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The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(4): 201-204

www.thepharmajournal.com
Received: 01-01-2023
Accepted: 05-02-2023

Resmi Paul
College of Agriculture, Vellanikkara, Kerala Agricultural University, KAU P.O., Thrissur, Kerala, India

MR Shylaja
College of Agriculture, Vellanikkara, Kerala Agricultural University, KAU P.O., Thrissur, Kerala, India

Corresponding Author:
Resmi Paul
College of Agriculture, Vellanikkara, Kerala Agricultural University, KAU P.O., Thrissur, Kerala, India

Growth analysis of ginger (*Zingiber officinale* Rosc.) regenerated through various routes of micropropagation during *ex vitro* establishment

Resmi Paul and MR Shylaja

Abstract
Ginger is an important spice used for flavouring foods and food-products. Genetic variability available in ginger is very limited due to lack of variability and absence of natural seed set. As natural variability stands limited, broadening the genetic base through mutagenesis or tissue culture techniques pave way for exploitation of induced variability. In the present study, plantlets of ginger cultivars Maran and Rio-de-Janeiro were produced through four routes *viz*., callus mediated indirect organogenesis, indirect somatic embryogenesis and *in vitro* mutagenesis of organogenic as well as embryogenic calli and regenerants were observed for morphological characters at plant out stage and also for a growth period upto three months. Regenerants derived through indirect organogenesis and irradiation of organogenic calli exhibited maximum number of roots, length of roots, number of tillers and length of pseudostem at plant out stage. Plantlets derived through indirect organogenesis, indirect somatic embryogenesis and irradiation of organogenic calli recorded highest plantlet establishment at three months after planting. Regenerants derived through irradiation of organogenic and embryogenic calli recorded highest length of longest leaf at three months after planting. There was no significant difference between regenerants derived through various routes in number of tillers and length of pseudostem at three months after plant out. Irrespective of the various routes of regeneration, there was no significant difference between the regenerants of two cultivars *viz*., Maran and Rio-de-Janeiro with respect to number of roots produced, length of roots, number of tillers and length of pseudostem at plant out and also on growth parameters for a period of three months. High somaclonal variation observed in regenerants derived through indirect organogenesis and irradiation of organogenic calli should be exploited.

Keywords: Gamma irradiation, ginger, indirect organogenesis, somatic embryogenesis

Introduction
Ginger (*Zingiber officinale* Rosc.), belonging to family Zingiberaceae is an important commercial crop in tropical and subtropical countries. The rhizome or modified underground stem of ginger is used worldwide as a spice for flavouring a multitude of foods and food-products. It is also used in medicines, particularly in traditional medicines of India. In the global scenario, India still continues to be the largest producer, consumer and exporter of ginger. Ginger is propagated vegetatively through underground rhizomes. As the crop is vegetatively propagated, genetic variability available in ginger is very limited. Breeding through selection and hybridization is not possible in ginger due to lack of variability and absence of natural seed set. Presence of spiny stigmatic surface, low pollen germination and low pollen tube growth prevent fertilization and seed set in ginger (Sathiabhama, 1998) [11]. Application of growth regulators for induction of flowering, effecting pollination and seed set was also not successful in ginger (Usha, 1984) [13]. Thus, ginger cannot be propagated sexually. As natural variability stands limited, broadening the genetic base through mutagenesis or tissue culture techniques pave way for exploitation of induced variability for isolating plant types with high yield, quality and resistance/ tolerance to diseases. Somaclonal variation can create different phenotypes and increase genetic variability in crops. Tissue culture-induced somaclonal variation has become important sources of variability for crop improvement, especially for asexually reproduced plant species (Krishna *et al*., 2016) [6]. Although the occurrence of somaclonal variation is an undesirable character in the commercial production of true to type disease-free seed rhizomes in ginger, the somaclonal variants are valuable sources to enrich genetic variability in this crop. Hence the present study was undertaken at College of Agriculture, Kerala Agricultural University, Thrissur, Kerala, India to induce variability in ginger and to observe the difference in growth characters of regenerants

produced through various routes of micropropagation during *ex vitro* establishment.

Materials and Methods

Seed rhizomes of the two cultivars of ginger were collected from Regional Agricultural Research Station, Ambalavayal, Kerala Agricultural University, Kerala, India. Plantlets of ginger were produced through callus mediated indirect organogenesis, indirect somatic embryogenesis and *in vitro* mutagenesis of organogenic as well as embryogenic calli. The experiment was laid out in a Randomized Block Design with three replications. Each replication consisted of 30 plants. Data were subjected to analysis of variance (ANOVA) using the GRAPES software developed by Kerala Agricultural University (Gopinath *et al*., 2021) [3].

