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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 5(2): 161-164, 2002

In vitro Shoot Multiplication and Rooting of Banana cv. Sabri

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Abstract: The effects of different cytokinins viz. BAP, 2iP and Kn each at 5 mg/l alone or in combination on shoot multiplication and auxins viz. IBA, NAA in different concentrations (i.e. 0, 1, 2, 3, 4 and 5 mg/l) on rooting of banana cv. Sabri were investigated. The rate of shoot multiplication varied in different cytokinin treatments. Among the treatments, MS medium supplemented with 5 mg/l each of BAP+ Kn produced the highest number of shoots/explant (3.11), while no shoot multiplication was observed in control (i.e. no cytokinin). On the other hand, the number of shoots responded for rooting and their survivability were higher with NAA than IBA. MS medium supplemented with 4 mg/l NAA produced the highest number of roots/shoot (20) whereas 1 mg/l IBA produced the minimum number of roots/shoot (2.4). Plantlets grown without any auxin in the medium gave the least number of root (1). The micro-propagated Sabri plantlets were successfully transferred from pot to the field and 100% plantlets survived in field environment.

Key words: *In vitro* shoot multiplication, rooting, cytokinin, auxin, Sabri

Introduction

Banana (*Musa* spp.) is one of the most important nutritious fruit crops of the world. It occupies second position in terms of area among the fruits comprising 42% of the total fruit production in Bangladesh (Haque, 1988). It is generally propagated vegetatively through suckers. But the traditional method is laborious, time consuming and not very efficient as far as production of homogeneous plant is concerned (Banerjee and De Langhe, 1985). Only 5 to 10 suckers can be obtained from a plant per year in conventional method. Furthermore, banana production sometimes becomes seriously affected by different fungal and viral diseases such as panama and bunchy top diseases. As a result, banana productivity decreases and the yield becomes very poor and static as well. Moreover, it is very difficult to carry the bulk volume of suckers from one place to another.

To overcome the situation, micro-propagation could be an alternative for production of planting materials of banana. A large quantity of uniform disease-free plants can be produced from a single plant or even a small piece of plant tissue showing good genetic potential in this method (Vuylsteke and De Langhe, 1985; Mantell *et al.*, 1985) and plant multiplication can be continued throughout the year irrespective the season (Razdan, 1993). Tissue cultured plants grow vigorously, establish more quickly and take a shorter time to bunch emergence and harvest (Drew and Smith, 1990; Vuylsteke and Ortiz, 1996). They have been reported to perform better and produce 39% higher yield than plants from conventional sword suckers (Pradeep *et al.*, 1992; Faisal *et al.*, 1998). Generally, cytokinins and auxins are used for multiplication of banana shoots and their rooting respectively. However, requirements of cytokinins and auxins depend upon the variety of banana and culture conditions used (Cronauer and Krikorian, 1984). Ali (1996) established the cultures of Amritsagar, Sabri, Anaje and Mehersagar for their micro-propagation but only the multiplication and rooting of Amritsagar were found to be successful. Sabri is the second leading commercial cultivar of banana in Bangladesh which is comparatively more susceptible to panama disease. To get the panama disease free healthy planting materials, it is very urgent to develop a protocol for micro-propagation of Sabri. Therefore, the present study was undertaken to identify the appropriate cytokinin for better shoot multiplication and to find the optimum concentration of auxin for better rooting of banana cv. Sabri.

Materials and Methods

The present study was carried out at the laboratory of Horticulture Department of Bangladesh Agricultural University, Mymensingh during January to December, 1998. Banana cv. Sabri (genome AAB) collected from Bangladesh Agricultural Research Institute, Gazipur was used as plant material for obtaining shoot tip (i.e. meristem and a few leaf primordia). The suckers were carefully removed from banana plants and washed thoroughly under running tap water. The explants were prepared by removing a number of outer layers of tissue from suckers with a sharp stainless steel knife. The pale white tissue blocks (10× 15 mm^2) containing shoot tip and rhizomatous bases were surface sterilized by immersing in 70% (v/v) ethanol, containing few drops of tween-80 for a minute and then rinsed twice in sterile distilled water. They were again surface sterilized with 0.1% mercuric chloride ($HgCl_2$) and a few drops of tween-80 for 15 minutes and then rinsed 4 times with sterile distilled water. The dead tissue of sterilized block was cut into a 5× 8 mm^2 portion together with 2-3 mm of rhizomatous base for inoculation.

