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ESTABLISHMENT OF A SUITABLE AND REPRODUCIBLE PROTOCOL FOR IN VITRO REGENERATION OF GINGER (*ZINGIBER OFFICINALE* ROSCOE)

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Abstract

Studies were carried out to find out a suitable and standard protocol for plant regeneration of 2 exotic and 6 locally cultivated varieties of ginger viz., Fulbaria, Syedpuri, Chittagongi, Jangli, Indian, Chaina, Sherpuri and BARI ada-1 were used as explants. For callus induction, comparing the varieties, Indian (51.84%) and among the treatments, selected MS media supplemented with 1 mgL^{-1} IAA + 3 mgL^{-1} BAP showed the maximum number of callus (2.681). For shoot regeneration, MS media supplemented with 4 mgL^{-1} BAP + 3 mgL^{-1} Kn + 1 mgL^{-1} IAA showed the best performance in ginger. Considering the combined effect of BAP, IAA, Kn and genotype, maximum regenerants (2.33) were found in Indian placed in selected media. In case of root induction, the root initiation potentiality was evaluated by testing on 5 different combinations of phytohormones. Among the genotypes Indian showed the best response (43.46%) on root initiation and it observed that half strength MS media supplemented with 2 mgL^{-1} IBA + 2 mgL^{-1} NAA was very effective. For the plant survival rate from sprout, leaf and root was about 86.67%, 80.00% and 85.71%, respectively. In case of plastic pots after the acclimatization of the plants in growth chamber whereas, in earthen pots it was 84.62%, 75.00% and 66.67% in case of Indian, Jongli and Chittagongi cultivars respectively.

Introduction

Ginger (*Zingiber officinales* Roscoe.) is one of the most important spice crops of the family Zingiberaceae. It is an important tropical horticultural plant, valued all over the world as an unparallel spice in culinary preparations and for its medicinal properties. It is rich in secondary metabolite such as Oleoresin (Bhagyalakshmi & Singh, 1988). *In vitro* culture techniques provide an alternative means of plant propagation and a tool for crop improvement (Vasil, 1987). Clonal multiplication of ginger through shoot multiplication has been reported (Hosoki & Sagawa, 1977; Wang, 1989; Balachandran *et al*., 1990; Rout & Das 1998). Plantlets derived from *In vitro* culture might exhibit somaclonal variation (Larkin *et al*., 1989), which is often heritable (Breiman *et al*., 1987). Ginger is vegetatively propagated through the underground rhizomes but, unfortunately, its multiplication rate is very low. In a vegetatively propagated plant like ginger, risk of systematic infections by root knot nematodes, bacterial wilt from the propagules is very high. Hosoki & Sagawa (1977) reported heavy losses in ginger production in some plantations due to some disease attacks caused by bacterial wilt (*Pseudomonas*

solanacearum), soft rot (*Pythium aphanidermatum*) and rhizome rot (*Fusarium oxysporum*). Because the diseases are mainly transmitted by rhizomes propagated every year, a production of disease-free clones is necessary in order to get a successful ginger cultivation. Micro propagation by using tissue culture technique can be a proper alternative to produce disease-free clones of ginger plant. Problems faced in ginger breeding have so far been the very low genetic variation in ginger plant. This is because ginger is vegetatively propagated crop and hybridization is not effective since its floral biology has not been properly observed yet (Simmonds, 1986). Ginger plays a vital role in our national economy. Therefore, to harvest the multifarious merits of sprout, leaf and root culture the present research work had been planned and carried out to establish a suitable and reproducible protocol for *in vitro* regeneration of Ginger plantlets through sprout, leaf and root culture, to optimize the suitable combination and concentration of hormones on selected media for regeneration of Ginger genotypes, to observe the suitability of various explants for callus induction and subsequent plantlet regeneration and screening of the better cultivar of ginger for good regeneration potentiality.

Materials and Methods

The experiment on establishment of a suitable and reproducible protocol for *In vitro* regeneration of ginger (*Zingiber officinale* Roscoe.) was conducted in Biotechnology and Genetic Engineering Laboratory of the Department of Genetics and Plant Breeding, Bangladesh Agricultural University, Mymensingh, Bangladesh during the period from July 2007 to June 2008.

