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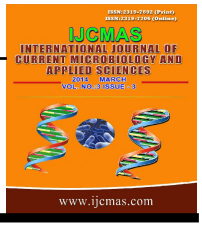

Original Research Article

In vitro microrhizome and minirhizome production in turmeric (*Curcuma longa* L.) cultivar *Alleppey Supreme* and its comparative anatomical and histochemical analysis

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A B S T R A C T

The present study was conducted to develop an efficient protocol for the development of microrhizome and minirhizome technology in high yielding variety of turmeric, *Alleppey Supreme*, using two media combinations and four types of culture vessels. It was observed that the variety showed highest response in liquid MS medium with 80gl^{-1} sucrose in Planton culture vessels. Microrhizome and minirhizome technologies were developed and comparative anatomical and histochemical studies were carried out. The microrhizome technology developed during the present study can be used for large scale production of planting materials in turmeric within a short period of time without compromising the quality and quantity.

Keywords

Alleppey Supreme; Anatomy; Field evaluation; Microrhizome; Tissue culture

Introduction

Turmeric, *Curcuma longa* L. is famed as the "Golden spice" and "spice of life". It was described as 'herb of sun' by the people of Vedic period. India is the world's largest producer, consumer and exporter of turmeric. Indian turmeric is regarded as the best in the world market because of its high curcumin content. Turmeric is used in the treatment of anorexia, cough, diabetic wounds, hepatic disorders, rheumatism and sinusitis. It can also cure smallpox and chickenpox lesions, jaundice, leprosy, arthritis, gallstone, cardio vascular diseases, cholesterol, platelet aggregation, HIV replication and multiple sclerosis (Ravindran, 2007; Valsala and Peter, 2007). The major production constraint in turmeric is the presence of certain soil born diseases which negatively affect the quality and quantity of rhizome. Among the diseases, rhizome rot and foliar diseases are the most serious ones (Devasahayam and Koya, 2007; Dohroo, 2007). Since the crop improvement programmes in turmeric is constrained due to low seed set, modern technologies like clonal selection, mutation breeding, induction of polyploidy and biotechnological tools are used for the improvement of this crop (Ravindran, 2007). Micropropagation of turmeric was first

reported by Nadgauda *et al*. (1978) and there are reports on the production of plants through micropropagation (Babu *et al*., 1997; Prathanturarug *et al.,* 2003; Rahman *et al.,* 2004).

In turmeric propagation by *in vitro* induced microrhizomes, produced independent of seasonal variations, is an ideal method both for the production of pathogen free planting material and for the conservation and exchange of germplasm. *In vitro* microrhizomes induction was reported earlier in turmeric (Rajan, 1997; Babu e*t al.,* 1997; Nayak, 2000; Sunitibala *et al.,* 2001; Shirgurkar *et al.,* 2001; Islam *et al*., 2002; Babu e*t al.,* 2003; Babu e*t al.,* 2007). Field evaluation of microrhizome derived plants of turmeric was also conducted by Babu *et al.* (2003).

The objective of the present study was to develop an efficient protocol for microrhizome and minirhizome induction in the high yielding variety of turmeric (*Alleppey Supreme)* using various media combinations and culture vessels and also their comparative anatomical and histochemical studies.

Materials and Methods

This work was carried out in the Crop Improvement and Biotechnology Division, Centre for Medicinal Plants Research, Arya Vaidya Sala, Kottakkal, Kerala, India.

Microrhizome induction

Microrhizome induction potential was studied using two media combinations such as solid and liquid ½MS (Murashige and Skoog, 1962) media containing 80gl^{-1} sucrose with four types of culture vessels (300ml and 350ml culture bottles, 300ml planton from Tarsons, Mumbai and 500ml Erlenmeyer culture flasks from Borosil, Mumbai). All the chemicals used for the present study were purchased from Himedia, Mumbai.

