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The Effect of 2, 4-Dichlorophenoxy Acetic Acid (2, 4-D) Concentration on Callus Induction in Sugarcane *(Saccharum officinarum)*

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ABSTRACT: Experiment was designed and conducted to determine the most appropriate concentration of 2, 4-Dichlorophenoxy Acetic Acid suitable for callus induction in sugarcane. Explants were obtained from apical meristems of two sugarcane cultivars (SP726180 and CO-001) and cultured in a modified MS medium supplemented with varying concentrations of 2,4-D. After sterilization, the explants were incubated in the growth chamber at 27^0C and monitored for callus induction for four Weeks. The resulting calli were sub cultured on a media with a reduced 2,4-D concentration to induce somatic embryogenesis. Percentages of callus formation, embryogenic callus as well as growth coefficient were the parameters monitored. Data generated were analyzed using Chi-square and showed a significant difference among the different 2,4-D concentrations ($P \leq 0.05$). Swelling of the explants was observed one week after inoculation while callus initiation begun after two weeks. The calli were found to be yellowish, compact and nodular. Callus induction was found to increase with increase in the concentration of 2,4-D.
Keywords: *Saccharum officinarum*, Explants, Callus Induction, 2,4-Dichlorophenoxy Acetic Acid.

INTRODUCTION

Sugarcane (*Saccharum officinarum*) belongs to the family graminae. It is commonly grown in the tropical and subtropical countries of the world. It grows in length up to about 4mt tall. Several horticultural varieties exist and differ in their stem color and length (Naidu, 1987). *S. officinarum* is polyploid (2n- 36-70) and account for about 65% of the raw materials needed for global Sugar production. The plant doesn't reproduce effectively through the seed. Therefore, it is widely cultivated through stem cuttings. This was found to be very slow and requires 6-8 years of field multiplication before a variety is released on commercial scale. Consequently, Plant tissue culture provides a very effective technique for rapid sugarcane seedling production. For example, a large number of Virus free and true to type sugarcane plantlets were reported to have been produced from a single plant per annum (Chen *et al.*, 1988, Hamish and Sue, 1998, Razdan, 2002)

Sugarcane cultivation in Nigeria was reported to have started as early as 15th century where it was cultivated for chewing and as a major raw material for sugar production. Indigenous sugar industries provide only 5% of our domestic sugar demand. Therefore, the country imports over 90% of its sugar demand. The implication of this import dependency *vis-à-vis* the nation's economy and food security of the country is a serious one (Naidu, 1987).

Several studies were carried out to develop protocols for *in vitro* micro propagation of sugarcane through callus culture from foliage leaves (Bugun *et al.*, 1995; Chengalraya and Gallo 2001; Abdullahi *et al*, 2002; Mamun *et al* 2004; Gandonuo, *et al.*, 2005a). However, due to regional differences in varietal demand and strong influence of genotype on *in vitro* regeneration of sugarcane, it becomes necessary to optimize regeneration protocol for specific genotypes.

Sucrose is the most important type of sugar produced from sugarcane. It is used as a sweetening agent for foods and in the manufacture of cake, candies, preservatives, soft drinks etc. It supplied about 13% of all energy derived from foods (Escalona *et al.*, 1995). Global over population, difficulty in conventional regeneration of sugarcane, coupled with increase in its industrial use has made scientists to begun its cultivation in laboratories *via* plant tissue culture technique (Lorenzo and Ganzalez, 1998). This allows for an extended growing season because

shoots can be regenerated *in vitro*, graduated into the field and harvested early. The system also reduces planting cost by 6% and yield plantlets that perform similar to the conventionally grown sugar cane (Lorenzo and Gonzalez, 1998). This paper presents results of a study carried out to determine the most suitable 2,4-D concentration for *in vitro* callus production in sugarcane with the view to establish a protocol for different sugarcane varieties.

MATERIALS AND METHODS

Two varieties of sugarcane cultivars SP726180 and CO- 001 were collected from Kazaure, Jigawa, Nigeria. It is situated in the north-western part of the country between latitudes 11.00°N to 13.00°N and longitudes 8.00°E to 10.15°E.