Plant materials and source of materials: Eight different locally cultivated varieties of ginger were used as plant materials in the present experiment such as Fulbaria, Syedpuri, Chittagongi, Jangli, Indian, China, Sherpuri and BARI ada-1 were collected from Bangladesh Agricultural Research Institute (BARI) and local market. The collected rhizomes were preserved in sandy soil in a pot in the laboratory to get growing buds or sprouts, leaves and roots for using them as explants.

Culture media: Medium supplemented with different phytohormones as per treatments were used as culture medium for callus induction, shoot regeneration and root formation from multiplied shoot for production of plantlet.

Callus induction media: For callus induction MS (Murashige & Skoog, 1962) medium was used with different phytohormone combinations such as, 1 mgL^{-1} 2, 4-D + 2 mgL^{-1} Kn (T_1); 2 mgL^{-1} 2, 4-D + 2 mgL^{-1} Kn (T_2); 3 mgL^{-1} 2, 4-D + 2 mgL^{-1} Kn (T_3); 1 mgL^{-1} IAA + 1 mgL^{-1} BAP (T_4); 1 mgL^{-1} IAA + 2 mgL^{-1} BAP (T_5); 1 mgL^{-1} IAA + 3 mgL^{-1} BAP (T_6); 2 mgL^{-1} IBA + 4 mgL^{-1} BAP (T_7); 2.5 mgL^{-1} IBA + 4 mgL^{-1} BAP (T_8); 3 mgL^{-1} IBA + 4 mgL^{-1} BAP (T_9); 2 mgL^{-1} 2, 4-D + 2 mgL^{-1} BAP + 2 mgL^{-1} Kn (T_{10}); 2.5 mgL^{-1} 2, 4-D + 2.5 mgL^{-1} BAP + 2.5 mgL^{-1} Kn (T_{11}) and 3 mgL^{-1} 2, 4-D + 3 mgL^{-1} BAP + 3 mgL^{-1} Kn (T_{12}).

Shoot regeneration media: For shoot regeneration MS (Murashige & Skoog, 1962) medium was used with different phytohormone combinations such as, 1 mgL^{-1} BAP + 1.5 mgL^{-1} Kn + 1 mgL^{-1} IAA(T_1); 2 mgL^{-1} BAP + 2 mgL^{-1} Kn + 1 mgL^{-1} IAA (T_2); 3 mgL^{-1} BAP + 2.5 mgL^{-1} Kn + 1 mgL^{-1} IAA (T_3); 4 mgL^{-1} BAP + 3 mgL^{-1} Kn + 1 mgL^{-1} IAA (T_4) and 5 mgL^{-1} BAP + 3.5 mgL^{-1} Kn + 1 mgL^{-1} IAA (T_5).

Root formation media: For root formation half strength MS (Murashige & Skoog, 1962) medium was used with different phytohormone combinations such as, 0.5 mgL^{-1} IBA + 0.5 mgL^{-1} NAA (T_1); 1 mgL^{-1} IBA + 1 mgL^{-1} NAA (T_2); 1.5 mgL^{-1} IBA + 1.5 mgL^{-1} NAA (T_3); 2 mgL^{-1} IBA + 2 mgL^{-1} NAA (T_4) and 2.5 mgL^{-1} IBA + 2.5 mgL^{-1} NAA (T_5).

