EFFECT OF SUGARCANE GENOTYPES AND 2, 4-DICHLOROPHENOXY ACETIC ACID ON CALLUS INDUCTION

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ABSTRACT

Callus induction is an important step in *in-vitro* culture valued for micropropagation, somaclonal variation, mutagenesis, synthetic seed production, transformation and genetic engineering. Callusing response of ten sugarcane genotypes viz., BO-110, BO-134, Cose-95422, Cose-98232, Cose-92423, UP-9742, BO-91, BO-135, and Cose-98255, were studied under three different doses (2, 3 & 4 mg L^{-1}) of 2,4-D supplemented in the callus induction media. Callus induction was found to depend on both genotype and 2,4-D concentration in the media. The genotype UP-9742 was most amenable to callus induction (67.50%) followed by Cose-97182 (56.25%) and Cose-92423 (51.00%). While, the media supplemented with 3 mg L^{-1} 2,4-D was found more effective to trigger callus induction in general. Yet, the optimum dose of 2,4-D to be used depends on the interaction of the specific genotype.

Key words: Sugarcane (*Saccharum spp.*), Callus, *in-vitro*, Murashige and Skoog (MS), Endogenous Hormone,

INTRODUCTION

No any sexual breeding program in sugarcane (*Saccharum spp.*), an industrial cum cash crop, has been carried out in Nepal till date. It is essentially due to unfavorable natural condition for flowering and/or successful seed set in the country. Even if possible, it is hindered by factors such as high ploidy, large genome, low fertility, complex environmental interaction, slow breeding advance, limited available information on its genetics and genomics, etc (Ali *et al.*, 2010). Not a single variety is being released over a decade and the released varieties (only four) are also degrading due to insect-pest and disease incidence. While, the acquisition of exotic genotypes of sugarcane is restricted, there is complete lack of varietal option in the country.

Callus culture is a basic *in-vitro* techniques which can aid in rapid development of disease-free propagating material *via* micropropagation (Dash *et al.*, 2011), generation of superior variants *via* somaclonal variation (Sobhakumari, 2012) and incorporation of specific desired genes *via* transformation and genetic engineering (Raza *et al.*, 2010). The variability generated *in-vitro* in together with chemical mutagenesis can aid in varietal improvement program since the resulting variants are reported to yield superior as well as are resistant to various diseases, insect-pests and abiotic stresses (Rajeswari *et al.*, 2009; Mallikarjun *et al.*, 2008; Bidabadi et al., 2011). *In-vitro* induced variability is reported to be enhanced during callus culture as it leads to the greater departure from the organized growth (Karp, 1995; Jain, 2001).

Callus induction in sugarcane is affected by several factors such as, genotype, explants type, explants age, sterilization protocol, media composition, hormonal dose, temperature, photoperiod, etc., of which the genotype of the parent and 2,4-D (an auxin) in the media has major effect and is studied by several researchers (Gandonou *et al.*, 2005; Goel *et al.,* 2010; Jahangir *et al.*, 2010; Sani

and Mustapha, 2010). Thus, it is important to study the callusing response of different sugarcane genotypes under varied hormonal dose supplementation so as to optimize the efficient protocol for future uses.

MATERIALS AND METHODS

Ten sugarcane genotypes *viz*., BO-110, BO-134, Cose-95422, Cose-98232, Cose-92423, UP-9742, BO-91, BO-135, and Cose-98255, were collected from the National Sugarcane Research Program, Jeetpur, Bara, Nepal and planted in the earthen pots (soil:FYM::2:1) in the glass house of Biotechnology Division, Khumaltar, Lalitpur, Nepal following standard agronomic practices. The shoot apices (about 2 cm) from the healthy, disease free and vigorously growing 5-6 month old plants were used as explants. Surface sterilization of the explants was carried out using 70% ethanol for 5 minutes followed by 2% sodium hypochloride for 5 minutes and 0.5% Plant preservative mixture (PPM^{TM}) for 10 minutes. The explants were cut into the final size (about 0.2 x 0.2 cm^2) under laminar air flow cabinet and were transferred to the callus induction media. MS basal salts were used to prepare the media with four different concentrations of 2,4-D as treatments as follows:

T_1: MS without 2,4-D (control) T_2: MS+ 2 mg L^{-1} 2,4-D
T_3: MS+ 3 mg L^{-1} 2,4-D T_4: MS+ 4 mg L^{-1} 2,4-D

Each treatment was replicated 10 times (a petriplate as a replication) with 4/5 explants per petriplate and were incubated in dark at 25±1°C. The callus induction percentage of each treatment in each replication was calculated as:

$$\text{Callus Induction (CI) \%} = \frac{\text{Number of explants showing callus (30 days after incubation) x 100}}{\text{Total number of explants plated per replication}}$$

The callus induction data in percentage were arcsine transformed and interpreted as Factorial Completely Randomized Design. MSTAT-C was used for analysis of variance and mean comparison through Duncan Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

Effect of genotypes on callus induction

The mean callus induction value (irrespective of the hormonal dose) reveals three distinct grouping of the genotypes *viz*., high response (>60%): UP 9742; medium response (25-60%): Cose-97182, Cose-92423, Cose-98422, Cose-98232, BO-134, BO-134, Cose-98422, BO-110; and low response (<20%): BO 110, to callus induction (Table 1). UP-9742 performed distinctly superior with 67.5 % mean callus induction while BO-91 was the poor one with only 13.75% mean callus induction (Table 1). Similar findings were reported by Gandonou *et al.* (2005) where they observed three distinct groups of genotypes as highest, intermediate and weakest behavior to callus induction.

Effect of 2,4-D concentration on callus induction

Highly significant difference between different 2,4-D supplementation (0 to 4 mg L^{-1}) treatments with respect to % callus induction was noticed (Table 1). The best response in terms of mean callus induction percentage (averaged over all genotypes) was observed in the media supplemented with 3 mg L^{-1} 2.4-D (64.3%) followed by 2 mg L^{-1} (52.8%) and 4 mg L^{-1} (39.8%). The media without 2,

4-D was found to be least effective in terms of callus induction with only 4% mean callus induction percentage (Table 1). In addition, no callus induction was observed in the media lacking hormonal supplement (control) in most of the genotypes except Cose-92423, UP-9742 and Cose-98255 which showed very little response *viz.*, 8%, 20% and 12% respectively. Ather *et al.* (2009) also reported 20% callus induction in the media without 2,4-D supplementation in sugarcane cv. Thatta-10.

Table 1. Callus induction percentage of ten sugarcane genotypes under four 2,4-D concentration†

Genotypes	2,4-D concentration				Mean CI %	F-value	LSD	SEm(±)
	Control	2 mg L^{-1}	3mg L^{-1}	4mg L^{-1}				
UP-9742	20^{b}	95^{a}	85^{a}	75^{a}	**68.75^{A}**			
Cose-97182	0^{c}	90^{a}	80^{a}	55^{b}	56.25^{B}			
Cose-92423	8^{c}	52^{b}	80^{a}	64^{ab}	51.00^{BC}			
Cose-98422	0^{b}	56^{a}	72^{a}	52^{a}	45.00^{BCD}			
Cose-98232	0^{c}	50^{b}	85^{a}	35^{b}	42.50^{CD}	39.23**	7.76	2.11
BO-134	0^{c}	52^{ab}	68^{a}	40^{b}	40.00^{DE}			
BO-135	0^{b}	40^{a}	44^{a}	40^{a}	32.00^{EF}			
Cose-98255	12^{b}	24^{b}	60^{a}	16^{b}	28.00^{EF}			
BO-110	0^{c}	44^{a}	40^{a}	16^{b}	25.00^{F}			
BO-91	0^{b}	25^{ab}	25^{a}	5^{ab}	13.75^{G}			
Mean CI %	4.00^{d}	52.80^{b}	**64.30^{a}**	39.80^{c}	40.22			
F-value	256.03**							
LSD	4.90							
SEm(±)	1.34							
C.V. (%)	36.76							

LSD: Least Significant Difference; SEm: Standard Error of mean; C.V.: Coefficient of Variation

† Means followed by same lower case letter in the row and capital letter in the column are not statistically significant ($p<0.01$) according to Duncan's multiple range test

But, considering individual genotypic response, callus induction % was significantly higher at 3 mg L^{-1} 2,4-D in Cose-98255 and Cose-98232 while non-significant yet higher response was observed in BO-134, Cose-98422, Cose-92423 and BO-135 at 3 mg L^{-1} compared to 2 and 4 mg L^{-1}. Similarly, callus induction % was non-significantly higher at 2 mg L^{-1} 2,4-D in three genotypes *viz.*, BO-110, Cose-97182 and UP-9742. While, similar response was observed at 2 and 3 mg L^{-1} 2,4-D in BO-91.

