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# Comparative studies on *in vitro* microrhizomeinduction in three varieties of *Curcuma longa* (Turmeric) – The role of two stress hormones

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**Abstract** Turmeric- *Curcuma longa* L. of zingiberaceae, is one of the major medicinal spice crop in India. Microrhizome induction is a novel biotechnological tool to produce disease free planting materials in this rhizomatous crop since other crop improvement programmes are failed due to low seed set. Pathogen free nature and the genetic stability of the resultant plantlets/ rhizomes increase the acceptance in the commercialization of this technique. In the present study disclosed an efficient method for enhanced microrhizome production in three high yielding varieties of turmeric.

***Key words: Abscicic acid, Jasmonic acid, Micropropagation, Zingiberaceae***

## INTRODUCTION

Turmeric- *Curcuma longa* L - belongs to the family zingiberaceae. It is known as the "Golden spice". The useful part is the rhizomatous stem. India is the world's largest producer, consumer, and exporter of the turmeric. Indian turmeric is regarded as the best in the world market because of its high curcumin content. The constituents of turmeric include starch, minerals, cellulose, gum, volatile oils and a yellow colorant curcumin. The medicinal uses of turmeric and curcumin ranging from cosmetic face cream to the preventive of Alzheimer's disease (Duke, 2003). It is used as an antioxidant, Chemotherapeutic, Chemo preventive and in the treatment of Liver injury, Arthritis, Gallstone, Cardio vascular diseases, cholesterol, platelet aggregation, HIV replication, Multiple sclerosis etc.

Turmeric is bitter in taste and its action is "pungent like" after digestion and metabolism. Being hot, light, and irritant it is able to reduce corpulence, and stimulate all functions in our body. According to Ayurveda turmeric has Rasa (taste), Tikta (bitter), Katu (pungent), Guna (property), Rooksha (irritant to make dry rough), veerya (potency), Ushna (hot), Vipaka (metabolic property) properties. The

use of turmeric as a spice, a dye, or a cosmetic is well known over the world. The Hindus, both tribal and civilized-consider turmeric as sacred and auspicious. It is associated with several rituals from ancient period and the traditions still goes on. This socio-religious aspect is very interesting and reveals how strongly turmeric is related to the Indian tradition (Remadevi and Ravindran, 2005; Warrier *et.al.,* 2000).

Now a days the productivity of turmeric is decreased due to many diseases that affect rhizome yield and quality. Among the diseases such as rhizome root, leaf blotch, leaf spots, rhizome scale, major and minor pests, rhizome rot and foliar diseases are the most serious (Dohroo, 2007; Devasahayam and Koya, 2007). So there is very important to develop an efficient *in vitro* protocol for the large-scale production of high quality microrhizomes of *Curcuma longa* L.

Micropropagation of turmeric was first reported by Nadgauda *et al*., (1978). Then many authors reported the production of multiple shoots by simple micropropagation (Babu *et al*., (1997), Rahman *et al.,* (2004), Prathanturarug *et al.,* (2003) *etc).* Adelberg and Cousins (2006) reported the superiority of liquid media over the solid media for increased biomass production. But Salvi *et al.,* (2002) and Prathanturarug *et al.,* (2005) preferred solid medium for a better response in turmeric. In turmeric micropropagation by *in vitro* microrhizomes is an ideal method for the production of disease free planting material and also for conservation and exchange of germplasm. Since minimal level of growth regulators are used and the number of subculture cycles are reduced in microrhizome production, the pathway may be better suited for the production of genetically stable planting material. Microrhizomes can be produced *in vitro*, independent of seasonal fluctuations. In turmeric *In vitro* induction of microrhizomes was reported by Rajan (1997), Babu e*t al.,* (1997, 2003), Nayak (2000), Sunitibala *et al.,* (2001), Shirgurkar *et al.,* (2001) *etc.* Field evaluation of microrhizome derived plants of turmeric was conducted by Babu *et al.,* (2003). Adelberg and cousins (2006) reported *in vitro* induction of functional storage organs (rhizomes) in turmeric using liquid cultures.

