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IN VITRO REGENERABILITY OF DIFFERENT SUGARCANE (*SACCHARUM OFFICINARUM* L.) VARIETIES THROUGH SHOOT TIP CULTURE

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ABSTRACT

An experiment was conducted to develop an efficient protocol for micropropagation of sugarcane using the method of growing shoot tips of three varieties of sugarcane viz. BL-4, Thatta-10, and Larkana-2001. A protocol for the regeneration of direct shoot without the intervention of the callus phase was developed using shoot tip culture in a basic medium of Murashige and Skoog (MS) supplemented with different concentrations of auxin (NAA) and cytokinin (BAP). The analysis of variance due to varieties, concentration and variety x concentration interaction was significant for all characters. In general, the best results were seen from the BL-4 and Thatta-10 varieties with 1.0 mg/L BAP and 3.0 mg/L NAA for various parameters. Different concentrations of BAP for shoot initiation and multiplication were used and 1.0 mg/L BAP showed the most effective concentration for induction and shoot multiplication, while MS medium supplemented with different concentrations of NAA for *in vitro* formation of roots from proliferated shoots, the maximum root formation on MS medium supplemented with 3.0 mg/L NAA was recorded. However, BL-4 had high power (85.3 %) of regenerating from explants took minimum (11.00) days for shoot induction and gave highest number of shootlets (6.50) with maximum length of shootlets (5.50 cm) by 1.0 mg/L BAP. For root induction BL-4 variety produced higher number of roots per shootlet (6.80) after minimum 9 days with maximum length of the roots (2.50 cm) at 3.0 mg/L NAA.

KEYWORDS: Sugarcane; Micropropagation, Shoot initiation, Root multiplication, Shootlets.

INTRODUCTION

Non economic performance and lower sugar recovery cause very high cost of production which makes Pakistan the least competitive in domestic and international sugar market (Khan et al., 2005). There are many causes of low yield, one of which is the lack of a rapid method of multiplying seed and once a desired clone is identified, it usually takes 6-7 years to produce enough quantity of improved seed material. This long duration causes a bottle neck in

important breeding program (Siddiqui et al., 1994).

Nowadays, the technique of plant tissue culture has become a powerful tool for studying, solving basic and applied problems in plant biotechnology (Yadav, et al., 2012). During the last thirty years, micro-propagation and *in vitro* techniques have become more widely used in commercial horticulture and agri-cultural fields. In-vitro multiplication of sugarcane has received considerable research attention because of its economic importance as a cash crop (Khan et al., 2004). As a result of the regeneration of plants through tissue culture technique could be a viable option for improving the quality and productivity of sugarcane. So far, a lot of reports have been published in the tissue culture of sugarcane from different countries (Dibax et al., 2011; Nawaz et al., 2013, Takahshi and Takamizo, 2013) but the first attempts to regenerate plants through *in vitro* technique were conducted by Heniz and Mee, (1969) and Naz, (2003). Standardization of protocols for *in vitro* multiplication of sugarcane through a callus, axillary bud and shoot tip culture has been reported by several authors (Beard et al., 1978; Nadar et al., 1978; Bhansali and Singh, 1984; Nagai, 1998; Anita et al., 2000). The rate of multiplication by the conventional method is 1-10 within a year (Gosal et al., 1998). They also reported rapid multiplication in liquid media in BAP (0.5 mg/ L) and kinetin (0.5 mg/ L) and NAA rooting (5 mg/L) and sucrose (7.0%). Jadhav et al., (2001) established a protocol for micropropagation of sugarcane on MS medium (Murashige and Skoog, 1962) supplemented with BAP, NAA and IBA and kept at 23°C under continuous light. This statement demonstrated an effective method with high frequency of regeneration that allows convenient micro plant multiplication that is easily set through *in vitro* shoot tip culture. Sugarcane has also been genetically modified (GM) through the introduction of genes that affect a number of features. Some of these lines of genetically modified sugar cane have been approved for limited and controlled release in Australia (Mitchell, 2011). Some of the features that have been improved through genetic modification include, altered sugar content, improved nitrogen efficiency, enhanced water use efficiency, altered plant architecture and resistance to pests (CRC SIIB 2006; Wu and Birch, 2007).

