

Original Research Article

Microrhizome and minirhizome production in three high yielding cultivars of ginger (*Zingiber officinale* Rosc.)

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A B S T R A C T

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Ginger (*Zingiber officinale* Rosc.) is one of the most important medicinal spice plants. Most important production constraint in this crop is the presence of soil borne pathogens which negatively affect the quality and the quantity of the produce. Microrhizome and minirhizome technologies are the promising technologies to develop pathogen free planting materials in the rhizomatous species including ginger. The present study is aimed to establish an efficient protocol for microrhizome and minirhizome production in ginger for the production of healthy planting material. Among the three experimental trials conducted using different media combinations and different types of culture vessels, medium with high sucrose in culture bottles was observed ideal for *in vitro* microrhizome production in ginger. Among the three cultivars taken for the study (cvs. *Mahima*, *Rejatha* and *Varada*), cv. *Rejatha* showed superiority in two trials and cv. *Mahima* responded more in the field condition. The pathogen free nature of the *in vitro* microrhizome was confirmed using disc culture method. The microrhizome and minirhizome technology developed in this study holds better promises for large scale production of pathogen free seed rhizomes in ginger.

Introduction

Ginger (*Zingiber officinale* Rosc.) is one of the most important medicinal spices. Ginger is a reputed remedy in curing a number of ailments and it is an indispensable ingredient in traditional systems of medicine. India is the largest producer of ginger, contributing approximately 30 to 40% of the world production (Ravindran *et al.*, 2005). The major constraints involved in the conservation and cultivation of ginger are

the soil borne diseases such as rhizome rot caused by *Pythium aphanidermatum* and the bacterial wilt caused by *Ralstonia solanacearum*. Various *in vitro* methods had been reported for induction of microrhizomes in ginger (Balachandran *et al.*, 1990; Babu, 1997; Bhat *et al.*, 1994; Sharma and Singh, 1995; Rout *et al.*, 2001; Geetha, 2002; Babu *et al.*, 2005; Zheng *et al.*, 2008).

The present paper reports an efficient method for enhanced microrhizome production using best media in terms of concentrations of sucrose (among which containing 30 or 80 or 90 or 100g/l sucrose), best culture vessels (among culture tubes, Erlenmeyer culture flasks and culture bottles) and the effect of two stress hormones ABA and JA and minirhizome production in three elite varieties of ginger, namely *Mahima*, *Rejatha* and *Varada*.

Materials and Methods

Three varieties of ginger (*Mahima*, *Rejatha* and *Varada*) released from Indian Institute of Spices Research, Calicut were used for the study. *In vitro* cultures of ginger were established and multiplied in MS medium with 2mg^l⁻¹ BA, 0.5mg^l⁻¹ NAA, 25mg^l⁻¹ Adenine sulphate along with 30g^l⁻¹ sucrose and 8g^l⁻¹ agar. Prior to the microrhizome induction experiments, the cultures were allowed to grow in growth regulator free MS medium for two weeks to minimize carryover effect of growth regulators in the multiplication medium into the experimental phase. These cultures served as the source of explants and single shoots were isolated from these multiplied cultures. Four experiments were conducted towards induction of microrhizomes from shoot cultures of ginger. Different media components, different types of culture vessels, stress hormones and supporting materials were used separately or in certain combinations. MS (Murashige and Skoog, 1962) basal medium with 8g^l⁻¹ agar was used in all the trials.

In the initial trial, effect of varying concentrations of sucrose (30, 80, 90 and 100g^l⁻¹) was studied. Effect of culture

vessels such as culture tubes (200mmx50mm), 500ml Erlenmeyer flasks (CF) and 350ml culture bottles (CB) containing MS medium with 90g^l⁻¹ sucrose and 8g^l⁻¹ agar was investigated in another trial. The quantity of medium used per vessel was 25ml, 100ml and 40ml in culture tubes, culture flasks and culture bottles respectively. In third trial, stress hormones such as Abscisic acid (ABA) and Jasmonic acid (JA) were tried separately at various concentrations (0.1, 1, 5 and 10mg^l⁻¹) in MS medium containing 90g^l⁻¹ sucrose and 8g^l⁻¹ agar to study their effect on microrhizome induction. In all the trials number of culture unit per vessel was single with ten to fifteen replicates.

