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IN VITRO PROPAGATION IN TURMERIC

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ABSTRACT

The research was conducted on *in vitro* propagation in turmeric (cv. Salem) at Tissue Culture Project, VNMKV Parbhani during the year 2023-24. The excised shoot tip 2-3 cm long was utilized as explants for *in vitro* studies. Among the many surface sterilisation treatments tested, Tween 20 solution @ 1 drop/100 ml for 10 min + 70% Alcohol dip for 1 min. + 0.1% Mercuric chloride for 11 min, gave better sterilization of the explants, which resulted in a lower percentage of contamination (13.33%) and a higher percentage of survival (80.67%). The MS media supplemented with 2.5 mg/l BAP + 0.5 mg/l NAA was the most effective treatment which achieved 100% shoot induction, with a shoot length of 5.07 cm and shoot initiation time of 12.35 days. The treatment, MS media augmented with 2.0 mg/l BAP + 0.5 mg/l NAA proved best for shoot proliferation, which produced the highest number of shoots per explant (4.67), maximum shoot length (3.19 cm), maximum number of leaves (4.33) and a shortest period (8.07) for shoot initiation. Among the different *in vitro* rooting treatments, the Half MS medium supplemented with 3.0 mg/l NAA resulted in maximum number of roots (8.20) and half- strength MS medium supplemented with 2.5 mg/l NAA produced highest length of roots (6.27 cm) and minimum root initiation time of 7.93 days. Out of various potting mixtures tried, Soil: FYM: Cocopeat (1:1:1) was evaluated to be the best, resulting in 100.00% plantlet survival.

***Keywords*:** Surface sterilisation, NAA, BAP, Shoot proliferation, *In vitro* rooting, Acclimatization.

Introduction

Turmeric (*Curcuma longa* L.) is a rhizomatous herbaceous perennial plant, but it is cultivated as an annual, belonging to the family Zingiberaceae. It is a widely used spice, found throughout the Indian subcontinent. It is one of the most significant spice crops, also known as Indian saffron, golden spice, or spice of life. Turmeric is a native of South East Asia and India (Sopher, 1964). Turmeric is extensively cultivated in South East Asia, particularly in India and China (Labban, 2014; Mehrotra *et al*., 2013).

Turmeric is constrained severely by several factors. (1) Turmeric normally propagates by rhizome with a low proliferation rate, and rhizome is also the economically used part of the Turmeric plant, which restricts the availability of turmeric seeds needed for cultivation, (2) Preservation of rhizome seeds is a hard job. It requires much attention, time and space, (3) these rhizomes are prone to damages due to different biotic and abiotic factors such as adverse environment, pathogen and insect infestation etc. The high susceptibility of this crop to rhizome rot (*Pythium aphanidermatum*) and bacterial wilt (*Pseudomonas solanacearum*) are the major constraint in the production of turmeric, (4) The multiplication rate of rhizomes is slow, and the conventional method of propagation is not sufficient to meet the demand for planting material. This will affect the final cost of rhizomes. (5) With limited availability of high yielding genotypes, expensive field maintenance of planting material in turmeric production may discourage

commercial growers (Khader, 1994; Nayak *et al.*, 2006; Nair, 2019). One way to relieve these limiting factors for turmeric establishment may be through the use of tissue culture technique.

The demand for high-quality planting material is being increasing rapidly. Plant tissue culture offers an efficient method for rapid propagation, production of disease free, true-to-type plants and germplasm preservation of plants irrespective of season and weather. The tissue culture technique may play a key role in this regard. Successful research work on *in vitro* micropropagation has been reported in *Curcuma longa* (Nadgauda *et al*., 1978; Sato *et al*., 1987; Salvi *et al*., 2000; Meenakshi *et al*., 2001; Sunitibara *et al.*, 2001; Kumar and Reddy, 2011; Nayak *et al*., 2011; Sarma *et al*., 2011; Singh *et al*., 2011; Sinchana *et al*., 2020). Moreover, tissue culture protocols are specific to particular cultivars. With this brief background the present research work "*In vitro* propagation in turmeric" using cv. Salem was carried out to establish an *in vitro* regeneration protocol for turmeric to ensure a year-round supply of disease-free planting material for future needs.

Materials and Methods

The experiments *in vitro* propagation of turmeric was carried out at Tissue Culture Project, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani (VNMKV) during 2023-24. Fully mature rhizome of turmeric (cv. Salem) procured from germplasm maintained by Horticulture Research Scheme (Vegetable), VNMKV, Parbhani was the source of explants for *in vitro* studies. Rhizomes were kept in sand for sprouting.

