Pak. J. Bot., 41(4): 1667-1676, 2009.

IN VITRO REGENERATION OF GINGER USING LEAF, SHOOT TIP AND ROOT EXPLANTS

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Abstract

MS medium supplemented with different combinations and concentrations of hormone were studied to obtain a suitable protocol of plantlet regeneration of ginger from calli. Three explants of two varieties of ginger viz., Suruchi and BARI ada-1 were cultured on MS medium supplemented with 0.5 mg/l Dicamba, 0.75 mg/l Dicamba and 1 mg/l 2,4 D. Assessments on callus induction were studied through five quantitative traits such as days required for callus initiation, size of callus, color of callus, nature of callus and percentage of callus induction. Suruchi showed 62.64% callus induction, 63.98% shoot induction and 68.76% root induction. Leaf explant gave best result over shoot tip and root explant. Leaf explant produced 67.07% callus, 67.77% shoot and 66.93% root. MS medium supplemented with 0.5 mg/l Dicamba was the best (70.20%) for callusing, MS + 1.0 mg/l Kn + 1.0 mg/l BAP best (72.03%) for shooting and Ms + 1 mg/l IBA gave best (66.43%) result for rooting over other treatments. The highest (73.60%) callus induction was found from the leaf x Suruchi interaction. Leaf x Suruchi gave 74% shooting and 74.13% rooting. Percent callus induction was maximum (76.47%) with Suruchi x 0.5 mg/l Dicamba interaction and it was significantly different from all other values. Percent shoot induction were highest (76.33%) by the interaction of Suruchi x 1.0 mg/l Kn +1.0 mg/l BAP. Highest (76.87%) percentage of root was produced by 1 mg/l IBA x Suruchi interaction. Highest percentage of callus induction (87.60%) was obtained from leaf explants of Suruchi and 0.5 Dicamba interactions. The interaction of Suruchi x leaf x 1.0 mg/l Kn + 1.0 mg/l BAP produced highest (87.60%) percentage of shoot. Root induction was best (85.40%) from Suruchi x leaf x MS + 1 mg/l IBA interaction. The regenerated plantlets were successfully established into pot after proper hardening.

Introduction

Ginger (*Zingiber officinale* Rosc.) is one of the important spice crops in Bangladesh under the family Zingiberaceae. It is a herbaceous perennial with underground tuberous aromatic stems (rhizomes) usually grown as an annual. This herb originated in India and was introduced to China at a very early date. The potential yield of ginger is as high as 30-35 tons per hectare. Bangladesh produces about 48,000 metric tons of ginger in an area of 6882 hectare of total land (Anon., 2004). Its yearly requirement is 122,000 metric tons. The country can produce only 40% of its requirement. The rest 60% demand for home consumption totally depends on import costing hard earned foreign exchange. Since ginger is vegetative propagated through small pieces of rhizomes, large amount of total production is utilized as seed in the next season. Vegetative propagation of ginger has the high risk of spreading systemic infections. It is reported that a three-fold increase

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in the production of rhizomes could be possible by the effective control of the diseases (Balachandran *et al*., 1990). The regeneration of plants from tissue culture is an important and essential component of biotechnological research. High frequency regeneration of plants from *In vitro* cultured tissues is a pre-requisite for successful application of tissue culture technique for crop improvement (Akter, 2001). The present research work has been planned and undertaken with the following objectives: a) To standardize the media and hormone composition for the regeneration of ginger. b) To test suitability and reducibility of various explants for callus induction and subsequent plantlet regeneration. c) To screen better cultivar of ginger for good regeneration potentiality. d) To develop a suitable and reproducible protocol of ginger regeneration using leaf, shoot tip and root as explants.