Production of regenerants through indirect organogenesis

Plantlets were produced through callus mediated indirect organogenesis as per the procedure reported by Paul and Shylaja (2010) [10]. Rhizome bits after seed treatment with 0.3 percent Indofil M 45 for 30 minutes were germinated in sterile sand. Pale yellow sprouts from rhizomes were excised, washed thoroughly, removed the scale leaves and dipped in teepol solution for 10 minutes and washed with distilled water. The sprouts, after treating with 0.1 percent Indofil M-45 for 30 minutes were surface sterilized with 0.1 percent $HgCl_2$ for 10 minutes, washed free off the sterilant, dried and inoculated to modified MS medium supplemented with BAP (3.00 mg l^{-1}). The protocol reported by Shylaja *et al*. (2003) [12] was followed for producing adventitious bud regenerants in ginger. Shoot tips from adventitious bud regenerants were then used as explant for callusing. For indirect organogenesis, calli were induced from shoot tips in half-strength MS medium supplemented with 1.00 mg l^{-1} 2,4-D. Half strength MS medium supplemented with 3.0 mg l^{-1} BAP was used for shoot morphogenesis and rooting of the organogenic calli.

Production of regenerants through indirect somatic embryogenesis

Regenerants were produced through indirect somatic embryogenesis as per the procedure reported by Paul (2006) [9]. Rhizome bud was used as explant for somatic embryogenesis. Embryogenic calli were induced on half strength MS medium supplemented with 2,4-D (0.50 - 1.00 mg l^{-1}) and BAP (0.50 - 1.00 mg l^{-1}). Cultures were incubated under dark for production of embryogenic calli. Somatic embryoids were matured and proliferated in half MS basal medium and germinated in basal half MS medium supplemented with BAP (3.00 mg l^{-1}).

Production of regenerants through *in vitro* mutagenesis

In vitro mutagenesis was attempted in ginger using gamma rays from a 60Co source (Gamma chamber 900 of BARC, Mumbai, India) with a dose rate of 306.8 Gy/h. Morphogenic cultures derived from organogenic/ embryogenic calli of two cultivars *viz*., Maran and Rio-de-Janeiro were subjected to gamma irradiation as per the procedure reported by Paul (2006) [9]. Organogenic cultures of Maran and Rio-de-Janeiro and embryogenic cultures of cultivar Rio-de-Janeiro were irradiated at gamma irradiation doze of 10 Gy. Embryogenic cultures of cultivar Maran were irradiated at gamma irradiation doze of 20 Gy. Irradiated cultures were inoculated to regeneration and rooting media (Half strength MS medium supplemented with BAP 3.00 mg l^{-1}).

Planting out, hardening, final survival and growth of regenerants

Plantlets with well developed pseudostem and roots were washed free off the medium and compared with respect to number of roots produced, length of roots, number of tillers and length of pseudostem. The plantlets were planted in bags of size 11 x 8 cm filled with sterile sand, kept under shade house and watered daily. After two weeks, the plantlets were transferred to big poly bags of size 12 x 8" filled with potting mixture in the proportion 1:1:1 sand, soil and cow dung. The polybags were kept in the net house and watered daily. The plantlets were observed for growth parameters for a period of three months and growth observations on number of tillers produced, height of pseudostem and length of the longest leaf were recorded.

Results and Discussion

Morphological characters in regenerants produced through various routes and two cultivars irrespective of the various routes of regeneration observed at plant out and three months after planting are presented in Table 1 and 2.

Morphological characters in plantlets regenerated through various routes in ginger

Regenerants derived through indirect organogenesis and irradiation of organogenic calli were on par with each other and recorded maximum number of roots (19.10, 18.10), length of roots (7.31 cm, 6.59 cm), length of longest root (9.26 cm, 8.50 cm), number of tillers (4.36, 5.43) and length of pseudostem (8.59 cm, 8.21 cm) at plant out stage. Regenerants derived through indirect somatic embryogenesis and irradiation of embryogenic calli were on par with each other and recorded lowest number of roots (14.83, 13.90), length of roots (5.20 cm, 4.03 cm), length of longest root (6.13 cm, 5.00 cm), number of tillers (3.54, 3.61) and length of pseudostem (6.93 cm, 5.86 cm) at plant out stage. The difference in characters of regenerants may be due to somaclonal variation that happened in cultures maintained for a long time under *in vitro* conditions (Kaeppler *et al*., 2000 [6]). In ginger, several workers also observed somaclonal variation in tissue culture-derived plant populations (Babu *et al.*, 1996 [1], Bhardwaj *et al.*, 2012 [2], Hameid *et al.*, 2020) [1, 2, 4].

