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# Micropropagation of Sugarcane Through Apical Bud and Axillary Bud

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## ABSTRACT

Micro propagation study of sugarcane (*Saccharum officinarum*) related that for multiplication of desirable clone apical or axillary bud might be used to get easy and faster shoot initiation. By keeping the cultured tube (media portion) under dark, phenolic exudation could be controlled. Genotypic response for shoot initiation, shoot length, shoot multiplication and rooting was different to different media formulation. Maximum survival of explant of SPF 234 and CP 77/400 was observed on M3. M2 was better media than other for CPF 237 and SPF 213. CP43/33 showed survival on wider range of cytokinin (M1 to M4). HSF240 performed well on M5. SPF 213 produced maximum root length (3.20 cm) after 30 days; whereas, minimum root length of (0.43 cm) was observed in varieties CP77/400, with 10 cm shoot length.

**Key Words:** Micro propagation*; Saccharum officinarum*; MS media; Sugarcane cultivars

## INTRODUCTION

Sugarcane (*Saccharum officinarum*) has a paramount importance among the cash crops of Pakistan. Commercially, sugarcane is propagated from stem cutting with each cutting or set having two or three buds. After the development of clone/variety, major bottleneck in spreading of clone or variety is slow propagation rate through conventional method, which takes years.

Hendre *et al.* (1983) described method for rapid multiplication of by using BAP (0.2 $mg^{-1}$) and (0.1 $mg^{-1}$) and 10% coconut milk in liquid media and rooting was achieved in simple medium without hormone. Multiplication rate through conventional method is 1 to 10 within a year (Gosal *et al.,* 1998). He also reported rapid multiplication in liquid media on BAP (0.5 $mg^{-1}$) and Kinetin (0.5$mg^{-1}$) and rooting on NAA (5 $mg^{-1}$) and sucrose (70%). Jadhav *et al.* (2001) established a protocol for micro propagation of sugarcane on MS medium supplemented with BAP, NAA, and IBA and maintained at 23°C under continues light. Lal *et al.* (1996) reported axillary bud proliferation from shoot meristem of sugarcane variety Colk-8102. A maximum of 247 axillary buds per clump was produced after 105 days of culture. Chattha *et al.* (2001) reported micro propagation by culturing axillary and apical buds on MS medium with 1.5 $mg^{-1}$ of BA and GA3. Almost 2500 seedling /plants could be generated from one bud in a 12 week period. At lower concentration of BAP, shoot proliferation was significant. Root development was achieved at IBA (2 $mg^{-1}$), sugar (6%) within 2-3 weeks. Rapid micro propagation is also achieved by Lee (1987) by producing 78408 plantlets in three months. So, through tissue culture new variety could be spread after 1-2 years of release and multiplication of shoot of sugarcane by using BAP (0.2 $mg^{-1}$) and Kinetin (0.1 $mg^{-1}$). Taylor (1994) reported that shoot development was faster from either apical bud /axillary bud than apical meristem, and shoot growth was more rapid from apical bud than apical meristem (Hendre *et al.,* 1983). Sauvaire and Glozy (1978) also used axillary bud for micropropagation of sugarcane as it gives shoot on wider range of media. Taylor and Duke (1993) produced *in vitro* plants of over 200 sugarcane cultivars from apical bud on same media (BAP and Kintin). Present studies were launched to standardize the media for shoot initiation, and evaluation of varieties for multiplication and rooting.

## MATERIALS AND METHODS

**Shoot initiation.** Six recommended varieties HSF-240, SPF-213, SPF-234, CP43/33, CP77/400, CPF237 received from Sugar Cane Research Institute Faisalabad were studied. Spindle explant measuring 0.5 to 1 cm (apical meristem, apical bud, and axillary bud) was excised and put on Murashige and Skoog (1962) media was supplemented with six combination of cytokinin (BAP and Kinetin) as:

| | |
|---|---|
| M1 | (BAP 0. 2 – KIN 0.1) |
| M2 | (BAP 0. 3 – KIN 0.1) |
| M3 | (BAP 0. 5 – KIN 0. 0) |
| M4 | (BAP 0. 5- KIN 0.1) |
| M5 | (BAP 1.0 –KIN 0. 0) |
| M6 | (BAP 1.5 –KIN 0.1) |

Two per cent sucrose added in the media as carbon source and media was autoclaved at 121°C and 15 lbs psi pressure for 20 min. Medium pH was adjusted to 5.7 to 5.8.

The culture were incubated under continues light (24 hrs photoperiod). Ten explants per replication were used. Single rooted plant was transplanted in plastic bags in controlled temperature (27ºC).

**Multiplication.** High cytokinin dose (BAP 0.4 mg $L^{-1}$ and Kinetine 0.4 $mg^{-1}$) was used for the multiplication of the clump of 3-5 shoot per tube.

**Root induction.** Half strength MS medium was used to get the root from six varieties on the same media to see the response of varieties for root length after 30 days.

