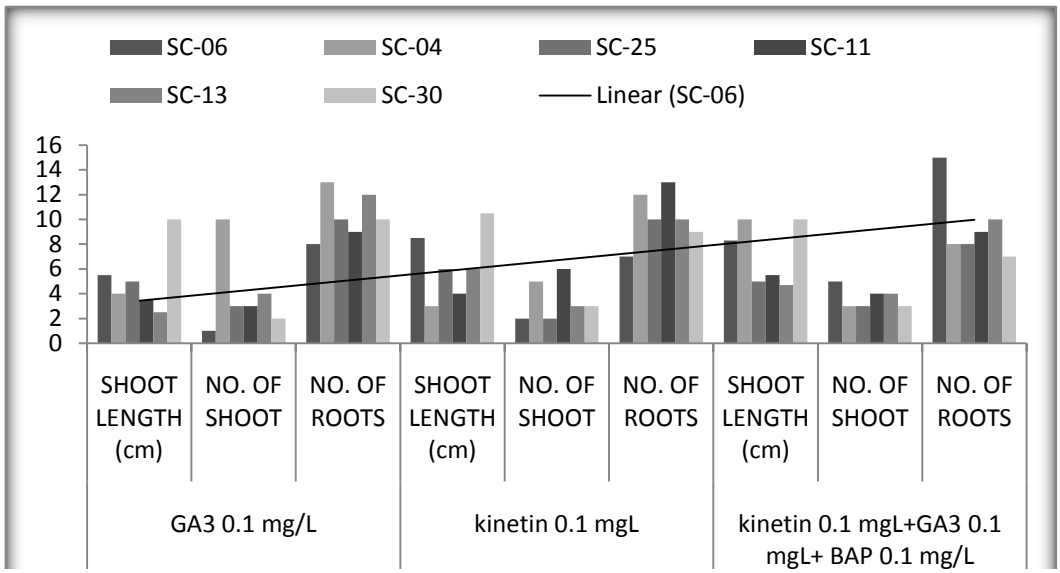

Figure 1: Performance of genotypes on treatments

Conclusions

It is concluded from this study that apical buds give better result as compared to lateral buds. Results indicate that SC 30 performed best among all genotypes. These genotypes available at the In Vitro Conservation Lab, Plant Genetic Resource Institute (PGRI), NARC which can be used any time for further multiplication, characterization and research.

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