All the cultures were observed periodically up to a maximum of three months. Bulging of basal portion of pseudostem was considered as an indication of microrhizome induction and was further confirmed by anatomical studies. Number of shoots produced with bulged basal portion was taken as the character for assessing the superiority of the medium. Data on the number of shoots, length of shoots, number of leaves, root formation and microrhizome induction were taken and analyzed statistically.

Diagnostic tests were carried out to detect the presence or absence of the pathogen in the seed material used for planting. In the case of microrhizome derived plants, the basal portion of the pseudostem and pieces of minirhizomes and normal rhizome (control) were transferred to Potato Dextrose Agar (PDA) and Plate Count Agar (PCA), cultured for 8–10 days for pathogen growth on the medium. Visual observation on presence / absence of disease symptoms was recorded throughout the growing season. The

minirhizomes were stored in river sand and the number of rotted and healthy rhizomes was recorded after six months of storage.

Microrhizome induced plants with various maturity classes were planted in nursery beds / polybags for studying the feasibility of mini-rhizome production technology for quality planting material production. The microrhizome induced plants were taken out from the lab and vessel, separated individually; roots were removed and planted in beds prepared out of a mixture of solarised soil, sand and farmyard manure in the ratio 2:2:1 or in big polybags (6"x10") filled with same mixture. Irrigation was done through misting once a day. Hoagland's solution was sprayed once in a week for initial two months. As the plant grows, the beds were raised by adding the same mixture and in the polybags also the mixture was added. Irrigation was stopped when the plants reached four to five months of growth to dry out the aerial portion and for the rhizome to attain maturity. Harvesting is done after six to seven months. On maturity the minirhizomes were harvested, cleaned, washed, cured in a clean dry area and used as planting material for field planting.

Results and Discussion

Cultures in MS basal medium solidified with 8g l⁻¹ Agar with various concentrations of sucrose (80, 90 and 100g l⁻¹) showed induction of microrhizome after one month of culture in all the media tried, except in control (30g l⁻¹ sucrose). After three months of culture, medium with either 80g l⁻¹ or 90g l⁻¹ sucrose showed best response in terms of number of shoots with bulged basal stem portion. Among the three varieties of ginger, cv. *Rejatha*

showed comparatively better response in terms of shoot production in both media, percentage of establishment in the field, microrhizome fresh weight followed by *Mahima* and *Varada*. After three months of *in vitro* growth, quantitative data collected on microrhizome production indicated variety wise and media-wise differences (Table 1; Figure. 1).

Number of shoots (4.35±3.04), microrhizome fresh weight (0.58±0.8g) and percentage of establishment (80.5±16.05) was higher in cv. *Rejatha*. Microrhizomes harvested from the medium with 10% sucrose were not of good quality as it exhibited vitrified nature and decaying of buds. Plants from all other media got established successfully in the soil with 80–100% survival, depending on the culture media used.

While comparing the effect of vessel size on microrhizome induction, it was found that after two to three months of incubation, higher number of shoots was produced in 500ml Erlenmeyer flasks, irrespective of varieties. Culture bottles (350ml) seemed to be the second highest for shoot number. In conical flasks, a maximum of 19-20 plantlets with microrhizome with an average of 15 microrhizomes per culture was produced, whereas in culture bottles the average number was 12. In the culture tubes (200mm x 50mm) an average of only 5 microrhizomes was produced ((Table 2, Figure. 1). In this trial more number of shoots (10.9±5.71) was produced in cv. *Mahima* when cultured in culture flasks. But the fresh microrhizome weight was higher in cv. *Rejatha* (0.84±0.31). Though the difference exists in number of microrhizomes in two preferred types of vessels, culture bottles are recommended for large-scale production as it facilitates

Table.1 Culture responses of ginger varieties after 3 months of *in vitro* growth in medium with varying concentration of sucrose*

Cultivar	Sucrose (gl ⁻¹)	Number of shoots	Length of shoot (cm)	Microrhizome weight per unit (g)	Establishment in the nursery
Mahima	30	2.0±1.2	8.0±2.2	0.36±0.1	33%
	80	4.8±1.4	3.9±1.1	1.38±0.3	67%
	90	3.5±1.5	2.2±0.5	1.02±0.1	100%
	100	3.4±1.1	2.0±0.8	0.10±0.0	73%
Rejatha	30	2.3±1.5	5.5±1.2	0.21±0.1	83%
	80	5.8±3.1	3.5±1.5	0.15±0.0	61%
	90	5.3±2.8	2.7±1.2	1.83±0.4	100%
	100	4.0±2.3	2.1±0.8	0.12±0.0	78%
Varada	30	3.5±1.8	4.6±1.4	0.50±0.02	60%
	80	4.2±1.82	3.3±1.3	1.29±0.05	100%
	90	3.25±2.87	2.4±0.4	0.55±0.10	65%
	100	4.67±1.15	1.9±0.5	0.12±0.03	71%

