International Journal of Innovative Horticulture. 6(2):141-146, 2017 **Original Article**

A simple method of induction of *in vitro* microrhizomes and their field performance in ginger (*Zingiber officinale* Rosc.)

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ABSTRACT

Microrhizomes were produced from tissue culture derived shoots of ginger (*Zingiber officinale* Rosc.) by culturing them in Murashige and Skoog (MS) medium with an enhanced concentration of sucrose (9–12%). Sucrose at 9% was most suitable for induction of microrhizomes. Microrhizomes were formed at the base of the shoots after 30 days of incubation at temperatures of 22±2°C and 25±3°C. The number of shoots produced from microrhizomes ranged from 5–10. Weight of microrhizome per explant ranged from 12–50 g fresh weight in 4–6 months. These microrhizomes could be directly planted in the field with 90-100% survival, thus eliminated the need for hardening. Microrhizomes gave good yields of about 500 g of fresh rhizomes/ plant (12-20 kg/3m^2) bed. The yields are similar to those when normal seed rhizomes of 50 g per plant are used. The seed rate requirement per 3 m^2 bed is about 400 g for microrhizomes compared to 1.5-2 kg in case of conventional planting thus saving huge amounts of seed. In addition microrhizomes are good source of disease free planting material due to its aseptic origin. Microrhizomes were produced independent of seasonal fluctuations. The microrhizome cultures could be stored up to 12–15months *in vitro* facilitating safe and disease free germplasm exchange.

Keywords: *In vitro* culture, microrhizomes, *Zingiber officinale,* disease free seed.

INTRODUCTION

Ginger (*Zingiber officinale* Rosc), a herbaceous perennial, is usually grown as an annual crop and is the third most important medicinal spice in the world. India is the largest producer and exporter of ginger. The National Conservatory of Ginger, at ICAR-Indian Institute of Spices Research, Kozhikode, has 530 accessions including indigenous collections, exotic cultivars, improved lines, mutants, tetraploids and related species and taxa. All the available germplasm are presently conserved as field clonal repositories. In the field the gemplasm is threatened by serious soil bone pathogens like *Pythium aphanidermatum, Ralstonia solanacearum* etc.

Ginger productivity in India is severely hampered by rhizome rot disease caused by *P. aphanidermatum* and *R. solanacearum*, which sometimes can reach the level of an epidemic. Infected seed rhizome is one of the major causes for the disease spread. Micropropagation is the most efficient method to produce disease free planting material, which is an important component in the disease management strategies. However tissue cultured plantlets have a disadvantage in that they need two generations in nursery before they are used for commercial cultivation. This could be overcome through *in vitro* microrhizome production similar to microtubers of potato. This is an important technology which can overcome the delay in the utilization of tissue cultured plantlets.

There are several reports on the investigations conducted on *in vitro* micro-tuber formation and their application in planting material production and medium-term to long-term conservation of potato germplasm (Sylvestre, 1983; Tovar *et al.*, 1985; Bohac and Miller, 1988; Kwiatkowshi *et al.*, 1988; Mitten *et al.*, 1988; Garner and Blake, 1989; Khuri and Moorby, 1995; Malaurie, 1998; Al-safadi *et al.,* 2000; Veramendi *et al.*, 2000). *In vitro* rhizome formation and their germination in turmeric has also been reported. Reghuarajan (1997) reported formation of microrhizomes in a medium containing ancymidol and Nayak (2000) could

induce microrhizomes in turmeric in a medium containing BAP and higher levels of sucrose. In the present study, sucrose at higher levels alone could induce microrhizomes in turmeric. Microrhizome formation in ginger has been reported earlier by Bhat *et al.* (1994), Sharma and Singh (1995) and Nirmal Babu *et al.* (1997). This paper reports efficient protocol for production of microrhizomes in ginger and studies on their field performance in comparison with normal rhizomes. The possibility of using microrhizomes in *in vitro* conservation of germplasm is also investigated.