Keeping in view the importance of the rapid multiplication of sugarcane for the growing world population, this study was planned to evaluate the *in vitro* regeneration capacity of three sugarcane varieties by shoot tip culture technique.

MATERIALS AND METHODS

Tree varieties of sugarcane like BL-4, Thathta-10, and Larkana-2001 were kindly provided by the Research Institute of Agriculture, Tando Jam and around the district of Hyderabad and were transferred to the

Laboratory of Department of Biotechnology, Sindh Agriculture University Tando Jam for micropropagation. Explants material were separated from 8 months old sugarcane plants. The shoot tips (1-4 innermost leaves and 2-3 mm in length) of sugarcane were taken from the top of the cane. The explants were thoroughly cleaned and the outer layers were removed to expose the tips of the shoots of sugarcane. The exposed region was excised and sterilized with 100% commercial bleach for 30 min and 70% ethanol for 1 minute followed by rinsing three times with sterile distilled water. Moreover, manipulation of the explants was carried out in a laminar air flow cabinet. Tissue culture of sugarcane was conducted according to technique developed by Khan et al., (2008). The explants were rinsed under the laminar air flow cabinet for removal of residual disinfectant. The shoot tips were excised from sugarcane and placed in each of MS basal medium supplemented with different concentrations (1.0, 1.5, 2.0, 2.5 and 3.0 mg/L) of BAP. Furthermore, the regenerated shoots were dissected and transferred to rooting medium containing MS basal medium with NAA 1.0, 1.5, 2.0, 2.5 and 3.0 mg/L.

RESULTS AND DISCUSSION

The analysis of variance due to varieties, concentration and variety x concentration interaction were significant for all traits. The study showed that shoot tips of sugarcane inoculated aseptically in BAP established the shoot induction and shoot multiplication at the same concentration, whereas at 1.0mg/L of BAP as high as the rate of 85.3% of that multiplication, which also resulted in the desirable quality, well developed and easily separable healthy seedlings of BL-4 variety, while the regeneration capacity was relatively low in Thatta-10 (80.9%) and Larkana-2001 (75.8%). These results showed in (Table 1) that concentrations of auxins and cytokinins affect shoot regene-ration as reported by Chengalrayan et al., (2005).

Table- 1: Survival rates (%) of different sugarcane varieties on various concentrations of BAP.

Concentrations of BAP (mg/L)	Sugarcane **varieties**			Means
	BL-4	Thatta-10	Larkana-2001	
	Survival rates (%)			
1.0	85.3	80.9	75.8	80.7a
1.5	79.1	74.2	70.6	74.6b
2.0	74.2	68.1	64.1	68.8c
2.5	72.1	69.1	65.6	68.9c
3.0	72.3	60.9	62.3	65.2d
Mean	76.6a	70.6b	67.7c	------
		Concentrations	Varieties	Concentrations x Varieties
LSD (0.05)		1.391	1.077	0.314

The results of days required for shoot induction suggested that various concentrations of BAP affected the shooting of sugarcane varieties (Table 2). During this investigation, shoot formation was greatly influenced by the concentration and type of growth regulator used in the experiment. Among the different concentrations, the best performance for days required for shoot induction was given by the variety BL-4 with the average number of days taken for shoot induction was 13.96 days, followed by Thatta-10 (14.98) and Larkana-2001 (16.60 days). With respect to concentration, MS medium supplemented with 1.00 mg/L of BAP to be optimal for all varieties of sugarcane was observed, and it has taken minimum days for shoot induction. However, the best performance was recorded in BL-4 with minimum days taken to shoot induction (11.0 days), while the second performance was good Thatta-10 (12.80) in the MS medium containing 1.00mg/l BAP and average day over varieties were 12.67. The best response in terms of multiple shoot formation was also observed in MS medium supplemented with MS medium containing 1.00 mg/L BAP, and was found to be effective in the production of buds. Similar results were reported by Khan et al., (2008) and Gopitha et al., (2010).

