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PROTOCOL OPTIMIZATION FOR EFFECTIVE IN VITRO ROOT FORMATION OF SUGAR CANE SOMA CLONES IN NIA-2012, NIA-105 AND GULABI-95 VARIETIES

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ABSTRACT

The aim of this study is to develop an efficient *In vitro* regeneration and root formation procedure from callus. Present work was conducted in 2015 at Nuclear Institute of Agriculture, Tando jam. Study relevant with three sugarcane varieties of NIA like NIA-2012, NIA-105 and GULABI-95. Root formation of plantlets was compared under different concentration of auxins IBA (1.0, 2.0, 2.5 mg1-1 and sucrose 2, 3 and 4 % respectively. Highly significant ($p<0.05$) variations were observed for all parameters of root formation. Interactive effect of variety treatment and concentration was non-significant for the number of root and length of root formation. Auxins 3% and sucrose 4% mgl^{-1} were highly effective for rooting. *In vitro* root formation for all genotypes of sugarcane showed the maximum root and highest length in ½ MS media supplemented with 2.5 $mg1^{-1}$ IBA and 4% sucrose, therefore this concentration of growth regulators was used for future *in vitro* culture of sugarcane.

Key words: Auxins, Roots, Sucrose, Sugarcane, growth regulators

INTRODUCTION

Sugarcane is the most useful agro-industrial crop, belongs to the Poaceae family with chromosome number = 80 (Khan et al., 2005). Clonally propagated crop Sugarcane is important for vulnerable to the diseases. The main advantage of tissue culture techniques is the rapid multiplication of new varieties, superior plant vigor and its efficacy in germplasm savings (Ali et al., 2008).

In vitro plant root techniques offers successful sugarcane propagation by controlling a lot of troubles which were faced during conventional breeding practices. The technique ensures disease free multiplication of elite varieties (Khan et al., 2006) and minimizes time span required for mass production.

There are so many researchers from different countries using tissue culture technique for genetic improvement of sugarcane (Dibax et al., 2011; 2013,Takahshi and Takamizo, 2013). MS medium supplemented with 2,4-dichlorophenoxyacetic acid (2,4-D) along with cytokinins (Sughra et al., 2014). Regeneration from callus culture creates genetic as well as epigenetic variations induced by enforced hormonal stimuli (Nawaz et al., 2013; Karim et al., 2015; Solangi et al., 2015). To fulfill demand of sugar for increasing population. This study was planned to induce somaclonal variation with improved in vitro rooting ability of three sugarcane varieties influenced by auxins and sucrose.

MATERIALS AND METHODS

Three varieties selected for this experiment, which were early maturing NIA-2012, mid maturing NIA-105 and late maturing Gulabi-95. Selected callus was shifted to the fresh media for regeneration of the plantlets.

Root formation: The mass of regenerable calli produced on the five callus induction media were transferred to 5 types of regeneration media. Shoot regeneration started with the appearance of green dots on callus within two weeks on regeneration medium and generally produced normal micro shooting. No response was observed in control or hormone-free MS medium. After two subcultures (4 weeks of each), When the regenerated plantlets reached 7-8cm height, the plantlets obtained were aseptically transferred to the 5 different rooting medium for 3 other weeks. Multiplication of roots continuously till healthy plantlets obtained for this purpose trimmed offshoots and transmission into fresh media. Data analysis by (ANOVA) collected number of roots, micro roots and length of roots. Computer software was used for analyzing data (Statistics version 8.1.). The complete randomized design (CRD) was used with three treatments and five different concentrations through two factorial designs.

Plantlets with at least five well-developed roots were transferred to a pot containing soil under high humidity (90%) by covering the plants with plastic envelopes after cutting their leaves (plantlet et al., Glaszmann, 1994).

RESULTS AND DISCUSSION

Root/regenerated plants: MS ½ strength medium supplemented with different concentrations of auxins and different combinations of sucrose were used to regenerate roots. Among all soma clones, the plentiful rooting is observed in 2.5mg/l IBA + 4% sucrose in NIA-2012 (14. 6), and minimum in Gulabi-95 (11.66). Highly significant variation ($P< 0.05$) in number of roots was detected for all genotypes. In IBA+3% sucrose a maximum number

of root was recorded in NIA-2012 (9.66 root/ explants), while minimum observed in Gulabi-95 (8.33root/explants). Overall highest mean variation in number of roots was observed in NIA-2012 (8.28) followed by NIA-105 (5.68) and minimum in Gulabi-95 (4.11). The highest number of root was documented in IBA 2.5mgl^{-1}+4% sucrose for all the genotypes.