Preparation of Explants

The healthy and disease free fully mature rhizome of turmeric (cv. Salem) was collected and washed thoroughly by repeated washing with distilled water. The shoot tip 2-3 cm long with small portions attached to the rhizome was excised and used as explants for *in vitro* studies. Then explants were surface sterilized and subjected to MS media by aseptic practices. The explants were transferred and kept in a 500 ml conical flask having 100 ml distilled water. Then 3 drops of Tween-20 were added to the flask for about 10 min with constant shaking in the clockwise and anticlockwise direction. Then explants were washed 4-5 times with distilled water to remove any dirt or residues. Subsequently, the explants were transferred to laminar airflow cabinet and kept in a 250 ml sterilized conical flask. The flask with explants were constantly shaken during sterilization. Then explants were treated with 70 per cent alcohol for 1 min and rinsed with sterilized distilled water for 3-4 times. Then, the explants were immersed in different concentration sterilants such as Mercuric chloride ($HgCl_2$) as per treatments concentration within a flask and shaken constantly in the clockwise and anticlockwise direction. Then explants were washed 3-4 times with sterilized distilled water to make free from chemicals and ready for inoculation in culture media.

Shoot initiation and multiplication

The surface sterilized sprouted rhizome buds were collected in sterilized petri plate and the shoot tip (2-3 cm) was cut along with the base outermost layer of tissue was removed with the help of sterile forceps and scalpel blade. The individual explants were quickly inoculated in the test tube containing initiation culture media with the help of sterilized forceps. All inoculation steps were performed in laminar air flow cabinet under aseptic conditions. After inoculation, the cultures were incubated in culture room for four weeks.

Subculturing of multiplied shoots were done on MS media with growth regulators at an interval of four weeks. The clumps of shoots from multiplied shoots were separated into groups and sub cultured to fresh multiplication medium after four weeks of the previous subculture.

Culture/ growth room conditions

The culture vessels (test tubes and culture bottles) were kept on the culture racks and allowed to grow in a controlled environment. The inoculated cultures vessels were incubated in culture room wherein temperature 25±2 ^{0}C, relative humidity 70 per cent and photoperiod of 16 hrs light and 8 hrs dark conditions were maintained.

In vitro rooting

To evaluate the *in vitro* rooting response, shoots multiplied and grown on MS medium supplemented with growth regulator showing the best results were selected. Shoots of length ≥ 2.5 cm were used for rooting medium. Active and uniform shoots were transferred from the shoot induction medium to the root induction medium.

Hardening of *in vitro* plantlets

To acclimatize the *in vitro* produced plantlets to the natural environment. The healthy *in vitro* regenerated plantlets which have good numbers of roots were selected for hardening. The plantlets were removed from the media and washed with double distilled water properly to get rid of the traces of agar sticking to the roots. Thereafter, roots of the *in vitro* raised plantlets were dipped in 2 per cent Carbendazim

solution for 15 min to avoid any fungal infection. Well-developed tissue culture plantlets were transferred to nursery bags according to treatment combination for acclimatization.

Statistical analysis

The data obtained in all the experiments were statistically analyzed (Panse and Sukhatme, 1985). The standard error (SE) and Critical difference (C.D.) was worked out and results obtained were compared statistically. All the data were analyzed by using OPSTAT programme.

Results and Discussion

Standardization of surface sterilization method for the explants of turmeric

The significant differences were noticed in the contamination with respect to concentration and duration of sterilant (Table 1). The minimum per cent contamination (13.33%) was found when explants were treated with Tween 20 solution @ 1 drop/100 ml for 10 min + 70% Alcohol dip for 1 min. + 0.1% Mercuric chloride for 11 min (T_5). Whereas, maximum per cent contamination (100.00%) was found in untreated control (T_8).

The per cent survival of explant of turmeric was recorded. It was found from the study that survival per cent was increased as the duration time of treatments increased. Significantly the maximum survival per cent (86.67%) was obtained when explants were treated with Tween 20 solution @ 1 drop/100 ml for 10 min + 70% Alcohol dip for 1 min. + 0.1% Mercuric chloride for 11 min (T_5). Whereas, zero per cent survival rate was found in untreated control (T_8).

The findings show that detergent treatment followed by alcohol and mercuric chloride is necessary for obtaining higher culture asepsis in turmeric shoot tip explants. Mercuric chloride is highly antimicrobial with action against both fungi and bacteria. Concentrated alcohol is a potent disinfectant that instantly dehydrates the majority of bacterial and fungal spores. Jagadev *et al*. (2008) discovered that 0.1 $HgCl_2$ for 13 minutes was the best surface sterilant for ginger cv. Suravi which dramatically reduced the infection to 3.3% and took the shortest time (9.3 days) for the development of buds in standard MS medium. With this sterilant, explant survival (96.70%) and bud per explant (2.7) were at their highest levels. Shirgurkar *et al*. (2001) carried out surface sterilization in turmeric as first the buds were washed with a 0.5 % detergent and 0.05 to 0.1% antiseptic for 3-5 minutes. The buds were treated aseptically with 0.07 % $HgCl_2$ for 7-8 minutes then rinsed with sterile distilled water. Kaewthip *et al*. (2021) conducted sterilisation techniques to obtain contamination free of *Curcuma longa* explants and the results showed that immersing rhizome buds in 70% ethanol for 5 min, followed by 0.10% sterilisation for 10 min offered approximately 66% survival rate.