Table -2: Days taken to shoot induction of different varieties of sugarcane at various concentrations of BAP.

Concentrations of BAP (mg/L)	Sugarcane **varieties**			Mean ± SE
	BL-4	Thatta-10	Larkana-2001	
	Days post incubation			
1.0	11.00	12.80	14.20	12.67±0.92e
1.5	12.80	13.90	15.80	14.17±0.88d
2.0	13.90	14.50	16.70	15.03±0.85c
2.5	15.80	16.10	17.80	16.57±0.62b
3.0	16.30	17.60	18.50	17.47±0.64a
Mean ± SE	13.96±0.97c	14.98±0.85b	16.60±0.76a	--------
		Concentrations	Varieties	Concentrations x Varieties
LSD (0.05)		0.295	0.229	0.066

The effect of different concentrations of BAP on forming shootlets per explant, indicated that on an average, maximum numbers of shootlets per explant were recorded in variety BL-4 (4.78) (Table 3) followed by Thatta-10 (3.80), while the minimum number of shootlets per explant were found (2.74) in Larkana-200. Among the media concentrations, the maximum capacity of shoot regeneration was recorded in 1.00 mg/L BAP for BL-4 (6.50). Whereas, the minimum number of shootlets per explant was (1.98) noted in 3.0 mg/L BAP in Larkana-2001 and was found to be less effective in producing number of shootlets per explant.

Table- 3: Number of shootlets per explant of different varieties of sugarcane at various concentrations of BAP.

Concentrations of BAP (mg/L)	Sugarcane varieties			Mean ± SE
	BL-4	Thatta-10	Larkana-2001	
	Number of shootlets per explant			
1.0	6.50	4.70	3.80	5.00±0.79a
1.5	5.30	4.40	3.20	4.30±0.61b
2.0	4.50	3.50	2.50	3.50±0.58c
2.5	4.10	3.30	2.20	3.20±0.55cd
3.0	3.50	3.10	1.98	2.86±0.46d
Mean ± SE	4.78±0.52a	3.80±0.32b	2.74±0.34c	--------
		Concentrations	Varieties	Concentrations x Varieties
LSD (0.05)		0.215	0.167	0.048

The BL-4 variety showed better response in comparison to variety Larkana-2001 and Thatta-10 to produce shootlets per explant. The results also suggest that shoot multiplication in sugarcane depends on the genotype and media concentration. However, the best shoot regeneration was achieved when cultured on MS medium supplemented with 1.0 mg/L BAP (Ali et al., 2008; Gopitha et al., 2010) and increased concentration decreased shootlets induction. As far as shoot length (Table 4) is concerned, the results suggest that among the three varieties of sugarcane, on an average, BL-4 measures longer shoots (5.22 cm), followed by (4.05 cm) of Thatta-10 and (3.58 cm) Larkana-2001. Between mass concentrations the longer shoots were obtained on BL-4 (5.50 cm) to 1.00 mg/L BAP, while smaller buds were developed by Larkana-2001 (3.0 cm) in 3.0 mg/L BAP. However, shoot length between varieties and hormones ranged from 3.00 to 5.50 cm. Overall, the results suggest that the preferred variety BL-4 low, however, all concentrations of cytokinin induce robust growth, while other varieties prefer the highest concentration of cytokinin. It can be concluded that basically phytohormones influence cell division, cell elongation and cell differentiation and integrates the overall development of shooting. Similar findings were reported by Babu (2003) and Khan et al., (2008) in their experiment.

Table-4: Length of shootlets (cm) of different varieties of sugarcane at various concentrations of BAP.

