

Original Research Article

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In vitro Studies in *Litchi chinensis* - Effect of Explant and Medium

Kumari Anjani* and Harsh Kumar

Department of Agricultural Biotechnology and Molecular Biology, Dr. Rajendra Prasad
Central Agricultural University, Pusa-848125, Bihar, India

*Corresponding author

ABSTRACT

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Litchi is a very important fruit of the Indian subcontinent and has a great demand in local and international market. But it is a very troublesome plant to cultivate and the current production rate cannot meet the growing demands of litchi. The *in vitro* techniques can be used to rapidly increase production. The present study was carried out to develop a rapid micropropagation protocol for litchi by studying the effect of medium and the explant used. The apical bud was found to be the most suitable explant among the four explants used. Out of the nine medium used for the study, MS 1, MS 2 MS 7 and MS 9 were the most effective medium for callogenesis in apical bud. The frequency of callogenesis was more on medium containing 2,4-D in higher concentration and low kinetin and also in medium with higher concentration of kinetin and low 2,4-D.

Introduction

Litchi, a native of China and Thailand, has been an important fruit in Southeastern Asia for over 2000 years. It is the second most important fruit crop after mango that is cultivated in most parts of the world (Das *et al.*, 2016). In India, it is cultivated in some part of Bihar, Himachal Pradesh, Uttar Pradesh and West Bengal. The litchi plant is a tree, belonging to the family Sapindaceae, which reaches a height of 35 to 40 feet and has a broad round topped crown and leathery shiny leaves. The fruits are very distinctive and their edible part is aril that covers the seed. Litchi fruit has deliciously flavored, sweet, juicy and pearly white flesh which is a rich source of vitamin C. The litchi fruit has strong antioxidant, anti-cancerous, anti-

diabetic, antimicrobial, anti-inflammatory activities along with free radical scavenging and hepatic protection properties (Naskar and Mazumder, 2015; Taak and Koul, 2016; Emanuele *et al.*, 2017). It is regarded as a functional fruit (Emanuele *et al.*, 2017). Litchi is also utilized as an excellent canned fruit. A highly flavored squash is prepared from inferior fruits which are liked by many during summer months.

The tree is also valuable for ornamental purposes apart from its fruits, various other products, such as pickles, jam, jelly, wine, candy, litchi nut etc. are also made from litchi (Goto, 1960). The peel extract of litchi is found to be a useful ingredient in anti-aging and skin lightening cosmetic products (Lourith *et al.*, 2017). The high demand and the high

prices paid for litchi has created new interest in the growing of this fruit with an increase in the demand for propagation materials.

Besides its economic importance, plantation of new litchi cultivars is very troublesome through conventional methods due to its long reproductive cycle and highly heterozygous genetic background. Litchi is basically a plant of tropics and is grown in areas where there is cool dry, frost free winter and long hot summer with high rainfall and humidity (Taak and Koul, 2016). Only 6 to 9 month old healthy plants with fine roots can be used for plantation. The plants are planted by making a small hole in the centre of the pit sufficient to the soil ball. The new plants are inoculated with mycorrhizal planting for good results of growth. The land should not be allowed to dry completely. Planting should be immediately followed by irrigation. In case of high wind plants are tied to the stake.

The most valued plants of litchi are those which produce ‘chicken tongue’ *i.e.* fruits with aborted seeds. But most of these plants are lost prior to harvest and cannot be propagated by seeds. For these reasons, *in vitro* propagation techniques can be used for the efficient propagation of the litchi plants.

Litchi is a problematic plant for tissue culture (Kantharajah *et al.*, 1989; Das *et al.*, 2016) but its tissue culture has many advantages like high and regular yield and which is free from showing biennial tendency; large fruit size and a large number of fruits per panicle; small seed size or higher aril percentage of aborted seeded plants; good fruit quality; resistance to physiological disorders and insect pests like fruit borers; wide adaptability to diverse ecological condition and desirable tree characteristics. Thus, this study was carried to standardize the protocol for micropropagation of litchi and develop a method of clonal propagation of this important plant.

Materials and Methods

Murashige and Skoog (1962) (MS) medium was used as basal medium during the study which was supplemented with different concentration and combination of phytohormones.

Nine different medium were used for the tissue culture of litchi. The composition of the medium used for the study is listed in Table 1.