Nayak (2000, 2002) and Nayak and Naik (2006) have reported factors such as concentration of sucrose and BA in the medium, photoperiod and their interaction for the induction of microrhizomes. Cousins and Adelberg, (2008) reported microrhizome development in the presence of methyl jasmonate (MeJa) and benzyladenine (BA).

## MATERIALS AND METHODS

Turmeric, *Curcuma longa* L. belonging to the family Zingibereacae was used in the present study. The three high yielding varieties of turmeric *Alleppey Supreme* and *Prabha*, released from Indian Institute of Spices Research (IISR), Kozhikode, Kerala and a norh east variety *Lakadong* (recieved from The Energy Research Institute, Delhi) were selected for the study of *in vitro* microrhizome induction responses in various media combinations. Multiplied stock cultures maintained in Crop Improvement and Biotechnology Facility of Centre for Medicinal Plant Research (CMPR), Arya Vaidya Sala Kottakkal, Kerala, India were used as the source material for explants in all experiments.

All the experimental works were done in the labs and the fields of Centre for Medicinal Plant Research (CMPR), Arya Vaidya Sala (AVS), Kottakkal, Kerala, India.

MS medium (Murashige and Skoog, 1962) supplemented with Abscisic acid (ABA) and Jasmonic Acid (JA) in four different concentrations ($0.1mgl^{-1}$, $1mgl^{-1}$, $5mgl^{-1}$, $10mgl^{-1}$) was used for the microrhizome induction trials. MS medium with 9% sucrose was used as control with all trials.

### Microrhizome induction trials

Single shoot explants were excised from the *in vitro* multiplied stock cultures maintained in the Crop Improvement and Biotechnology Facility of CMPR were inoculated into growth regulator free medium, for one week as a pre-culture method to make the shoots withstand the stress due to high sucrose levels in the media for microrhizome induction trials. After 1–2 weeks of growth, the cultures were transferred into various combinations of microrhizome induction media (Table 1).

**Table 1: Media combination used for microrhizome trial**

| Sl. No. | Composition |
|---|---|
| 1 | MS+ 9%Sucrose +8gl$^{-1}$ Agar (Control). |
| 2 | MS+ 0.1mg mgl$^{-1}$ ABA + 9%Sucrose +8gl$^{-1}$ Agar. |
| 3 | MS+ 1mg mgl$^{-1}$ ABA + 9%Sucrose +8gl$^{-1}$ Agar. |
| 4 | MS+ 5 mg mgl$^{-1}$ ABA + 9%Sucrose +8gl$^{-1}$ Agar. |
| 5 | MS+10 mg mgl$^{-1}$ ABA +9%Sucrose +8gl$^{-1}$ Agar. |
| 6 | MS+ 0.1mgl$^{-1}$ JA+ 9%Sucrose +8gl$^{-1}$ Agar. |
| 7 | MS+ 1 mgl$^{-1}$ JA + 9%Sucrose +8gl$^{-1}$ Agar. |
| 8 | MS+ 5 mgl$^{-1}$ JA + 9%Sucrose +8gl$^{-1}$ Agar. |
| 9 | MS+10 mgl$^{-1}$ JA + 9%Sucrose +8gl$^{-1}$ Agar. |

The cultures were maintained at 24± 2$^0$C with a photoperiod of 12 hours at 2500-3000 lux and observed for shoots development and microrhizome induction periodically and data collected. Lower portions of the shoots were used for the anatomical studies to analyze the developmental stages of microrhizome development and to compare the oil and starch content to the *in vitro* formed rhizome. Well rooted plantlets after 30, 45, 60, 75, 90 and 120 days of growth from various treatments were separated in to single units and planted in polythene bags filled with sand, soil and farmyard manure in the ratio 2:2:1. The plants were kept in a nursery with 75% shade.

## RESULT

### Microrhizome induction

Microrhizome induction trials indicated that the three varieties showed specific differences in microrhizome induction responses in eight media combinations tried. The observations were done at regular intervals of 30, 45, 60, 75, 90 and 120 days and the results of three months are given below.

