IN VITRO REGENERATION OF GINGER (*Zingiber officinale* Rosc.) THROUGH CALLUS CULTURE

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ABSTRACT

The study was conducted in Plant Tissue Culture Laboratory at Department of Genetics and Plant Breeding, N. M. Collage of Agriculture, Navsari Agricultural University, Navsari. A protocol for formation of callus and regeneration of ginger (Zingiber officinale Rosc.) shoots cv. Santarampur Local was achieved during investigation. MS medium supplemented with 1 mg/l 2, 4-D proved to be the best for the induction of a good quality callus i.e. creamy white and friable from the ginger leaf explants. The maximum photosynthetic callus (80.25) with parrot green colored spots was obtained on MS medium containing 1 mg/l BAP + 0.5 mg/l NAA and gave highest multiple micro-shoot ratio 1:8.25. Maximum shoot regeneration per gram of callus was achieved from the MS media containing 1 mg/l BAP with 1 mg/l KN giving 82.24 per cent shooting with 8.62 number of shoots under 2000 lux light intensity. A good quality of callus induced under dark condition, while dark gray or brown and sticky callus observed under light.

KEY WORDS: *Callus, ginger (Aadu), growth regulators, light intensity, micro-propagation, medium, micro-shoots, regeneration*

INTRODUCTION

Ginger is the rhizome of the plant *Zingiber officinale* Rosc and family (*Zingiberaceae*). Ginger is a tuber that is consumed whole as a delicacy, medicine or herb. Other notable members of this plant family are turmeric, cardamom and galangal. Ginger cultivation began in South Asia and has spread to East Africa and the Caribbean. The method of micro-propagation of ginger is through pieces of underground rhizomes. But, this is slow process. Rhizome has dormancy period and only sprouts during the monsoon that to only 5 to 6 plants can be obtained from one single rhizome in a year. So, rapid method of multiplication is needed especially of newly developed high yielding varieties, which are available in small quantities. But, through tissue culture, dormancy problem could be overcome and it would be possible to cultivate the crop under favourable conditions. Ginger cultivation is threatened by rhizome rot diseases caused by *Pseudomonas solanacearum* and *Phythium sp.* These are spread through infected seed rhizome. The major hindrance for crop improvement in ginger is due to lack of seed set. So, rapid multiplication of diseases free propagules is needed on a large scale. It is possible only through *in vitro* culture. It is estimated that 3 fold increases in the production of rhizomes could be possible by effective control of diseases and pests (Hosoki and Sagawa, 1977).

MATERIALS AND METHODS

The study was conducted in Plant Tissue Culture Laboratory at Department. of Genetics and Plant Breeding, N. M. Collage of Agriculture, Navsari Agricultural University, Navsari. Fully mature rhizome of ginger *(Zingiber officinale* Rosc., cv. Santrampur Local) available in the local markets of Santrampur village in Panch Mahal district was used in present experimentation. The tubers and leaves were surface sterilized by 0.5 per cnent (v/v) NaOCl for 15 minutes followed by dipping in 70 per cent (v/v) ethyl alcohol for 30 seconds and finally treating with 0.1 per cent (w/v) $HgCl_2$ solution for 12 minutes. The leaf explants were excised under laminar airflow and placed on the medium. For shoot-tips culture of tuber, Murashige and Skoog (1962) medium supplemented with different concentrations of 2, 4-D was used for callus induction. The media were autoclaved at 121 °C for 20 min. The pH of the medium was adjusted to 5.8 with 1 N NaOH before autoclaving. For further induction of photosynthetic callus, it was grown on media supplemented with BAP and NAA. Regeneration of shoots from healthy callus was carried out by keeping it on the media containing different concentrations of BAP in combination with KN containing in the MS medium. Callus regenerated from leaf explants was kept in the different light intensities (Extremely high light (3000 Lux), high light (2000 Lux), medium light (1000 Lux), low light (500 Lux), extremely low light (100 Lux) and Dark), so as to understand the requisite light intensity for the better induction of callus and shoot proliferation from it. Fully rooted shoots were taken out of laboratory and planted in the poly house where five different formulations of potting mixtures *viz*., Cocopeat + Clay + FYM, FYM + Clay, Vermiculite, Sand + Clay and Sand + Vermiculite were tried for hardening of plantlets.

Data on number of days taken for callus induction, quality of callus and shoot regeneration frequency were recorded. Data analysis was subjected to analyze statistically in Completely Randomized Design (CRD), wherever necessary as prescribed by Panse and Sukhatme (1985).

RESULTS AND DISCUSSION

Effect of 2, 4-D on the induction of the callus is well known and largely appreciated by so many workers, who have pursued on the formation of the callus from the various plant parts like leaf, stem etc. Leaf explants were taken as subject for the induction of the callus *in vitro* (Table 1). Among four different concentrations of 2, 4-D (0.5, 1.0, 1.5 and 2.0 mg/l) were studied along with control, 1 mg/l of 2, 4-D produced creamy white, usable and friable callus within considerably short period of time (21.68 days) with 3.39 gm weight, which was significantly superior over the rest of the treatments (Fig.A). Our findings are in harmony with Malmug *et al*. (1991), Samsudeen *et al.* (2000), Nirmalbabu *et al.* (2004) and Guo and Zhang (2005).

