**SHORT COMMUNICATION**

# EFFECT OF SUBCULTURING PERIOD ON SHOOT MULTIPLICATION RATE IN SUGARCANE MICROPROPAGATION

**Swapanil Yadav[1*], Aquil Ahmad[1], G.N. Gupta[2] and Madan Lal[2]**

**Abstract**

An experiment was carried out to study the effect of subculturing period on shoot multiplication rate in micropropagation of sugarcane variety CoSe 01235. The established shoot cultures were subcultured at 5, 10, 15 and 20 days intervals upto 60 days. The results showed that maximum shoots (1744) were produced after 60 days of multiplication when the cultures were subcultured at 15 days interval. Similarly, the average shoot length recorded in cultures subcultured at 15 and 20 days intervals was higher compared to other treatments. The results indicated that 15 days interval is the optimum period of subculturing to enhance the rate of multiplication and produce more number of elongated and healthy shoots in a seed multiplication programme through in vitro micropropagation.

**Key words:** Sugarcane, micropropagation, subculturing

Conventionally, sugarcane is propagated through setts containing two or three buds. A number of high yielding and high sugared varieties have been developed which have the yield and recovery potential greater than the national averages. However, due to slower seed multiplication ratio through conventional sett propagation method, it takes several years for the varieties to spread in large areas. By the time the varieties attain their peak area, they start deteriorating in yield and quality traits due to degeneration. In vitro micropropagation technique is emerging as a complimentary tool to conventional methods of seed multiplication and currently there is considerable interest in applying this technique for not only the multiplication of newly released varieties of sugarcane for rapid spread over a large area but also the production of disease free seedlings. Various protocols for in vitro micropropagation of sugarcane have been described by several investigators during the past decades (Sauvaire and Glazy 1978; Hendre et al. 1983; Lee 1987; Sreenivasan and Sreenivasan 1992; Lal and Singh 1994; Shukla et al. 1994; Ramanand and Lal 2004; Vinayak et al. 2009; Yadav et al. 2014). Earlier studies have indicated that the in vitro morphogenetic responses in plants, being under the influence of plant growth regulators, are cultivar dependent. The regeneration responses are highly influenced by varied microclimate in growth room and also by different subculturing methods and practices which enormously affect the rate of shoot multiplication. Work has been done on some of the factors mentioned above (Ramanand 2006; Yadav et al. 2014) but information on the effect of subculturing period on the rate of shoot multiplication is scanty. The present work was undertaken to investigate the effect of period of subculturing on the rate of shoot multiplication and shoot growth

Swapanil Yadav[1*], Aquil Ahmad[1], G.N. Gupta[2] and Madan Lal[2]
[1]Department of Botany, Gandhi Faiz-e-Aam (P.G.) College, Shahjahanpur, India
[2]U.P. Council of Sugarcane Research, Shahjahanpur, India
*Email : swapanilgfc@gmail.com

during in vitro micropropagation of the sugarcane variety CoSe 01235.

Fresh tops of the early maturing sugarcane variety CoSe 01235, popularly known as *Rapti*, were collected from 8-10 month old healthy plants at the Sugarcane Research Institute, Shahjahanpur farm. After removing all the open green leaves from the tops, about 6 cm long spindle segments were dissected out and washed under running tap water for about half an hour. The segments were rinsed with 1% aqueous detergent solution for 5 min followed by thorough washing with tap water. The washed segments were dipped in 70% ethanol for 10 sec and again rinsed with sterile distilled water several times. Finally, these segments were surface sterilized with 0.1% aqueous mercuric chloride ($HgCl_2$) solution for 10 min followed by several washings with sterile distilled water. About 1.0 cm long shoot tip explants containing apical dome alongwith one to two leaf primordia were carefully excised from the sterilized segments and immediately inoculated on agar solidified (0.8%) MS medium (Murashige and Skoog 1962) supplemented with BAP (0.5 mg/l), Kinetin (0.5 mg/l) and sucrose (30 g/l) for shoot initiation. The pH of the medium was adjusted to 5.8 before autoclaving. Cultures were incubated at 25±2ºC under 16 h illumination of 4000 lux provided by cool white fluorescent tubes. The established shoot cultures were multiplied and used for experimentation. Four sets of 20 bottles each were made and shoot cultures were subcultured at 5, 10, 15 and 20 days interval in 12, 6, 4 and 3 cycles, respectively. Thus, the total period of multiplication in each set was 60 days. The cumulative number of shoots produced after 60 days was recorded and the multiplication ratio was calculated in terms of number of shoots produced from one shoot per cycle considering 4 cycles of 15 days in each case.