Pre-sterilization of explants

The explants used for the study were leaves, nodes, internodes and apical bud. The explants were first cut (size about 1-2 cm) with the help of sharp knife.

These were first washed gently with 1% setavelon solution for 2-3 minute and then washed with running tap water to remove the detergent.

Pre-treatment of explants

One of the problems in the micropropagation of litchi is the secretion of polyphenols into the medium by the tissues. The polyphenols undergo oxidation to produce substances that kill the tissues (Kantharajah *et al.*, 1992). Another problem is that field grown litchi plant, used as a source explant, is heavily contaminated with organisms which are difficult to remove prior to culture.

Thus, before inoculation the explants are treated with pretreatment solution. The pretreatment solution contains free oxygen which makes the phenol inactive. The composition of pretreatment solution is as given in Table 2. After washing with tap water, the explants were transferred to sterilized beaker and then kept in pre-treatment solution for about 15 min. These explants were then washed with sterilized distilled water.

Surface sterilization of explants and inoculation

The pre-sterilized explants were treated with 0.1% mercuric chloride solution for 10 min in the laminar air flow hood for surface sterilization. The explants were then washed with sterilized distilled water three times to remove the excess mercuric chloride. The pre-treated and surface sterilized explants were inoculated in media containing culture tubes over the flame of spirit lamp. The inoculation was carried out in the laminar air flow.

The culture tubes were transferred to a thermal insulated temperature controlled tissue culture room with temperature around 25⁰C and relative humidity 60-80%. The continuous light of about 2000-4000 lux was provided through fluorescent tubes. Three types of response namely establishment, swelling and callogenesis were observed in the cultured tubes.

Results and Discussion

The four explants namely leaf, node, internode and apical bud of shoot were inoculated on nine different MS media with varying concentrations of phytohormones (2, 4-D and kinetin). The explants showed swelling after 6-7 days of inoculation while callus formation was visible only after 10-12 days. Some tubes also exhibited browning of the explants and media. The contamination mainly fungal was also present in some of the cultured tubes which can be attributed to mycorrhizal associations. The callus formation was seen mostly in the areas which were in direct contact of the media. The callus obtained in all the cultures was friable callus which were either creamish or greenish in colour.

Effect of medium on response of leaf

Most of the medium showed a 100% establishment in leaf. For frequency of

swelling of explant, the most responsive medium were MS 1 and MS 8, while no swelling was observed on MS 4 and MS 5. For callogenesis, only MS 2, MS 3, MS 8 and MS 9 were effective. MS 8 was found to be the most suitable medium for all responses in leaf (Table 3 and Fig. 1).

Effect of medium on response of node

The node showed establishment on all medium with the highest on MS 1, MS 2, MS 4, MS 8 and MS 9. The swelling of the explant was observed on all medium except MS 2, MS 4, MS 8 and MS9 with the highest on MS 1. All media were not very responsive for callogenesis and it was only observed on the media MS 1, MS 3, MS 5 and MS 6 (Table 4 and Fig. 2).

Effect of medium on response of internode

The internode was not a very responsive explant and did not show very significant response on any medium. However, establishment of internode was observed on medium MS 3, MS 4, MS 5, MS 7, MS 8 and MS 9 (Table 5 and Fig. 3).

Effect of medium on response of apical bud

The apical bud was very responsive on all medium and showed a 100% establishment on all medium except MS 5. The swelling of the explant was observed on all media except MS 5, MS 6 and MS 8. The highest frequency of swelling was observed on MS 2 and MS 3. The callogenesis was observed on MS 1, MS 2 MS 7 and MS 9. For all responses, MS 2 was the best medium for the explant apical bud (Table 6 and Fig. 4).

Cumulative effect of medium and explant

The highest establishment frequency was observed in apical bud (97.78%) and lowest in internode (62.22%).