Maximum green coloured callus was noticed on MS medium fortified with 0.5 mg/l NAA and 1.0 mg/l BAP (80.25 %) presented in Table 2 and Fig. B. There is no such report found in the literature in case of ginger but similar line of work has been notified by Samsudeen et al. (2000) and Patel (2007). When 2, 4-D induced leaf callus were transferred to the new MS medium containing 1 mg/l BAP + 1 mg/l KN, showed significantly highest shooting percentage i.e. 82.24 per cent within 22.40 days (Table 3 and Fig. C). As displayed in Table 2, multiple shoot ratios was observed maximum (1:8.25) in treatment combination MS medium fortified with 0.5 mg/l NAA, 1.0 mg/l BAP and 20 g/l sucrose. Moreover, close positive correlation was observed between the percentage of green coloration of callus and multiple shoot ratios. These results are in conformity with those of

Malmug *et al*. (1991), Samsudeen *et al.* (2000) and Patel (2007).

Light intensity exhibited profound effect on callus differentiation and height of shoots and plantlets survival was eight time higher as they developed under high light than low light (Barba *et al*., 1969). High light intensity (2000 lux) was found more desirable for conversion of callus into proliferated shoots of ginger i.e. 69-76 per cent (Table 4), which is also been in connivance with the recent findings of Patel (2007) and Lincy *et al.* (2009).

In vitro raised plantlets needed hardening for acclimatization to the natural environment, which is a critical process due to their anatomical and physiological peculiarities. On transplanting, excessive water loss from the plantlets has been recorded which was attributed to the improper development of cuticle and slowness of stomatal response to water stress (Brainerd and Fuchigami, 1981). Inside culture vessels which in this experiment, was prevented by covering the plantlets in polycarbonate trapezoidal tray and kept inside net house. Perusal of data in Table 5 indicated that potting mixture having Cocopeat + Clay + FYM was best with most survival of the plants (86.50 %) during first hardening and 84.50 per cent during second hardening among all the tested mixtures followed by FYM + Clay and vermiculite only. Besides, the potting mixture Sand + Vermiculite poorly supported to plant survival and growth during first (60.50 %) and second hardening (46.50 %). Cocopeat: Clay : FYM (1:1:1 v/v/v) was superior to others, mixing Clay and FYM might have helped in giving better grip for roots, ample aeration and sufficient organic matter. However, our results are in accordance with Sit and Tiwari (1997), Hosoki and Sagawa (1977) and Hiremath (2006), who used basically same potting mixture.

CONCLUSION

From the present study, it can be concluded that MS medium supplemented with 1 mg/l 2, 4- D proved to be the best for the induction of a good quality callus from the ginger leaf explants. The maximum photosynthetic callus (80.25) with parrot green colored spots was obtained on MS medium containing 1 mg/l BAP + 0.5 mg/l NAA and gave highest multiple micro-shoot ratio 1:8.25. Maximum shoot regeneration per gram of callus was achieved from the MS media containing 1 mg/l BAP with 1 mg/l KN giving 82.24 per cent shooting with 8.62 number of shoots under 2000 lux light intensity.

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Table 1: Effect of different levels of 2, 4-D on callus induction in ginger in vitro

2,4-D (mg/l)	Days for Callus Induction	Wt. of Callus (g)	Quality of Callus (Visual)
0.5	26.74	1.98	Yellowish, compact
1.0	21.68	3.39	Creamy white, friable and globular
1.5	25.58	2.70	Creamy white, compact
2.0	27.76	1.39	Yellowish brown, slimy
S.Em. ±	0.22	0.05	
C.D. at 5%	0.66	0.16	
C.V.%	2.16	5.68	

Medium-MS, Incubation: 4 weeks, No. of Repetitions: 4

Table 2: Multiple shoot ratio observed in various combinations of growth regulators BAP and NAA from 1 gm of callus

BAP + NAA (mg/l)	Green Coloration of Callus (%)	Multiple Shoot Ratio Obtained
0.5+0.5	44.00	1:3.25
1.0+0. 5	80.25	1:8.25
1.5+0.5	69.00	1:6.25
2.0+0.5	56.00	1: 4.75
S.Em. ±	1.49	0.43
C.D. at 5%	4.60	1.33
C.V.%	4.79	15.40

Medium-MS, Incubation: 4 weeks, No. of Repetitions: 4

Table 3: Effect of BAP and KN combinations on shoot regeneration from callus in ginger in vitro

BAP (mg/l)	KN (mg/l)	Days for Shooting	Shooting (%)	No. of Shoots
0.5	0.5	28.42	54.33	3.74
1.0	0.5	30.32	47.62	2.52
0.5	1.0	26.19	64.96	6.75
1.0	1.0	22.40	82.24	8.62
S.Em. ±		0.26	1.08	0.16
C.D. at 5%		0.79	3.31	0.48
C.V.%		1.92	3.45	5.78

Medium-MS, Incubation: 4 weeks, No. of Repetitions: 4

Table 4: Effect of light intensity on conversion of callus cells in to micro-shoots