MATERIALS AND METHODS

In vitro Multiplication

In vitro cultures of ginger were established and multiplied using the method standardized at author's laboratory (Nirmal Babu *et al.,* 1997). Rhizome bud explants were initiated in MS (Murashige and Skoog, 1962) medium supplemented with 0.5 mgl^{-1} kinetin and multiplied in MS basal medium with 1.0 mgl^{-1} BAP (6-Benzyl aminopurine) and 0.5 mgl^{-1} NAA (α- Naphthalene acetic acid).

Induction of Microrhizomes

Healthy plantlets with a small emerging axillary bud from *in vitro* cultures were used as explants for microrhizome induction experiments. The aerial portion of the plantlet was cut removed at 2 cm from the base without damaging the side shoot and inoculated into the medium. MS basal medium devoid of growth regulators supplemented with sucrose alone and sucrose and mannitol at various concentrations were used (Table 1).

Table 1: The various carbon sources and their concentrations used in the media for induction of microrhizomes

Sl. No.	Carbon source	
	Sucrose (%)	Mannitol (%)
1.	3	-
2.	9.0	-
3.	10.0	-
4.	12.0	-
5.	1.0	1.0
6.	1.5	1.5
7.	2.0	1.0
8.	3.0	3.0
9.	3.0	6.0
10.	5.0	5.0
11.	6.0	6.0

The media were solidified with 0.8% agar and 200 ml of media was dispensed per culture vessel. Cultures were incubated at two different temperature levels (22±2°C and 25±3°C) at 2500–3000 lux. Two types of culture vessels, 500 ml Erlenmeyer flasks and 500 ml wide mouthed borosilicate culture bottles, were used. Single explant was used per culture vessel. Observations were made on time taken for induction, percentage of miccrorhizome induction, number of shoots per culture, fresh weight of microrhizomes and the number of planting units produced per culture. Rhizome morphology and anatomy was also studied. Transverse sections of microrhizomes were taken and stained in 1% saffranin and observed under Olympus binocular microscope for rhizome anatomy.

Field Planting of Microrhizomes

Microrhizomes were harvested from culture, cleaned by removing roots, partially died aerial shoots and planted directly on 1x1 m^2 beds. Two planting densities were tried, one with normal @ 40 planting units per 3x1m^2 bed and the other with doubled planting density. Observations were taken on time taken for germination, percentage of establishment, plant height, number of tillers, height of tillers, number of leaflets, disease incidence, yield and rhizome characters like size and number of primaries and secondaries, internodal distance etc. and compared with the normal rhizome.

RESULTS AND DISCUSSION

In vitro Multiplication

Multiple shoots at the rate of 1:7 were produced in MS basal medium supplemented with 30gl^{-1} sucrose, 1.0 mgl^{-1} BAP and 0.5 mgl^{-1} NAA , solidified with 8 gl^{-1} agar. In this medium rooting was also achieved (Fig. 1). An average of five shoots could be harvested from a culture after one month of culture and used as explants for microrhizome induction.

Fig. 1: Cultures forming multiple shoots

Induction of Microrhizomes

MS medium with various concentrations of sucrose with or with out mannitol in different combinations were tried for their ability to induce microrhizomes in ginger and the responses and observations are given in Table 2. Temperature ranging from 22±2°C to 25±3°C was suitable for microrhizome formation. No variations were observed in the ability of microrhizome formation in two different incubation temperatures.

Microrhizomes were formed at the base of the shoots after 30 days of incubation, in all the media combinations with sucrose alone. In MS with 3% sucrose microrhizome formation was observed in 30-40% cultures in 6–12 months in culture. Microrhizomes were produced in 80-100% of cultures in 1–6 m, when sucrose concentrations were at 9–12%. The most suitable combination was MS supplemented with 9% sucrose. No significant difference was noticed in medium with 10% sucrose. Cultures producing microrhizomes exhibited a peculiar pattern of growth with healthy, sturdy aerial shoots and profuse rooting (Fig. 2). The microrhizomes from these cultures were comparatively larger.

