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Effects of Liquid Medium on Rooting and Acclimation of Regenerated Microshoots of Banana (*Musa sapientum* L.) cv. Sagar

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Abstract

A regeneration protocol ensuring a high frequency rooting of micro-shoots derived from apical and axillary buds of suckers of banana cv. Sagar was achieved by using liquid medium. When the explants were cultured on MS medium supplemented with 0.5 mg/l each of BA, Kn and NAA, a large number of shoots developed. With the progression of the number of subcultures, shoot proliferation was enhanced. Addition of 10 % coconut water to the medium increased the number of shoots per culture and growth of individual shoots. Shoots rooted well within two weeks, when they were separated individually and sub-cultured in 1.0 mg/l IBA supplemented half strength MS liquid and agar-gelled medium but rooting percentage and their growth were superior in the liquid medium to agar-gelled medium. Rooted plantlets were transferred to small polythene bags containing sterile sand, soil and humus (1 : 2 : 1) and maintained with a high humidity for acclimation. Acclimation and transplantation performances were found to be superior in plants that were rooted in the liquid medium.

Introduction

Bananas (*Musa* spp.) constitute one of the oldest fruits of the world (Singh 1969). For millions of people in developing countries in the tropics and subtropics, plantains and bananas (*Musa* spp.) provide a basic food source of great importance (Daniels 2002). The largest banana-producing regions in the world are Africa and Latin America, which represent 74.2 and 22.5 % of the world production, respectively followed by Asia with 3.3 % (Rodriguez and Rodriguez 2001). In Bangladesh bananas are grown all over the country and the variety Sagar is very popular and grown widely in the greater districts of Jessore, Kushtia, Dhaka, Rajshahi, Mymenshingh, Rangpur and Dinajpur. But its production per unit area is low in comparison with other banana producing countries of the world due to the diseases and lack of high yielding strains. Moreover, every year a large number of banana crop fields are destroyed due to flood or heavy rainfall. In Bangladesh, farmers usually use conventional banana

suckers as planting materials. Generally four-five suckers are obtained per plant which are insufficient to replace the infected or affected farms with healthy and disease free planting materials. The area under cultivation as well as per hectare production could be increased if appropriate technologies supplement conventional practices. Tissue culture has been proven as a potential technology to produce millions of identical plantlets, which are disease free and true to parental type. Through this technology, (*Musa sapientum* cv. Sagar) from a single shoot tip or an axillary bud, a large quantity of uniform and disease free plants with good genetic potential can be produced within a short time (Vuylsteke and Langhe 1985; Wong 1986). The protocol for micropropagation of bananas has been achieved during the past two decades. While Banerjee and De Langhe (1985); Vuylsteke 1989 and Israili et al. (1995) used shoot tips, others chose floral apices for *en masse* multiplication (Cronauer and Krikorian 1984a; Cronauer and Krikorian 1985a, 1985b; Dore and Shijiram 1989 and Azad and Amin 2001). There are also reports of somatic embryogenesis and regeneration in liquid medium (Novak et al. 1989; Daniels et al. 2002). Due to its soft and delicate root system, the rate of acclimation of *in vitro* regenerated banana plants is not satisfactory. This paper reports results of studies of an important parameter, namely a high rate of acclimation, using liquid medium in inducing rooting in *in vitro* regenerated local banana cultivars.

Materials and Methods

Four-six weeks old suckers were collected from healthy plants growing in cultivated fields in Kaliganja Upajila of Jhenaidah district. The suckers were brought to the laboratory and washed thoroughly under running tap water. Outer leaves with leaf-sheaths of the suckers were peeled off until the size became to 5-6´ × 2-3 cm. Surface sterilization was carried out with 70% (v/v) alcohol for 15 min, after which they were rinsed three times with sterile water in a laminar air-flow cabinet. The plant materials were then dipped in 0.2 % $HgCl_2$ solution for ten min. followed by washing three times with sterile distilled water to wash out any trace of $HgCl_2$. After surface sterilization these were trimmed 2 - 3 mm from both ends and the outer sheath was peeled off. The plant materials were then longitudinally dissected into two unequal halves of which one half contained apical and axillary shoot buds and the other half contained only axillary shoot buds. The individual explants were cultured in agar gelled (0.7%) MS nutrient medium containing different concentrations and combinations of cytokinins, auxins and CW. For rooting, half strength MS supplemented with auxin was used individually in two supporting materials, one with 0.7% agar and another in liquid substratum. Three per cent sucrose was used in all media preparations. The media were adjusted to pH 5.8, and autoclaved for 20 min at

121^{0}C under 1.1 kg/cm^2 pressure. Cultures were incubated at 25 ± 1^{0}C under 16 h photoperiod with a light intensity of 3000 - 4000 lux.

