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Effect of Auxin, Sucrose and pH Level on *in vitro* Rooting of Callus Induced Micro Shoots of Sugarcane (*Saccharum officinarum*)

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Abstract: Microshoots were cultured on MS medium supplemented with different combinations of auxin, sucrose and pH level for ***in vitro*** rooting of callus induced sugarcane (***Saccharum officinarum)*** variety namely Isd 31. Among the types and concentrations of auxin used the best rooting in all the microshoots was obtained with half strength (major salts) MS basal medium supplemented with NAA at 5.0 mg l^{-1}, sucrose at 50 g l^{-1} and pH level 5.7 were the optimum conditions for rooting when microshoots were incubated 25± 2°C under 16 h photo period regime. The regenerated plantlets were successfully transferred to soil and the percentage of survivability under ***ex vitro*** conditions was 75.

Key words: *In vitro*, microshoot, auxin, sucrose, root, plantlets

Introduction

Sugarcane (***Saccharum officinarum***) is mainly propagated vegetatively by stem cuttings, this conventional process of propagation (stem cutting) is very slow. Moreover, different types of diseases make the cultivars degenerate gradually. Sugarcane seed (seed cane) production through micropropagation method is suitable and effective for rapid propagation in comparison to conventional method. Initial attempts to regenerate plants through ***in vitro*** technique were made on sugarcane by Nickell (1964) and Heinz and Mee (1969). Protocols for ***in vitro*** plant regeneration of sugarcane through callus culture, axillary bud and shoot tip culture have been developed by many authors (Barba ***et al.***, 1977; Sauvaire and Glazy 1978; Heinz ***et al.***, 1977, Lee 1987; Baksha ***et al.***, 2002). One of the major obstacles to the micropropagation of plants in ***in vitro*** is the rooting of the plantlet. Under proper conditions, the differentiation of either shoots or roots from callus of most sugarcane varieties is easily obtained (Heinz and Mee, 1969). However, obtaining roots from the differentiated shoots is more difficult. The failure of callus-derived plantlets to root results in a loss of 20 to 30% of all plants produced (Heinz ***et al.***,1977). Although, considerable advancement in tissue culture systems for micropropagation has been achieved, but there are a few information regarding to the factors that influence ***in vitro*** plant growth and development and such information could provide successfully established commercial micropropagation of sugarcane. Therefore, the present investigation has been undertaken to determine the optimum doses of auxin, sucrose and pH level of the rooting medium for inducing healthy roots and makes micropropagation method economically viable and technically feasible.

Materials and Methods

The experiment was conducted at Breeding Division (Germplasm Laboratory) in Bangladesh Sugarcane Research Institute (BSRI), Ishurdi, Pabna, Bangladesh during the period of 2001-2002. The plants of sugarcane variety Isd 31 developed by BSRI, Ishurdi, Pabna, Bangladesh was used as experimental material in the present study. MS (Murashige and Skoog, 1962) medium with half strength of major salts and minor salts and vitamins was used for different rooting experiments. Microshoots prepare from proliferating shoot cultures, established ***in vitro*** from leaf sheath explants were cultured. Filter paper bridges were used in all cases. Media used for experiments on sugar concentrations was supplemented with 10-60 g l^{-1} and for experiments on pH level were adjusted at 4.0 to 6.0 before sterilization and autoclaved at 120°C for 15 min. All Experiments were incubated at 25±2°C under 16 h photo period regime. There were 15 to 20 replicates per treatment and the experiment was replicated two times and the means and standard errors of the results were calculated.

