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# Studies on Somatic Embryogenesis in Sugarcane

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**Abstract:** Somatic embryogenesis was studied in sugarcane cultivars CPF-237 and SPF-213. Explants used were leaves, lateral buds and pith. MS medium along with NAA and 2,4-D in various concentrations were used and it was observed that 1.0 mg NAA and 3-mg/l 2,4-D was optimal for embryogenesis. Leaf portion showed maximum embryogenesis and proved a better explant source than pith.

**Key words:** *Saccharum officinarum*, Naphthalene acetic acid, 2, 4 dichlorophenoxy acetic acid, apical meristem

## Introduction

Sugarcane (*Saccharum officinarum*) is an important cash crop of Pakistan cultivated over an area of 1029.7 ha (Anonymous, 1999-00). Due to lack of breeding facilities, new varieties of sugarcane are not available and farmers are relying upon imported germplasm, which has already exhausted. Research in Pakistan is handicapped by way of cane breeding facilities. During the past few years, biotechnology has opened new avenues for crop improvement and somaclonal variation is one of these techniques, which may be induced by *in vitro* culturing of tissues or through somatic embryogenesis.

With the rapid expansion of tissue culture technologies came the observation that genetic off-types were occurring in plants regenerated from somatic cells and this was seen as a novel source of variation for plant breeders (Larkin and Scowcroft, 1981). The frequency of off-types occurring in culture varies with species, culture type and number of sub-cultures and has been attributed to a number of alterations that occur within cultured cells (Scowcroft *et al.*, 1987). Somatic embryogenesis in sugarcane has been reportd by many scientist including Kharinarain *et al.* (1996), Pan *et al.* (1989) Somashekharet *et al.* (2000). Leaf segments, lateral buds and pith of sugarcane were used to raise embryogenic calli (Faheem *et al.*, 1999). The cultures media in many instances contained MS salts along with 2,4-D; NAA in various concentration ( Prajapati *et al.*, 2000; Kharinarain *et al.*, 1996) .The importance of plant regeneration through callus culture in sugarcane cannot be emphasized as is that it can provide new ways of improving the plant for salt tolerance, cold hardiness and high yield. Leal *et al.* (1996) reported the somaclonal variation as a source of resistance to eyespot disease of sugarcane. The objective of this study was to investigate the frequency of somatic embryogenesis and the response of different genotypes to callus induction in the local cultivars of sugarcane.

## Materials and Methods

Two sugarcane cultivars SPF-213 and CPF-237 were used in this study. This experiment has been done in Agriculture Biotechnology Institute (ABI) at the National Agricultural Research Centre (NARC), Islamabad, during Sep. to Nov. Different explants i.e., apical meristem, pith and spindle leaves were cultures *in vitro* to study somatic embryogenesis. Size of the apical meristem was 4-5 mm, pith i.e., sub apical region was 6-8 mm in diameter and that of spindle leaves was 10 mm in length and 4-5 mm in diameter.

Explants were sterilized using 20% Clorox (commercial bleach containing 5% v/v sodium hypochlorite) for 20 minutes after which the solution was decanted. The source materials were washed three times with sterilized distilled water for 5 minutes each.

Murashige and Skoog' s medium was used in the present study supplemented with NAA 1.0 mg and 2,4-D in various concentrations i.e., in the range of zero to 5.0 mg/l. Sucrose was added at the rate of 2% w/v. Table 1 shows different concentrations of 2,4-D employed in this study.

Table 1: Concentrations of 2,4-D used and the code given to the media

| Concentration of 2,4-D (mg/l) + NAA (1.0 mg/l) | Media code |
|---|---|
| --- | M1 |
| 0.5 | M2 |
| 1.0 | M3 |
| 2.0 | M4 |
| 3.0 | M5 |
| 4.0 | M6 |
| 5.0 | M7 |

## Results and Discussion

Callus initiation started 18-20 days after inoculation in both the cultivars and variable callus induction frequency was recorded. Callus formed on all combination except M1 and M2 medium (Table 1). At 2 and 3 mg/l, excellent callus induction resulted which showed that callus induction gradually enhanced with increase concentration of 2,4 D.

When leaves were used as explant source in CV SPF –213, callus initiation frequency was excellent on M4 and M5 while percentage frequency of embryo formation was higher in M4 (80%) followed by M5 (70%). In case of CV CPF-237 M4 and M5 showed excellent callus initiation. Somatic embryo formation was higher on M 5(90) than M4 (70) respectively. (Table 2). In both cultivars, a considerable mass of callus was accumulated after five weeks (Fig. 1). The calli, which were compact in texture cremish white in color, were maintained by sub culturing on fresh medium every third week. It has been observed that callus initiated from cut edges of the leaf explants and developed into a full-grown callus with small embryos within 25 days of culture (Fig. 2). Callus formation were noted in all the combination tested except M 1 and M2 which are supplemented with low levels of 2,4-D. Culture media enriched with 2,4-D at the rate of 2 and 3 mg/l showed profuse callus growth. It was also observed that immature leaves have greater ability to produce proliferating embryogenic calli as compared to mature one. Similar results has been reported by Manickavasagam and Ganapathi (1998).

Apical meristem when used as explant source in CV SPF –213 showed initiated callus, which was observed on M4 followed by M5 medium. Higher percentage of somatic embryo formation was seen on M 4(80%) medium than M5 (60%) respectively (Table 3). In case of *CV.* CPF-237, a whitish to cremish calli were observed on M4 (80%) followed by M 5 (60 %). However in case of CV SPF-213, pith failed to produce embryos in any concentration . It was due to excretion of phenols, which turned the whole pith, brown after 15-18 days (Table 2).