Results and Discussion

Apical and axillary buds of *Musa sapientum* cv. Sagar were excised aseptically and cultured on MS nutrient medium supplemented with different concentrations and combinations of cytokinins and auxins for induction and multiplication of shoots. Induction of shoots were observed in media containing BA or Kn alone and in combination with auxin but the number of induced shoots in explants varied according to the concentration of cytokinin or auxin. Among different concentrations and combinations used, the combination 0.5 mg/l BA + 0.5 mg/l Kn + 0.5 mg/l NAA was found to be the best; that is 90% explants developed shoots within eight weeks with 8 shoots per culture. The average shoot length was 6.5 cm (Table 1, Fig. 1). The induced shoots were subcultured individually and it was observed that with the progression of the number of subcultures, the number of shoot proliferation increased. The shoots continued to proliferate through eight or more subcultures with an average of 12 shoots per transfer. For further improvement of the medium coconut water (5 - 15 %) was added. When 10% coconut water was added to the medium, the number of shoots increased up to 18 and the length of the shoots was found to be 7.2 cm (Table 2, Fig. 2).

Regenerated shoots were taken out carefully from the culture vessels and individual shoots were separated and implanted in the rooting media containing half strength MS with different concentrations (0.2 - 2.0 mg/l) and combinations of auxins (IBA and NAA) individually in liquid and agar-gelled media. Effects of supporting materials and auxins on percentage of root formation, number of roots per shoot and days required for root induction were recorded up to four weeks of culture. The rooting response differed according to supporting materials and concentration and combination of auxins used (Table 3). Among the two supporting materials used, the liquid medium was found to be superior considering the frequency of rooted shoots and the number of roots per shoot. Among the auxins used, IBA was found to be the best for root induction and among various concentrations, 1.0 mg/l IBA proved to be optimum in which 100 shoots rooted within 10 - 15 days with 5 - 6 and 10 - 12 roots per shoot cultured in agar-gelled and liquid media, respectively (Table 3, Fig. 3). For acclimation and transplanting in the field the culture vessels containing rooted shoots were kept in room temperature with indirect day light for seven days. The plantlets were then taken out from the culture vessels and washed thoroughly under running tap water and planted in small polythene bags containing sterile sand, soil and humus (1 : 2 : 1). The plantlets were covered by transparent polythene sheet to maintain high humidity.

Table 1. Effects of different concentrations and combinations of cytokinins and auxins on shoot induction and proliferation of banana from apical and axillary shoot buds of suckers. Data were recorded after eight weeks of culture.

Growth regulators (mg/l)	% of explants producing shoots	No. of shoots per culture*	Average length of shoots (cm)*
BA + Kn + NAA			
0.5+ 00 + 00	26.0	2.4 (0.82)	3.2 (0.65)
1.0+ 00 + 00	38.0	2.2 (0.85)	3.1 (0.85)
2.0+ 00 + 00	32.0	3.3 (1.58)	4.5 (0.69)
0.0+ 0.5 + 00	20.0	4.5 (0.95)	4.8 (0.59)
0.0+ 1.0 + 00	28.0	4.3 (1.42)	4.7 (0.85)
.00 + 2.0 + 00	25.0	3.7 (0.85)	4.3 (0.75)
0.5+ 0.5 + 00	40.0	4.5 (1.95)	5.8 (0.65)
0.5+ 0.5 + 0.5	90.0	8.5 (3.55)	6.5 (0.95)
1.0 + 00 + 0.5	75.0	6.5 (2.85)	5.2 (0.75)
00 + 1.0 + 0.5	60.0	5.2 (2.35)	4.9 (0.85)
BA + Kn + IAA			
0.5 + 0.5 + 0.5	65.0	4.5 (1.85)	5.2 (0.84)
1.0 + 00 + 0.5	40.0	3.5 (1.25)	4.2 (0.75)
0.0 + 1.0 + 0.5	35.0	3.5 (0.85)	4.0 (0.96)
BA + Kn + IBA			
0.5 + 0.5 + 0.5	60.0	4.5 (1.25)	4.6 (0.87)
1.0 + 00 + 0.5	45.0	2.5 (1.09)	4.3 (0.63)
0.0 + 1.0 + 0.5	40.0	2.2 (0.89)	4.1 (0.79)

*Standard error in the parenthesis.

Table 2. Effects of different concentrations of CW in MS medium with 0.5 mg/l each of BA, Kn and NAA on number and growth of shoots proliferated from explants of banana. Data were recorded after four weeks of culture.

Conc. of CW (%)	No. of shoots per culture*	Average length of shoot (cm)*
5	14.5 (2.56)	6.8 (0.75)
10	18.4 (2.86)	7.2 (0.96)
15	15.7 (2.93)	6.3 (0.64)

*Standard error in the parenthesis.

Under this condition, within 15 - 20 days new leaves emerged from about 55 and 95% of the plantlets. These were rooted in agar-gelled and liquid media, respectively. In a few days new growth resumed. After three months the plants were transplanted in the open field. Nearly all transplanted survived and grew satisfactorily.