In the present study observed that root formation of sugarcane is affected by the different concentration of Indole butyric acid and their interactive effects with sucrose percentage. It is obvious from the results (Table -1 & 2, Figure 1) that the number of roots increases with increasing concentration of IBA + sucrose. All the genotypes gave best results in 2.5mg/l IBA for the number of roots. Present results are similar to the finding of Alam et al., 2003; Seema et al., 2011; Alves et al., 2011; Mekonnen et al., 2013 and Karim et al., (2015). However, current work is quite different from the previous work of Khan et al., (2008). Sabaz et al., (2008) have obtained 41 roots /explants at 1.0mg/l of IBA.

Table -1: ANOVA for root formation in plantlets of sugar cane genotypes modulated by different concentration of different auxin and Sucrose

Source	DF	Mean square	
		Number of root /plant	Length of root / plants
Varieties	2	20.230**	47.030 **
Treatment	4	233.656 **	430.359**
Concentrations	2	200.274**	321.896**
V x T	4	3.711**	6.076**
V x C	8	1.419 ns	7.507**
T x C	8	10.033 **	30.526**
V x T x C	16	2.247 ns	2.234 ns
Error	88		
Total	134	**CV 23.23**	**CV 14.88**

In each column, means followed by common letter are not significantly different at 5% probability level

Table -2: Effect of different concentration of ½ MS supplemented with auxins and sucrose on number of roots in regeneration of plantlets of three different genotype.

Conc (mg l^{-1})		Varieties			Mean
Sucrose (%)	IBA	NIA-2012	NIA-105	Gulabi-95	
4	0.0	1.66 s-t	3.00 p-t	3.00 p-q	1.77 j
4	1.0	7.66 f-i	5.66 i-n	6.33 h-l	5.55 g-h
4	1.5	8.33 he	7.66 f-i	8.66 e-g	6.66 e-g
4	2.0	12.0 b-c	11.0 b-c	10.0 c-e	8.66 b-c
4	2.5	14.66 a	13.0 a-b	11.66 b-d	11.33 a
3	0.0	2.33 r-t	1.66 s-t	1.33 t	2.00 j
3	1.0	6.33 h-l	4.66 k-q	3.33 o-t	4.44 h-i
3	1.5	7.33 g-j	5.00 k-p	5.66 i-n	5.44 g-h
3	2.0	8.66 e-g	5.66 i-n	6.66 g-k	7.55 c-e
3	2.5	9.66 d-f	8.66 e-g	8.33 e-h	9.11 b
2	0.0	1.33 t	1.33 t	1.66 s-t	2.00 j
2	1.0	2.66 q-t	3.00 p-t	2.66 q-t	4.11 i
2	1.5	4.33 l-r	3.66 n-s	4.00 m-r	6.11 f-g
2	2.0	5.33 j-o	6.00 i-m	5.33 j-o	7.33 d-f
2	2.5	9.66 d-f	5.66 i-n	5.00 k-p	8.33 b-d
		8.28 a	5.68 b	4.11c	

In each column, means followed by common letter are not significantly different at 5% probability level.

Varieties	SE 0.2953)	LSD 5%) 0.5868)
Concentrations	SE 0.6603)	LSD 5%) 1.3122)
V x C	SE 1.1437)	LSD 5%) 2.2728)

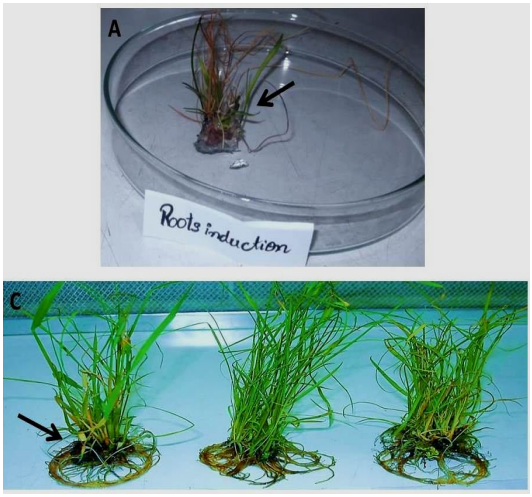

Figure -1: Root induction in three genotype of sugarcane in ½ MS supplemented by different concentration of IBA and sucrose