In the first case *i. e.*, the cultures grown in ABA, vitrification was observed within 7days. But in the case of cultures grown in JA, within 7days itself, the vitrification rate was maximum. The cultures showed slow growth rate and development.

In this study variety wise difference was observed in both ABA and JA. After three months of incubation, in case of *Alleppey Supreme* more response was noticed in the media with MS+0.1mgl$^{-1}$ABA+8gl$^{-1}$agar (2.5±0.71, 7.82±1.44 and 4.43±0.47 for number of shoots, length of shoots and number of leaves respectively) and MS+0.1mgl$^{-1}$JA+8gl$^{-1}$agar (2.4±1.14, 6.69±2.01 and 3.9±2.97 for number of shoots, length of shoots and number of leaves respectively) i.e., the least concentrations of both ABA and JA. Variety *Prabha* showed better response in the media with MS+1mgl$^{-1}$ABA+8gl$^{-1}$agar (4±2, 5.19±1.13 and 3.11±1.14 for number of shoots, length of shoots and number of leaves respectively) and MS+0.1mgl$^{-1}$JA+8gl$^{-1}$agar (3±1.41, 4.8±1.15 and 2.89±0.58 for number of shoots, length of shoots and number of leaves respectively). The variety *Lakadong* responded maximum to media with MS+1mgl$^{-1}$ ABA or JA+8gl$^{-1}$agar (3.17±1.47, 7.90±2.54 and 3.71±1.21 in ABA and 3.17±1.94, 9.98±2.73 and 4.74±1.83 in JA media for number of shoots, length of shoots and number of leaves respectively). Variety *Lakadong* showed more resistance against high concentrations of ABA and JA than the other three varieties (Table 2; Fig.1&2).

**Table 2: Observation after 3 months**

| **Combination on MS+9% sucrose+8gl$^{-1}$ agar** | **Number of shoots** | | |
|---|---|---|---|
| | ***Alleppey Supreme*** | ***Lakadong*** | ***Prabha*** |
| Control medium | 4.0±0.02 | 3.17±1.33 | 3.75±0.96 |
| 0.1mgl$^{-1}$ ABA | 2.5±0.71 | 2.33±1.21 | 3.5±1.91 |
| 1.0mgl ABA | 1.67±1.15 | 3.17±1.47 | 4.0±2.0 |
| 5.0mgl ABA | 1.0±0.00 | 1.33±0.52 | 1.25±0.5 |
| 10mgl ABA | 1.0±0.00 | 1.67±0.82 | 1.0±0.0 |
| 0.1mgl JA | 2.4±1.14 | 2.6±1.34 | 3±1.41 |
| 1.0mgl JA | 1.33±0.58 | 3.17±1.94 | 1.67±0.58 |
| 5.0mgl JA | 1.75±0.96 | 2.8±1.48 | 2.2±2.17 |
| 10mgl JA | 1.8±0.84 | 1.8±0.84 | 1.0±0.0 |

**Figure 1: Cultures after three months growth in media with various concentrations of ABA (a- 0mg/l, b- 0.1mg/l, c- 1mg/l, d- 5mg/l and e- 10mg/l of ABA; A&D- *Alleppey Supreme*, B&E- *Lakadong* and C&F- *Prabha*)**