Table 2: Frequency of somatic embryos formation from leaf explants of sugarcane cvs. SPF 213 and CPF 237

| Explant | Replicates | Media | Callus initiation frequency | Frequency of somatic embryo formation(%) | Texture |
|---|---|---|---|---|---|
| SPF 213 | 20 | M1 | - | --- | -- |
| | | M2 | - | --- | -- |
| | | M3 | + | 10 | N. C. Yel |
| | | M4 | + + + | 80 | Com W. Col |
| | | M5 | + + + | 70 | Com, W. Co |
| | | M6 | + + | 40 | Com W. Col |
| | | M7 | + + | 10 | N. C. Yel |
| CPF 237 | 20 | M1 | - | --- | N. C |
| | | M2 | - | --- | N. C |
| | | M3 | + + | 40 | Com W. Col |
| | | M4 | + + | 70 | Com, W. Col |
| | | M5 | + + + | 90 | Com, W. Col |
| | | M6 | + + | 60 | Com, W. Col |
| | | M7 | + + | 40 | Com, W. Col |

Table 3: Frequency of somatic embryos formation from apical meristem explants of sugarcane cvs. SPF 213 and CPF 237

| Explant | Media | Replicates | Callus initiation frequency | Frequency of somatic embryo formation(%) | Texture |
|---|---|---|---|---|---|
| SPF 213 | M1 | 20 | - | --- | N. C. Yel |
| | M2 | | + + | 10 | N. C. Yel |
| | M3 | | + | 40 | Com W. Col |
| | M4 | | + + + | 80 | Com, W. Col |
| | M5 | | + + + | 60 | Com, W. Col |
| | M6 | | + + | 40 | Com, W. Col |
| | M7 | | + + | 40 | Com, W. Col |
| CPF 237 | M1 | 20 | - | --- | -- |
| | M2 | | - | --- | -- |
| | M3 | | + | 10 | N. C, Yel |
| | M4 | | + + + | 80 | Com, W. Col |
| | M5 | | + + + | 60 | Com, W. Col |
| | M6 | | + + + | 60 | Com, W. ol |
| | M7 | | + + | 40 | Com, W. Col |

+ + + = Excellent callus + + =Good callus + =Average callus N. C = Non compect, W. Col = White to creme in color, Yel = Yellow in color

Table 4: Frequency of somatic embryos formation from pith explants of sugarcane cvs. SPF 213 and CPF 237

| Explant | Media | Replicates | Callus initiation frequency | Frequency of somatic embryo formation(%) | Texture |
|---|---|---|---|---|---|
| SPF 213 | M1 | 20 | -- | -- | -- |
| | M2 | | -- | -- | -- |
| | M3 | | -- | -- | -- |
| | M4 | | -- | -- | -- |
| | M5 | | -- | -- | -- |
| | M6 | | -- | -- | -- |
| | M7 | | -- | -- | -- |
| CPF 237 | M1 | 20 | -- | --- | -- |
| | M2 | | -- | --- | -- |
| | M3 | | -- | --- | -- |
| | M4 | | + + | 50 | Com, brown |
| | M5 | | + + | 60 | Com, brown |
| | M6 | | + | --- | -- |
| | M7 | | -- | --- | -- |

+ + + = Excellent callus + + =Good callus + =Average callus N. C = Non compect, C. Col =Cremish Color

Sustained multiplication of cultures when pith tissue was used as the explant source was difficult as browning greatly hindered the proliferation (Fig. 3). In CV CPF-237, pith initiated embryogenic calli on M 4(60%) followed by M 5(50%) medium . This difference may be attributed to the cultivars differences. The response of different genotype to callus induction was also studied by Cheema *et al.* (1992).

Leaf explants and buds gave the highest number of somatic

Fig. 1: Leaf callus of SPF-213 after two weeks of culturing on MS medium supplemented with 2 mg/l 2,4-D and 1.0 mg /l NAA

Fig. 2: Embryo formation in CPF-237 leaf on MS medium supplemented with 3.0 mg/l 2,4-D and 1.0 mg /l NAA

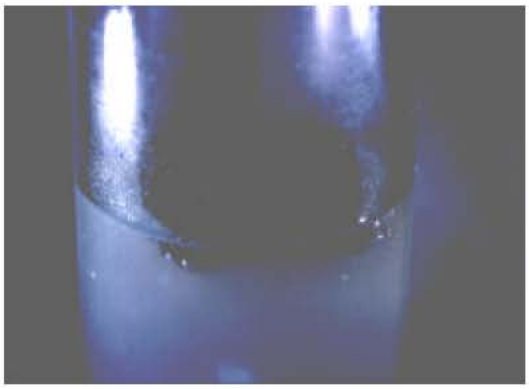

Fig. 3: Pith on MS medium supplemented with 3.0 mg/l 2,4-D and 1.0 mg/l NAA after two week of inoculation

embryos, on M5 and M4 medium respectively (Table 3). It can also be conferred that concentration of 2,4 –D from 2.0 –4.0 mg/l was considered to be the best for somatic embryogenesis resulting white, compact embryogenic calli. Same results have been reported by Himanshu *et al.* (2000). It was also observed that embryogenic calli initiation and proliferation was better in dark while greening of calli and shoots production promoted by 16 h photoperiod light, which means that light, may have a profound effect on metabolism of embryos formation.

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