The results enumerated in Table 1 showed that a maximum of 1744 shoots were produced after 60 days of multiplication when the cultures were subcultured at 15 days interval followed by those subcultured at 20 days interval (1636 shoots). The subculturing of shoot cultures at 5 and 10 days intervals gave 1232 and 1560 shoots, respectively after 60 days of micropropagation indicating that frequent subculturing of cultures reduces the total number of shoots significantly (Table 1). Daily observations of shoot cultures indicated active growth of shoots and proliferation of new shoots between 6 and 15 days of subculturing in most of

**Table 1. Effect of days of subculturing on rate of shoot multiplication in the sugarcane variety CoSe 01235**

| Parameter | Days of subculturing | | | |
|---|---|---|---|---|
| | 5 | 10 | 15 | 20 |
| Total number of subcultures | 12 | 6 | 4 | 3 |
| No. of cycles of 15 days each | 4 | 4 | 4 | 4 |
| Total number of shoots produced after 4 cycles (60 days) of multiplication | 1232 ± 46 | 1560 ± 58 | 1744 ± 72 | 1636 ± 68 |
| Multiplication ratio per cycle | 1 : 4.98 | 1 : 5.28 | 1 : 5.43 | 1 : 5.34 |
| Average shoot length* at the last subculture (cm) | 4.1 ± 0.4 | 4.6 ± 0.5 | 5.9 ± 0.5 | 5.8 ± 0.4 |
| Shoot vigour at the last subculture | Poor | Moderate | Good | Good |

*Shoots less than 3.0 cm in length were not considered

the cultures. This indicated that the cultures require 4 - 5 days to withstand the stresses of separation and media change after subculturing and thereafter start rapid growth and proliferation of new shoots which last up to about 15 days. Gradual yellowing of media as well as some leaves of shoot cultures was observed after 15 days of subculture. This indicated that the medium gets exhausted within 15 days and the shoot cultures need to be transferred to fresh media (Ramanand 2006).

A perusal of data revealed that the highest multiplication ratio (1:5.43) was obtained in cultures subcultured at 15 days interval followed by a ratio of 1:5.34 in cultures subcultured at 20 days interval. Similarly, the highest average shoot length (5.9 cm) was recorded in cultures subcultured at 15 days interval and was on par with that observed in those subcultured at 20 days interval (5.8 cm). The results revealed that frequent subculturing reduced shoot length significantly. Shoots produced in cultures subcultured at 15 or 20 days interval were more vigorous as compared to those subcultured at 5 and 10 days intervals.

Days of subculturing or the incubation period of a subculture had noticeable effect on rate of shoot multiplication. The highest number of shoots could be produced after 60 days of multiplication when the cultures were subcultured at 15 days interval followed by 20 days interval. These results showed that frequent subculturing of shoot cultures reduces the total number of shoots, shoot length and multiplication ratio. The optimum incubation period was 15 days to enhance the rate of multiplication and produce more number of elongated healthy shoots in a seed multiplication programme through in vitro micropropagation.

## Acknowledgement

Authors are thankful to the Director, U.P. Council of Sugarcane Research, Shahjahanpur, for providing the laboratory facilities.

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