* MS basal medium solidified with 8gl⁻¹ agar in 200mmx50mm culture tubes

Table.2 Effect of different culture vessels on *in vitro* microrhizome induction in shoot cultures of three cultivar varieties of ginger*

Variety	Vessel type	Number of shoots	Length of shoots (cm)	Average fresh weight of microrhizomes
Mahima	CT	5.0±1.1	3.4±0.9	0.44±0.2
	CF	15.3±3.9	7.9±1.3	0.8±0.1
	CB	12.1±3.0	6.1±1.1	0.6±0
	Mean	10.9±5.71	5.8±2.36	0.62±0.24
Rejatha	CT	5.4±1.3	4.2±1.0	0.5±0.2
	CF	12.0±2.7	8.6±1.0	1.05±0.1
	CB	9.3±2.2	6.6±1.1	0.98±0.1
	Mean	8.9±3.79	6.47±2.27	0.84±0.31
Varada	CT	6.2±1.5	3.8±0.9	0.2±0.2
	CF	10.4±2.9	6.7±1.1	1.0±0
	CB	9.0±2.0	6.0±1.6	0.8±0.0
	Mean	8.53±3.09	5.5±1.91	0.67±0.39

*In MS basal medium with 90gl⁻¹ sucrose and 8gl⁻¹ agar

CT – Culture tubes, CF – 500ml Erlenmeyer conical flasks, B – 350ml culture bottles

easy handling during harvesting when compared to culture flasks in which harvesting after 4 months was found difficult due to the size of microrrhizomes and the narrow neck. Microrrhizome production in 350ml culture bottles is most ideal in terms of quantity of media (40ml) used per vessel. In 500ml flasks the culture medium used was 100ml, whereas in the bottles it was less than half the volume i.e., 40ml. When comparing these factors with the rate of microrrhizome production the bottles are more economical. Effect of the vessel volume on growth induction was studied by Kavanagh *et al.*, (1991).

Various media with ABA and JA showed difference in responses with respect to variety used (Table 3; Figure 1), but no significantly superior response than control medium was observed. Highest shoot induction response in *cv. Varada* was noticed in both ABA (8.5 ± 2.1) and JA (10 ± 1.0) at 5.0mg l^{-1} . Ginger *cv. Rejatha* was a poor performer i.e., it was second best in control medium. After four months of incubation cultures showed yellowing. Two to three months old plantlets of all the cultivars from all the media showed high rate of establishment (98-100%) in the nursery. This study indicated that addition of either ABA or JA did not help in increasing the microrrhizome induction than the control, but the plantlets from ABA and JA containing media were sturdier and they exhibited faster establishment than control. Compared to the three cultivars, *Rejatha* produced more number of shoots (6.47 ± 5.71) by taking all the media combinations and cultivars in to consideration. All the cultivars produced more number of shoots in the control medium (higher media mean for the number of shoots for all the three cultivars was 10.47 ± 3.3) than the media supplemented with ABA or JA. As in case

of many earlier reports in various plants (Gopal *et al.*, in 2004; George, 1993; Sarkar *et al.*, in 2006; Pruski *et al.*, in 2002), JA or ABA containing media induced microrrhizome in ginger, but addition of any of these plant hormones did not help in enhancing the microrrhizome induction effect.

The microrrhizome induced cultures were subjected to *in vitro* pathological screening using Potato Dextrose Agar (PDA) and Plate Count Agar (PCA) media to check their pathogen free nature. No bacterial or fungal growth was observed in these microrrhizome induced cultures.

Direct planting of microrrhizomes in the field was successful, but the mortality was high (more than 60%), if utmost care is not taken upto the establishment stage and forward. Even slight drying up will adversely affect germination of microrrhizome induced plants. Microrrhizome induced plants, possessing microrrhizomes with fresh weight ranging from 0.1 to 1.5g at the basal portion (0.1g for smaller plants and 1.5g for larger plants), showed 90–100% establishment on planting out in the nursery. Within one week the rhizome buds emerged out and developed to shoots. An average of 4-5 tillers was produced within one month and the number of tillers increased with maturity. Average number of tillers/clump, plant height and rhizome yield varied with species and also with the size of the initial planting unit used. Higher tiller formation and plant height were noticed in *cv. Mahima* (1.55 ± 1.1 and $14.83 \pm 6.97 \text{cm}$ respectively for number of tillers and length of shoot). The aerial portion dried out within 5–6 months after planting.