Culture techniques: Three culture techniques were employed in the present investigation as explants culture, subculture or transfer and rooting

i. Explants culture: Properly sterilized sprouts were cut into 3-6 mm length of longitudinal sections inside the laminar airflow cabinet and directly cultured on shoot regeneration media containing 20 ml of MS medium supplemented with different concentrations of hormones as per treatment, but in case of leaves and roots each of the sterilized explant were cut into 2-3 mm pieces using sterile scalpel. Five pieces were inoculated in each Petri dish/vial (Figs. 1, 2 and 3) containing sterile culture medium with different concentration and combination of growth regulators for callus induction and then transferred to growth room and allowed to grow in controlled environment. The temperature of the growth room was maintained within $25\pm2^{o}C$ by an air conditioner. A 16 hour light period was maintained with light intensity of 2000 lux for the growth and development of culture. All petridishes/vials were marked with permanent marker to indicate specific treatment.

ii. Subculture or transfer of the callus for shooting: Three to four weeks after inoculation of explants, the calli attained to a convenient size. Then those were removed from the existing media and again placed them into small vials with shoot regeneration media under laminar airflow cabinet.

iii. Rooting: When the shoots grew about 2-3 cm 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. Day to day observations was carried out to note the response of growing plantlets.

Preparation of pots and transplantation: Pot containing mixture of garden soil, sand and cow dung in the ratio of 1:2:3 was mixed properly and autoclaved for one hour at 121°C at 1.16 Kg/cm^2. Then the soil mixtures were allowed to cool in normal temperature and were taken into 10 cm plastic pots for growing the plantlets at *In vivo* condition.

When the plantlets became 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 transferred to pots containing the potting mixture. Immediately after transplantation, the plants along with the pots were covered with polythene bag to prevent desiccation. To reduce sudden shock, the pots were kept in growth room for 7-15 days under controlled environment.

Transfer of plantlets from plastic pots to earthen pots/soil: After two and half months the culture vessels with well developed plantlets were transferred to laboratory at room temperature and kept for 2-3 days. After that rooted plantlet were removed from culture vessel and the medium attached to root was gently wash out with tap water and individually transplanted to plastic pots containing the mixed soil. Immediately after transplantation, the plantlets along with pot were covered with moist and transparent poly bag to prevent desiccation. To reduce sudden shock, the pots were removed and the plantlets were kept in glass house. After 12 days these were transferred to the field.

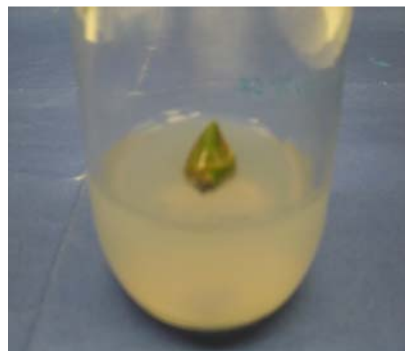

Fig. 1. Inoculation of sprout for direct shooting.

Fig. 2. Inoculation of root segments for callus induction.

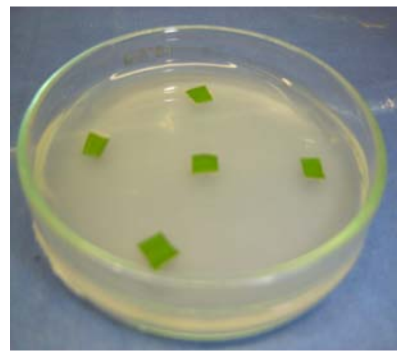

Fig. 3. Inoculation of leaf segments for callus induction.

Results and Discussion

Callus induction: The callusing performance of Indian variety was the highest (51.84%) and the lowest was found in Sherpuri (12.00%) and maximum sized (7.35mm) greenish to creamy colored and almost compact in nature callus was also produced from Indian variety (Table 1).

Similar results were also found by Babu *et al*., (1992). They found best response on callusing when young leaf of *Zingiber officinale cv.* Muran was cultured with various concentrations of growth regulators.

Callusing ability of MS + 1 mg/l IAA + 3 mg/l BAP supplemented medium was maximum (53.5%) than other hormones and also produced maximum sized (7.81 mm), creamy and nearly compact calli. Callusing ability was minimum on MS medium supplemented with 2.5 mg/l 2,4-D+2.5 mg/l BAP+2.5 mg/l Kinetin and produced minimum sized (5.34 mm), creamy and nearly compact calli (Table 2).

Effect of variety and explant interactions on callus induction: Performance of the cultivars x explants for callus induction have been presented in Table 3.