Single shoots isolated from the multiplied cultures maintained at Crop Improvement and Biotechnology Division of Centre for Medicinal Plants Research (CMPR) was used as explants. The inoculated cultures were maintained at 24±2^{0}C with a photoperiod of 12 hours at 2500-3000lux provided by white fluorescent lamp. Observations were taken on number of shoots, length of shoots and microrhizome induction at regular intervals for a period of three months.

Anatomical and histochemical studies

Anatomical and histochemical studies were conducted to confirm the rhizome formation *in vitro* and to compare rhizome development. For anatomical studies, thin transverse sections of the microrhizomes, minirhizomes and conventional rhizomes were taken, stained with safranine (0.3%), (Himedia, India) and mounted in glycerin (Merck, India). Thin sections were also taken and stained with Lugol's iodine solution (Nice Chemicals, Kerala) for 2-3 minutes and mounted on a glass slide for histochemical localization of starch. The amount of oil cells and starch were observed under trinocular research microscope with microphotographic attachment (Olympus, Japan) and documented the differences.

Growth responses in the field and the yield attribute

After three months of *in vitro* growth microrhizome induced plants were taken out, cleaned and planted in the nursery. After a season of growth, these microrhizomes produced minirhizomes. The

rhizome pieces from both conventional rhizomes and minirhizomes (15g) with one or two viable buds were used as seed rhizomes for field planting. Rhizomes were planted in the field beds (3 X 1m) with a spacing of 25cm. Prior to planting, the pits were lined with cowdung. After planting, organic matter was applied @ 4kg/bed for mulching. After 60 and 120 days of planting organic matter was applied @ 2kg/bed. After a season (April-December) of growth, the rhizomes were harvested and the data was documented on various yield attributes.

Results and Discussion

Cultures in all media showed prominent basal bulging within one month of growth as an indication of mocrorhizome induction irrespective of vessels used. In solid medium shoot number ranged from 1.0 to 5.55±0.71 and in liquid medium it was ranged from 3.67±1.15 to 5.5±2.12. In both the medium, cultures grown in planton vessel showed higher number of shoots. Length of shoot did not exhibit any prominent difference in any of the media and vessel except for cultures grown in 350ml bottle (B1) in solid medium where maximum shoot length (11.6±0.57) was observed. In this case the resources available in the medium must have used for elongation of the single shoot instead of forming multiple shoots (Table 1; Fig. 1). Adelberg and Cousins (2006) reported the superiority of liquid medium over the solid medium for increased biomass production in turmeric. But Salvi *et al.* (2002) and Prathanturarug *et al.* (2005) preferred solid medium for better response in turmeric Kavanagh *et al*. (1991) reported effect of culture vessels in plant growth, development and acclimatization in grapevine and they found that liquid culture in large vessels yielded higher number of taller shoots. Islam *et al*. (2005) reported magenta vessel GA-7 better than Erlenmeyer flask or tubes for the culturing of mint.

Anatomical and histochemical studies

Microrhizome induction was further configured by anatomical and histochemical studies. Transverse sections of microrhizomes, minirhizomes and conventional rhizomes indicated high amount of oil cells. The colour of oil cells ranged between light yellow to dark orange. Starch content was high throughout the cells (Table 2; Fig. 2).