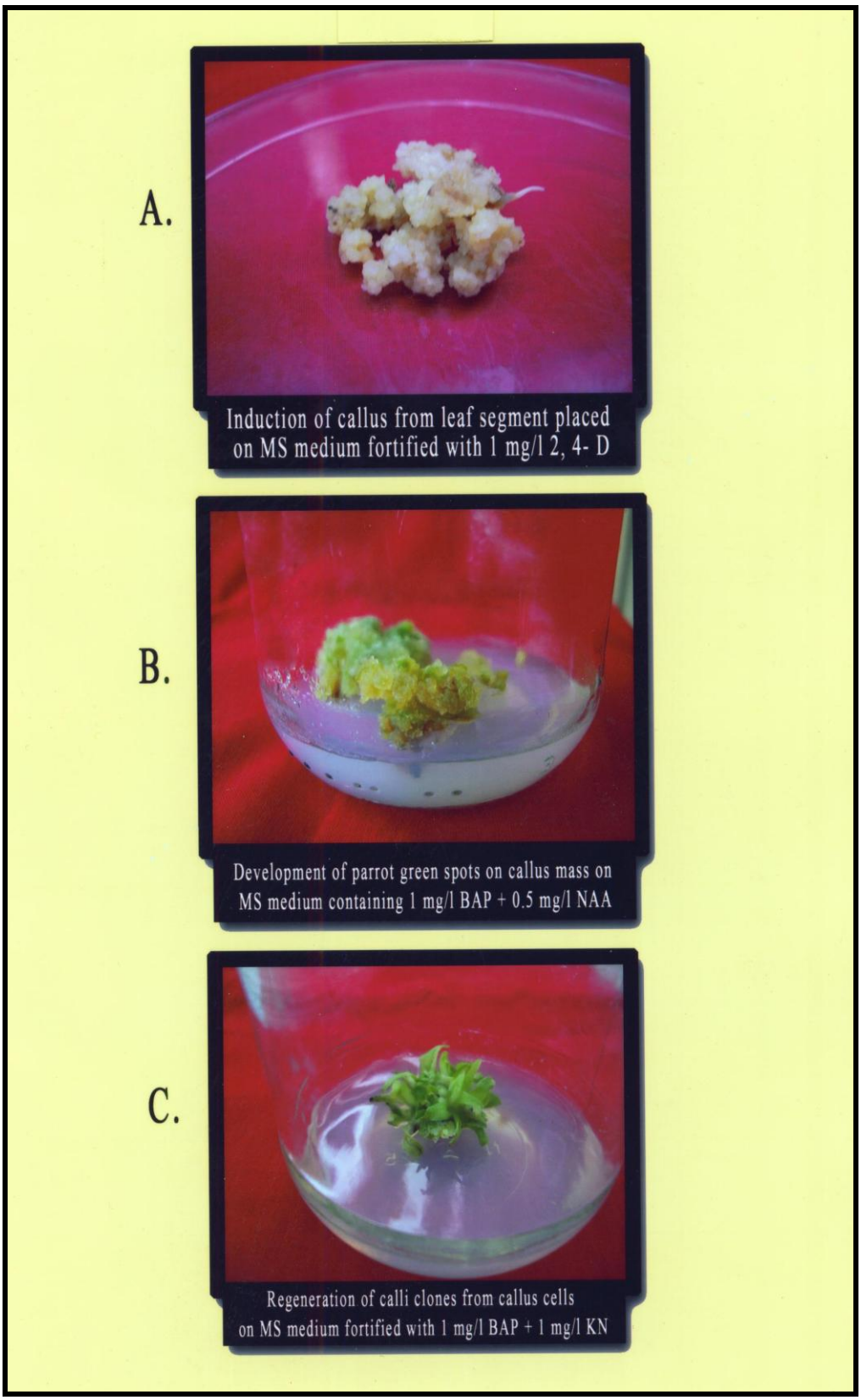
Light Intensity	**Conversion of Proliferated Callus in to Shoots (%) from 1g of callus**
Extremely high light (3000)	58-64 %
High light (2000)	69-76%
Medium light (1000)	48-54%
Low light (500)	30-35%
Extremely low light (100)	10-15%
Dark	No

Medium: MS medium + 0.5 mg/l NAA+ 1.0 mg/l BAP, Incubation: 4 weeks, No. of Repetitions: 4

Table 5: Effect of potting mixture on survival percentage of plantlets during hardening in ginger

Medium	**No. of Plants Survived**		**Survival%**	
	15 DAT	**30 DAT**	**15 DAT**	**30 DAT**
Cocopeat + Clay + FYM	43.25	42.25	86.50	84.50
FYM + Clay	41.25	39.75	82.50	79.50
Vermiculite	37.50	36.25	75.00	70.50
Sand + Clay	36.25	34.75	72.50	69.50
Sand + Vermiculite	30.25	23.25	60.50	46.50
S.Em. ±	0.73	0.89	1.46	1.78
C.D. at 5%	2.20	2.69	4.40	5.38
C.V.%	3.87	5.06	3.87	5.06

No. of Repetitions: 4



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