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Effect of carbohydrate source, pH and supporting media on *in vitro* rooting of banana (*musa spp*.) cv. Grand naine plantlets

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The present study was conducted in the Division of Fruit Science, SKUAST-Jammu during the year 2012 to 2013 to investigate the effect of different carbohydrate source, pH and supporting media on *in vitro* rooting of banana plantlets using MS medium with 0.1 mg/L IBA and activated charcoal. Sucrose in the medium remarkably influences the rooting of plantlets. In the absence of sucrose, culture could not survive after 3 weeks of incubation. In the sucrose containing media, 30 g/L gave the best result. Out of different pH levels tested, minimum time for root initiation with longest length of root was obtained on pH 5.5. The reduction of agar concentration from 0.8 to 0.4% in the medium improve the *in vitro* root and shoot characters as compare to other supporting structures viz., Whatman No. 1 filter paper, ordinary filter paper and brown paper.

Key words: Micropropagation, *Musa* spp, Grand Naine, *in vitro* rooting.

INTRODUCTION

Banana is the premier fruit of Asia and the pacific. It is widely grown in the tropics and sub-tropics in all types of agricultural system from small, mixed, subsistence gardens to large commercial monocultures. The crop serves in many countries as a staple food or the cornerstone of the country's economy. For commercialization, it is important that consistent supplies of good quality bananas are produced. This is achieved through clonal planting material obtained through tissue culture propagation technique. This technique provides high rates of multiplying, genetically uniform and year around availability of pest and disease-free planting material. However, before exploiting such a technique for commercial purposes, gathering information regarding the requirements for each of micropropagation and then developing an industrially practicable procedure for the best culture conditions are necessary (Anderson, 1980; Lakshmi et al., 1982). The survival of the micropropagated plants in *ex vitro* conditions largely dependant on the efficient rooting of shoots. For this reason, an optimum requirement for maximum rooting response needs to be determined for each plant.

The factors which greatly influenced *in vitro* rooting of shoot are carbohydrate sources, pH of the medium and

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supporting structures (Kitto and young, 1981). The role of pH on adventitious root formation has been associated with acidic pH (Lee et al., 1976; Stone, 1963; Williams et al., 1985), alkaline pH (Lee et al., 1976) and near-neutral pH (Mellor and Stace-Smith, 1969). Therefore, the present investigation was undertaken to evaluate the effect of carbohydrate sources, pH and supporting structures on *in vitro* rooting of banana cv. Grand Naine.

MATERIALS AND METHODS

The present study was conducted in the Division of Fruit Science, SKUAST-Jammu during the year 2012 to 2013. Sher-e-Kashmir University of Agricultural Sciences and Technology, Chatha is situated in the sub-tropical zone at latitude of 32°.4'N and 74°.58'E longitude. The altitude of the place is 332 m above sea level. Annual precipitation is about 1000 to 1200 mm. Most of the rains are received during July to October (about 70%). The mean annual maximum and minimum temperatures are 45 and10°C respectively. Summer months are hot with temperature and humidity ranging from 23.50 to 35.50°C and 53.00 to 73.50%, respectively. The winter months experience mild to severe cold conditions with an average temperature ranging from 6.50 to 21.70°C. January is the coldest month, when minimum temperature touches at 3.8°C. The highest temperature 45.0°C is recorded in the month of June. The planting material of four week old suckers of *Musa* cultivar Grand Naine was collected from the healthy true to type mother plants. For obtaining the explants material, the suckers were removed from the mother plant with a straight flat bar having a sharpened point that could be inserted between plant and the sucker. The suckers were excised and surface sterilized by using different combinations and concentration of mercuric chloride, sodium hypochlorite and ethanol individually or in combination is presented in Table 1**,** which was followed by washing of explants thoroughly in running tap water for 30 min and treatment with 10% detergent solution (Teepol, BDH) for 10 min. Suckers were cultured on agar gelled Murashige and Skoog basal medium (MS) with full strength salts supplemented with specific concentration of growth regulators [6-Benzylamino purine, Indole-3-acetic acid and Naphthalene acetic acid (NAA)] singly or in combination and 3% sucrose was used for culture establishment and shoot multiplication and MS half strength salts containing various concentration of auxins (IBA/NAA) were used for adventitious root formation. All the treatments, except those for different levels of pH, were adjusted to pH 5.8 ± 0.1 fortified with 30 g/L sucrose and 0.8% agar. Media were supplemented with 1.0 mg/L IBA and 200 mg/L activated charcoal. These were added to the media before autoclaving for 20 min. All the cultures were incubated in the culture room at 26 ± 2°C under 16 h photoperiod and about 1.5 k lux light intensity. Data recorded for different parameters viz. surface sterilization of explants, culture establishment and multiplication (Stage I & II), shoot proliferation, *in vitro* rooting (Stage III) and acclimatization to normal conditions (Stage IV) were subjected to completely randomized design (CRD). Statistical analysis based on mean values per treatment was made using analysis of variance (ANOVA) technique of CRD.