Concentrations of BAP (mg/L)	Sugarcane **varieties**			Mean ± SE
	BL-4	Thatta-10	Larkana-2001	
	Length of shootlets (cm)			
1.0	5.50	4.50	3.70	4.57±0.52a
1.5	5.20	4.10	3.90	4.40±0.40ab
2.0	5.10	3.90	3.60	4.20±0.46b
2.5	5.40	4.00	3.70	4.37±0.52ab
3.0	4.90	3.75	3.00	3.88±0.55c
Mean ± SE	5.22±0.11a	4.05±0.13b	3.58±0.15c	-------
		Concentrations	Varieties	Concentrations x Varieties
LSD (0.05)		0.127	0.098	0.029

The results of days required for the induction of root suggested that rooting was highly influenced by different auxin concentrations used (Table 5). However, the appropriate amount of auxin in the rooting medium is crucial for the induction of the root. Among three varieties of sugarcane, on average, BL-4 requires minimum days (9.38) for the induction of the root, while the maximum days were recorded in Thatta-10 (10.62) and Larkana-2001 (12.16). However, more vigorous root development was achieved when the plantlets were separated and cultured on MS medium supplemented root induction with 3 mg/ L of NAA in BL-4 (9.0 days), followed by Thatta-10 (10.00 days) and Larkana-2001 (11.5 days). It was also observed that the variety BL-4 expressed excellent results in 3 mg/L of NAA from all other varieties of sugarcane. These results confirm previous findings with Behra and Sahoo (2009), while Lal and Singh (1994) also reported that the most efficient for root initiation was auxin NAA. Schenk and Hildebrandt (1972) also observed a high concentration of auxin rooting in sugarcane.

Table-5: Days taken to root induction of different varieties of sugarcane at various concentrations of NAA.

Concentrations of NAA (mg/L)	Sugarcane **varieties**			Mean ± SE
	BL-4	Thatta-10	Larkana-2001	
	Days taken to root induction			
1.0	10.00	11.50	12.90	11.47±0.84a
1.5	9.60	10.90	12.40	10.97±0.81b
2.0	9.20	10.50	12.10	10.60±0.84c
2.5	9.10	10.20	11.90	10.40±0.82c
3.0	9.00	10.00	11.50	10.17±0.73d
Mean ± SE	9.38±0.19c	10.62±0.27b	12.16±0.24a	-------
		concentrations	Varieties	Concentrations x Varieties
LSD (0.05)		0.089	0.068	0.021

The results shown in (Table 6) indicate that the number of roots produced by shootlet medium varies from 3.70 to 6.80. However, the maximum number of roots was recorded in variety BL-4 (6.12), while the minimum number of roots was observed in Larkana-2001 (4.30) and medium in Thatta-10 (4.76). Moreover, the maximum number of roots per shootlet was observed in BL-4 (6.8) to 3.0 m/L of NNA, while the minimum number of roots were observed in Larkana-2001 (4.0) to 1.0 mg/L NAA. These results suggest that the optimal amount of auxin in the rooting medium is crucial for the development of roots by shootlet. Five concentrations of auxin, NAA at doses greater than 3 mg/L produced the highest number of roots in all varieties. This agreed with the results obtained by Biradar et al., (2009), Behera and Sahoo (2009) and Gopitha et al., (2010).

Table-6: Number of roots per shootlet of different varieties of sugarcane at various concentrations of NAA.

Concentrations of NAA (mg/L)	Sugarcane **varieties**			Mean ± SE
	BL-4	Thatta-10	Larkana-2001	
	Average number of roots			
1.0	5.50	0.6	3.70	4.40±0.56d
1.5	5.90	1.0	4.00	4.90±0.55c
2.0	6.00	1.5	4.20	5.07±0.52bc
2.5	6.40	2.0	4.70	5.43±0.50ab
3.0	6.80	2.5	4.90	5.50±0.65a
Mean ± SE	6.12±0.22a	4.76±0.20b	4.30±0.22c	-------
		concentrations	Varieties	Concentrations x Varieties
LSD (0.05)		0.178	0.138	0.04

It was evident from (Table 7) that almost all varieties had a better response on MS medium supplemented with NAA at different concentrations. Among the varieties, BL-4 measured an average longer roots (2.08 cm) followed Thatta-10 (1.32 cm) and Larkana-2001 (1.10 cm). The results also suggest that higher doses of NAA are needed for the efficient development of root length in sugarcane because the variety BL-4 expressed best answer (2.50cm) of 3.0 mg/L NAA. However, the poor response was observed in Thatta-10 (1.20 cm) to 1.0 and 2.0 mg/L of NAA Larkana-2001 followed by (0.9 cm) to 1.0 mg/L NAA. Similar results were reported by Behera and Sahoo (2009) who mentioned that MS basal media supplemented with 3.0 mg/L NAA, rooting was more profuse.