The findings of this study are consistent with those of Phoung *et al*. (2020), Sinchana *et al.* (2020), Bandara *et al*. (2023) and Kalawong *et al*. (2024) who reported better culture asepsis in turmeric with combined sterilization treatments. An increase in sterilant treatment duration from 7 to 11 min resulted in increased culture asepsis.

Standardization of establishment of medium

There was a significant variation in per cent shoot induction, among the different concentrations of growth regulators tested (Table 2). Incorporation of BAP in combination with NAA into MS media has either a positive or negative effect on per cent shoot induction. Among the different concentrations of growth regulators tested, the significantly highest percentage of shoot induction (100.00%) was noticed in T_4 Treatment (MS + 2.5 mg/l BAP + 0.5 mg/l NAA). Control recorded the lowest percentage of shoot induction (40.00 %). The significantly minimum time for shoot initiation (12.35 days) was taken by the treatment combination MS + BAP 2.5 mg/l + NAA 0.5 mg/l (T_4). Control recorded significantly the highest days for initiation of shoot (20.25 days). The data pertaining to the response of emerging shoot tip explant with respect to the length of the main shoot revealed that significantly the highest length of shoot (5.07 cm) was recorded in treatment (T_4) MS + BAP 2.5 mg/l + NAA 0.5 mg/l. Control recorded the lowest shoot length (2.31 cm).

All the two plant growth regulator combinations tested during the investigation resulted in successful culture establishment. MS media augmented with BAP 2.5 mg/l + NAA 0.5 mg/l proved best for culture establishment. It also took a smaller number of days to initiate culture along with high length of shoot. According to Raut (2016) MS basal media augmented with 1.0 mg/l BAP + 0.5 mg/l NAA resulted in the best culture establishment in turmeric. Anik (2018) reported that the highest percentage (81.00%) of shoot induction occurred with BA 2.0 mg/l for a minimum of 14.0 days. Kasilingam *et al*. (2018) revealed that ginger shoot tips grown on MS medium supplemented with 1.0 mg/l NAA and 3.0 mg/l BAP provided the best various of shoot regeneration. Low NAA levels and high BAP concentrations are demonstrated to produce the optimal shoot and bud production. Thakur *et al.*

(2018) recorded that MS + 1.0 mg/l BAP + 0.1 mg/l NAA was an appropriate medium for inducing shoots in the ginger var. Himgiri (80.19%) and Solan Ginger-44 (78.29%). Similar results were reported by George *et al*. (1998), Taghavi *et al*. (2021) and Sang *et al*. (2024).

Effects of growth regulators on multiple shoot formation

There was significant variation among the treatments for number of shoots produced per explant (Table 3). The data were recorded at the end of the first subculture cycle roughly 28 days after culturing. The significantly maximum number of shoots per explant (4.67) was recorded when multiple shoot clumps were incubated onto MS + 2.0 mg/l BAP + 0.5 mg/l NAA (T_2). Whereas, significantly lowest number of shoots per explant (1.87) was recorded in the control. The initial response of cytokinin may be mediated by an increase in the cytosolic calcium concentration by promoting calcium uptake from the medium. Calcium affects the cytoskeleton which can regulate exocytosis (Hager *et al*., 1991).

Significant differences were observed among the different concentrations of growth regulators tested with respect to the number of days taken for initiation of shoot (Table 3). The significantly minimum time for shoot initiation (8.07 days) was taken by the treatment combination MS + 2.0 mg/l BAP + 0.5 mg/l NAA (T_2). Control recorded significantly the highest days for initiation of shoot (12.33 days).The data revealed significant differences among the treatments with respect to the length of the shoot (Table 3). Significantly highest length of the shoot (3.19 cm) was recorded with MS + 2.0 mg/l BAP + 0.5 mg/l NAA (T_2). Whereas, significantly lowest length of shoot (1.56 cm) was recorded in control. The data on mean number of leaves per shoot as influenced by plant growth regulators are presented in (Table 3). The number of leaves per shoot influenced by plant growth regulators differed significantly. The significantly maximum number of leaves per shoot (4.33) was recorded with MS + 2.0 mg/l BAP + 0.5 mg/l NAA (T_2). Whereas, significantly lowest number of leaves per shoot (1.47) was recorded in the control. For calculating shoot multiplication ratio, the best treatment MS + 2.0 mg/l BAP + 0.5 mg/l NAA (T_2) was subcultured for 4 times at 28 days interval. The initial 100 No. of shoots were used, after 4th subculturing we got 678 No. of shoots. Based on this shoot multiplication ratio 6.78 % was achieved.