The values under each character of callus are the mean response of each variety in two different explants. Depending on varieties and explants the callus size varies from 4 mm to 9 mm depending on varieties and explants. The highest sized callus (8.98mm) was produced from leaf explant of Indian variety (Fig. 4). Second highest size of callus was derived from leaf of Chittagongi and that was 8.56 mm. Size of callus was found to be lowest (4.27mm) from root of Sherpuri variety. Callus produced from leaf of Jongli was almost greenish in color, but in case of compactness Indian variety produced the highly compact callus. Leaf and root of both Indian and Jongli variety produced greenish to creamy colored, compact to friable natured callus. Creamy colored and compact to friable natured callus was produced by root of BARI ada-1.

Performance for percent callus induction was also influenced by the variety and explant. The highest (57.66%) callus induction was found from the leaf of Indian variety followed by leaf of Jongli variety (46.00%). In case of BARI ada-1 variety the lowest percentage (9.32%) of callus induction was observed in the root explants followed by root of Sherpuri (14.67%) and these values were observed significantly different.

Table 1. Response of different varieties on callusing characters of ginger.

Variety	Characters of callus				
	No. of explants producing callus	Size of callus (mm)	Color of callus	Nature of callus	Callus Induction (%)
Fulbaria	1.49bc	6.70bc	2.40a	2.03a	29.84
China	0.76d	6.09de	1.82ef	1.76ef	15.17
Syedpuri	1.11cd	6.44b-d	2.04cd	1.90cd	22.16
Chittagongi	1.53bc	6.99ab	2.25a-c	1.96a-c	30.50
Jongli	2.08ab	6.93ab	2.35ab	2.22ab	41.66
Indian	2.59a	7.35a	2.15bc	2.27bc	51.84
Sherpuri	0.60d	5.81e	1.68f	1.66f	12.00
BARI ada-1	0.93cd	6.28c-e	1.92de	1.85de	18.50

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

Table 2. Response of different treatments on callusing characters of ginger.

Treatment, MS medium supplemented with	Characters of callus				
	No. of callus Induction	Size of callus (mm)	Color of callus	Nature of callus	Callus Induction (%)
1 mgL^{-1} 2, 4-D + 2 mgL^{-1} Kn	0.39gh	5.57hi	1.83gh	1.70gh	7.75
2 mgL^{-1} 2, 4-D + 2 mgL^{-1} Kn	1.24d-f	6.43ef	2.05c-g	1.93c-g	24.74
3 mgL^{-1} 2, 4-D + 2 mgL^{-1} Kn	1.46c-e	6.67d-f	2.10b-f	1.99b-f	20.00
1 mgL^{-1} IAA + 1 mgL^{-1} BAP	1.01e-g	6.24fg	2.00d-g	1.86d-g	20.26
1 mgL^{-1} IAA + 2 mgL^{-1} BAP	2.40ab	7.60ab	2.31ab	2.22ab	45.00
1 mgL^{-1} IAA + 3 mgL^{-1} BAP	2.681a	7.81a	2.37a	2.27a	53.50
2 mgL^{-1} IBA + 4 mgL^{-1} BAP	2.14a-c	7.37a-c	2.25a-c	2.19a-c	42.75
2.5 mgL^{-1} IBA + 4 mgL^{-1} BAP	1.94bc	7.15b-d	2.21a-d	2.13a-d	38.75
3 mgL^{-1} IBA + 4 mgL^{-1} BAP	1.78b-d	6.85c-e	2.18a-e	2.07a-e	34.74
2 mgL^{-1} 2, 4-D+2 mgL^{-1} BAP + 2 mgL^{-1} Kn	0.81e-h	6.08f-h	1.98e-h	1.86e-h	16.25
2.5 mgL^{-1} 2, 4-D+2.5 mgL^{-1} BAP+2.5 mgL^{-1} Kn	0.28h	5.34i	1.76h	1.15h	4.75
3 mgL^{-1} 2, 4-D+3 mgL^{-1} BAP + 3 mgL^{-1} Kn	0.59f-h	5.78g-i	1.88f-h	1.76f-h	11.75

Table 3. Performance of variety and explants interaction on different callus characters of ginger.