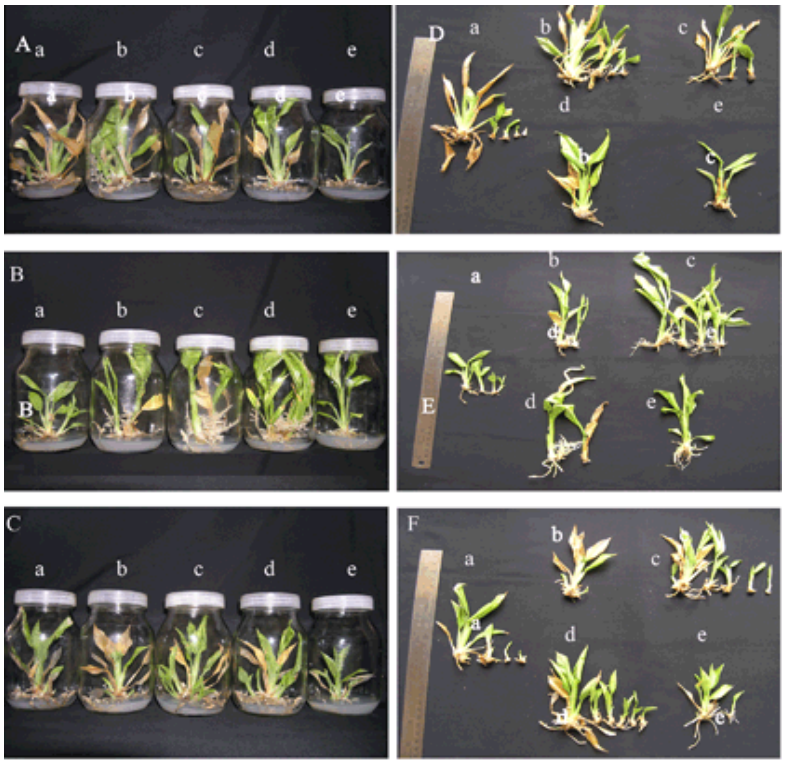
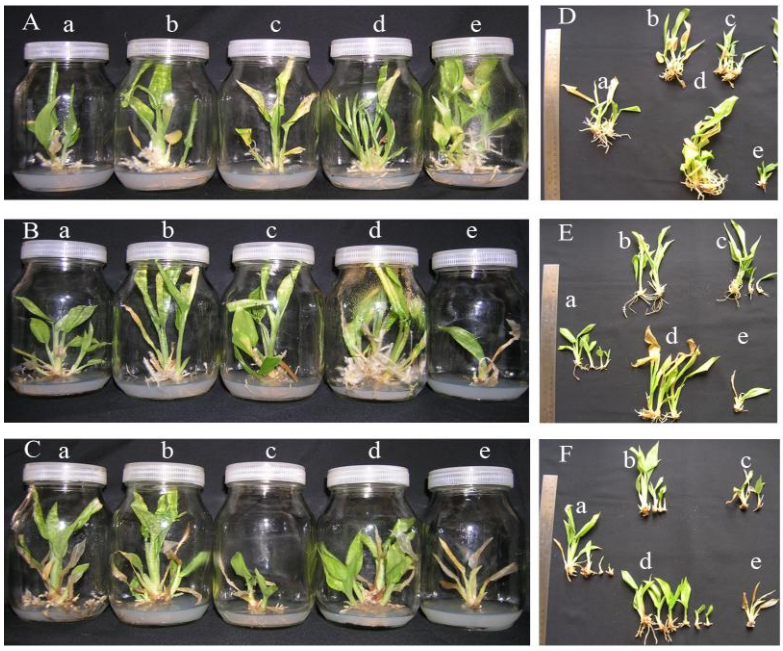



**Figure 2: Cultures after three months growth in media with various concentrations of JA (a- 0mg/l, b- 0.1mg/l, c- 1mg/l, d- 5mg/l and e- 10mg/l of JA; A&D- *Alleppey Supreme*, B&E- *Lakadong* and C&F- *Prabha*)**




*In vitro* induction of microrhizomes in media with higher levels of sucrose observed in the present study could be supported by work done by Rajan1997; Babu *et al.*, 1997, 2003; Nayak, 2000; Sunitbala *et al*., 2001; Shirgukar *et al*., 2001; Peter *et al*., 2002.

Abscisic acid (ABA) and Jasmonic acid (JA) were the two stress hormones used in the present study. Of the two stress hormones used, ABA exhibited superiority over JA. Mrudul *et al*., (2001) studied that ABA failed to promote microrhizome induction in *Curcuma longa* L. where as in the present study it was seen that lower concentrations of ABA promoted the formation of microrhizomes in turmeric in a minimum level is support to the work done by Riera *et al*., (2005), Kim *et al*., (1994) and Xu *et al.*, (1998) who studied that ABA promotes the formation of tubers, bulbs and corms.