In the present study culture proliferation in turmeric, results indicated that low auxin to cytokinin ratio favoured the proliferation of turmeric cultures. This may be due to the fact that suppression of apical dominance leads to the production of more number of multiple shoots and reduced shoot length. According to Shirgurkar *et al*. (2001), micropropagation of turmeric using sprouted buds as explants cultured on MS medium augmented with 0.88 µM BAP + 0.92 µM kinetin + 5% coconut water + 2% sucrose + 0.5% agar and incubated for roughly 20 days at 25±1˚C in 16-h light (at 11.7 mol/m^{2}s intensity)/8-h dark cycles resulted in maximum proliferation. MS + 3.0 mg/l BAP + 1.0 mg/l NAA was the appropriate medium for inducing shoots in the ginger and turmeric from shoot tip explant (Raihana *et al*., 2011 and Kasilingam *et al.,* 2018). Shahinozzaman *et al*. (2013) discovered that MS media augmented with 3.0 µM BAP + 0.5 µM NAA was most successful for shoot initiation and proliferation in *Curcuma caesia* Roxb using rhizome buds as explants. After 8 weeks of culture, explants with a maximum success rate of 99.97% produced 10.38 shoots measuring 4.53 cm in length. Patel (2006) observed shoot initiation after days of inoculation in media containing 2.0 mg/l BAP and 1.0 mg/l NAA, while Raut (2016) noticed shoot initiation in turmeric 13.33, 12.33 and 12.00 after days of inoculation in media containing 1.0 mg/l BAP + 0.5 mg/l NAA in turmeric cv. Salem, Rajapuri, and Kadapa respectively.

Gaur (2021) observed that maximum number of shoots per explants (7.67) and average shoot length (10.46 cm) treatment- MS medium supplemented with 2.5 mg/l BAP and 0.5mg/l NAA. Estouka *et al.* (2021) used mixtures of BAP (2 or 3 mg/l) and NAA (Naphthalene acetic acid) (0.5 or 1 mg/l) for shoot induction. With an average of 5.4 shoots per explant and a maximum number of shoots of 3 mg/l BAP in MS media, each shoot on average has 10 leaves and 17.7 roots. Similar results were reported by Swarnathilaka and Nilantha (2012), Zuraida *et al.* (2016) and Taghavi *et al.* (2021). When present in sufficient amounts, cytokinins are known to inhibit terminal buds and encourage the proliferation of axillary shoots. Thus, determining the optimal concentration of cytokinins for optimal growth of shoots is a prerequisite for the rapid multiplication of any species.

***In vitro* root development**

The results of number of roots per plantlet is presented in Table 4. Significantly maximum number of roots per plantlet (8.20) was observed when *in vitro* shoots were incubated onto Half MS + 3.0 mg/l NAA (T_6). While the number of roots per plantlet was minimum with control (5.87). Data presented in Table 4 revealed that, there was significant difference with

respect to initiation of roots among the auxins used. The time taken for root initiation was minimum (7.93 days) in Half MS + 2.5 mg/l NAA (T_5) treated shoots. Control recorded significantly the highest days for initiation of roots (12.47 days). The data revealed significant differences among the treatments with respect to the length of the root (Table 4). Significantly highest length of root (6.27 cm) was recorded in T_5 (Half MS + NAA 2.5 mg/l). Control recorded significantly the lowest root length (4.83 cm). The formation of roots on auxin-free media may be attributed to the presence of endogenous auxin in *in vitro* raised shoots.

Effect of different dosages of NAA on the root activity of turmeric cv. Salem was studied in the current investigation. Kambaska *et al*. (2010) also reported better performance of NAA than IBA in rooting of micro shoots of black turmeric with 2.0 mg/l NAA mixed in half-strength MS medium (95.00% culture established with an average of 7.3 roots per plantlets and 4.5 cm on average length). This was in confirmation with the results of Meenakshi *et al*. (2001), who reported that maximum rooting was observed in NAA 0.3 mg/l with maximum root length. According to Phoung *et al*. (2020) observed the highest numbers of roots 13.57 in the MS media containing 2.0 mg/l NAA, have proven the positive effects of NAA on root induction of multiplied white turmeric shoots. Sang *et al*. (2024) discovered in turmeric that there were variations in the number of roots in rooting studies conducted with varying concentrations of NAA. According to the findings, the MS control medium without NAA generated the lowest number of roots. There were more roots as the concentration of NAA increased.

Ex-vitro acclimatization of plantlets

Hardening of the *in vitro* raised plantlets to make them adapted to the natural environment is a critical step during micropropagation. The ultimate success of *in vitro* propagation lies in the successful acclimatization of plants in the soil with less mortality (Saxena and Dhawan, 1998). After sufficient shoot and root development of culture, the healthy *in vitro* regenerated plantlets which have good numbers of roots were taken out from the culture vessel carefully without damaging any roots.