Variety	Explants	Characters of callus			
		No. of explants producing callus	Size of Callus(mm)	Color of callus	Nature of callus
Fulbaria	Leaf	1.68b-d	8.14b-d	2.57a	2.23a
	Root	1.30d-g	5.26f-h	2.23bc	1.84bc
China	Leaf	0.93e-h	7.56de	1.92e-h	2.06e-h
	Root	0.58gh	4.62ij	1.70hi	1.66hi
Syedpuri	Leaf	1.30d-g	7.99b-d	2.12c-e	2.10c-e
	Root	0.92e-h	4.90g-i	1.96d-g	1.70d-g
Chittagongi	Leaf	1.63b-e	8.56ab	2.33bc	2.14bc
	Root	1.42c-f	5.42fg	2.17cd	1.78cd
Jongli	Leaf	2.10bc	8.35bc	2.40ab	2.41ab
	Root	2.07bc	5.51f	2.31bc	2.02bc
Indian	Leaf	2.89a	8.98a	2.17cd	2.49cd
	Root	2.30ab	5.71f	2.13c-e	2.05c-e
Sherpuri	Leaf	0.73f-h	7.36e	1.76g-i	1.82g-i
	Root	0.47h	4.27j	1.60i	1.62hi
BARI ada-1	Leaf	1.10d-h	7.79c-e	2.00d-f	2.02d-f
	Root	0.75f-h	4.76h-j	1.86f-h	1.50i

Table 4. Response of variety, explant and treatment on shoot regeneration.

Variety	China	Fulbaria	Indian	Syedpuri	Sherpuri	Jongli	Chittagongi	BARI ada-1
Shoot regeneration %	19.20	30.40	35.20	27.46	16.27	35.74	32.00	24.54
Treatments	Treatment-1		Treatment-2	Treatment-3		Treatment-4		Treatment-5
Shoot regeneration %	21.16		24.16	31.00		33.66		28.00
Explants	Sprout		Leaf			Root		
Shoot regeneration %	31.40		27.49			24.00		

Response of variety, explant and treatment on shoot regeneration: Influence of variety, explants and treatment on number of shoots/callus, length of shoots (cm) and number of leaves per shoot was statistically significant. Highest percentage (35.74%) of shoot induction was observed at Jongli variety and the lowest shoot induction was performed by Sherpuri (16.27%) (Table 4). Highest percentage (31.40%) of shoot induction was produced by sprouting buds (Fig. 6) followed by leaf (27.40%) and root (24.00%) explants respectively (Table 4). Kanker *et al*., (1993) observed that callus culture of ginger was induced on MS medium from young leaf segments gave best shooting response. Percent shoot induction (33.66%) were highest on Treatment_4 (Table 4).

Effect of variety and treatment interactions on shoot induction: Among the 8 varieties Indian variety produced highest number of shoots/explant (2.33) with Treatment_4 phytohormone combination followed by Jongli (2.28), Chittagongi respectively (Table 5). Kacker *et al*., (1993) found similar results when cultures were transferred to MS medium containing 8.9 mg/l BAP (Table 5). In case of length of shoot and number of leaves/shoot, the Indian variety also produced the highest length (1.34 cm) of shoots and highest number of leaves/shoot (1.21) followed by Jongli and Chittagongi varieties with Treatment_4 phytohormone interactions (Table 5).

Root induction: Well root induction is very important for establishment of *In vitro* regenerated plantlets. The shoots just after the initiation from the calli, were subcultured to another hormone supplemented media for root formation. The influence of variety and explant on the characters of root like; No. of roots/shoot and Length of root (cm) have been presented in Table 6.

From the table it was found that the interaction of different explants and varieties showed variations on rooting. The interaction of Indian variety and sprouting bud explants produced maximum number (2.28) of roots per shoot and length of roots (1.01 cm) followed by Jongli (2.20), Chittagongi (2.12) respectively (Table 6). The rooting performance from leaf explants was also satisfactory as well as the length of roots. The number of root initiation and length of roots from root explants was minimum in number roots/shoot (1.64) and also the minimum length (0.71) of roots.