Figs. 1-3: *In vitro* regeneration of banana (cv. Sagar) plantlets from apical and axillary buds of suckers. 1. Shoot bud induction in MS with 0.5 mg/l each of BA, Kn and NAA in eight weeks. 2. Enhanced shoot proliferation subcultured in MS + 0.5 mg/l BA + 0.5 mg/l Kn + 0.5 mg/l NAA + 10% CW in four weeks. 3. Rooting of *in vitro* regenerated shoots cultured in half strength MS liquid medium with 1.0 mg/l IBA in four weeks.

Tissue culture from apical and axillary buds is currently being used for clonal propagation of horticultural plants. The results of the present investigation clearly show that apical and axillary buds of suckers from selected plants are capable of producing multiple shoots *in vitro,* which can be rooted to form complete plantlets. Superiority of BA over other cytokinins for multiple shoot

formation in banana has been reported (Cronauer and Krikorian 1984a, 1984b; Novak et al. 1989; Abdullah et al. 1998; Mohammad et al. 2000). Habiba et al (2002) reported that MS medium in combination with 4 mg/l BA + 2.0 mg/l NAA + 13% CW was optimum to generate highest number of shoot regenerants in banana from sucker explants, whereas Azad and Amin (2001) developed a medium for regeneration of banana from excised floral apices. The medium they used was MS + 2.0 mg/l BA + 1.0 mg/l Kn + 1.0 mg/l IAA + 15% CW. In the present experiments, it was observed that MS with 0.5 mg/l BA + 0.5 mg/l Kn + 0.5 mg/l NAA + 10% (v/v) CW was optimum for maximum number (18) of shoot regeneration from sucker explants. Different results obtained by different authors might be due to differences in their genotypes and explants.

Table 3. Effects of various concentrations of different auxins in half strength MS on adventitious root formation of *in vitro* raised banana shoots. Twenty explants were taken for each treatment and data were recorded four weeks after culture.

Auxins (mg/l)		Liquid medium			Agar gelled-medium		
IBA	NAA	% of shoots rooted	No. of roots/ shoot*	Days required for rooting	% of shoots rooted	No. of roots/ shoot*	Days required for rooting
0.2	00	55.0	5.1 (0.35)	20-25	45.0	3.5 (0.35)	20-25
0.5	00	81.0	5.6 (0.42)	15-20	75.0	3.9 (0.65)	15-20
1.0	00	**100**	**10.3(1.09)**	**10-15**	**100**	**5.2 (1.34)**	**10-15**
1.5	00	95.0	5.3 (1.03)	10-15	85.0	4.1 (1.23)	10-15
2.0	00	71.0	5.2 (0.98)	15-20	65.0	4.2 (0.92)	15-20
00	0.2	45.0	4.2 (0.76)	20-25	30.0	2.9 (0.54)	20-25
00	0.5	48.0	4.6 (0.85)	20-25	45.0	3.2 (0.83)	20-24
00	1.0	75.0	5.4 (0.75)	15-20	70.0	3.9 (0.91)	15-20
00	1.5	95.0	5.4 (0.77)	10-15	90.0	4.8 (1.22)	10-15
00	2.0	80.0	5.7 (0.78)	15-20	75.0	4.1 (0.82)	15-20
0.2	0.2	75.0	5.8 (0.68)	15-20	60.0	3.6 (0.61)	15-20
0.5	0.2	80.0	5.1 (0.57)	15-20	75.0	4.0 (0.33)	15-20
0.5	0.5	65.0	4.8 (0.65)	15-20	50.0	3.2 (0.87)	15-20
1.0	0.5	55.0	4.5 (0.74)	20-25	45.0	3.0 (0.91)	20-25
1.0	1.0	45.0	3.8 (0.68)	20-25	40.0	3.0 (0.51)	20-25
1.5	1.0	45.0	3.8 (0.86)	20-25	30.0	2.8 (0.82)	20-25

*Standard error in the parenthesis.

For the induction of roots in *in vitro* raised shoots of banana, De Langhe (1985) and Novak et al. (1990) used half strength MS + 1.0 mg/l IBA, whereas Cronauer and Krikorian (1994a) used auxin-free MS for rooting of banana microshoots. On the other hand, Banerjee et al. (1986) and Azad and Amin (2001) obtained rooted banana shoots in half strength MS agar-gelled medium

supplemented with 0.2 mg/l IBA. In the present investigation it was observed 1.0 mg/l IBA in half strength MS used both in agar-gelled and liquid medium was optimal for root induction but liquid medium was superior to agar-gelled medium in some respects (Table 3). Previous researchers did not use liquid medium for root induction in banana micro-shoots. Mohammad *et al.* (2000) used the liquid medium for shoot multiplication only but did not mention the supporting material used in the rooting medium, nor did they report survival rate of the plants transferred to field. In our experiments we observed that the survival rate of the plants, which were rooted in liquid medium was higher. When the rooted plantlets were taken out from the rooting medium and washed, injury of root system generally occurred in case of agar-gelled medium, whereas in liquid medium no such injury was observed. Lack of injury during transplantation of plantlets was probably the cause of higher rate of survival. Moreover, use of liquid medium for root induction in rooting was found to be highly economical. The protocol described here is promising for the large scale production of disease free planting materials of the Sagar variety of banana.

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