Treatments

Isolation and Sterilization

Apical meristems of the two sugarcane varieties were excised from intact plant. Surface sterilization of the explants was carried out in the following steps:

i. Explants were first washed on a running tap water.
ii. They were treated with house hold detergent for five minutes
iii. They were washed again on a running tap water to remove all traces of detergent.
iv. They were then washed with double distilled water in the laminar flow hood.
v. They were further sterilized by dipping into 70% ethanol for 2 minutes.
vi. They were then treated with a commercial bleach containing 5% Sodium hypochlorite for 15 minutes.
vii. They were finally rinsed 3 times with sterile double distilled water. (Hamish and Sue, 1998; Razdan, 2002)

Media Preparation

One liter of Murashige & Skoog (MS) medium supplemented with different concentrations of 2,4-Dichlorophenoxy acetic acid (2.5,3.0,3.5,4.0,and 0.0mg/l) and 30% sucrose was prepared. The media was solidified using 8g agar. The pH of the media was adjusted to 5.8 using potassium hydroxide (KOH). The prepared media was autoclaved at 121°C for 15 minutes (Hamish and Sue, 1998; Razdan, 2002; Qin *et al.*, 2005).

Inoculation

Under sterile condition, leaf rolls were pealed to cylinder forms and cut to the size of about 10mm long. Three explants were inoculated in each bottle containing 35ml of the prepared callus induction media (Hamish and Sue, 1998; Razdan, 2002)

Incubation

Explants were incubated at 27°C and kept in darkness for 4 weeks. The resulting callus materials were sub cultured on the same type of media but with a reduced concentration of 2,4-D to induce somatic embryogenesis for another 4weeks (Hamish and Sue, 1998; Razdan, 2002).

Observations and Data Collection

Percentage of callus formation, embryonic callus and Growth coefficient were the parameters studied and determined using the following formula:

1. $$\text{Percentage of callus formation} = \frac{\text{Number of explants forming callus}}{\text{Total number of explants}}$$

2. $$\text{Percentage embryonic callus} = \frac{\text{Number of embryonic callus}}{\text{Total number of callus}}$$

3. $$\text{Growth coefficient} = \frac{\text{Weight after sub culturing}}{\text{Weight before sub culturing}}$$

Data Analysis

Data generated from this study was subjected to Chi square statistical method.

RESULTS

Swelling of the explants was observed one week after inoculation. However, callus initiation was observed in the second week after inoculation. Four weeks later, callus initiated at the cut edge of the explants and developed into a full grown callus. The callus was morphologically found to be yellowish-white, compact, dry and nodular (Plate I). The response of the two cultivars to

treatments and subsequent callus induction varied, with cultivar CO-001 having the highest performance.

The mean result for SP 726180 genotype indicated that for percentage of callus formation, 2,4-D concentration of 4.00 g/l gave the best result followed by 3.50 mg/l. Higher percentage of embryonic callus was observed at 3.00 mg/L 2,4-D concentration followed by 2.50mg/L. Similarly, highest growth coefficient was performed by 2.50mg/l 2,4-D concentration followed by 3.00mg/L (Table 1).

However, the mean result of CO-001 genotype showed that highest percentage of callus formation was observed at 3.50mg/L 2,4-D concentration followed by 4.00mg/L. The lowest callus formation was observed at 2.50mg/L 2,4-D concentration. For embryonic callus, 3.00mg/l was found to be the best treatment followed by 2.50mg/l concentration. However, results for growth co-efficient indicated that, 2.50mg/l was the best concentration followed by 3.00mg/l. (Table 2).

Table 1: Result for SP726180 genotype.

2,4-D mg/l)	Callus Formation (%)	Embryogenic Callus (%)	Growth Coefficient	Degree of Callus
0.00	0.00	0.00	0.00	-
2.50	41.7	54.3	2.34	Xx
3.00	45.9	62.0	2.23	Xx
3.50	80.7	39.1	1.88	Xxx
4.00	82.8	18.2	1.90	Xxx

Table 2: Result for CO- 001 genotype.