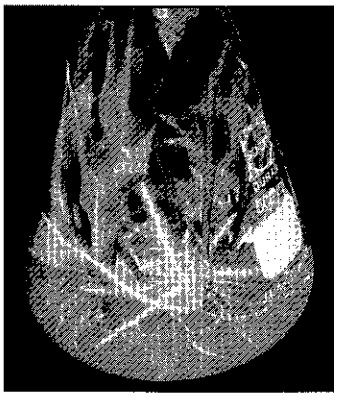

Fig. 2: Culture producing microrhizome

Table 2: The effect of various concentrations of carbon source on microrhizome induction

Sucrose (%)	Mannitol (%)	Percentage response*	Time for microrhizome production (months)	No. of shoots per culture	Fresh weight (g)*
3.0	-	30-40	6–12m	7.5	0.30
9.0	-	80-100	1–6m	6.0	33.94
10.0	-	80-100	1–6m	6.5	30.54
12.0	-	80-100	1–6m	12.0	18.40
1.0	1.0	50-60	6–8m	3.0	0.50
1.5	1.5	50-60	6–8m	3.5	0.84
2.0	1.0	30–40	6–8m	3.2	0.35
3.0	3.0	-	No induction	20.0	-
3.0	6.0	-	No induction	5.0	-
5.0	5.0	-	No induction	15.0	-
6.0	6.0	-	No induction	5.0	-

* Average of 20 replicates

Fig. 3: and 4: Abnormal growth patterns in higher levels of sucrose and mannitol

In the media with sucrose and mannitol in combination, only lover concentrations (1%, 1.5% 2%) favoured microrhizome formation. The time taken for microrhizome formation was 4–8 months with 30-60% of the cultures responding. In these combinations comparatively smaller microrhizomes, weighed up to 0.05-1.2g were produced. Other combinations of sucrose and mannitol failed to induce microrhizomes and the culture showed varying growth patterns with profuse shooting with miniature plants, callusing, vitrification etc (Fig. 3 & 4).

Presence of higher levels of sucrose alone was sufficient for production of microrhizomes in ginger. Sucrose is the most effective carbon source for potato microtuber induction. An increase in sucrose concentration from 1 to 8 % induces early tuberization (Wang and Hu, 1982; Hussey and Stacey, 1984), but at sucrose levels above 8% the tuberization was inhibited (Garner and Blake, 1989). It has been reported that as high as 60% sucrose is utilized during *in vitro* tuberization, and there is no quantitative relation between sucrose absorption and reducing sugar appearance in the medium (Khuri and Moorby, 1995). Increased sucrose utilization results in an increase in microtuber weight and yield, but does not have any effect on microtuber number.

The culture vessels played a major role in the number and weight of microrhizomes. The effect of different types of culture vessels on the production of microrhizomes were given in Table 3. In 500ml Erlenmeyer flasks the fresh weight of the microrhizomes ranged from 20.5g to 50.2g with an average of 33.9g (Fig. 5 & 6), whereas in the culture bottles the weight ranged from 5g to 20g with an average of 11.7g (Fig. 7).

An average of 20 planting units with not less than five buds were obtained from a culture in Erlenmeyer flask. The cultures in the bottles produced an average of 10 planting units. The only disadvantage of Erlenmeyer flask is the difficulty in harvesting the microrhizomes without damage due to the narrow neck.

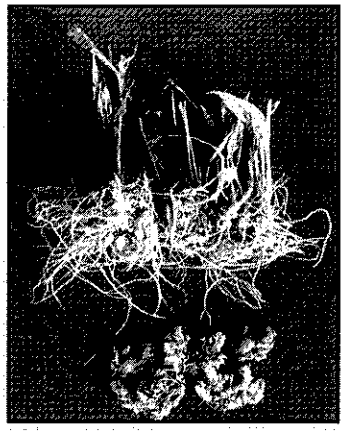

Fig. 5: Microrhizome harvested from 500 ml Erlenmeyer flask

Fig. 6: Microrhizome harvested from 500 ml culture bottle

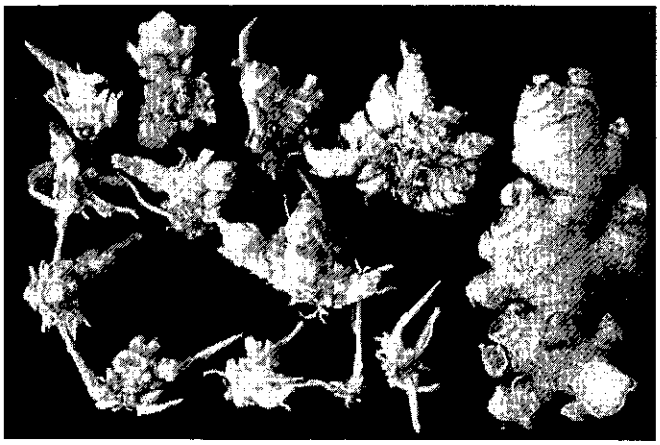

Fig. 7: Morphological similarity of microrhizomes with normal rhizome

Table 3: The effect of various culture vessels on the production of microrhizomes