At three months after planting, regenerants derived through indirect organogenesis recorded highest plantlet establishment (68.48%) which was on par with regenerants derived through irradiation of organogenic calli (62.95%) and regenerants derived through indirect somatic embryogenesis (61.04%). There was no significant difference between regenerants derived through various routes in number of tillers and length of pseudostem at three months after planting. Regenerants derived through irradiation of embryogenic calli recorded highest length of longest leaf (15.49 cm) which was on par with regenerants derived through irradiation of organogenic calli (14.90 cm). Regenerants derived through regeneration of organogenic and embryogenic calli exhibited lowest length of longest leaf (13.98 cm, 13.90 cm). Similar difference in growth parameters in regenerants produced through various routes was reported in kacholam by Joseph (1997) [5]. Somaclonal variations arise from the mutations that occur during tissue culture. Somaclonal variations in tissue culture

crops may depend upon explant source, media components, genotype, regeneration systems, duration and number of culture cycles. Intermediary callus phase can produce more variations. Plant hormones and their concentrations are considered to be one of the major causes of somaclonal variation (Larkin and Scowcroft, 1981) [8].

Morphological characters in plantlets of two cultivars regenerated through various routes in ginger

Irrespective of the various routes of regeneration, there was no significant difference between the regenerants of two cultivars *viz*., Maran and Rio-de-Janeiro with respect to number of roots produced, length of roots, number of tillers and length of pseudostem at plant out stage and also on growth parameters for a period of three months (Table 2). Krishna *et al.*, (2016) [6] reported that the frequency of somaclonal variation is determined by genotype. But in the present study no variation was observed between regenerants of two cultivars at plant out stage as well as upto three months after plant out.

In the present study, regenerants derived through indirect organogenesis and irradiation of organogenic calli exhibited maximum number of roots, length of roots, number of tillers and length of pseudostem at plant out stage. Regenerants derived through indirect organogenesis, embryogenesis and irradiation of organogenic calli recorded highest plantlet establishment at three months after planting. Regenerants derived through irradiation of organogenic and embryogenic calli recorded highest length of longest leaf at three months after planting. The high somaclonal variation observed in regenerants derived through indirect organogenesis and irradiation of organogenic calli should be exploited in further studies.

Table 1: Morphological characters in plantlets regenerated through various routes in ginger

Sl. No.	Group	At plant out					At 3 MAP			
		Tiller no.	Length of pseudostem (cm)	No. of roots	Length of roots (cm)	Length of longest root (cm)	Establishment (%)	Tiller no.	Length of pseudostem (cm)	Length of longest leaf (cm)
1	C	4.36^{ab}	8.59^{a}	19.10^{a}	7.31^{a}	9.26^{a}	68.48^{a}	5.25	35.72	13.98^{b}
2	SE	3.54^{b}	6.93^{ab}	14.83^{b}	5.20^{b}	6.13^{b}	61.04^{ab}	5.97	38.29	13.90^{b}
3	C (Irradiated)	5.43^{a}	8.21^{a}	18.10^{a}	6.59^{a}	8.50^{a}	62.95^{ab}	6.43	30.80	14.90^{a}
4	SE (Irradiated)	3.61^{b}	5.86^{b}	13.90^{b}	4.03^{b}	5.00^{b}	55.08^{b}	6.29	39.12	15.49^{a}
	C.D (0.05)	1.17	1.72	2.21	1.18	1.54	8.38	NS	NS	0.67
	CV (%)	13.87	11.65	6.71	10.22	10.65	6.78	17.90	8.36	2.28

C - Plantlets regenerated through indirect organogenesis
SE - Plantlets regenerated through indirect somatic embryogenesis
C (Irradiated) - Plantlets regenerated through irradiation of calli derived through indirect organogenesis
SE (Irradiated) - Plantlets regenerated through irradiation of calli derived through indirect somatic embryogenesis
MAP - Months After Planting

Table 2: Morphological characters in plantlets of two cultivars regenerated through various routes in ginger

Sl. No.	Cultivars	At plant out					At 3 MAP			
		Tiller no.	Length of pseudostem (cm)	No. of roots	Length of roots (cm)	Length of longest root (cm)	Establishment (%)	Tiller no.	Length of pseudostem (cm)	Length of longest leaf (cm)
1	Maran	4.04	7.59	17.50	6.25	7.91	65.108	6.108	37.89	14.428
2	Rio-de-Janeiro	4.43	7.20	15.46	5.31	6.53	58.670	5.860	34.07	14.703
	C.D (0.05)	NS	NS	NS	NS	NS	NS	NS	NS	NS
	CV (%)	19.62	16.48	9.49	14.46	15.06	9.58	25.31	11.83	3.22

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