Table.3 Effect of different concentrations of Abscissic acid (ABA) and Jasmonic acid (JA) on *in vitro* microrhizome induction in shoot cultures of three cultivar varieties of ginger

Media	Number of microrhizome induced shoots			Media mean
	Mahima	Rejatha	Varada	
MS + 9% Sucrose + 8gl ⁻¹ Agar (Control)	12.8±2.4	10.6±1.6	9.5±2.0	10.47±3.3
MS + 0.1mg ⁻¹ ABA + 9% Sucrose + 8gl ⁻¹ Agar	8.3±2.3	3.3±0.8	6.7±0.6	6.1±2.8
MS + 1.0mg ⁻¹ ABA + 9% Sucrose + 8gl ⁻¹ Agar	7.1±2.2	5.6±1.8	6.0±0.0	6.23±1.9
MS + 5.0mg ⁻¹ ABA + 9% Sucrose + 8gl ⁻¹ Agar	6.9±4.2	6.0±1.7	8.5±2.1	7.13±3.4
MS + 10mg ⁻¹ ABA + 9% Sucrose + 8gl ⁻¹ Agar	6.1±4.5	3.0±2.8	1.3±0.6	4.67±4.6
MS + 0.1mg ⁻¹ JA + 9% Sucrose + 8gl ⁻¹ Agar	6.6±2.1	3.0±0.0	2.5±2.1	3.98±2.6
MS + 1.0mg ⁻¹ JA + 9% Sucrose + 8gl ⁻¹ Agar	6.3±2.1	1.0±0.0	6.0±0.0	4.32±3.7
MS + 5.0mg ⁻¹ JA + 9% Sucrose + 8gl ⁻¹ Agar	7.2±3.5	3.0±0.0	10.0±0.01	6.57±3.7
MS + 10mg ⁻¹ JA + 9% Sucrose + 8gl ⁻¹ Agar	4.8±4.9	1.0±0.0	1.0±0.0	0.95±0.48
Average freshweight of microrhiozmes (g)	1.78±0.5	1.35±0.9	0.98±0.6	

The term 'minirhizome' depicts the rhizomes produced from microrhizomes under partially controlled nursery conditions. Microrhizome induced plants from all the trial media were planted out for minirhizome production. The minirhizome fresh weight ranged from 13g to 145g, depending on the size of the initial planting material (microrhizome) and the variety used. Plantlets with 0.1g microrhizome yielded an average of 18.2±5.2g fresh minirhizome (in cv. *Rejatha*) and plantlets with 1.5g microrhizome gave an average higher yield of 124.5±13.6g fresh minirhizome

(in cv. *Mahima*). In ginger cv. *Mahima* was superior in minirhizome production and *Rejatha* was the poor performer. Higher minirhizome yield was achieved from 2–3 months old microrhizomes harvested from MS with 90gl⁻¹ sucrose and 8gl⁻¹ agar. It was observed that only a few treatments yielded minirhizomes on par with the control. The minirhizomes produced from the microrhizome induced plants generated in ABA and JA containing media gave comparatively good yield on par with control and the rhizomes were bolder than all the other media.