Table.1 Various medium used for the investigation

MEDIUM NO.	CONCENTRATION OF KINETIN (KIN)(mg/L)	CONCENTRATION OF 2,4-D (mg/L)
MS 1	0	0
MS 2	2.0	0
MS 3	4.0	2.0
MS 4	0	2.0
MS 5	2.0	2.0
MS 6	4.0	2.0
MS 7	0	4
MS 8	2.0	4.0
MS 9	4.0	4.0

Table.2 Pretreatment solution of litchi

Ascorbic acid	5 gm
Citric acid	5 gm
Bavistin	1 gm
Streptomycin	1 gm
Distilled water	500 ml

Table.3 Effect of medium on response of leaf

Medium	ESTABLISHMENT FREQUENCY (%)	FREQUENCY OF SWELLING OF EXPLANT (%)	FREQUENCY OF CALLOGENESIS (%)
MS 1	100	100	–
MS 2	100	80	60
MS3	100	33	33
MS 4	100	–	–
MS 5	100	–	–
MS 6	75	25	–
MS 7	100	50	–
MS 8	100	100	100
MS 9	100	75	75

Table.4 Effect of medium on response of node

MEDIUM	ESTABLISHMENT FREQUENCY (%)	FREQUENCY OF SWELLING OF EXPLANT (%)	FREQUENCY OF CALLOGENESIS (%)
MS 1	100	100	66
MS 2	100	–	–
MS3	75	50	25
MS 4	100	–	–
MS 5	75	50	25
MS 6	83	50	50
MS 7	50	50	–
MS 8	100	–	–
MS 9	100	–	–

Table.5 Effect of medium on response of internode

MEDIUM	ESTABLISHMENT FREQUENCY (%)	FREQUENCY OF SWELLING OF EXPLANT (%)	FREQUENCY OF CALLOGENESIS (%)
MS 1	–	–	–
MS 2	–	–	–
MS3	100	–	–
MS 4	100	–	–
MS 5	100	–	–
MS 6	–	–	–
MS 7	60	–	–
MS 8	100	–	–
MS 9	100	–	–

Table.6 Effect of medium on response of apical bud

MEDIUM	ESTABLISHMENT FREQUENCY (%)	FREQUENCY OF SWELLING OF EXPLANT (%)	FREQUENCY OF CALLOGENESIS (%)
MS 1	100	50	50
MS 2	100	100	66
MS3	100	100	–
MS 4	100	20	–
MS 5	80	–	–
MS 6	100	–	–
MS 7	100	75	50
MS 8	100	–	–
MS 9	100	50	50

Table.7 Cumulative effect of medium and explant

EXPLANT	ESTABLISHMENT FREQUENCY (%)	FREQUENCY OF SWELLING OF EXPLANT (%)	FREQUENCY OF CALLOGENESIS (%)
LEAF	97.22	51.44	18.67
NODE	87	33.33	18.44
INTERNODE	62.22	0	0
APICAL BUD	97.78	43.89	24