Cousins and Adelberg (2008) found that JA induced microrhizome development in *Curcuma longa* L. Similarly in the present study, it was seen that lower concentrations of JA favored an acceptable growth of the explants and induction of microrhizomes. Vitrification/ hyperhydrocity of the *in vitro* cultured plantlets were seen high concentrations of ABA and JA. It was also seen that ABA and JA showed senescence and abscission effect on the *in vitro* grown plantlets as was reported by Srivastava (2002).

Anatomical and phytochemical studies were conducted to confirm the microrhizome development. The presence of oil cells and starch content indicate the rhizome development.

**Field establishment**

The plants were successfully established (100%) in the nursery and they were observed for further growth. The *in vitro* cultured plants of turmeric were hardened and acclimatized to the field conditions with relative 100% success. Earlier studies in turmeric and other Zingiberaceous crops like turmeric, cardamom, and *Kaempferia* support this view (Hosoki and Sagawa, 1977; Nadgauda *et al*., 1980; Bhagyalakshmi and Singh, 1988; Vincent *et al*., 1992; Babu *et al.* 2007).

Thus the study revealed the effect of ABA and JA on *in vitro* responses in terms of microrhizome induction in turmeric shoot cultures. The attempt provided the possibility of exploiting these factors for enhanced production of microrhizomes.

## SUMMARY AND CONCLUSION

Many diseases like rhizome rot and leaf diseases now caused production decline in turmeric, so disease free planting material is one of the prerequisite in turmeric cultivation strategy. The rarity of seed set hampers

recombination breeding. Crop improvement programmes in turmeric resulted in release of many varieties.

Biotechnological tools proved to be good in solving many crop specific problems in turmeric. Direct *in vitro* regeneration of disease free planting material through tissue culture is possible in turmeric. But the micropropagated plants require three crop seasons in the nursery to form sufficient quantity of rhizomes to be planted out. This problem could be overcome through the induction of *in vitro* microrhizomes in zingiberaceous species. Microrhizome technology can be adopted for large-scale planting material production and conservation of these species. They are good propagules and hence could be used for production and exchange of disease free planting material.

The trials were conducted using media with varying concentrations of ABA and JA at various levels to develop a protocol for the mass production of disease free planting material.

All the cultures tested in the study responded to respective media combinations. Resemblance of the *in vitro* cultured plants to the field grown ones was confirmed by Anatomical studies of the rhizomes but they were smaller in size. Anatomical studies further confirmed rhizome formation at the base of the shoots in respective media.