The culture medium was prepared by supplementing the 4.4 g/l Murashige and Skoog (MS) medium (1962) with 40 g/l sucrose, 1 mg/l thiamin hydrochloride and 100 mg/l myo-inositol. Various cytokinins viz. BAP (6-Benzylaminopurine), 2iP (2-isopentyladenine) and Kn (Kinetin) were also added 5 mg/l each alone or in combination, to the medium to investigate their effects on shoot proliferation. The 8 treatments were: control (i.e. no cytokinin), BAP, 2iP, Kn, BAP+ 2iP, BAP+ Kn, 2iP+ Kn and BAP+ 2iP+ Kn. The media were adjusted to pH 5.8 and 8 g/l 'agar technical-3' was added. All the culture media, glassware and instruments were sterilized in an autoclave at 121 °C for 20 minutes at 1.16 kg/cm^2 pressure.

The explant about 0.5 cm long with 2-3 leaf primordia was directly inoculated to each culture vessel containing 20 ml culture medium with different cytokinins as per treatments. The brown tissues on the explant were removed and tip was transferred to a similar fresh medium every week up to 4 weeks to minimize the tissue browning. The shoots were subcultured at 4 weeks interval in the same fresh medium to produce multiple shoots. For subculturing the entire samples of shoot *in vitro* were cut into small pieces containing one shoot. All cultures were incubated at 25± 1 °C with 16 hr photoperiod (2000 lux) provided by cool white fluorescent tubes. The experiment was arranged in completely randomized design (CRD) with 3 replications, each consisted of 3 culture vessels. Shoot proliferation (%), number of shoots/explant, length of shoot and number of leaves/explant were recorded up to second subculture.

The regenerated shoots (3-5 cm in length with 2-3 well-developed leaves) were then cultured in prepared culture medium supplemented with different concentrations of IBA (Indole-3-butyric acid) and NAA (α-naphthalene acetic acid) viz. 0, 1, 2, 3, 4 and 5 mg/l. The experiment was arranged in CRD with 5 replications, each unit in the bottle was considered as a replicate. Number of roots/shoot and their length were recorded up to 4 weeks of culture. After rooting the culture vessels were kept in growth room opening the lids slightly for 5 days for hardening. Then the vessels were transferred to laboratory at room

temperature for 2 days. After removing the agar from roots by washing, the rooted plantlets were transferred to small earthen pots containing sterilized soil and sand and kept for 7 days by covering the plantlets with transparent polythene sheet to maintain the high humidity. The plantlets were then planted in earthen pots (15 cm) containing sand, soil and cowdung (1:1:1) and kept in net house. After 30 days the plantlets attained a height of about 25 cm and were transferred to the field.

Results and Discussion

Colour of explants: The colour of explant changed from very light green to dark green depending on duration and type of treatments (Table 1). After 2 weeks of inoculation (WAI), the explants became light green in media containing 2iP and 2iP+ Kn while in other treatments the color of explants was green in both subcultures. In control (i.e. no cytokinin) on the other hand, the colour was brown in both subcultures. After four weeks of inoculation, the colour of explants became green in media containing 2iP and 2iP+ Kn and dark green in other treatments except BAP+ 2iP+ Kn in both subcultures. But in control the brown explants became light green at both subcultures.