Root length: The effect of different concentration of IBA and sugar was important for root length. Overall highest mean variation in number of roots was observed in NIA-2012 (12.08cm) followed by Gulabi-95 (6.75cm) and minimum in NIA- 105 (9.06cm). Maximum length of root for IBA + 3% sucrose was detected in NIA-2012 (16.00cm) and minimum in NIA-105 (12.00cm). In IBA + 4% sucrose maximum length of root was recorded in NIA-2012 (15.00cm), while minimum observed in Gulabi-95 (13.33cm). In case of IBA + 2% sucrose length of root was notable for NIA-2012 (10.33cm) and minimum in Gulabi-95 (8.00cm). The highest length of root was documented at 2.5mg l^{-1} for all the entire genotypes. Significant variation ($P< 0.05$) in length of root was detected for all genotypes (Table -3, Figure 2).

Table -3: Effect of different concentration of auxins and sucrose on length of roots in regeneration of plantlets of three different genotype.

Conc (mg l^{-1})		Varieties			Mean
Sucrose (%)	IBA	NIA-2012	NIA-105	Gulabi-95	
4	0.0	4.00n-p	4.11n-m	4.22n-p	4.09 h
4	1.0	4.00 n-p	4.66 n-o	4.00 n-p	4.11 g
4	1.5	11.33 f-h	10.00 g-k	8.33 j-l	8.44 e
4	2.0	14.00 c-e	11.66 f-h	10.33 g-j	10.88 c
4	2.5	15.0 c-d	15.33 c-d	13.33 d-f	12.66 b
3	0.0	3.00 o-p	3.00 o-p	4.00 n-p	3.44 g
3	1.0	8.00 k-m	7.66 l-m	6.00 m-n	8.55 e
3	1.5	10.66 g-i	9.00 i-l	8.00 k-m	9.44 d-e
3	2.0	14.3 c-d	9.66 h-l	11.33 f-h	10.88 c
3	2.5	16.00 c	12.00 e-g	13.33 d-f	13.44 b
2	0.0	3.33 o-p	4.66 n-o	2.00 p	3.33 g

2	1.0	6.00 m-n	8.00 k-m	3.66 o-p	6.00 f
2	1.5	8.00 k-m	7.66 l-m	7.66 l-m	8.66 e
2	2.0	8.66 i-l	7.66 l-m	6.00 m-n	10.22 c-d
2	2.5	10.33 g-j	9.66 h-l	8.00 k-m	13.22 b
		12.08 a	9.06 b	6.75 c	

In each column, means followed by common letter are not significantly different at 5% probability level.

Varieties	SE 0.2919)	LSD 5%) 0.5801)
Concentrations	SE 0.6527)	LSD 5%) 1.2971)
V x C	SE 1.1305)	LSD 5%) 2.2466)

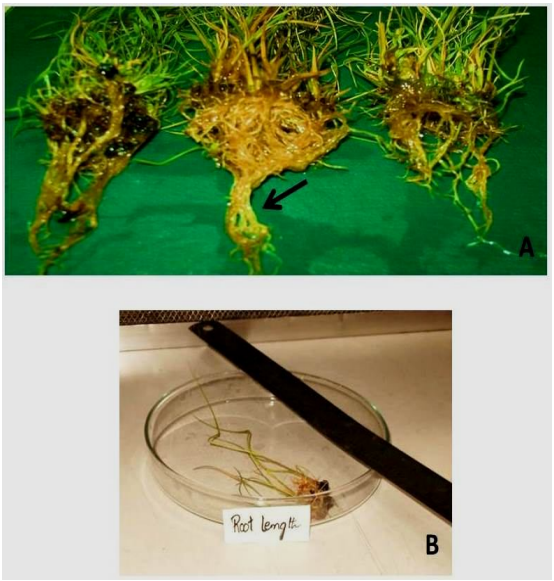

Figure -2: Root length in three genotypes of sugarcane in ½ MS supplemented by different

It is obvious from the results that length of roots/shoot increases with increase in the application of IBA+ sucrose applied. All the treatment gave best results in 2.5mg/l for the length of roots. Present results are concerning with the finding of Mamun et al., (2004); Franklin et al., (2006); Lakshmanan et al., (2006); Garcia et al., (2007) and Sughra et al., (2014) and Simair et al., (2013). On the contrary work of Ali and Afghan (2001) and Baksha et al., (2002) obtained highest number of root formation and length of root with 2.0mg/l IBA+ 6% sucrose.

CONCLUSION

Regeneration potential of callus and albino mutant mainly depends on type and concentration of auxins and cytokinins. Increasing concentrations of Auxin and sucrose has additive effect on in vitro root formation.

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