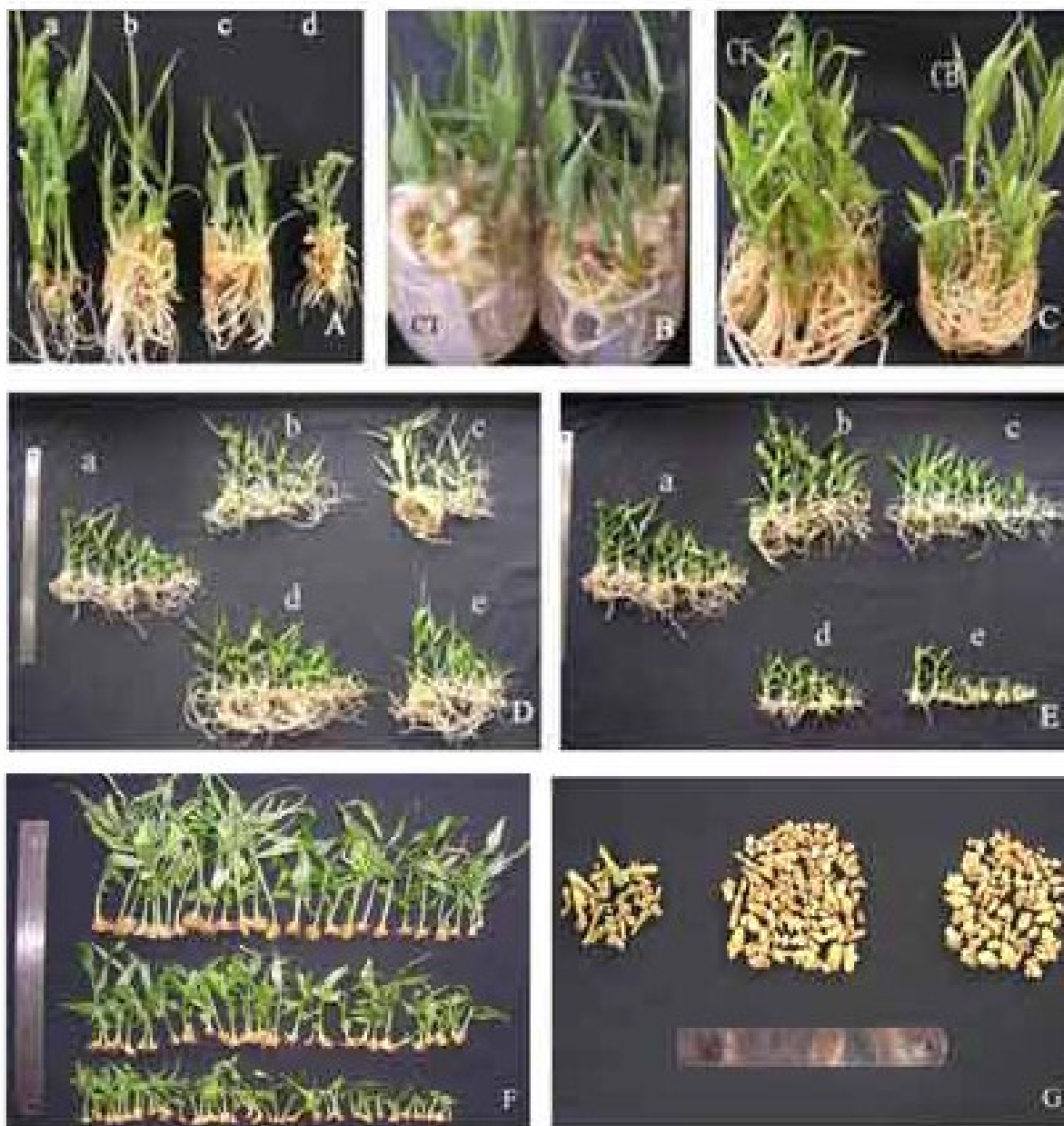


Figure 1. Culture responses in various media combinations A- Cultivar *Rejatha* in a- 30g/l sucrose, b- 80g/l sucrose, c- 90g/l sucrose and d- 100g/l sucrose; B- Cultivar *Mahima* grown in culture tubes (CT); C- Cultivar *Mahima* grown in Erlenmeyer culture flask (CF) and culture bottle (CB); D- Cultivar *Rejatha* grown in a- control medium (MS+90g/l sucrose), b- in 0.1mg/l ABA, c- in 1mg/l ABA, d- in 5mg/l ABA and e- in 10mg/l ABA; E- Cultivar *Rejatha* grown in a- control medium (MS+90g/l sucrose), b- in 0.1mg/l JA, c- in 1mg/l JA, d- in 5mg/l JA and e- in 10mg/l JA; F- Planting units; G- Microrhizomes

From the present study, it was observed that the use of medium with high sucrose in culture bottles was ideal for *in vitro* microrhizome production in ginger. Though cultivar *Rejatha* responded best in two trials it responded poorly in the field. Minirhizomes production was more in cv. *Mahima* compared to the other two cultivars. The pathogen free nature of the *in vitro* microrhizome was confirmed using disc culture method. The microrhizome and minirhizome technology developed in this study could be used for large scale production of pathogen free seed rhizomes in ginger.

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