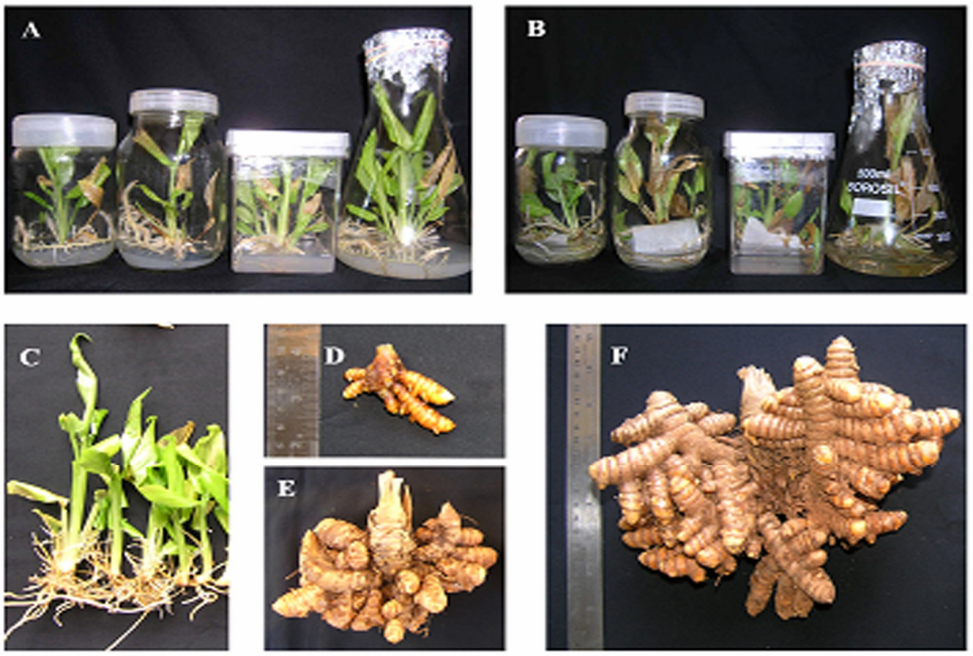
Growth response in the field and yield attributes

On field planting minirhizomes showed 89% establishment, when conventional rhizome showed 100% establishment. Number of tillers was higher in conventional rhizomes compared to minirhizomes where as length of shoots was higher in minirhizomes. In case of conventional rhizomes the percentage of germination was 100% (Table 3).

Yield was nearly three times higher in minirhizome seed material (526.67±9.5) compared to the conventional seed rhizome (183.0±8.30). Conventional seed rhizome produced lengthier (8.28±1.22cm) and thicker primary fingers (4.0±0.38cm) and secondary fingers (1.95±0.13cm) (Table 4) (Fig. 2).

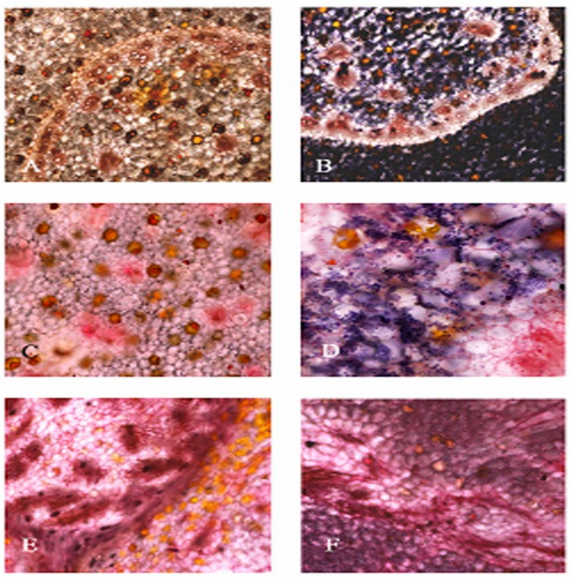
Jilani *et al*. (2012) reported field performance of turmeric cultivars and in the basis of better response in plant height, number of leaves, leaf width, and leaf length, they recommended variety Krishna for cultivating in Dera Ismail Khan Region of Pakistan. Manhas and Gill (2010)

Figure.1 Microrhizome induction and minirhizome production in turmeric



A-Cultures grown in ½ strength MS medium supplemented with 80gl^{-1} sucrose and 8gl^{-1} agar; B-Cultures grown in ½ strength MS medium supplemented with 80gl^{-1} sucrose; C- Planting units; D- Minirhizome harvested from greenhouse; E- Minirhizomes harvested from field bed; F-Conventional rhizome harvested from field.

Figure.2 Anatomical and histochemical comparison of rhizomes of turmeric



Section of conventional rhizomes showing A- oil cells and B-starch granules, Section of minirhizomes showing C- oil cells and D-starch granules and sections of microrhizomes showing E- oil cells and F- starch granules.