2,4-D mg/l)	Callus Formation (%)	Embryogenic Callus (%)	Growth Coefficient	Degree of Callus
0.00	0.00	0.00	0.00	-
2.50	36.0	65.0	2.29	Xx
3.00	44.0	55.8	1.56	Xx
3.50	85.7	38.0	1.67	Xxx
4.00	85.4	17.9	1.82	Xxx

DISCUSSION

Young sugarcane leaves were reported to provide good explants source for callus induction within 2-8 weeks. This may be as a result of their physiological state which provide actively dividing cells (Hamish and Sue, 1989; Cheu *et al*, 1988; Fitch and Moore, 1990; Brisibe *et al*, 1994). The development of callus from immature leaf explants is directly related to the presence of 2,4-D which is a suitable growth hormone responsible for callus induction in most plant Species in plant tissue culture work. This is similar to the findings of Mamun *et al*. (2004) and Baskaran *et al*. (2005). The production of a yellowish, compact and nodular callus at cut edge of explant may be due to the wound caused during the process of cutting which resulted in a synchronous cell division. This is considered as a process of de-differentiation of organized tissue and is similar to the work of Hamish and Sue (1989); Pellegrinechi *et al*. (2004); Qin *et al*. (2005) and Xing *et al*. (2010). Thus, there was optimum Callus induction with increase in 2,4-D concentration (Qin *et al*., 2005; Xing *et al*., 2010).With regards to percentage Callus formation, the two genotypes responded in different ways. Therefore, respond to callus formation might be genotype dependent. However, lower 2,4-D concentration gave better embryonic callus and callus growth among the two genotypes (Gandonuo, 2005a, Xing *et al*., 2010).

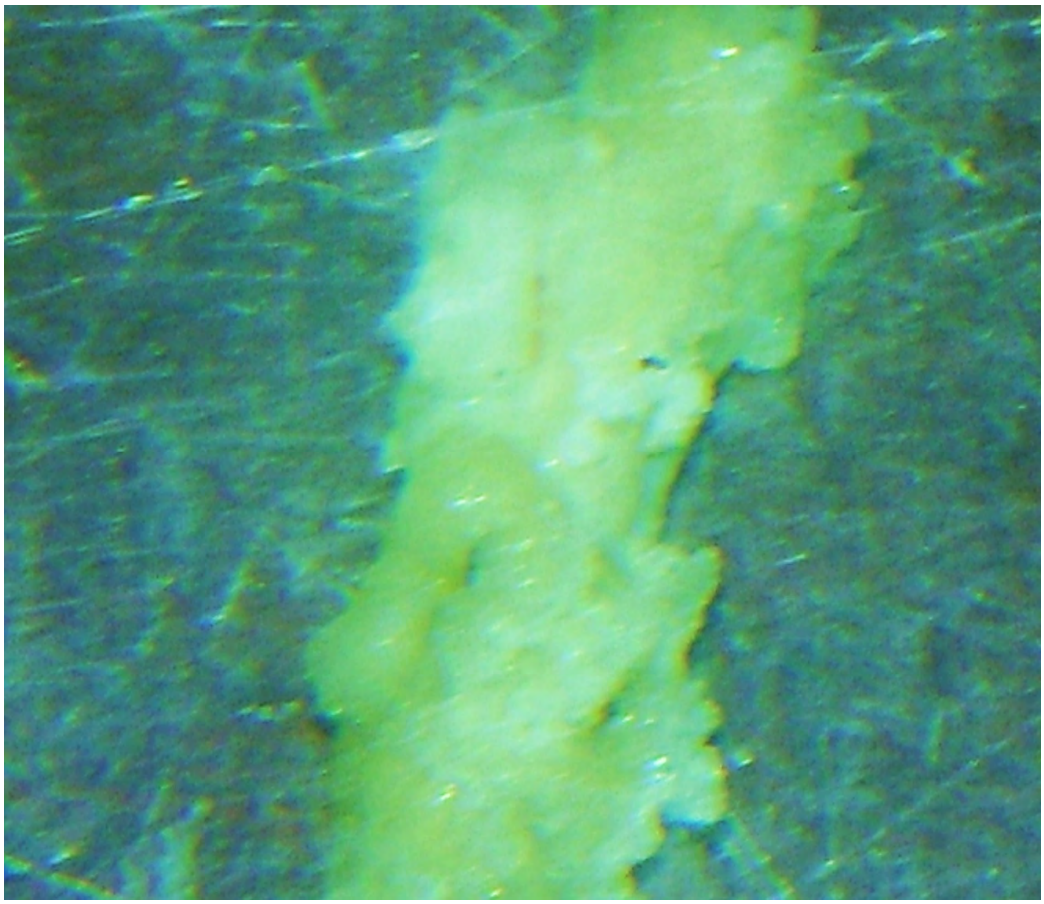

Plate: Showing Callus formed from Cultivar

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REFERENCES

Abdullahi, K.,Imtaz, A.K., Muhammad, A.J., Muhaamad, A.S., Muhammad, K.R.K., Muhammad, H.K., Nasir., A.D., and Raziullah, K. (2002) Studies on Callusing and Regeneration Potential of Indigenous and Exotic Sugarcane Clones. *Asian Journal of Plant Science*. **1:** 41-43.