RESULTS

Effect of different carbohydrate source

The carbon energy source is inevitable in any culture medium. Sucrose is the most widely accepted carbon source. The growth of the culture is not only affected by the particular type of carbon source used, but also by its concentration (Mehta, 1980). Out of various carbohydrates tested at different levels (Table 2 and Figure 1), rooting was observed only on media containing sucrose 1 to 3%, whereas in other medium containing different carbohydrate sources cultures were dried after 3 weeks of incubation. In the sucrose containing media, cent percent rooting was observed on sucrose 3%. The maximum number of roots/shoot (6.00 cm) was obtained in sucrose 3%. The length of shoot and length of root increased with increase in concentration of sucrose whereas in case of number of leaves per shoot, the treatments did not show much variation.

Effect of pH

The pH of the medium affected substantially the *in vitro* rooting of banana shoots (Table 3 and Figure 2). Cent percent rooting was obtained in pH 5.5 and 5.8. Regarding, the number of days taken for rooting, the minimum days (6.33) were required at pH 5.5, which was closely followed by medium having pH 5.8 (6.66 days), pH 6.0 (7.66), pH 5.0 (9.33) and pH 6.5 (10.66). Likewise, maximum number of roots (7.66), length of root (8.50 cm) and length of shoot (7.10 cm) were recorded at pH 5.5, which was significantly superior to rest of the treatments. In case of number of leaves, no significant differences were obtained among different pH levels.

Effect of supporting media

The reduction of agar concentration from 0.8 to 0.4% in the medium was found to improve the *in vitro* rooting and shoot characters. The data related to the effect of supporting media on *in vitro* root and shoot growth characteristics as shown in Table 4 and Figure 3 revealed that cent percent cultures showed rooting on medium gelled with 0.4% agar which varied significantly with 0.8% agar, whereas all other supporting media such as Whatman No. filter paper, ordinary filter paper and brown paper were found unsuitable for rooting.

The time taken for root initiation was minimum (5.66 days) in medium gelled with agar 0.4% which was closely followed by treatment agar 0.8% (6.33 days) and both of them were at par. Maximum number of roots (7.00) per culture with maximum length of root (6.00 cm) was observed in the cultures rooted on medium gelled with agar 0.4%. Similar trend was obtained regarding length of shoot whereas there was insignificant effect on number of leaves obtained on shoot.

DISCUSSION

Effect of different carbohydrate source

The results obtained in the present study are in

Table 1. Standardization of surface sterilization method for banana explants.

S/No	Sterilant	Concentration (%)	Duration
1	Dipping in mercuric chloride	0.1	2 min
2	Dipping in mercuric chloride	0.1	4 min
3.	Dipping in mercuric chloride	0.1	6 min
4.	Dipping in mercuric chloride and subsequent dipping in Ethanol	0.1 70	2 min 30 s
5.	Dipping in mercuric chloride and subsequent dipping in Ethanol	0.1 70	4 min 30 s
6.	Dipping in mercuric chloride and subsequent dipping in Ethanol	0.1 70	6 min 30 s
7.	Dipping in sodium hypochlorite	5	5 min
8.	Dipping in sodium hypochlorite	5	10 min
9.	Dipping in sodium hypochlorite and subsequent dipping in ethanol and mercuric chloride	5 70 0.1	5 min + 30 s + 2 min
10.	Dipping in sodium hypochlorite and subsequent dipping in ethanol and mercuric chloride	5 70 0.1	5 min + 30 s + 4 min
11.	Dipping in sodium hypochlorite and subsequent dipping in ethanol and mercuric chloride	5 70 0.1	5 min + 30 s + 6 min
12.	Dipping in sodium hypochlorite and subsequent dipping in ethanol and mercuric chloride	5 70 0.1	10 min + 30 s + 2 min
13.	Dipping in sodium hypochlorite and subsequent dipping in ethanol and mercuric chloride	5 70 0.1	10 min + 30 s + 4 min
14.	Dipping in sodium hypochlorite and subsequent dipping in ethanol and mercuric chloride	5 70 0.1	10 min + 30 s + 6 min

agreement with the result of Amin and Jaiswal (1989a) who reported that in the absence of sucrose culture could not survive after 3 weeks of incubation and in the sucrose containing media 30 to 40 g/L gave the best result. The similar positive effect of increased level of sucrose on *in vitro* rooting has been reported for sour cherry (Snir, 1983) and walnut (Driver and Kuniyuki, 1984). Likewise, Kabir et al. (2007) reported that sucrose in 30 g/L concentration as carbon source was proved to be best regarding the growth of shoot tip explant of papaya.