Materials and Methods

The experiments were conducted during the period from August 2005 to September 2006, in the Tissue Culture Laboratory and the Biotechnology and Genetic Engineering (BEG) Laboratory of the Department of Genetics and Plant Breeding, Bangladesh Agricultural University, Mymensingh. Two varieties *viz.,* Suruchi and BARI ada-1 and three explants *viz*., leaf, shoot tip and root were used in the research. For the induction of callus and regeneration of plantlets in ginger, MS (Murashige & Skoog, 1962) medium was used in present investigation. The following culture media were used in the present investigation depending on specific purposes as mentioned:

A. For callus induction:

i. MS medium containing 0.5 mg/l Dicamba
ii. MS medium containing 0.75 mg/l Dicamba
iii. MS medium containing 1 mg/l 2,4-D

B. For shoot initiation:

i. MS medium containing 1 mg/l BAP + 1 mg/l Kinetin
ii. MS medium containing 1 mg/l Kinetin
iii..MS medium containing 1 mg/l Kinetin + 1 mg/l IAA

C. For root formation:

i. MS medium containing 0.5 mg/l IBA
ii. MS medium containing 1 mg/l IBA
iii. MS medium containing 1.5 mg/l IBA

Each of the sterilized explants was cut into 2-3 mm pieces using sterile scalpel. Four pieces were inoculated in each vial containing sterile culture medium with different concentration and combination of growth regulators for callus induction. Three to four weeks after inoculation of explants, the calli attained convenient size. Then those were removed aseptically from the existing media and again placed them into the small vials with regeneration media. When the shoots grew about 4-5cm in length, they were rescued aseptically from the cultured vials and separated from each other and again cultured on freshly prepared root induction medium to induce root. The vials containing plantlets were incubated under continuous light. Day to day observations was carried out to note the response of growing plantlets. When the plantlets become 6-10 cm in length with sufficient root system, they were taken out from the vials. Medium attached to the roots

were gently washed out with running tap water. The plantlets were transplanted to pots containing the potting mixture containing garden soil, sand and cow dung in the ratio of 1:2:3. To investigate the effect of different treatments and response of different varieties on callus induction and plant regeneration, data were recorded on the following parameters; days required for callus initiation, percent callus induction, size of callus, color of callus, nature of callus, days to shoot initiation, percent shoot initiation, days to root formation, percent root formation. The diameter of callus was measured in millimeters (mm), nature of callus was recorded and graded as 3 for highly compact, 2.5 for compact, 1.5 for friable and 1 for loose in texture. Color of callus was observed visually and graded the marks as 3 for deep greenish, 2 for creamy and 1 for whitish color. The analysis of variances for different parameters was performed and the means were compared by the Least Significant Difference (LSD) Test (Meier, 2006).

Results and Discussion

Callus induction: The results of the experiment revealed a wide range of variation in days required for callus initiation, size of callus, color of callus, nature of callus and percentage of callus induction. The callus induction ability of Suruchi was higher (62.64%) than BARI ada-1 (52.18%) (Fig. 1). The highest (67.07%) percentage of callus was performed by leaf explant while root explant produced lowest (47.97%) callus (Fig. 2). Similar results were also found by Babu *et al*., (1992). They found best response on callusing when young leaf of *Zingiber officinle* cv. *Muran* was cultured with various concentrations of growth regulators. Callusing ability of 0.5 mg/l Dicamba supplemented medium was maximum (70.2%) than other hormones (Fig. 3).

The highest (73.60%) callus induction was found from the leaf of Suruchi followed the (60.80%) callus induction performed by shoot tip of Suruchi. Minimum time of 20.73 days was recorded for callus induction from Suruchi x leaf explant interaction (Table 1). Percent callus induction was maximum (76.47%) with Suruchi x 0.5 mg/l Dicamba interaction. Shortest time of 19.80 days was required for callus initiation by the interaction of Suruchi and media supplemented with the 0.5 mg/l Dicamba (Table 2).

Suruchi x leaf x 0.5 mg/l Dicamba in media took minimum period (17.40 days) for callus induction (Table 3). Highest percentage of callus induction (87.60%) was obtained from leaf explants of Suruchi and 0.5 Dicamba interactions (Fig. 4).

Shoot induction: The highest number of shoots (3.8) per callus and highest percentage (74%) of shoot induction were observed at Suruchi x leaf explant interaction (Table 4).

Number of shoots per callus (3.933) and percent shoot induction (76.33%) were highest by the interaction with Suruchi and 1.0 mg/l Kn +1.0 mg/l BAP (Table 5).