The variation in callus induction capacity with respect to genotypes has been reported by several researchers (Gandonou *et al.*, 2005; Tiel *et al.*, 2006; Raza et al, 2010; Sani and Mustapha, 2010; Begum *et al.*, 2011; Smiullah *et al.*, 2012). Significant differences to callus induction percentage ranging from 69.23 to 95.87% (Gandonou *et al.*, 2005), 77 to 91% (Raza *et al.*, 2010) and 43.6 to 75.8% (Begum *et al.*, 2011) was reported among different sugarcane genotypes tested. Such variation in response to *in-vitro* callogenesis is attributed to their intrinsic physiological differences, particularly the endogenous hormones levels in the sugarcane genotypes under investigation (Kumari and Verma, 2001; Sani and Mustapha, 2010).

In sugarcane, 3 mg L^{-1} 2, 4-D supplementation is repeatedly proved to be best (Sani and Mustapha, 2010; Dash *et al.*, 2011, Smiullah *et al.*, 2012) and is widely being followed for callus induction (Mallikarjun *et al.*, 2008; Sobhakumari, 2012; Mahlanza *et al.*, 2013). But, Ather *et al.* (2009), Goel *et al.* (2010) and Jahangir *et al.* (2010) reported non-significant callus induction and growth at 3 and 4 mg L^{-1} 2, 4-D but, comparatively better along with high regeneration potency at 3 mg L^{-1} in various sugarcane genotypes. Goel *et al.* (2010) also reported active callus induction and growth at higher 2,4-D concentration (3 & 4 mg l^{-1}) as compared to low concentration (1 and 2 mg L^{-1}). Tuladhar and Rajbhandari (1994) also reported 2 mg L^{-1} 2,4-D supplementation in the MS media (with 15% coconut milk) as the most optimum concentration for callus induction in 11 sugarcane genotypes.

Callus induction in sugarcane seems not solely dependent on quantity of hormonal supplementation in the media, but determined by the interaction of several other factors *viz.*, explants type, parent genotype, age of the explants, growth room environment, etc. Thus, it is worthwhile to examine the genotypic interaction with the specific concentration of the 2,4-D, since the indigenous hormonal composition of the genotype itself seems to play a vital role in determining callusing response (Sani and Mustapha, 2010). Since, the overall performance of all the genotypes was consistently superior in the MS media supplemented with 3 mg L^{-1} 2,4-D (Table 1), it might be suggested further to be used as a default hormonal dose in the media for other callus culture projects on sugarcane.

CONCLUSION

Callusing response in sugarcane is found to be associated with the genotype under consideration as well as the concentration of 2,4-D supplemented in the media. Some genotypes viz., UP-9742, Cose-97182, Cose-92423, seems more amenable to callus induction (with more than 50% mean callus induction) while BO-91 seems hardier in nature. Similarly, 2,4-D supplementation has significant role in triggering callus induction in sugarcane. Although, 2,4-D @ 3 mg L^{-1} seems conducive for callus induction in general, yet the response is more dependent on the interaction with specific genotype.

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REFRENCES CITED

Ather, A., S. Khan, A. Rehman and M. Nazir. 2009. Optimization of the protocols for the callus induction, regeneration and acclimatization of sugarcane cv. Thatta-10. Pakistan Journal of Botany. 41(2): 815-820.

Begum, M. K., M. O. Islam, M. A. S. Miah, M. A. Hossain and N. Islam. 2011. Production of somaclone *in-vitro* for drought stress tolerant plantlet selection in sugarcane (*Saccharum officinarum* L.). The Agriculturists. 9(1&2): 18-28.