Effect of pH

Though, the importance of pH in tissue culture studies was reported by Gautheret (1947), who observed pH drift

Figure 1. Effect of different carbohydrate source on in vitro rooting of banana plantlets.

Table 2. Effect of different carbohydrates on *in vitro* rooting and shoot growth of banana plantlets.

S/No	Treatment	Time taken for root initiation (days)	Culture rooted (%)	No. of roots per shoot	Length of longest root (cm)	Length of shoot (cm)	No. of leaves per shoot
1.	Sucrose 1%	13.00	75.66 (60.41)	4.00	5.66	4.90	5.66
2.	Sucrose 2%	12.00	83.33 (65.87)	5.66	6.00	5.70	6.33
3.	Sucrose 3%	11.00	100.00 (90.00)	6.00	7.00	6.20	7.00
4.	Commercial sugar 1%	-	-	-	-	-	-
5.	Commercial sugar 2%	-	-	-	-	-	-
6.	Commercial sugar 3%	-	-	-	-	-	-
7.	Glucose 2%	-	-	-	-	-	-
8.	Fructose 2%	-	-	-	-	-	-
9.	Lactose 2%	-	-	-	-	-	-
10.	Maltose 2%	-	-	-	-	-	-
11.	Glucose + Fructose 2 (1% each)	-	-	-	-	-	-
	SE (m) ±	0.30	0.70	0.07	0.12	0.10	0.13
	CD (0.05)	0.89	0.24	0.25	0.41	0.35	0.48

-Observation not recorded as no shoot produced rooted plantlet.

during growth of a culture. The usual practice is to adjust the pH of the medium within the range of 5.5 to 6.0 during the preparation of the medium. However, there are few reports on the effect of media pH on the growth of culture. Wali (1996) obtained best root and shoot growth at pH 5.5. The poor behaviour of pH 4.5 and 7.0 may be due to differential availability of various nutrients or due to some toxic effect attributed by high and low pH of the medium. In guava Amin and Jaiswal (1989b) observed that comparatively les acidic (pH 5.5 to 6.0) medium was better than more (pH 4.5 to 5.0) acidic medium for *in vitro* rooting.

Table 3. Influence of pH on *in vitro* rooting and shoot growth of Banana plantlets cv. Grand naine.

S/No	Treatments	Time taken for root initiation (days)	Cultures rooted (%)	No. of roots per shoot	Length of longest root (cm)	Length of shoot (cm)	No. of leaves per shoot
1.	pH 4.5	13.66	50.66 (45.36)	1.66	2.30	3.40	2.00
2.	pH 5.0	9.33	82.33 (65.14)	4.33	5.60	4.40	4.33
3.	pH 5.5	6.33	98.66 (83.70)	7.66	8.50	7.10	6.33
4.	pH 5.8	6.66	95.33 (78.68)	6.00	7.00	6.90	6.00
5.	pH 6.0	7.66	91.66 (73.26)	5.66	6.70	6.10	5.66
6.	pH 6.5	10.66	54.66 (47.66)	2.66	3.40	2.40	1.33
7.	pH 7.0	14.66	52.66 (46.51)	2.33	3.10	4.00	1.66
SE (m) ±		0.19	1.77	0.18	0.31	0.12	0.18
CD (0.05)		0.58	5.41	0.56	0.40	0.37	0.55

Table 4. Effect of supporting media on *in vitro* root and shoot growth of banana plantlets.

S/No	Treatments	Time taken for root initiation (days)	Cultures rooted (%)	No. of roots per shoot	Length of longest root (cm)	Length of shoot (cm)	No. of leaves per shoot
1.	Agar 0.8%	6.33	98.66 (83.32)	6.33	5.00	5.30	5.66
2.	Agar 0.4%	5.66	100.00 (90.00)	7.00	6.00	6.50	6.33
3.	Whatman No. 1 filter paper	-	-	-	-	-	-
4.	Ordinary filter paper	-	-	-	-	-	-
5.	Brown paper	-	-	-	-	-	-
SE (m) ±		0.05	4.65	0.09	0.11	0.06	0.10
CD (0.05)		0.18	14.86	0.37	0.47	0.23	0.41

- Observation not recorded as no shoot produced rooted plantlet.

Figure 2. Effect of different pH levels on *in vitro* rooting of banana plantlets.

Figure 3. Effect of supporting medium on *in vitro* rooting of banana plantlets.

Effect of supporting media

These results are in confirmation with those of Kitto and Young (1981) who reported increased response with the decrease in agar from 2.0 to 0.5%. Reports of Anderson (1980) support the result as they obtained best rooting by lowering the agar concentration.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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