Establishment of the plantlets: The small plantlets with the sufficient root system were taken off from the culture vessels without damaging roots. Adhered media around the roots was washed off 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. 8). After that polythene bag was removed and transferred the plantlets to earthen pots as well as in the field (Fig. 9). The survival rate of regenerants from sprout, leaf and root of ginger cultivars after transfer in soil is presented in Table 7. Chittagongi is the best performer followed by Jongli variety in plastic pots as well as in earthen pots.

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

Variety	Treatments (MS medium supplemented with)	Characters of shoot		
		No. of shoots/ explant	Length of shoot (cm)	No. of leaves/ shoot
Fulbaria	1 mgL^{-1} BAP + 1.5 mgL^{-1} Kn + 1 mgL^{-1} IAA	1.73n-p	1.13cd	0.53f
	2 mgL^{-1} BAP + 2 mgL^{-1} Kn + 1 mgL^{-1} IAA	1.80m-o	1.10de	0.60e
	3 mgL^{-1} BAP + 2.5 mgL^{-1} Kn + 1 mgL^{-1} IAA	2.00j	1.07c-e	0.73cd
	4 mgL^{-1} BAP + 3 mgL^{-1} Kn + 1 mgL^{-1} IAA	2.13c-f	1.17cd	0.80c
	5 mgL^{-1} BAP + 3.5 mgL^{-1} Kn + 1 mgL^{-1} IAA	1.87lm	1.04c-e	0.67de
China	1 mgL^{-1} BAP + 1.5 mgL^{-1} Kn + 1 mgL^{-1} IAA	1.40s-w	0.90ef	0.47fg
	2 mgL^{-1} BAP + 2 mgL^{-1} Kn + 1 mgL^{-1} IAA	1.53o-t	0.93de	0.47fg
	3 mgL^{-1} BAP + 2.5 mgL^{-1} Kn + 1 mgL^{-1} IAA	1.67op	0.98ef	o.53f
	4 mgL^{-1} BAP + 3 mgL^{-1} Kn + 1 mgL^{-1} IAA	1.73n-p	1.04c-e	0.60e
	5 mgL^{-1} BAP + 3.5 mgL^{-1} Kn + 1 mgL^{-1} IAA	1.60p	0.95ef	0.63d-f
Syedruri	1 mgL^{-1} BAP + 1.5 mgL^{-1} Kn + 1 mgL^{-1} IAA	1.80m-o	0.96ef	0.68ef
	2 mgL^{-1} BAP + 2 mgL^{-1} Kn + 1 mgL^{-1} IAA	1.73n-p	0.98ef	0.60e
	3 mgL^{-1} BAP + 2.5 mgL^{-1} Kn + 1 mgL^{-1} IAA	1.73n-p	1.04ef	0.60e
	4 mgL^{-1} BAP + 3 mgL^{-1} Kn + 1 mgL^{-1} IAA	1.93k-m	1.08cd	0.87bc
	5 mgL^{-1} BAP + 3.5 mgL^{-1} Kn + 1 mgL^{-1} IAA	1.87lm	1.14cd	0.47fg