Baskaran, P.B., Raja,R., Jayabalan,N. (2006). Development of an In vitro Regeneration System in Sorghum (*Sorghum bicholar* L) (2006). Using Root Tranverse Thin Layers (tTCLs). *Turk Journal of Botany.* **30:** 1-9.

Brisibe, E.A., Miyake, H., Tanighuchi, T., Maeda, E. (1994). Regulation of Somatic Embryogenesis in Long term Callus Cultures of Sugarcane (*Sachharum officinarum* L). *New Phytology* **126:** 301-30.

Chen, W.H., Davey, M.R., Power, J.B. and Cocking, E.C. (1988). Control of Plant Regenerationin Sugarcane Callus cultures. *Journal of Experimental Botany.* **39:** 251-261.

Chengalrayan, K and Gallo-Meagher, M (2001). Effect of various growth regulators on shoot regeneration of sugarcane. *In vitro* Cell and Devel. *Biology of Plant* **37:** 434-439.

Escalona, M., Castillo, R., Cocepcion, O., Bonoto, C.G., Lurenzo, J.C., Daquint, M.A., Sanchez, M., Puentents, C. and Chung-shed, K. (1995). Influence of two calt types on the establishment of Cell suspension in Sugarcane. *Centro Agricola.* **22:** 63-70.

Fitch,M.M, and Moore, P.H. (1990). Comparison of 2,4-D and Picloram for selection of long term Totipotent green callus cultures of Sugarcane. *Plant Cell Tissue and Organ Culture* **20:**157-163.

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

Gandonuo, C.H., Errabil, T., Abrini, J., Idaomar, M., Chibi, F. and Senhaji, N.S. (2005b). Effect of Genotype on callus induction from leaf explant of Sugarcane(*Saccharum spp*). *African Journal of Biotechnology* **4(11):**1250-1255.

Hamish, A.C. and Sue, E. (1998). *Plant Science Culture*. BIOS Scientific Publishers limited. Oxford, London. P157

Hess, J R., Caman. J.G. (1998) Embryogenic competence of immature wheat embryos: genotype donor plant environment and endogenous hormones levels. *Crop Science* **38:** 24-253.

Lorenzo, J.C. and Gonzalez, B.I. (1998). New Sugarcane shoot formation in temporary immersion system. *Plant Cell Tissue and Organ Culture.* **54:** 35-36.

Mamun, M.A., Sikdar, M.B.H., Dipak, K.P., Rahman, M. and Rezuanul Islam, M.D. (2004). In vitro micropropagation of some important Sugarcane Varieties of Bangladash. *Asian Journal of Plant Science*. **3(6):** 666-669.

Murashige, T. and Skoog, F.A. (1962). A revised medium for rapid growth and Bioassay with Tobacco Tissue Cultures. *Physiology Plant* **15:** *473-497*

Naidu, K.M. (1987). Potential yield in sugarcane and its realisation through variety improvement. Pp.1-17 *in* Sugarcane Varietal Improvement (M.K. Naidu, J.V. Screenivasan and M.N. Premachandran, eds.). Sugarcane Breeding Institute, Coimbatore, India.

Qin, C., Dong, Z., Lin, W. Deng,. and Tang, L. (2005). Effect of exogenous Plant growth regulators on *in vitro* regeration of Cotyledonary explants in Pepper. *Not. Bot.Hort. Agrobot.Cluji XXXIII*.

Razdan, M.K. (2002). *Introduction to plant Tissue Culture*. Second Edition. Oxford & IBH Publishing Co. Pvt. Ltd. New Delhi, India. P375.

Xing, Z.Y., Yuan, Y.H., Wang, L.F. and Zheng, L.P. (2010). Regenerating Plants from in vitro culture of *Erigeron Breviscapus* leaves. *African Journal of Biotechnology* **9(26):** 4022-4024.