Table.1 Culture responses of turmeric cv. *Alleppey Supreme* after 3 months of *in vitro* growth

½MS+80gl^{-1} sucrose	Vessel	Number of shoots	Length of shoots (cm)
Solid medium	B1	1.0±0	11.6±0.57
	B2	3.5±2.12	7.4±1.56
	PL	5.5±0.71	6.73±1.59
	CF	4.67±0.58	8.86±1.16
Liquid medium	B1	4.33±0.58	8.05±1.13
	B2	4.5±0.71	7.22±0.45
	PL	5.5±2.12	8.31±0.08
	CF	3.67±1.15	7.61±0.42

B1-350ml bottle; B2-300ml bottle; PL-300ml Planton; CF-500ml Erlenmeyer flask

Table.2 Oil and starch content in conventional rhizomes, microrhizomes and minirhizomes

Material/Sample	Number of oil cells		Amount of starch	
	Medulla	Cortex	Medulla	Cortex
Conventional rhizomes	864.5±7.8	1520.2±3.1	High	High
Microrhizomes	259±12.8	616±19.9	High	High
Minirhizomes	980.5±10.8	1820.2±2.7	High	High

Table.3 Growth response of cultivars in the field beds

Material	% of Germination	Number of shoots	Length of shoots (cm)
Conventional rhizomes	100%	2.41±1.46	38.62±10.66
Minirhizomes	89%	1.0±0	47.0±5.5

Table.4 Characters of rhizomes produced from conventional and minirhizomes grown in the field beds after harvesting

Material		Conventional rhizomes	Minirhizomes
Weight/clump (g)		183.0±8.30	526.67±9.50
PF	No	1.00±0.00	01.00±0.00
	Length (cm)	9.27±2.19	08.28±1.22
	No. of nodes	14.0±2.00	11.00±1.25
	Internodal length (cm)	0.59±0.05	00.63±0.11
	Thickness (cm)	3.73±0.25	04.00±0.38
SF	No	10.33±2.08	06.30±1.42
	Length (cm)	6.97±1.70	06.08±1.02
	No. of nodes	9.70±2.45	07.22±1.01
	Internodal length (cm)	0.77±0.13	00.76±0.09
	Thickness (cm)	1.95±0.13	01.87±0.33
TF	No	5.63±0.99	14.20±4.94

PF- Primary finger; SF- Secondary finger; TF- tertiary finger; No- Number,

reported the effect of different planting materials, mulch levels and farmyard manure on growth, yield and quality of turmeric. From the study they confirmed that the use of mother rhizome resulted in better yield and quality. The increased availability of moisture, nutrients and favorable temperature with mulch application (@ 9.38tonnes/ha) have resulted in better yield. Pandey *et al*. (2003) reported the effect of plant height, weight of primary rhizome, weight of secondary rhizomes and number of secondary rhizome per plant had direct positive effect on rhizome yield in genotypic level. While the length of clump, number of tillers per clump, number of secondary rhizomes per plant, weight of mother rhizome per plant and weight of primary rhizome per plant had significant positive association with yield at the phenotypic level. There are many reports on the effects of planting materials and mulch type on the yield of turmeric (Verma and Sarnaik, 2006; Nwokocha *et al*., 2007; Manhas *et al*., 2010). Hazarika (2006) reported the abnormalities in micropropagated plants in their morphology, anatomy and physiology compared to conventional plants. In the present study, there was no abnormalities observed in conventional, microrhizome induced or minirhizome derived plants. However minirhizomes gave threefold increase in yield over conventional rhizomes.

In the present study an efficient protocol was developed for the microrhizome and minirhizome production in turmeric. The microrhizome and minirhizome technologies developed can be used for the large scale production of pathogen free planting materials in turmeric within a short period of time without compromising the quality and quantity of the produce. These high quality rhizomes can be used in various field of research for the identification, comparison and isolation of different components and for the conservation and exchange of pure germplasm.

Acknowledgement

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