Physical, chemical and biological properties of potting mixture are important for the hardening of *in vitro* produced plantlets (Kansara *et al*., 2013). Out of various potting mixtures tried, Soil:FYM:Cocopeat (1:1:1) was evaluated to be the best, resulting in 100.00% plantlet survival with average increase in number of leaves per plant (5.20), plant height (53.48 cm), number of roots per plant (11.40) and root length (17.46 cm) after 60 days of transfer (Table 5).

These results are similar to various workers: Sit and Tiwari (1997) found that the survival rate during the hardening of turmeric plantlets in a mixture of soil: sand: FYM (1:1:1) was highest (93.33%). According Kambaska and Santilata (2009), the maximum survival rate of turmeric plantlets was (95.00%) noticed in a mixture of soil: sand: FYM (1:1:1). Soil:FYM:Cocopeat (1:1:1) to be the best potting mixture with high survival percentage in tissue culture raised plants of ginger (Parekh and Patel, 2014; Sharma, 2019). Mixing cocopeat, sand and FYM (1:1:1) might have provided sufficient organic matter to the plantlets with better grip and sufficient aeration to the roots, which is essential for healthy growth of the plants (Parekh and Patel, 2014).

Conclusion

The findings of present study demonstrate that the *in vitro* propagation technique play a crucial role in the rapid propagation of plants. It is concluded from present investigation that, MS medium supplemented with BAP 2.5 mg/l + 0.5 mg/l NAA found better with maximum percentage of shoot induction with least days. However growth regulator half-strength MS medium supplemented with 3.0 mg/l NAA produced maximum number of root and, half-strength MS supplemented with 2.5 mg/l NAA produced highest length of roots, and a minimum number of days for root initiation. The survival percentage of plantlets found maximum in Soil: FYM: Cocopeat at (1:1:1) ratio, with maximum plant height, more number of leaves per plantlets and highest number and length of roots to plantlets, but this need further confirmation, Therefore, for quick regeneration of turmeric *in vitro* propagation has been regarded as an effective alternative strategy.

Table 1: Effect of surface sterilants and duration of time on explants of turmeric after 21 days of culture.

Tr. No.	Treatment Details	Per cent contamination	Per cent survival of explants
T_1	Tween 20 solution @ 1 drop/100 ml (for 10 min.) + 70% Ethanol dip (for 1 min.) + 0.1% Mercuric chloride (for 7 min.)	40.00 (39.23)*	60.00 (50.77)
T_2	Tween 20 solution @ 1 drop/100 ml (for 10 min.) + 70% Ethanol dip (for 1 min.) + 0.1% Mercuric chloride (for 8 min.)	33.33 (35.01)	66.67 (54.75)
T_3	Tween 20 solution @ 1 drop/100 ml (for 10 min.) + 70% Ethanol dip (for 1 min.) + 0.1% Mercuric chloride (for 9 min.)	26.67 (30.79)	73.33 (58.93)
T_4	Tween 20 solution @ 1 drop/100 ml (for 10 min.) + 70% Ethanol dip (for 1 min.) + 0.1% Mercuric chloride (for 10min.)	26.67 (30.79)	73.33 (58.93)
T_5	Tween 20 solution @ 1 drop/100 ml (for 10 min.) + 70% Ethanol dip (for 1 min.) + 0.1% Mercuric chloride (for 11min.)	13.33 (21.34)	86.67 (68.66)
T_6	Tween 20 solution @ 1 drop/100 ml (for 10 min.) + 70% Ethanol dip (for 1 min.) + 0.1% Mercuric chloride (for 12 min.)	20.00 (26.57)	80.00 (63.43)
T_7	Tween 20 solution @ 1 drop/100 ml (for 10 min.) + 70% Ethanol dip (for 1 min.) + 0.1% Mercuric chloride (for 13 min.)	20.00 (26.57)	80.00 (63.43)
T_8	Untreated Control	100 (90.00)	0.00 (0.00)
	SE(m)	0.74	0.74
	C.D. at 1%	3.13	3.13
	C.V. %	3.42	2.46
* Figures in parenthesis are Arcsine transformed values			

Table 2: Effect of growth regulators on the shoot initiation of turmeric

Tr. No.	Treatment Details	Per cent shoot induction	Number of days taken for initiation	Mean length of shoot (cm)
T_1	MS + BAP 1.0 mg/l + NAA 0.5 mg/l	70.00 (57.10)*	15.70	2.40
T_2	MS + BAP 1.5 mg/l + NAA 0.5 mg/l	75.00 (60.27)	14.15	2.62
T_3	MS + BAP 2.0 mg/l + NAA 0.5 mg/l	80.00 (63.43)	13.00	3.05
T_4	MS + BAP 2.5 mg/l + NAA 0.5 mg/l	100.00 (90.00)	12.35	5.07
T_5	MS + BAP 3.0 mg/l + NAA 0.5 mg/l	95.00 (83.36)	13.55	3.83
T_6	MS only (Control)	40.00 (39.23)	20.25	2.31
	SE(m)	3.22	0.33	0.06
	C.D. at 1%	13.40	1.37	0.26
	C.V. %	9.81	4.44	3.94
* Figures in parenthesis are Arcsine transformed values				

Table 3: Effect of growth regulators on number of shoot/explant and length of the shoot in turmeric.