Bidabadi, S. S., M. Mahmood, S. Meon, Z. Wahab and C. Ghobadi. 2011. Evaluation of *in vitro* water stress tolerance among EMS-induced variants of banana (*Musa* spp., AAA),

using "morphological, physiological and molecular" traits. Journal of Crop Science and Biotechnology. 14(4): 255-263.

Dash, M., P. K. Mishra and D. Mohapatra. 2011. Mass propagation via shoot tip culture and detection of genetic variability of *Saccharum officinarum* clones using biochemical markers. Asian Journal of Biotechnology. 3(4): 378-387.

Gandonou, Ch., J. Abrini, M. Idaomar and N. S. Senhaji. 2005. Response of sugarcane (*Saccharum sp.*) varieties to embryogenic callus induction and *in vitro* salt stress. African Journal of Biotechnology. 4(4): 350-354.

Goel, Y., V. P. Singh, M. Lal and M. L. Sharma. 2010. *In-vitro* morphogenesis in leaf sheath explants of sugarcane hybrid var. CoS 99259. Sugar Tech. 12(2): 172-175.

Jahangir, G. Z., I. A. Nasir, R. A. Sial, M. A. Javid and T. Husnain. 2010. Various hormonal supplementations activate sugarcane regeneration *in vitro*. Journal of Agricultural Science. 2(4): 232-236.

Jain, S. M. 2001. Tissue culture-derived variation in crop improvement. Euphytica. 118: 153-166.

Karp, A. 1995. Somaclonal variation as a tool for crop improvement. Euphytica. 85: 295-302.

Kumari, R. and D. K. Verma. 2001. Development of micropropagation protocol for sugarcane (*Saccharum officinarum* L.)- A review. Agricultural Reviews. 22(2): 87-94.

Mahlanza, T., R. S. Rutherford, S. J. Snyman and M. P. Watt. 2013. *In-vitro* generation of somaclonal variant plants of sugarcane for tolerance to *Fusarium sacchari*. Plant Cell Reports. 32: 249-262.

Mallikarjun, K., R. R. Hanchinal and H. L. Nadaf. 2008. Ethyl methane sulphonate (EMS) induced mutation and selection for salt tolerance in sugarcane *in-vitro*. Indian Journal of Plant Physiology. 13(4): 405-410.

Rajeswari, S., S. Thirugnanakumar, A. Anandan and M. Krishnamurthi. 2009. Somaclonal variation in sugarcane through tissue culture and evaluation for quantitative and quality traits. Euphytica. 168: 71-80.

Raza, G., K. Ali, Z. Mukhtar, S. Mansoor, M. Arshad and S. Asad. 2010. The response of sugarcane (*Saccharum officinarum* L.) genotypes to callus induction, regeneration and different concentration of selective agent (geneticin-418). African Journal of Biotechnology. 9(51): 8739-8747.

Sani, L. A. and Y. Mustapha. 2010. Effect of genotype and 2,4-D concentration on callogenesis in sugarcane (*Saccharum spp.* Hybrids). Bayero Journal of Pure and Applied Sciences. 3(1): 238-240.

Smiullah, F. A. Khan, Abdullah, A. Afzal, M. A. Javed, Z. Iqbal, R. Iftikhar and J. I. Wattoo. 2012. *In-vitro* regeneration, detection of somaclonal variation and screening for mosaic virus in sugarcane somaclones. International Journal of the Physical Science. 11(48): 10841-10850.

Sobhakumari, V. P. 2012. Assessment of somaclonal variation in sugarcane. African Journal of Biotechnology. 11(87): 15303-15309.

Tiel, K., G. A. Enriquez, Y. Ceballo, N. Soto, A. D. Fuentes, A. Ferreira, Y. Coll and M. Pujol. 2006. Development of a system for rapid plant regeneration from *in-vitro* sugarcane (*Saccharum officinarum* L.) meristematic tissue. Biotecnología Aplicada. 23: 22-24.

Tuladhar, P. S. and S. B. Rajbhandari. 1994. Tissue culture of sugarcane (*Saccharum spp.*) for mass production. *In*: Proceedings of II[nd] National Conference on Science and Technology June 8-11, 1994. Royal Nepal Academy of Science and Technology, Kathmandu, Nepal. pp. 380-383.