Results and Discussion

The percentage of microshoots rooted, number of roots and length of roots per culture was influenced by the different types and concentrations of the auxin used (Table 1). Appropriate amounts of auxin in the rooting medium are crucial for root induction. Among three auxins, NAA at 5 mg l^{-1} produced highest percentage of rooting. The frequencies of root formation for the variety Isd 31 were 95% on the medium with 5 mg l^{-1} of NAA (Fig. 1). The highest number of roots per shoot 17.0±0.3 and the average length of the root was 4.5±0.5 cm in variety Isd 31. Among the concentrations tested, all the three auxins showed better rooting response within the range of 2.5 to 5.0 mg l^{-1}. Beyond this range the auxins either produced very week root or excessive callus formation at the cutting bases (Fig.2). This effect was more remarkable in the cases of IAA and IBA. These results agree well with the previous findings of Nadar and Heinz (1977), Lal (1992) who reported that preferred auxin for root initiation was NAA. Root developed in medium containing lower concentration (1,0 mg l^{-1}) of NAA was poor in quality. Shenk and Hildebrandt (1972) have also reported requirement of high concentration of auxin for rooting in sugarcane.

Besides serving as carbohydrate source sucrose regulate the osmomolarity of the culture media and also plays a role during morphogenesis (Sopory, 1979). Results of the experiments on different concentrations of sucrose (Table 2) show that sugarcane micro shoots originated from callus inducted tissue could be rooted on media containing a wide range (0 to 60 g l^{-1}) of sucrose concentration. However, the rooting percentages on media containing no sucrose or very high level concentrations (60 g l^{-1}) of sucrose were lower. Among the various concentration tested 5% sucrose appeared to be optimum for rooting. Subculture on full or half strength MS medium containing 6 to 8% sucrose was found sufficient for obtaining intense root formation (Maretzki and Hiraki 1980). Barba ***et al.*** (1977) also reported that for root development requires higher sugar levels in nutrition media.

Another phase of experiment was to determine the effect of different pH level on induction and growth of adventitious roots. The pH of medium may be a limiting factor for growth and in general should be between 5.5 and 5.8. A downward shift of the pH during autoclaving should

Fig. 1: A plantlet with well developed roots formed in medium containing 5.0 mg l^{-1} NAA and 50 g l^{-1} sucrose after three to two weeks of culture

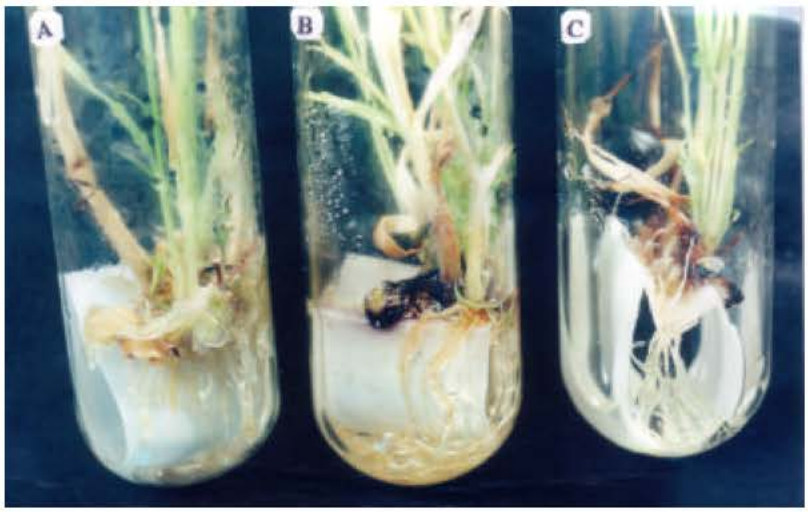

Fig. 2: Effects of different type of auxin and 60 g l^{-1} sucrose on root formation from microshoots. (A) Root formation on medium with 7.5 mg l^{-1} of IBA (B) with 7.5 mg l^{-1} of IAA and © with 7.5 mg l^{-1} of NAA after two to three weeks of culture

be taken in to account (Quak, 1977). After 2 to 3 weeks of culture at different levels of media pH, days to rooting, percentages of culture rooted, length of root per culture were recorded (Table 3). Among these pH levels the highest percentage of explant showing (100%) rooting was recorded on the medium adjusted to pH 5.5 to 5.7 and plants showed poor quality at pH 4.0-5.0. Mellor and Stace-Smith (1969) observed that the pH of the medium dropped with in a week from