Chittagongi	1 mgL^{-1} BAP + 1.5 mgL^{-1} Kn + 1 mgL^{-1} IAA	1.93k-m	0.98ef	0.60e
	2 mgL^{-1} BAP + 2 mgL^{-1} Kn + 1 mgL^{-1} IAA	1.87lm	1.01c-e	0.67de
	3 mgL^{-1} BAP + 2.5 mgL^{-1} Kn + 1 mgL^{-1} IAA	2.07e-h	1.08ef	0.87bc
	4 mgL^{-1} BAP + 3 mgL^{-1} Kn + 1 mgL^{-1} IAA	2.13c-f	1.12d	1.00b
	5 mgL^{-1} BAP + 3.5 mgL^{-1} Kn + 1 mgL^{-1} IAA	2.00j	1.04cd	0.73cd
Jongli	1 mgL^{-1} BAP + 1.5 mgL^{-1} Kn + 1 mgL^{-1} IAA	1.93k-m	1.00c-e	0.60e
	2 mgL^{-1} BAP + 2 mgL^{-1} Kn + 1 mgL^{-1} IAA	2.07ef	1.03cd	0.80c
	3 mgL^{-1} BAP + 2.5 mgL^{-1} Kn + 1 mgL^{-1} IAA	2.13ef	1.15a-d	1.00b
	4 mgL^{-1} BAP + 3 mgL^{-1} Kn + 1 mgL^{-1} IAA	2.28a-d	1.13c	1.20a
	5 mgL^{-1} BAP + 3.5 mgL^{-1} Kn + 1 mgL^{-1} IAA	2.13c-f	1.07c-e	0.87b-d
Indian	1 mgL^{-1} BAP + 1.5 mgL^{-1} Kn + 1 mgL^{-1} IAA	2.07e-f	1.04cd	0.87b-d
	2 mgL^{-1} BAP + 2 mgL^{-1} Kn + 1 mgL^{-1} IAA	2.07e-f	1.09b-d	0.93bc
	3 mgL^{-1} BAP + 2.5 mgL^{-1} Kn + 1 mgL^{-1} IAA	2.27a-d	1.26b	1.13ab
	4 mgL^{-1} BAP + 3 mgL^{-1} Kn + 1 mgL^{-1} IAA	2.33a	1.34a	1.21a
	5 mgL^{-1} BAP + 3.5 mgL^{-1} Kn + 1 mgL^{-1} IAA	2.13c-f	1.17c	1.07ab
Sherpuri	1 mgL^{-1} BAP + 1.5 mgL^{-1} Kn + 1 mgL^{-1} IAA	1.27z	0.88g	0.40g
	2 mgL^{-1} BAP + 2 mgL^{-1} Kn + 1 mgL^{-1} IAA	1.33v-z	0.91e-g	0.47fg
	3 mgL^{-1} BAP + 2.5 mgL^{-1} Kn + 1 mgL^{-1} IAA	1.53o-t	0.98ef	0.53f
	4 mgL^{-1} BAP + 3 mgL^{-1} Kn + 1 mgL^{-1} IAA	1.67o-p	1.01de	0.53f
	5 mgL^{-1} BAP + 3.5 mgL^{-1} Kn + 1 mgL^{-1} IAA	1.40s-w	0.95fg	0.47fg
BARI ada-1	1 mgL^{-1} BAP + 1.5 mgL^{-1} Kn + 1 mgL^{-1} IAA (T_1)	1.53o-t	0.95fg	0.40g
	2 mgL^{-1} BAP + 2 mgL^{-1} Kn + 1 mgL^{-1} IAA (T_2)	1.60p	0.97e-g	0.53f
	3 mgL^{-1} BAP + 2.5 mgL^{-1} Kn + 1 mgL^{-1} IAA (T_3)	1.80m-o	1.03cd	0.60e
	4 mgL^{-1} BAP + 3 mgL^{-1} Kn + 1 mgL^{-1} IAA (T_4)	1.87lm	1.05cd	0.67d
	5 mgL^{-1} BAP + 3.5 mgL^{-1} Kn + 1 mgL^{-1} IAA (T_5)	1.73n-p	1.00c-e	0.53f