Culture vessel	Explant/ vessel	Medium/ vessel (ml)	Weight of microrhizome /explant (g)	No. of planting units per vessel
Erlenmeyer flask (500 ml)	1	500	33.9	15
Culture bottles (500 ml)	1	500	11.7	8

Microrhizomes resembled the normal rhizomes in all respects, except for their small size (Fig. 8). The microrhizomes had the aromatic flavour of ginger and they resembled the normal rhizome in anatomical features. Presence of well-developed oil cells, fibres, starch grains and curcumin cells was also observed (Fig. 9). The rhizomes germinated *in vitro* as well as *ex vitro*. The microrhizomes on transfer to MS medium without any growth regulators, germinated within 5 to 7 days with 100% success.

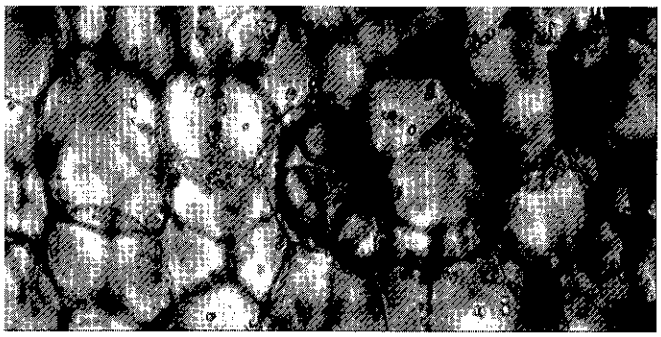

Fig. 8: Transverse section of microrhizomes showing oil cells and starch grains

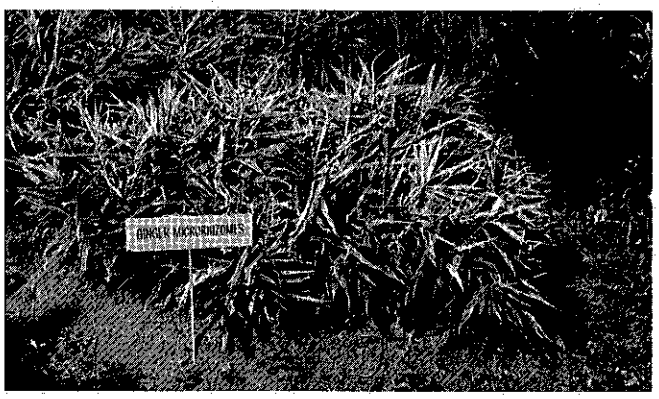

Fig. 9: Establishment of microrhizomes in field

Field Evaluation of Microrhizomes

Microrhizomes could be directly planted in the field with out hardening and established with 90-100% success (Fig. 10). Microrhizomes were field evaluated and the morphological data on field evaluation of microrhizomes is given in Table 4. The microrhizomes had more tillers per plant though the plant height was less compared to plants derived from normal seed rhizomes. Field trials showed that the microrhizomes gave commercially viable yields ie 200-500 g of fresh rhizomes per plant with an estimated 12-20 kg of fresh rhizomes per 3 m^2 bed (Fig. 11) in comparison with control. The seed rate requirement per 3 m^2 bed is about 800 g (@ 40 plants per bed) for the normal seed rhizomes and in case of microrhizomes it is less than half that amount.

