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
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 (PPMTM) for 10 minutes. The explants were cut into the final size (about 0.2 x 0.2 cm²) 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:

- **T₁**: MS without 2,4-D (control)
- **T₂**: MS+ 2 mg L⁻¹ 2,4-D
- **T₃**: MS+ 3 mg L⁻¹ 2,4-D
- **T₄**: 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:

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

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-98222, 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>
<thead>
<tr>
<th>Genotypes</th>
<th>2,4-D concentration</th>
<th>Mean CI %</th>
<th>F-value</th>
<th>LSD</th>
<th>SEM(±)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>2 mg L⁻¹</td>
<td>3 mg L⁻¹</td>
<td>4 mg L⁻¹</td>
<td></td>
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<td>UP-9742</td>
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<td>95⁺</td>
<td>85⁺</td>
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<td>28.00⁺</td>
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<td>25ab⁺</td>
<td>25⁺</td>
<td>5⁺ab</td>
<td>13.75⁺</td>
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<tr>
<td>Mean CI %</td>
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<td>52.80b</td>
<td>64.30a</td>
<td>39.80c</td>
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<td>LSD</td>
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<td>C.V. (%)</td>
<td>36.76</td>
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</table>

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 \textit{et al.}, 2011, Smillah \textit{et al.}, 2012) and is widely being followed for callus induction (Mallikarjun \textit{et al.}, 2008; Sobhakumari, 2012; Mahlanza \textit{et al.}, 2013). But, Ather \textit{et al.} (2009), Goel \textit{et al.} (2010) and Jahangir \textit{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 \textit{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 \textit{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 \textit{viz.}, UP-9742, Cose-97182, Cose-92423, seems more amenable to callus induction (with more than 50% mean callus induction) while BO-91 seems harder 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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