Table-7: Duration of roots (cm) of different varieties of sugarcane to various concentrations of NAA.

Concentrations of NAA (mg/L)	Sugarcane **varieties**			Mean ± SE
	BL-4	Thatta-10	Larkana-2001	
	Average longer roots (cm)			
1.0	1.80	1.20	0.90	1.30±0.27c
1.5	1.90	1.40	1.00	1.43±0.26bc
2.0	2.00	1.20	1.10	1.43±0.29bc
2.5	2.20	1.30	1.20	1.57±0.32ab
3.0	2.50	1.50	1.30	1.77±0.37a
Mean ± SE	2.08±0.12a	1.32±0.06b	1.10±0.07c	-------
		Concentrations	Varieties	Concentrations x Varieties
LSD (0.05)		0.138	0.088	0.05

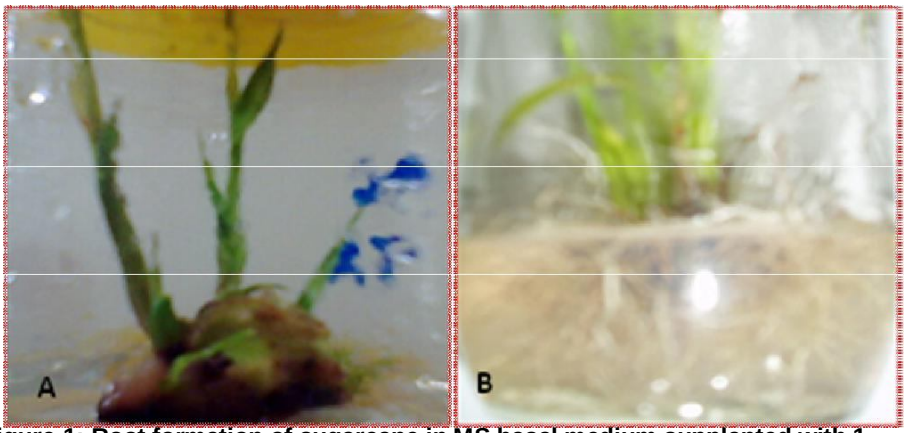

Figure-1: Root formation of sugarcane in MS basal medium supplanted with 1 mg/L (A) of BAP and 3 mg/L (B) of NAA, respectively.

CONCLUSION

This study concluded that the MS medium supplemented with 1.0 mg/L BAP showed significantly high (85.3%) regeneration capacity in BL-4 followed by Thatta-10 (80.9%). While among varieties, BL-4 showed moderately high regeneration rate (76.6%) among other varieties of sugarcane and variety BL-4 took minimum 13.62 days for shoot initiation, followed by Tatta-10 with 14.98 days. It was also found that MS medium supplemented with 1.0 mg/L gave the best results for shoot formation in BL-4 variety. The frequency of shoot proliferation was reduced with the increase of BAP concentration and the time required for the formation of shoots was also delayed. The maximum number of shootlets observed 4.78 per explants plant with length of 5.22 cm in BL-4 followed variety of Thatta-10. One can also assume that BL-4 gave better response for the number of shootlets (6.50) per explant compared to all other varieties of sugarcane on MS

medium supplemented with 1.0 mg/L BAP and It takes minimum 9 days for the induction of the root by 3 mg/L of NAA. Although we also observed the maximum number and length of the longest root on MS medium supplemented with 3.0 mg/L of NAA in BL-4 variety.

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