The interaction of leaf x 1.0 mg/l Kn + 1.0 mg/l BAP x Suruchi took minimum (20.20 days) time and produced maximum number (4.8) (Table 6). This interaction gave highest (87.60%) percentage of shoot induction (Fig. 5).

Root induction: The interaction of Suruchi and leaf explants produced maximum number (8.87) of roots per shoot and highest percentage (74.13%) of root (Table 7).

It was observed that the media supplemented with 1 mg/l IBA x Suruchi took minimum time (13.07 days). Maximum number (9.07) of roots per shoot and highest percentage (76.87%) of root was produced by 1 mg/l IBA x Suruchi interaction (Table 8).

Maximum number (9.6) of roots/shoot and highest (85.40%) percentage of root was also produced by Suruchi x leaf x 1 mg/l IBA interactions (Table 9). The results have been presented in Fig. 6.

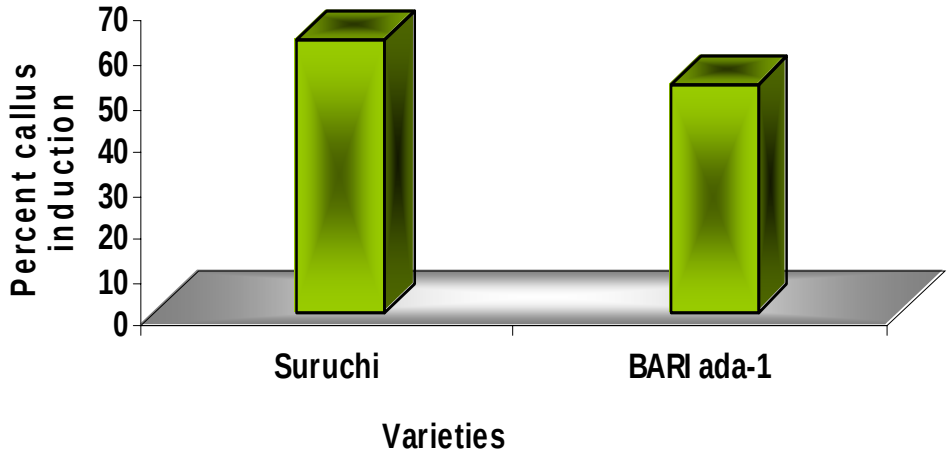

Fig. 1. Response of different varieties on percent callus induction of ginger.

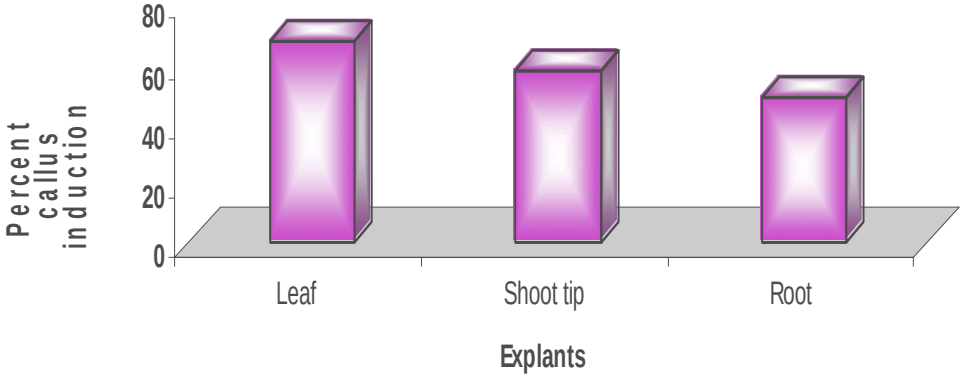

Fig. 2. Response of different explants on percent callus induction of ginger.