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**REFERENCE**

1. Adelberg, J., and Cousins, M. Thin film of liquid media for heteromorphic growth and storage organ development: Turmeric (*Curcuma longa*) as plant. *Hort Sci*. 2006.41:539-542.
2. Ali, A., Munawar, A. and Siddiqui, F. A. *In vitro* propagation of turmeric, Curcuma longa L. *Int. J. Biol Biotechnol*. 2004.1:511-518.
3. Babu, K. N. and Minoo, D. Commerical Micropropagation of Spices. In: Chandra, R. and Misra, M. (eds). *Micropropagtion of horticultural Crops*. International Book Distribution Company, Lucknow. 2003.345.
4. Babu, K. N., Minoo, D., Geetha, S. P., Sumathi, V. and Praveen, K. Biotechnology of Turmeric and related species. In: Ravindran, P. N., Babu, K. N. and Sivaraman, K. (eds). *Turmeric the genus Curcuma.* CRC press. Boca Raton. 2007.107-127.
5. Babu, K. N., Ravindran, P. N., and Peter, K. V. *Protocols For Micropropagation of spices and aromatic crops*. Indian Institute of Spices Research. Calicut. Kerala. India. 1997.35.
6. Babu, K. N., Ravindran, P. N., and Sasikumar, B. Field evaluation of tissue ciltured plants of species and assessment of their genetic stability using molecular markers. *Final report submited to Dpt of Biotechnology Government of India*. 2003.94.
7. Bhagyalakshmi, N. and Singh, N. S. Meristem culture and micropropagation of a variety of ginger (*Zingiber officinale* Rosc.) with a high yield of oleoresin. *Journal of Horticultural Science*. 1988.63(2): 321-327.
8. Cousins, M. M., and Adelberg, J. W. Short- term and long-term time course studies of turmeric (*Curcuma longa* L.) microrhizome development *in vitro*. *Plant Cell Tissue Organ Cult*. 2008.93: 283-293.
9. Devasahayam, S., and Koya, A. Insect pest of Turmeric. In: Ravindran, P.N., Babu, K. N., and Sivaraman, K., (eds). *Turmeric the genus Curcuma*. CRC press. Boca Raton. 2007.169-191.
10. Dohroo,N. P. Diseases of turmeric and ginger. *Advances in Spices research. History and Achievements of Spices research in India since Independence. Agrobios* .Pub. India. 2007.493-513.
11. Duke, T. A. *CRC handbook of Medicinal Spices*. Boca Raton. CRC Press. London. 2003.34-36.
12. Geetha, S. P. *In vitro technology for genetic conservation of some genera of zingiberaceae.* Ph. D. Thesis. Calicut. University. Kerala. India. 2002.

13. Hosoki, T. and Sagawa, Y. Clonal propagation of ginger (*Zingiber officinale* Rosc.) through tissue culture. *Hort Science*. 1977.12 (6): 451-452.
14. IISR, 2004. Annual report. Indian Institute of Spices Research, Calicut, Kerala, India. 2003-2004.
15. Keshavachandran, R., and Khader, M. D. A. Tissue culture propagation of turmeric. *South Indian Horticulture.* 1989.37:101-102.
16. Kim, K. S., Davelaar, E. and Klerk, G. J. D. Abscisic acid controls dormacy development and bulb formation in lily plantlets regenerated *in vitro*. *Physiol. Plant*. 1994.90: 59-64.
17. Meenakshi, N., Suliker, G. S., Krishnamoorthy, V., and Hegde, R. V. Standardization of chemical environment for multiple shoot induction of turmeric (*Curcuma longa*) for *in vitro* clonal propagation. *Crop Research Hissar.* 2001. 22:449-453.
18. Mrudul, V., Shirgurkar, C. K., John and Nadgauda, R. S. Factors affecting *in vitro* microrhizome production in Turmeric. *Plant Cell, Tissue and Organ Culture*. 2001.64: 5-11.
19. Murashige, T., and Skoog, F., A reviced medium for rapid growth and bioassays with tobacco cultures. *Physiologia*. 1962.15: 473-497.
20. Nadgauda, R. S., Kulkarni, D. D., Mascarenhas, A. F. and Jagannathan, V. Developments of plantlets from cultured tissues of ginger (*Zingiber officinale* Rosc.). In: Rao, P. S., Heble, M. R. and Chadha, M. S. (eds). *Proc. Natl. Sym. Plant Tiss. Cult. Genetic manipulation and somatic hybridization of plant cells. BARC*, Bombay. 1980.358-365.
21. Nadgauda, R. S., Mascrenhas, A. F., Hendre, R. R., and Jagannathan. V. Rapid clonal multiplication of turmeric *Curuma longa* L. Plants by tissue culture. *Ind. J. Exp Bot*. 1978.16: 120-122.
22. Nayak, S. *In vitro* microrhizome production in four cultivars of turmeric *(Curcuma longa* L.) as regulated by different factors from: *Spices-and-aromatic-plants: -challenges and opportunities in the new century Contributory papers Centennial conference on spices and aromatic plants,-Calicut,-Kerala,-India*. 2000.3-9.
23. Nayak, S. High Frequency *In vitro* Production of Microrhizomes of *Curcuma amada*. *Indian Jounel of Experimental Biology*. 2002.230-232
24. Nayak, S., and Naik, P. K. Factors effecting *In vitro* formation and growth in *Curcuma longa* L. and improved field performance of Micropropagated plants. *In J. Science Asia.* 2006.32: 31-37.
25. Peter, K. V., Ravindran, P. N., Babu, K. N. and Geetha. S. P. Establishing *in vitro* conservation of spices germplasam. *ICAR project report.* Indian Institute of Spices Researech. Calicut. Kerala. India. 2002.131.
26. Prathanturarug, S., Soonthornchareonnin, N., Chukul, W., Phaidee, Y. and Saralamp. Rapid micropropagation of Curcuma longa using bud explants pre-cultured in thidiazuron- supplemented liquid medium. Plant cell tissue and organ culture. 2005.80:347-351.
27. Prathanturarug, S., Soonthornchareonnon, N., Chuakul. W., Phaidee, Y. and Saralamp, P. High frequency shoot multiplication in *Curcuma longa* L. using thidiazuron. *Plant Cell Rep*. 2003.21: 1054 - 1059.
28. Praveen, K. Variability in Somaclones of Turmeric (*Curcuma longa* L.). Ph D Thesis. Calicut University, Kerala, India. 2005.108-110.
29. Rahman, M. M., Amin, M. N., Jahan, H. S., and Ahamed, R. *In vitro* regeneration of plantlets of *curcuma longa* L. a valuable spice plant in Bangladesh. *Asian J Plant Science.* 2004.3:306-309.
30. Rajan, V. R. Micropropagation of turmeric (*Curcuma longa* L) by *in vitro* microrhizomes. Biotechnology of species, Medicinal and Aromatic crops. *Indian society for spices*. India. 1997.25-28.
31. Ramadevi, R. and Ravindran, P. N. Turmeric :myths and traditions. *Spice India*. 2005.18(9): 11-17.