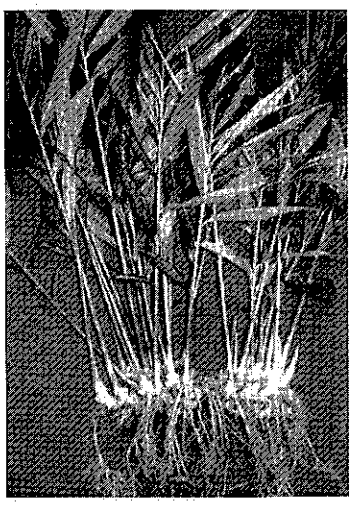

Fig. 10: Microrhizome production from a single planting unit

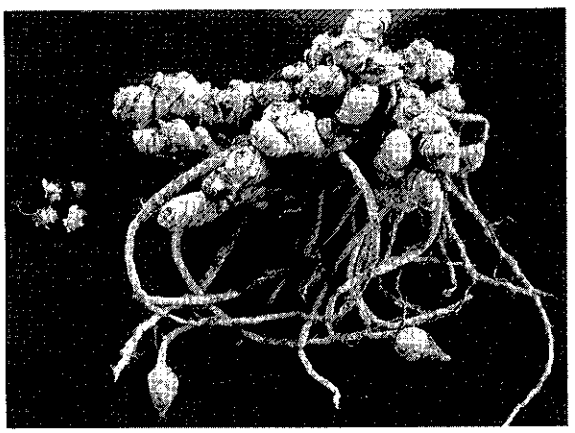

Fig. 11: Per plant yield of microrhizomes

Microrhizomes being very small and weighing an average of 0.8 to 1.5g per planting unit, the requirement of seed rhizome is less compared to normal rhizome. The multiplication rate when microrhizomes were planted was 80 to 90 times. This may be due to the presence of more sprouting buds in microrhizomes than in normal rhizomes.

Incidence of diseases were not noticed in microrhizome derived plants, where as 5 to 10 % of rhizome rot was noticed in control. This may be due to disease free nature of microrhizomes, while normal seed rhizomes may carry increased amount of inoculam.

In vitro rhizome formation and their germination in ginger was reported by various workers also (Sakamura *et al.*, 1986; Sakamura and Suga, 1989; Bhat *et al.*, 1994). Bhat *et al.* (1994) reported *in vitro* induction of rhizomes in ginger at higher sucrose concentrations (9-12%). Quality analysis of *in vitro* developed rhizomes indicated that they contain the same constituents as the original rhizome but with quantitative differences. The composition of basal medium seems to affect the composition of oil (Sakamura *et al.*, 1986; Sakamura and Suga, 1989; Charlwood *et al.*, 1988). Sharma and Singh reported *in vitro* microrhizome formation. Microrhizomes weighed up to 459 mg and 80% of them germinated after storage for two months in moist sand at room temperature.

In vitro Storage of Microrhizomes

The microrhizome producing cultures could be stored up to 12–15 months and within this period the aerial shoots completely dried. The rhizomes on culture to MS medium, germinated within one week. There are several reports on *in vitro* microtuber formation and their application in medium-term to long-term conservation of potato germplasm (Sylvestre, 1983; Tovar *et al.*, 1985; Bohec and Miller, 1988; Kwiatkowshi *et al.*, 1988; Mitten *et al.*, 1988). Miniature storage organs like microtubers in potato have great advantage as they can be readily removed from culture flask in a dormant condition and stored *ex vitro.* When planted in soil they behave as normal tubers and produce plants from axillary shoots. If they are produced *in vitro* from virus free stocks, the microtubers provide an ideal method for propagating and distributing virus free planting material to farmers.

In the present study a highly efficient low cost system for microrhizome induction developed in ginger. This is the first report of direct utilization of microrhizomes in field planting. Their utility in disease free planting material for large scale cultivation, germplasm storage and exchange have been established.

Acknowledgements

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Table 4: Morphological characters of microrhizome plants in the field in comparison with control

Plant species	Plant Height (cm)	No. of tillers	No. leaves/ plant	Width of rhizome (cm)	No. nodes/ finger	Internode distance (cm)	Mean yield/ plant (g)
Control*	90.0	12.5	12.0	2.0	6.8	6.3	278
Microrhizome plants**	46.5	14.5	14.4	1.57	6.6	5.2	260

* 800g of planting material used, ** 200 g of seed material used

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