Tr. No.	Treatment Details	Number of shoots per explant	Number of days taken for initiation of shoot	Mean length of shoot (cm)	Number of leaves per shoot
T_1	MS + 1.0 mg/l BAP + 0.5 mg/l NAA	2.47	8.67	2.75	1.67
T_2	MS + 2.0 mg/l BAP + 0.5 mg/l NAA	4.67	8.07	3.19	4.33
T_3	MS + 3.0 mg/l BAP + 0.5 mg/l NAA	3.73	8.93	2.77	3.13
T_4	MS + 4.0 mg/l BAP + 0.5 mg/l NAA	3.27	9.40	2.96	2.27
T_5	MS + 1.0 mg/l BAP + 1.0 mg/l NAA	2.80	9.20	2.45	1.80
T_6	MS + 2.0 mg/l BAP + 1.0 mg/l NAA	3.33	9.80	1.80	2.73
T_7	MS + 3.0 mg/l BAP + 1.0 mg/l NAA	2.53	10.60	1.93	1.73
T_8	MS + 4.0 mg/l BAP + 1.0 mg/l NAA	3.33	10.80	2.24	2.80
T_9	MS only (Control)	1.87	12.33	1.56	1.47
	SE(m)	0.10	0.16	0.10	0.08
	C.D. at 1%	0.43	0.68	0.42	0.33
	C.V. %	5.82	2.92	7.28	5.61

Table 4: Effect of growth regulators on number of roots and length of the root in turmeric under *in vitro* condition

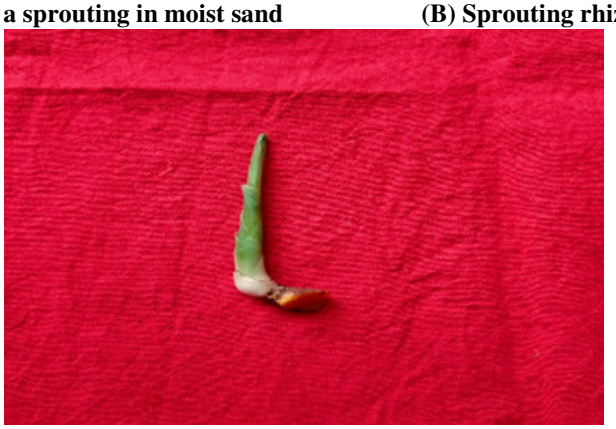
Tr. No.	Treatment Details	Number of roots per plantlet	Number of days taken for initiation of root	Mean length of root (cm)
T_1	Half MS + NAA 0.5 mg/l	5.93	11.20	4.94
T_2	Half MS + NAA 1.0 mg/l	5.93	10.33	5.10
T_3	Half MS + NAA 1.5 mg/l	6.40	9.80	5.20
T_4	Half MS + NAA 2.0 mg/l	7.07	9.13	5.47
T_5	Half MS + NAA 2.5 mg/l	7.67	7.93	6.27
T_6	Half MS + NAA 3.0 mg/l	8.20	8.47	5.05
T_7	Half MS only (Control)	5.87	12.47	4.83
	SE(m)	0.20	0.25	0.13
	C.D. at 1%	0.88	1.06	0.57
	C.V. %	5.22	4.31	4.35

Table 5: Effect of different potting mixtures on hardening of *in vitro* raised plants of turmeric

Tr. No.	Treatment details	Survival of plantlets (%)	No of leaves per plant	plant height (cm)	No of roots per plant	Root length (cm)
T_1	Soil:FYM:Sand (Ratio 1:1:1)	97.14 (84.73)	4.40	39.41	8.23	13.04
T_2	Soil:FYM:Cocopeat (Ratio 1:1:1)	100.00 (90.00)	5.20	53.48	11.40	17.46
T_3	Soil:FYM:Perlite (Ratio 1:1:1)	91.43 (77.14)	3.74	31.61	6.34	11.44
	SE(m)	2.91	0.12	0.80	0.19	0.18
	C.D. at 5%	8.95	0.38	2.46	0.58	0.55
	C.V. %	9.16	7.30	5.08	5.74	3.35
* Figures in parenthesis are Arcsine transformed values						

(A) Turmeric rhizome kept for a sprouting in moist sand

(B) Sprouting rhizome bud of turmeric



(C) Shoot tip explant

Fig. 1: Turmeric explant preparation (A) Turmeric rhizome kept for a sprouting in moist sand, (B) Sprouting rhizome bud of turmeric & (C) Shoot tip explant