Shoot proliferation (%): The highest percentage of explants or shoot (94%) proliferated in media supplemented with BAP or BAP+ 2iP+ Kn followed by BAP+ Kn (89%) in both subcultures (Table 1). The lowest response (61%) on the other hand, was noticed in media containing 2iP+ Kn. In control, shoot did not proliferate at all. The results of the present study indicate that BAP was more efficient in shoot proliferation than Kn and 2iP alone or in combination.Wareing and Phillips (1981) also reported BAP as the most effective cytokinin in shoot proliferation of a number of plants. The results of the present study agree with the findings of Ali (1996), who reported higher shoot proliferation in banana on MS media supplemented with 5 mg/l BAP.

Number of shoots/explant: During the culture period, the number of shoot/explant increased gradually in all the media supplemented with different cytokinins alone or in combination (Table 1). The media containing BAP+ Kn produced the highest average number of shoots/explant in both the subcultures though it was statistically similar with the treatments of BAP, Kn, BAP+ 2iP and BAP+ 2iP+ Kn. However, maximum 14 shoots were produced from a single explant at BAP+ Kn treatment after 4 weeks of culture in the first subculture (Plate C). The lowest rate of shoot proliferation was observed in the medium supplemented with 2iP or 2iP+ Kn in both subcultures. Shoots on the other hand, did not proliferate on the medium without cytokinin (i.e. control). The results therefore, indicated the necessity of a cytokinin for shoot proliferation of banana. However, the variation in responses of banana explants to cytokinin treatments might be due to the different cytokinins.

Cronauer and Krikorian (1984a) reported that culture media containing 5 mg/l Kn or BAP increased the number of banana shoots/explant. Multiple shoots/explant were also observed in banana on MS medium supplemented with 2.5 or 5 mg/l BAP (Balakrishnamurthy and Sree Rangasamy, 1988). The results of the

Table 1: Effects of different cytokinins on in vitro shoot multiplication of banana cv. Sabri

Cytokinins*	Color of explants		Shoot proliferation (After 4 wks) (%)	No. of shoots/explant ± SE**		No. of shoots/explant ± SE**		No. of leaves/explant ± SE**	
	After 2 weeks	After 4 weeks		After 2 Weeks	After 4 weeks	After 2 weeks	After 4 weeks	After 2 weeks	After 4 weeks
1st subculture									
Control	B	+	-	-	-	-	-	-	-
BAP	+ +	+ + +	94.4	1.67± 0.33	2.22± 0.47	2.72± 0.52	3.22± 0.55	1.71± 0.49	2.20± 0.56
2iP	+	+ +	66.0	1.22± 0.22	1.33± 0.29	1.44± 0.42	2.94± 0.99	0.89± 0.22	1.35± 0.41
Kn	+ +	+ + +	80.0	2.11± 0.56	2.44± 0.67	2.50± 0.19	2.83± 0.25	2.94± 0.56	3.00± 0.56
BAP+ 2iP	+ +	+ + +	69.0	1.78± 0.62	1.89± 0.63	1.11± 0.32	1.61± 0.43	1.50± 0.50	1.64± 0.50
BAP+ Kn	+ +	+ + +	89.0	2.33± 0.55	3.11± 0.66	1.72± 0.27	2.50± 0.38	1.39± 0.46	2.14± 0.65
2iP+ Kn	+	+ +	60.6	1.33± 0.44	1.33± 0.44	1.39± 0.39	2.22± 0.75	1.00± 0.58	1.28± 0.60
BAP+ 2iP+ Kn	+ +	+ + +	94.0	1.56± 0.34	2.33± 0.47	1.50± 0.29	2.22± 0.30	2.28± 0.84	2.80± 1.05
2nd subculture									
Control	B	+	-	-	-	-	-	-	-
BAP	+ +	+ + +	94.0	1.83± 0.60	2.67± 0.99	2.42± 0.84	2.83± 0.87	1.50± 0.70	2.14± 1.05
2iP	+	+	64.0	1.17± 0.48	1.33± 0.49	1.33± 0.48	1.75± 0.64	0.36± 0.21	1.35± 0.20
Kn	+ +	+ + +	81.2	1.50± 0.76	1.50± 0.76	1.25± 0.34	1.67± 0.46	0.71± 0.39	2.68± 0.85
BAP+ 2iP	+ +	+ + +	70.0	1.17± 0.40	1.50± 0.62	0.83± 0.40	1.17± 0.53	0.42± 0.18	0.85± 0.50
BAP+ Kn	+ +	+ + +	88.8	2.17± 0.60	3.00± 0.97	1.33± 0.49	1.50± 0.59	0.85± 0.28	1.57± 0.92
2iP+ Kn	+	+	61.0	0.83± 0.40	0.83± 0.40	1.25± 0.50	1.33± 0.54	0.50± 0.22	0.71± 0.28
BAP+ 2iP+ Kn	+ +	+ + +	94.0	1.50± 0.56	1.67± 0.72	1.17± 0.60	1.17± 0.60	1.00± 0.64	1.00± 0.64