Table 1: Effects of auxin type and concentrations on rooting of *in vitro* proliferated micro shoots of sugarcane variety lsd31

Auxin	conc. mg l^{-1}	Days to rooting	% of cultures rooted	Mean of root/culture(±S.E)	Mean length (cm) of root/ culture(±S.E)
NAA	1.0	10	55	5±0.2	1.5±0.1
	2.5	7	75	12±0.5	3.3±0.5
	5.0	5	95	17±0.3	4.5±0.5
	7.5	8	70	8±0.2	3.1±0.1
IAA	1.0	14	30	3±0.5	1.2±0.5
	2.5	12	60	10±0.2	2.3±0.5
	5.0	10	70	12±0.2	2.8±0.1
	7.5	12	+++	-	-
IBA	1.0	12	45	5±0.5	1.5±0.5
	2.5	10	75	10±0.4	2.8±0.1
	5.0	8	85	14±0.5	3.5±0.5
	7.5	10	+	-	-

Sucrose and pH level were at 50 g l^{-1} and 5.7 respectively

Rating scale of callus: + = Slight, ++ += Considerable

Table 2: Effect of sucrose concentrations on rooting of *in vitro* differentiated shoots of sugarcane

Sucrose conc. g l^{-1}	Days to rooting	% of cultures rooted	Mean of root/ culture(±S.E)	Mean length (cm) of root/culture(±S.E)
Nil	12	20	1±0.2	1.0±0.2
10	12	30	3±0.5	1.5±0.5
20	10	45	5±0.3	3.0±0.2
30	7	75	8±0.2	3.8±0.4
40	7	83	12±0.5	4.0±0.2
50	5	95	17±0.2	4.5±0.2
60	8	75	10±0.2	3.7±0.2

NAA and pH level were 5.0 mg l^{-1} and 5.7 respectively

Table 3: Effect of different pH level on rooting of *in vitro* differentiated shoots of sugarcane

pH level	Days to rooting	% of cultures rooted	Mean of root/culture(±S.E)	Mean length (cm) of root/culture(±S.E)
4.0	12	30	1±0.2	1.0±0.5
4.5	12	45	2±0.5	1.3±0.5
5.0	10	75	7±0.3	1.5±0.2
5.5	7	83	12±0.5	3.0±0.5
5.7	5	95	16±0.5	4.5±0.2
6.0	8	78	8±0.3	1.8±0.2

Sucrose and NAA used were 50 g l^{-1} and 5.0 mg l^{-1} respectively

5.7 to 5.4. Rooting was inhibited on the media with a low initial pH. Dannis and James (1993) also reported that for rooting of shoots may be affected by pH level and auxin level in the root induction media.

Root generally requires two-three weeks to achieve healthy plantlets. Healthy and well-established ***in vitro*** regenerated plantlets were transferred to small pots containing mixture of soil and sand (2:1) for future establishment. Among the regenerates transplanted 70% of them survived and acclimatized successfully on the soil. Induction and development of roots at the

base of ***in vitro*** grown microshoots is an indispensable step to establish tissue culture derived plantlets on the soil. Although, auxin plays important role, other factors like appropriate sucrose and pH level on the nutrient media enhanced the induction and root formation affecting the percentage of root and the number of root per explant suggesting the possible involvement. From the present report showed that the MS medium containing 5.0 mg l^{-1} NAA, sucrose 50 g l^{-1} and pH at 5.7 proved more effective for healthy and profuse root formation. The protocol described here may be used for commercial purpose as it reduced time on root formation, development and higher establishment percentage of ***in vitro*** derived planlet at a faster rate in a shorter period time.

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