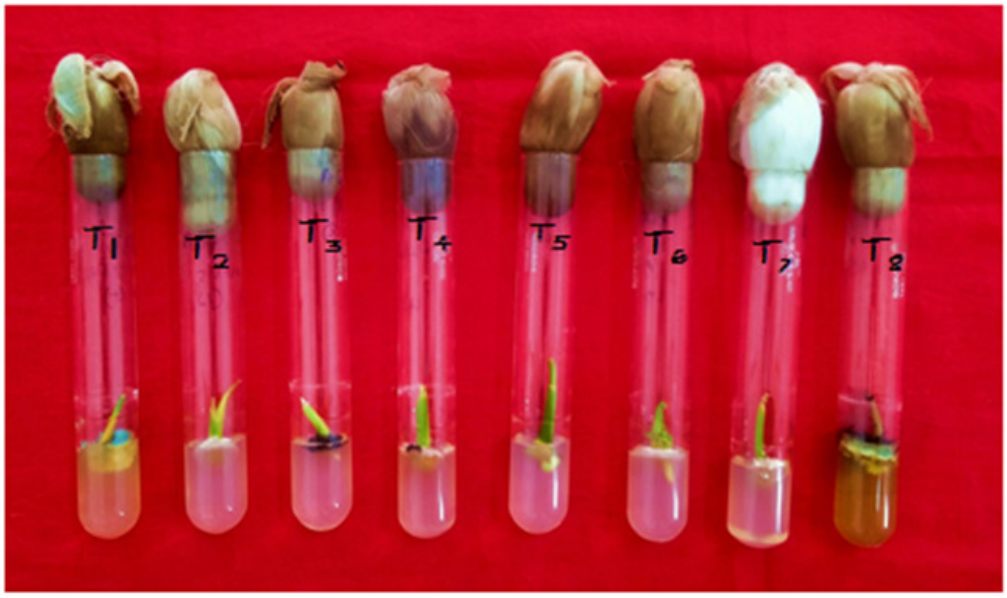
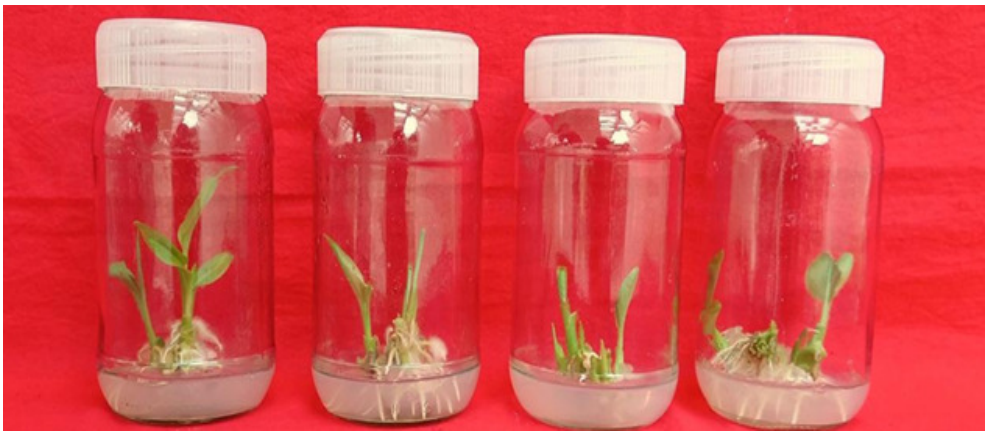


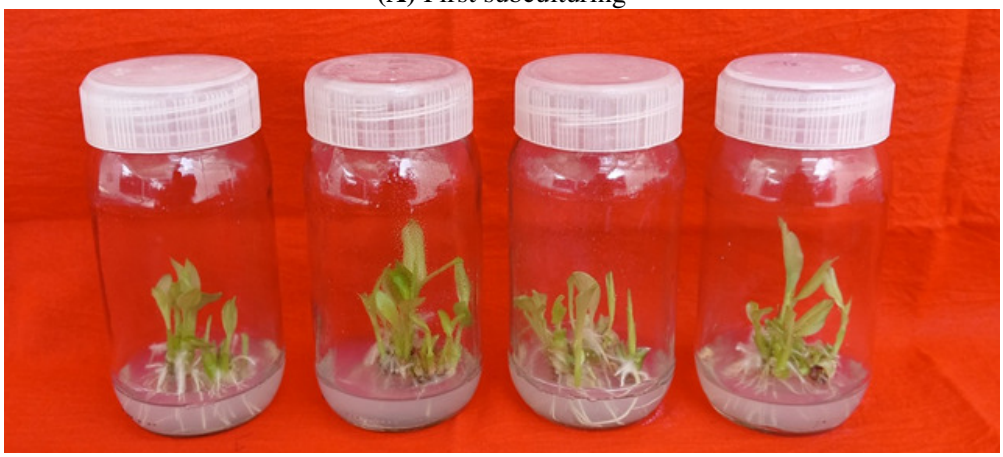
Fig. 2: Effect of surface sterilants and duration of time on explants of turmeric