* 5mg/l each alone or combination B= brown, + = light green, + + = green, + + + = dark green, - = no response

** Each value represents the means of 3 replicates of 9 explants ± standard error (SE)

Table 2: Effects of IBA and NAA on in vitro rooting of banana cv. Sabri and their survivability in pots

Auxin mg/l		Number of roots/ plantlet ± SE*		Root length(cm) ± SE*		Survivability of plantlet in pot (%)
		After 2 weeks	After 4 weeks	After 2 weeks	After 4 weeks	
Control		0.60± 0.40	1.00± 0.78	0.36± 0.17	1.22± 0.75	50
IBA:	1	1.40± 0.75	2.40± 0.68	0.36± 0.22	2.14± 1.00	60
	2	1.20± 0.49	7.00± 2.17	1.02± 0.22	4.26± 1.26	65
	3	4.00± 1.27	6.60± 1.63	1.14± 0.81	3.52± 0.96	76
	4	1.40± 1.17	3.40± 1.57	0.76± 0.58	2.22± 1.96	80
	5	0.80± 0.58	2.60± 1.25	0.36± 0.22	1.26± 0.88	67
NAA:	1	3.20± 1.10	9.80± 4.86	0.74± 0.10	1.64± 0.38	76
	2	4.00± 2.41	11.40± 3.72	0.72± 0.31	2.64± 0.97	76
	3	1.40± 0.98	13.60± 5.68	1.44± 0.37	3.12± 1.10	80
	4	2.60± 0.93	20.00± 3.15	0.70± 0.20	2.00± 0.37	67
	5	6.60± 3.31	17.40± 6.45	0.42± 0.11	1.00± 0.40	67

* Mean of 5 replicants ± standard error (each unit in vials was considered as a replicate)

A

B

C

D

E

F

Plates A-F: A) Prepared shoot tip explant for culture B) Shoot multiplied on MS medium containing BAP (after 20 days of culture initiation) C) Production of shoots (max. 14) from a single explant at BAP+ Kn treatment (after 4 weeks of culture) D) Root initiation on MS medium supplemented with 4 mg/l NAA at 4 weeks of culture E) Plantlet established in pot (after 15 days of transfer) F) Plantlets established in field (after 75 days of transfer from pot).

present experiment are more or less similar with the findings of the above authors.

Length of shoot: The mean length of banana shoot was influenced by different cytokinins up to second subcultures and it ranged from 1.17 to 3.22 cm (Table 1). The highest shoot length (3.22 cm in first and 2.83 cm in second subculture) was observed in medium supplemented with BAP in both subcultures. The lowest shoot length was noted in the treatment of BAP+ 2iP (1.61 cm) in first subculture and BAP+ 2iP or BAP+ 2iP+ Kn (1.17 cm) in second subculture. The shoot length although increased gradually with time in all cytokinin treatments, the rate of increase was faster in BAP treatment. The result of present experiment agree with the findings of Khanam *et al.* (1996), who obtained longest shoot in banana on MS medium supplemented with 25 µM BAP.