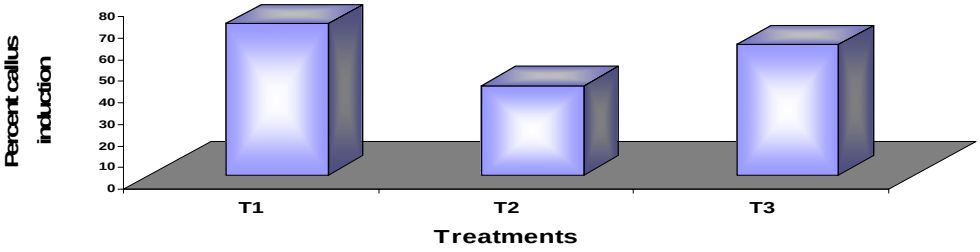

Fig. 3. Response of different treatments on percent callus induction of ginger.
T_1 = 0.5 mg l^{-1} Dicamba, T_2 = 0.75 mg l^{-1} Dicamba, T_3 = 1 mg l^{-1} 2, 4-D

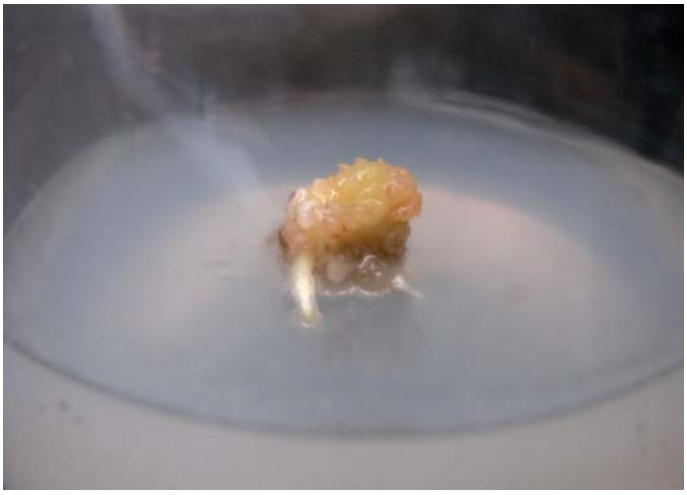

Fig. 4. Callus formation from leaf explants derived from Suruchi of ginger on MS medium supplemented with 0.5 mg/l Dicamba.

Table 1. Performance of variety and explant interaction on different callus characters of ginger.

Variety	Explants	Characters of callus				
		Days to callus induction	Size of callus (mm)	Color of callus	Nature of callus	% Callus formation
Suruchi	Leaf	20.7c	9.400a	2.700	2.593a	73.60a
	Shoot tip	20.80c	8.900b	2.480	2.407b	60.80b
	Root	27.27b	6.933c	2.253	2.120c	53.53c
BARI ada-1	Leaf	20.80c	8.900b	2.467	2.400b	60.53b
	Shoot tip	27.27b	5.967d	2.260	2.153c	53.60c
	Root	34.07a	5.767d	2.033	1.767d	42.40d

Note: Mean values in a column having common letter are statistically identical and those having different letters are statistically different.

Table 2. Performance of variety and treatment interaction on different callus characters of ginger.

Variety	Hormonal conc. (mg/l)	Characters of callus				
		Days to callus induction	Size of callus (mm)	Color of callus	Nature of callus	%Callus formation
Suruchi	0.5 Dicamba	19.80f	9.267a	2.573	2.453a	76.47a
	0.75 Dicamba	26.40c	7.567c	2.373	2.267d	44.87e
	1.0 2,4 D	22.60e	8.400b	2.487	2.400a	66.60b
BARI ada-1	0.5 Dicamba	24.93d	7.433c	2.360	2.307c	63.93c
	0.75 Dicamba	30.07a	6.067e	2.160	1.993e	37.93f
	1.0 2,4 D	27.13b	7.133d	2.240	2.020e	54.67d

Note: Mean values in a column having common letter are statistically identical and those having different letters are statistically different.

Table 3. Performance of variety, explant and treatment interaction on different callus characters of ginger.