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

Table 6. Effect of variety and explant interaction on root initiation.

Variety	Explants	Characters of root	
		No. of roots/shoot	Length of root (cm)
	Sprouts	1.96i-n	0.92 d-g
Fulbaria	Leaf	1.92j-p	0.89e-g
	Root	1.84l-p	0.87gh
	Sprouts	1.64q-v	0.82h-i
China	Leaf	1.60r-w	0.79ij
	Root	1.52s-y	0.74jk
	Sprouts	1.80mn	0.88f-h
Syedruri	Leaf	1.80mn	0.86gh
	Root	1.84l-p	0.82hi
	Sprouts	2.12d	0.94c-f
Chittagongi	Leaf	1.96i-n	0.93c-f
	Root	1.92j-p	0.91d-g
	Sprouts	2.20a-e	1.01ab
Jongli	Leaf	2.12d-i	0.98a-c
	Root	2.00g-m	0.95b-e
	Sprouts	2.28a	1.01a
Indian	Leaf	2.16b-g	0.99a-c
	Root	2.08e-j	0.96a-d
	Sprouts	1.56rs	0.780ij
Sherpuri	Leaf	1.44v-y	0.74jk
	Root	1.32z	0.71k
	Sprouts	1.76p-v	0.87gh
BARI ada-1	Leaf	1.72p-t	0.82h-i
	Root	1.64n-v	0.78ij

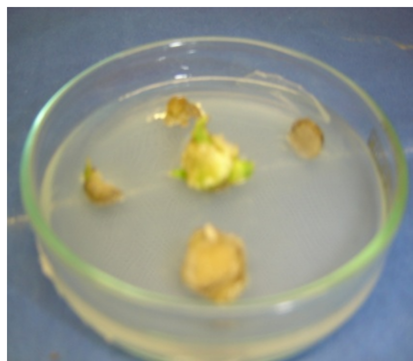

Fig. 4. Callus induction from leaf of Indian cultivar after 20 days of inoculation.

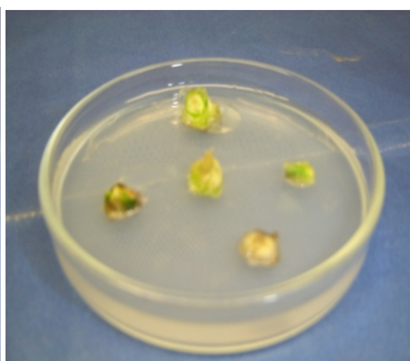

Fig. 5. Callus induction from leaf explant of Jongli cultivar after 20 days of inoculation.

Fig. 6. Shoot regeneration from sprouts of Jongli cultivar after 25days of inoculation.

Fig. 7. Root initiation from Indian cultivar on ½ MS+2 mgL^{-1} IBA + 2 mgL^{-1} NAA.

Fig. 8. Regenerated plant of Indian cultivar from sprout in plastic pot covered with polythene bag kept in net house for hardening.

Fig. 9. Regenerated plant of Indian cultivar of Ginger in earthen pot.

Table 7. Survival rate of regenerants from sprout, leaf and root of Ginger cultivars after transfer in soil.

	Genotypes	Sprout			Leaf			Root		
		No. of plantlets transplanted	No. of plant survived	Survival rate (%)	No. of plantlets transplanted	No. of plant survived	Survival rate (%)	No. of plantlets transplanted	No. of plant survived	Survival rate (%)
In plastic pot	Fulbaria	9	7	77.78	5	3	60.00	4	2	50.00
	China	4	2	50.00	4	2	50.00	1	0	0.00
	Syedpuri	7	5	71.43	6	4	66.67	1	0	0.00
	Chittagongi	11	9	81.82	8	6	75.00	7	6	85.71
	Jongli	13	11	84.62	10	8	80.00	7	5	71.42
	Indian	15	13	86.67	10	7	70.00	6	4	66.67
	Sherpuri	3	1	33.33	3	1	33.33	5	3	60.00
	BARI ada-1	5	3	60.00	7	5	71.43	4	2	50.00
In earthen pot/soil	Fulbaria	7	5	71.43	3	1	33.33	2	0	0.00
	China	2	1	50.00	2	0	0.00	0	0	0.00
	Syedpuri	5	3	60.00	4	2	50.00	0	0	0.00
	Chittagongi	9	7	77.78	6	4	66.67	6	4	66.67
	Jongli	11	9	81.82	8	6	75.00	5	3	60.00
	Indian	13	11	84.62	7	5	71.43	4	2	50.00
	Sherpuri	1	0	0.00	1	0	0.00	3	1	33.33
	BARI ada-1	3	2	66.67	5	3	60.00	2	0	0.00

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