Number of leaves/explant: After 4 weeks the medium supplemented with Kn produced the maximum number of leaves/explant (3.0) in first subculture (Fig. 2). Statistically identical results were also obtained in this regard on media supplemented with BAP, BAP+ Kn or BAP+ 2iP+ Kn. The minimum number of leaves was observed in media supplemented with 2iP, 2iP+ Kn or BAP+ 2iP. More or less similar results were found in second subculture.

Number of roots/explant: Since the number of roots developed from the multiple shoots was not enough for individual shoot, the excise shoots were allowed to develop roots on MS medium supplemented with different levels of two auxins viz. IBA and NAA (i.e. 0, 1, 2, 3, 4 and 5 mg/l). The number of roots produced/plantlet increased with increasing concentration up to 2 mg/l IBA and declined thereafter up to 5 mg/l (Table 2). Among the IBA treatments, 2 mg/l was found to be the most effective in rooting of banana plantlets. After 4 weeks, the highest number of roots/plantlet (7) was obtained in 2 mg/l IBA which was statistically identical with that obtained in 1, 3 or 4 mg/l IBA while the lowest number of roots/plantlet (1) was recorded in control. But in case of NAA, the number of roots increased with the increase in concentration up to 4 mg/l but decreased at 5 mg/l NAA. At 4 weeks of culture, the banana plantlets produced the highest number of roots/plantlet (20) in the medium supplemented with 4 mg/l NAA (Plate D). Statistically similar results were also observed in 3 or 5 mg/l NAA whereas lowest number of roots was produced at lower dose of NAA (1mg/l). Though IBA produced the highest number of roots/plantlet at 2 mg/l, it was lower than the lowest number of roots/plantlet (9.8) produced by NAA at 1 mg/l. Therefore, the present study revealed that NAA was superior to IBA in respect of root formation of banana cultivar.

The present finding is partially in conformity with that of Habib (1994), Raut and Lokhande (1989), Khanam *et al.* (1996) and Dore Swamy *et al.* (1983), who used MS medium supplemented with IBA in different concentration for good rooting of banana. On the other hand, Bhaskar *et al.* (1993) observed best *in vitro* rooting on Knudson's medium supplemented with 5 mg/l NAA. The effects of NAA in the present experiment also agree with the findings of Raut and Lokhande (1989) and Habib (1994).

Length of root: A significant variation in root length was also noticed among different concentration of IBA and NAA (Table 2). After 4 weeks, the highest root length (4.26 cm) was recorded in the media containing 2 mg/l IBA though it was not significantly different from that obtained in 1, 3 or 4 mg/l IBA whereas the lowest root length (1.22 cm) was obtained in control. The rate of increase in root length was rapid in the media with 2 or 3 mg/l IBA but was slower in the media with 5 mg/l and control. In case of NAA, the root length was the highest in the medium supplemented with 3 mg/l NAA (3.12 cm) which was statistically identical with 1, 2 or 4 mg/l NAA. The root length on the other hand, was the lowest in the medium containing 5 mg/l NAA (1 cm) but smaller than that of control (1.22 cm). The result agrees with the findings of Khanam *et al.* (1996), who obtained 4.2 cm long roots in banana cv. Amritsagar grown with MS medium containing 2 mg/l IBA and Berg and Bustamante (1974), who obtained about 1 cm long roots on medium containing 1mg/l NAA.

Survivability (%): Table 2 shows that the survivability rate of plantlets in pots was relatively higher in NAA than IBA. The survivability was the highest (80%) at 3 mg/l NAA or 4 mg/l IBA and the lowest (50%) in control treatment. Hundred per cent of the tissue culture derived plantlets survived when they were transferred to the field from the pots. The present result is consistent with the finding of Cronauer and Krikoria (1984) who reported successful establishment of *in vitro* rooted shoots of banana cultivars on pots.

Therefore, it could be concluded that MS medium supplemented with 5 mg/l each of BAP+ Kn was the best for rapid multiplication of shoots of banana cv. Sabri and MS medium supplemented with 4 mg/l NAA was suitable for rooting of the micro shoots. The protocol developed through the present study may be useful for production of large number of disease free and healthy planting materials of banana cv. Sabri.

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