Variety	Explant	Hormonal conc. (mg/l)	Characters of Callus				
			Days to callus initiation	Size of callus (mm)	Color of callus	Nature of callus	%Callus induction
Suruchi	Leaf	0.5 Dicamba	17.40h	9.900a	2.760	2.660a	87.60a
		0.75 Dicamba	24.60f	8.700b	2.600	2.480b	55.60g
		1.0 2,4 D	20.20Gg	9.600a	2.740	2.640a	77.60b
	Shoot tip	0.5 Dicamba	17.40h	9.700a	2.580	2.480b	75.40c
		0.75 Dicamba	24.60f	8.200c	2.380	2.300c	43.40i
		1.0 2,4 D	20.40g	8.800b	2.480	2.440b	63.60e
	Root	0.5 Dicamba	24.60f	8.200c	2.380	2.220de	66.40d
		0.75 Dicamba	30.00d	5.800e	2.140	2.020g	35.60k
		1.0 2,4 D	27.20e	6.800d	2.240	2.120f	58.60f
BARI ada-1	Leaf	0.5 Dicamba	17.40h	9.700a	2.560	2.480b	74.60c
		0.75 Dicamba	24.60f	8.200c	2.380	2.260cd	43.60i
		1.0 2,4 D	20.40g	8.800b	2.460	2.460b	63.40e
	Shoot tip	0.5 Dicamba	24.60f	6.800d	2.360	2.260cd	66.60d
		0.75 Dicamba	30.00d	5.300f	2.180	2.040g	35.40k
		1.0 2,4 D	27.20e	5.800e	2.240	2.160ef	58.80f
	Root	0.5 Dicamba	32.80c	5.800e	2.160	2.180ef	50.60h
		0.75 Dicamba	35.60a	4.700g	1.920	1.680h	34.80k
		1.0 2,4 D	33.80b	6.800d	2.020	1.440i	41.80j

Note: Mean values in a column having common letter are statistically identical and those having different letters are statistically different.

Table 4. Influence of variety and explants interaction on shooting.

Variety	Explants	Characters of shoot		
		Days to shoot induction	No. of shoot/callus	% Shoot induction
Suruchi	Leaf	21.53e	3.800	74.00a
	Shoot tip	23.27d	2.867	61.20b
	Root	25.33c	2.733	56.73c
BARI ada-1	Leaf	27.80b	2.467	61.53b
	Shoot tip	28.87a	2.067	49.67d
	Root	29.27a	1.533	46.27e

Note: Mean values in a column having common letter are statistically identical and those having different letters are statistically different.

Table 5. Influence of variety and treatment interaction on shooting.

Variety	Hormonal conc. (mg/l)	Characters of shoot		
		Days to shoot induction	No. of shoot/callus	% Shoot induction
Suruchi	1.0 Kn +1.0 BAP	22.40	3.933a	76.33a
	1.0 Kn	23.40	3.000b	66.93c
	1.0 Kn +1.0 IAA	24.33	2.467c	48.67e
BARI ada-1	1.0 Kn +1.0 BAP	27.73	2.333c	67.73b
	1.0 Kn	28.93	2.000d	50.47d
	1.0 Kn +1.0 IAA	29.27	1.733d	39.27f

Note: Mean values in a column having common letter are statistically identical and those having different letters are statistically different.

Table 6. Influence of variety, explant and treatment interaction on shooting.

Variety	Explants	Hormonal conc. (mg/l)	Characters of shoot		
			Days to shoot initiation	No. of shoot/callus	%Shoot induction
Suruchi	Leaf	1.0 Kn +1.0 BAP	20.20	4.800	87.60a
		1.0 Kn	21.60	3.800	77.80b
		1.0 Kn +1.0 IAA	22.80	2.800	56.60i
	Shoot tip	1.0 Kn +1.0 BAP	22.20	3.600	74.60c
		1.0 Kn	23.60	2.600	64.40f
		1.0 Kn +1.0 IAA	24.00	2.400	44.60j
	Root	1.0 Kn +1.0 BAP	24.80	3.400	66.80e
		1.0 Kn	25.00	2.600	58.60h
		1.0 Kn +1.0 IAA	26.20	2.200	44.80j
BARI ada-1	Leaf	1.0 Kn +1.0 BAP	26.40	2.800	74.80c
		1.0 Kn	28.20	2.400	65.00f
		1.0 Kn +1.0 IAA	28.80	2.200	44.80j
	Shoot tip	1.0 Kn +1.0 BAP	28.20	2.000	67.80d
		1.0 Kn	28.80	1.800	44.80j
		1.0 Kn +1.0 IAA	29.60	1.800	36.40l
	Root	1.0 Kn +1.0 BAP	28.60	1.600	60.60g
		1.0 Kn	29.80	1.600	41.60k
		1.0 Kn +1.0 IAA	29.40	1.200	36.60l

Note: Mean values in a column having common letter are statistically identical and those having different letters are statistically different.