32. Riera, M., Valon, C., Fenzi F., Giraudat, J and Leung, J. The genetics of adaptive responses to drought stress abscisic acid- dependent and abscisic acid- independent signaling components. *Physiol. Plant*. 2005.123: 111-119.
33. Salvi, N. D., George, L., and Eapen, S. Micropropation and field evaluation of microprotagated plants of turmeric. *Plant cell Tiss. Org. Cult*. 2002.68: 143-151.
34. Shetty, M. S. K., Hariharan, P. and Iyer, R. D. Tissue culture studies in turmeric. In: Nair, M. K., Premkumar, T., Ravindran, P. N and Sarma, Y. R. (eds.). *Proceedings of National seminar on Ginger and Turmeric.* Calicut: CPCRI, Kasargode. 1982.39-41.
35. Shirgurkar, M. V., John, C. K., and Nagdgauda, R. S. Factors affecting *in vitro* microrhizome production in turmeric, *Plant Cell Tissue & organ culture.* 2001.64:5-11.
36. Srivastava, L. M. (2002). *Plant growth and development. Hormones and Environment*. Academic Press, California, USA. 2001.217-520.
37. Sunitibala, H., Damayanti, M., and Sharma, G. *In vitro* propagation and rhizome formation in *Curcuma longa* Linn. *Cytobios.* 2001.105:71-82.
42. 78.
38. Vincent, K. A., Mary, M. and Molly, H. Micropropagation of *Kaempferia galanga* L., a medicinal plant. *Plant Cell Tiss. Org. Cult*. 1992.28: 229-230.
39. Warrier, P. K., Nambiar, V. P. K., and Ganapathy, P. M. *Some important medicinal plants of Western Ghats*. India: a profile published by Medicinal and Aromatic plants programme in Asia (MAPPA) New Delhi.1. 2000.
40. Xu, X., Lammeren, A. A. M. V. and Vereugdenhil, D. The role of gibberellins, abscisic acid and sucrose in the regulation of potato tuber Formation *in vitro. Plant Physiol*. 1998.117: 575-584.
41. Yasuda, K., Tsuda, T., Shimizu, H., Sugaya, A. Multiplication of curcuma species by tissue culture. *Plant Med*. 1988.75-