Fig. 3: Effect of growth regulators on the shoot initiation of turmeric

Fig. 4: Effect of growth regulators on the shoot multiplication of turmeric



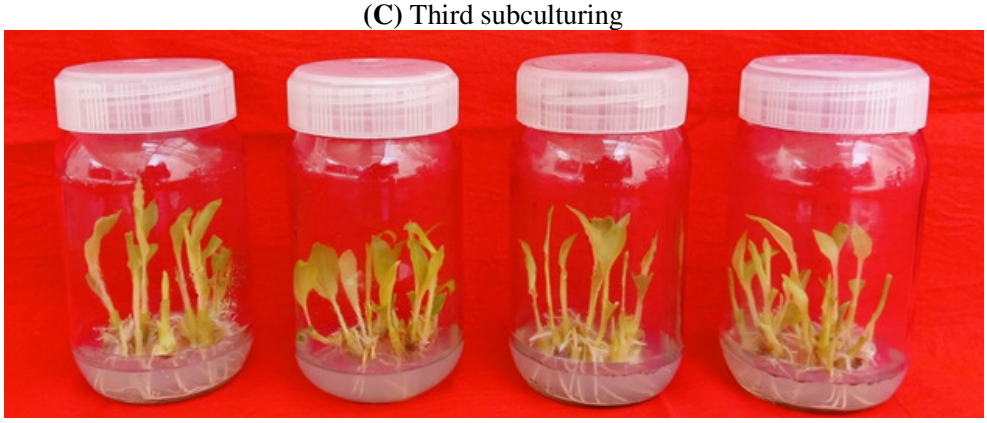
(A) First subculturing



(B) Second subculturing

Fig. 5: Effect of best treatment on shoot multiplication of turmeric (A) First sub culturing (B) Second subculturing

(C) Third subculturing



(D) Fourth subculturing

Fig. 6: Effect of best treatment on shoot multiplication of turmeric (C) Third sub culturing (D) Fourth sub culturing

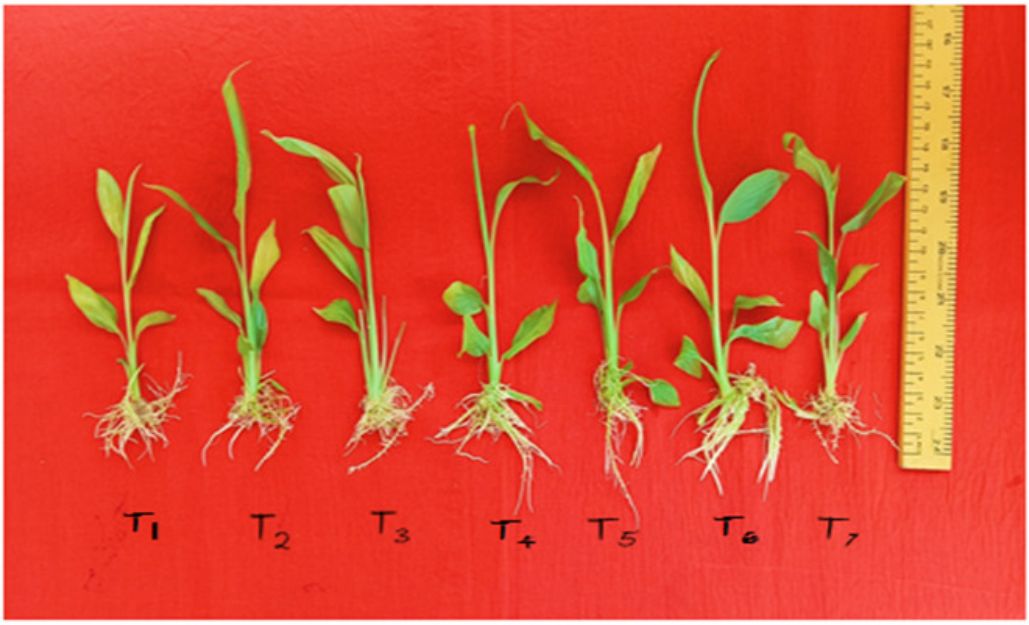


Fig. 7: Effect of growth regulators on *in vitro* root development

Fig. 8 : Plantlets transplanted in nursery bags filled with different media combination

Fig. 9 : *Ex-vitro* hardening of turmeric cv. Salem: Best treatment Soil: FYM: Cocopeat (1:1:1)

Fig.10: Effect of different potting mixtures on growth of plantlets

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Conflict of Interest

The authors declare no conflicts of interest. They bear sole responsibility for the content and composition of the paper.

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