## RESULTS AND DISCUSSION

Response of different varieties to different cytokinin ratio was significant by different at 0.05 levels, which showed range of variability (12.06 to 23.32%) for CPF 237 and HSF- 240, respectively. This different response might be due to: (1) Juvenile or old tissue (2) Position of explant on the plant (3) Time of the year (4), Endogenous level of the hormones (5) Size of explant (6) Physical growth factor (Pierik, 1997). In addition to that interaction of different factors (light and cytokinin, darkness and temperature) makes the over all picture very complicated. Hendre *et al.* (1983) obtained shoot of sugarcane cultivars on different BAP and kinitin concentrations. Maximum survival of explant of SPF 234 and CP 77/400 was observed on M3. M 2 was better media than other for CPF 237 and SPF 213. CP43/33 showed survival on wider range of cytokinin (M1 to M4). HSF240 performed well onM5 (BAP 1.0 - kin 0.1). Different varieties give shoot on different media with different survivability rate. It may be due to the variable presence of cytokinin in bud. George (1993) reported that young tissue had more cytokinin than the old one with other factors mentioned above. It is possible variable quantity of cytokinin plays the major role in the adjustment of cytokinin in e media for getting the shoot (Table I). Another problem of getting shoot is concentration of phenolic excreted by explants, which restrict the nutrient availability. Phenolic was controlled as mentioned in material and method. But different varieties give different response to media for phenolic excretion.

**Shoot intiation.** CP237 took minimum days (13) to give shoot initiation (Table II) and maximum days were taken by variety HSF240 (19) to give shoot. Variation in shoot initiation may be due to factors mentioned above and may be adjusted by cytokinin and other factors needed to get shoot. Chattha *et al*. (2001) and Jadhav *et al.* (2001) reported also different genotype gives shoot on different media.

**Shoot elongation.** Maximum shoot length (7 cm) was measured in varieties CP 43/33; where as minimum shoot length after 30 days was observed in SPF 234 (2 cm). Different shoot length was observed in different varieties after 30 days (Table II). It has been observed that varieties with shorter shoot length, formed more shoots per tube in a cycle. It might be due to the excess of cytokinin, which suppressed shoot elongation.

**Multiplication of shoot.** Maximum shoots after 30 days was observed in the variety SPF 234 (29 shoot) per cycle and minimum shoot was observed SPF 77/400 (18 shoot). It was observed that SPF 234 proliferated rapidly but observed stunted growth. For such varieties, shoot clump should be cultured on half strength MS to get longer shoot. Gosal *et al.* (1998) used high dose of BAP and kinitin to get faster multiplications. In present study, it is observed that different genotypes responded differently to same media. These results are in line with Lal *et al.* (1996) who also reported potential axillary bud proliferation of variety Colk- 8102.

**Effect of shoot length on rooting.** To study root induction, two types of shoot lengths were placed on half strength MS media. SPF 213 produced maximum root length (3.20 cm) after 30 days; whereas, minimum root length of (0.43 cm) was observed in varieties CP77/400 with 10 cm shoot length (Table III). On the other hand, when 5 cm length shoots were cultured on media, maximum roots were given by same variety SPF213; while minimum root length (0.16 cm)

**Table I. Response of different varieties to different Cytokinin concentration for survival (number)**

| Media | HSF240 | CPF237 | SPF213 | SPF234 | CP77/400 | CP43/33 |
|---|---|---|---|---|---|---|
| M1 | 3ab | 2ab | 5b | 7ab | 2b | 6a |
| M2 | 2b | 3a | 6a | 6bc | 2b | 6a |
| M3 | 2b | 2ab | 5b | 8a | 3a | 6a |
| M4 | 3ab | 1b | 2c | 5cd | 2b | 6a |
| M5 | 4a | 2ab | 2c | 4d | 3a | 4b |
| M6 | 3ab | 1b | 1d | 5cd | 2b | 5ab |
| LSD | 1.02 | 1.39 | 0.72 | 1.18 | 0.75 | 1.09 |
| C.V | 23.33 | 19.73 | 17.32 | 17.20 | 11.13 | 12.06 |

M1 (BAP0.2 –Kin 0.1); M2 (BAP0.5 – Kin 0.0); M3 (BAP 1.0 – Kin 0.0) M4 (BAP 0.3 – Kin 0.0) M5 (BAP 0.5 – Kin 0.1); M6 (BAP 1.5 – Kin 0.1)

**Table II. Effect of high dose of Cytokinin on different varieties for root initiation, shoot length and multiplication**

| Varieties | Root initiation (days) | Shoot length (cm) after 30days | Multiplication After30days (number) |
|---|---|---|---|
| HSF240 | 19 a | 4d | 28ab |
| SPF213 | 18ab | 3e | 26b |
| SPF234 | 17b | 2f | 29a |
| CP43/33 | 15c | 7a | 20c |
| CP77/400 | 14cd | 6b | 18c |
| CPF237 | 13d | 5c | 19c |
| LSD | 1.13 | 0.72 | 2.54 |

**Table III. Response of different varieties to shoot length on half strength MS media**

| Varieties | 10 cm | 5 cm |
|---|---|---|
| HSF 240 | 1.33 bc | 0.26 d |
| SPF213 | 3.20 a | 1.33bc |
| SPF237 | 0.96c | 0.70d |
| CP77/400 | 0.43d | 0.20d |
| SPF234 | 1.36b | 0.16d |
| CP43/33 | 1.5b | 0.46d |
| LSD | 0.18 | |

was observed in variety SPF 234. Hendre *et al.* (1983) also got roots on media without any hormone.

## CONCLUSION

From the present studies, it is concluded that an efficient protocol is needed for every new variety or clone to get rapid shoot initiation, shoot multiplication and root induction and elongation. Maximum survival of explant of SPF 234 and CP 77/400 was observed on M3. M2 was better media than other for CPF 237 and SPF 213. CP43/33 showed survival on wider range of cytokinin (M1 to M4). HSF240 performed well on M5. SPF 213 produced maximum root length (3.20 cm) after 30 days; whereas, minimum root length of (0.43 cm) was observed in varieties CP77/400, with 10 cm shoot length.

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