Fig. 5. Initiation of shoot from callus derived from leaf explant of Suruchi of ginger on MS medium supplemented with 1 mg/l Kinetin + 1 mg/l BAP.

Fig. 6. Rooting of *in vitro* grown shoots of ginger on MS medium supplemented with 1 mg/l IBA.

Table 7. Influence of variety and explant interactions on rooting.

Variety	Explants	Characters of root		
		Days to root induction	No. of root/shoot	% Root induction
Suruchi	Leaf	13.53	8.867a	74.13a
	Shoot tip	14.47	8.133b	67.67b
	Root	15.40	7.733c	64.47c
BARI ada-1	Leaf	15.73	4.533d	59.73d
	Shoot tip	16.47	3.400e	49.40e
	Root	17.27	1.867f	41.07f

Note: Mean values in a column having common letter are statistically identical and those having different letters are statistically different.

Table 8. Influence of variety and treatment interaction on rooting.

Variety	Hormonal conc. (mg/l)	Characters of root		
		Days to root induction	Number of root/shoot	Percent root induction
Suruchi	0.5 IBA	14.67d	8.400b	68.40b
	1.0 IBA	13.07e	9.067a	76.87a
	1.5 IBA	15.67c	7.267c	61.00c
BARI ada-1	0.5 IBA	17.00b	3.067e	49.60e
	1.0 IBA	14.53d	4.600d	56.00d
	1.5 IBA	17.93a	2.133f	44.60f

Note: Mean values in a column having common letter are statistically identical and those having different letters are statistically different.

Table 9. Influence of variety, explant and treatment interactions on rooting.

Variety	Explants	Hormonal conc. (mg/l)	Characters of root		
			Days to root induction	No. of roots/shoot	% Root induction
Suruchi	Leaf	0.5 IBA	13.80k	9.200	72.20c
		1.0 IBA	12.20m	9.600	85.40a
		1.5 IBA	14.60ij	7.800	64.80f
	Shoot tip	0.5 IBA	14.80hij	8.200	67.60e
		1.0 IBA	12.80l	9.000	74.80b
		1.5IBA	15.80fg	7.200	60.60g
	Root	0.5 IBA	15.40gh	7.800	65.40f
		1.0 IBA	14.20jk	8.600	70.40d
		1.5 IBA	16.60de	6.800	57.60h
BARI ada-1	Leaf	0.5 IBA	16.20ef	4.400	60.60g
		1.0 IBA	13.80k	5.800	64.80f
		1.5 IBA	17.20c	3.400	53.80j
	Shoot tip	0.5 IBA	16.80cd	3.200	47.80k
		1.0 IBA	14.80ij	4.600	55.60i
		1.5 IBA	17.80b	2.400	44.80l
	Root	0.5 IBA	18.00b	1.600	40.40m
		1.0 IBA	15.00hi	3.400	47.60k
		1.5 IBA	18.80a	0.600	35.20n

Note: Mean values in a column having common letter are statistically identical and those having different letters are statistically different.

Establishment of the plantlets: The small plantlets with the sufficient root system were taken of from the culture vessels without damaging roots. Adhered media around the roots was washed of by running tap water. The plantlets were then transplanted to plastic pots having soil, sand and cowdung in 1:2:1 ratio and were covered with perforated polythene bag and kept into a hardening chamber for proper hardening for 8-10 days (Fig. 7). After that polythen bag was removed and transferred the plantlets to open air (Fig. 8).

Fig. 7. Hardening stage of the regenerated plantlet in the growth chamber.

Fig. 8. Established plant in earthen pot.

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(Received for publication 20 October 2008)