Fig.1 Effect of medium on response of leaf

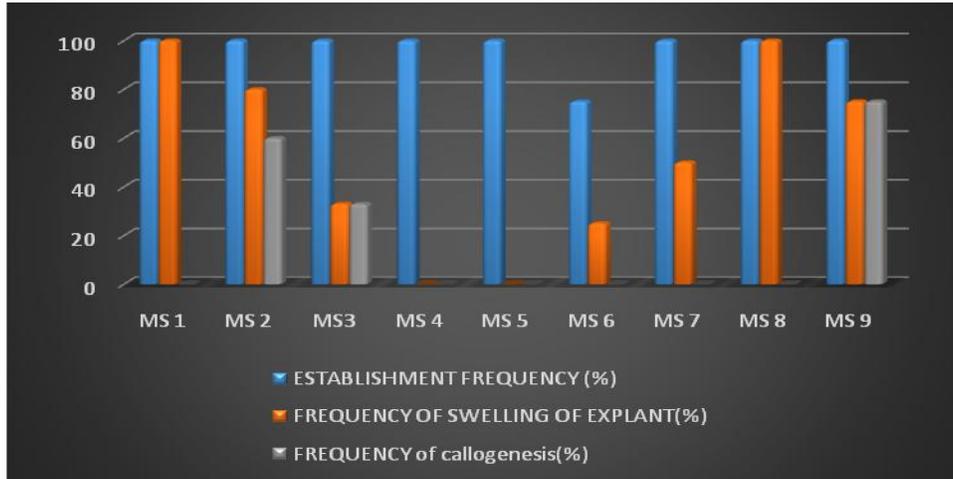


Fig.2 Effect of medium on response of node

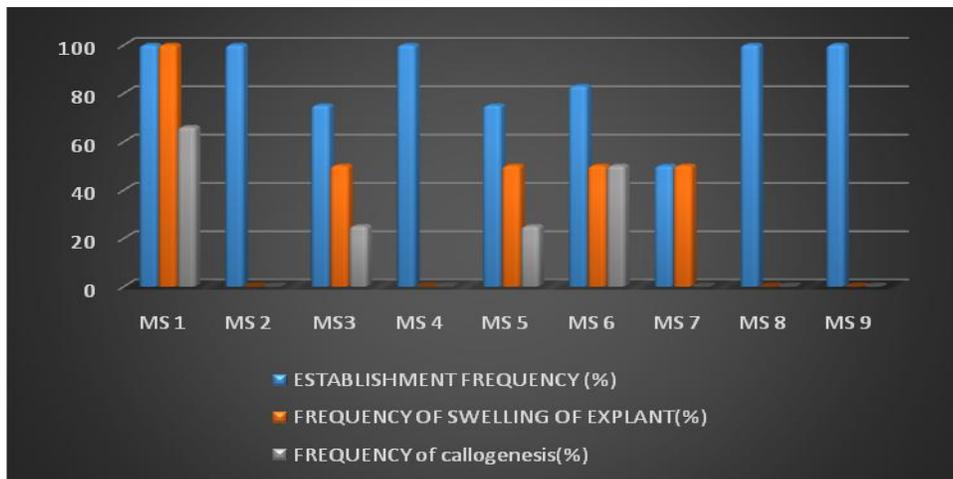


Fig.3 Effect of medium on response of internode

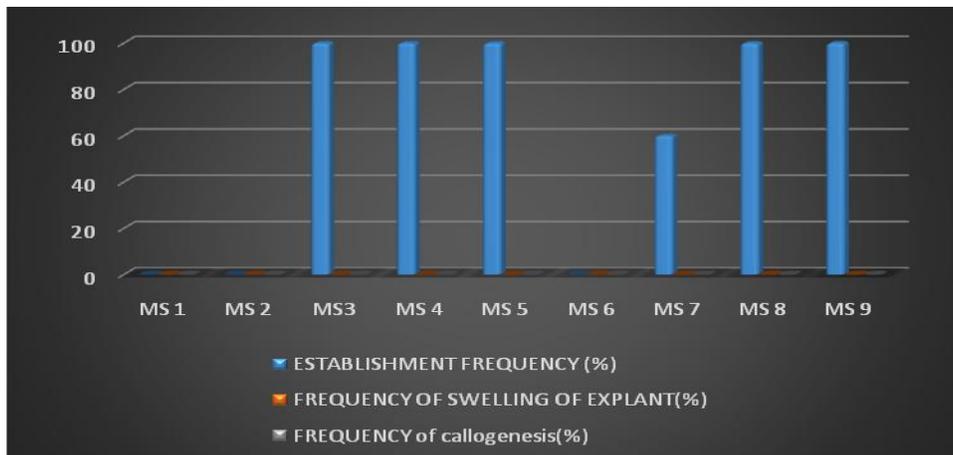


Fig.4 Effect of medium on response of apical bud

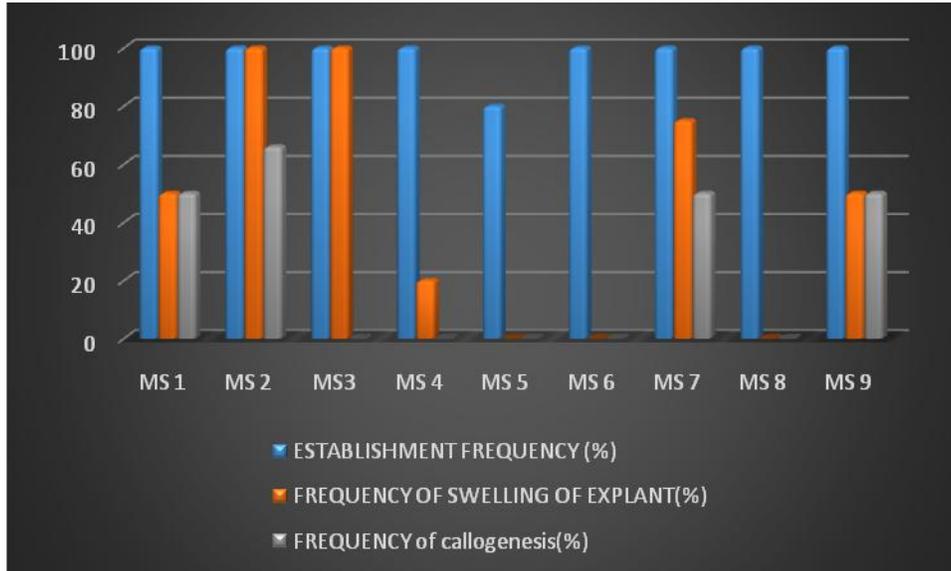
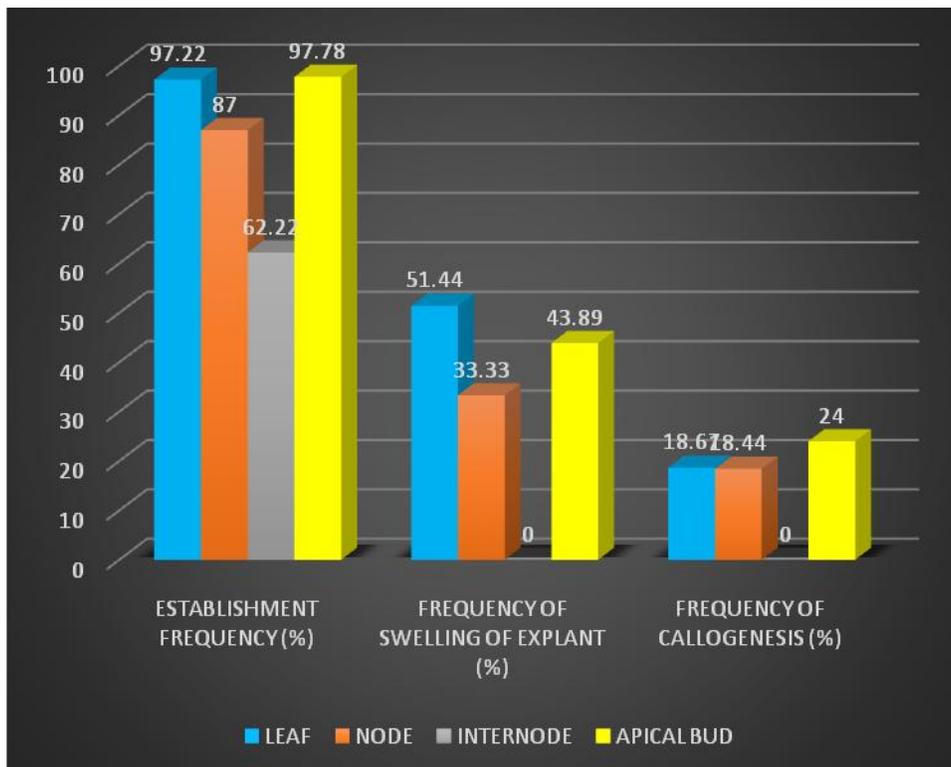


Fig.5 Cumulative effect of medium and explant



While the node as explant showed highest frequency of swelling of explant (51.44%), internode did not showed this response. The frequency of callogenesis was the least

observed response in all the explants with highest frequency being 24% in apical bud. The internode did not show this response also (Table 7 and Fig. 5).

Litchi is believed to be a problematic plant for tissue culture and this was indicated by the results of the present study also. However, contradictory to previous findings certain significant results were observed in this study. Frequency of establishment of cultures did not show any regular pattern. However, the highest establishment frequency was observed in apical bud (97.78%) and lowest in internode (62.22%). This can be explained on the fact that establishment of culture is defined only on the basis of presence or absence of infection, the healthy state of explants *i.e.*, whether it has died or exhibited browning. The component of the media has no effect on these aspects and thus do not affect frequency which was similar with the finding of earlier workers (Yu, 1991; Zhou *et al.*, 1996; Das *et al.*, 1999).

The node as explant showed highest frequency of swelling of explant (51.44%) but the internode did not showed this response. For the leaf, the most responsive medium were MS 1 and MS 8, while no swelling was observed on MS 4 and MS 5. The swelling of the node explant was observed on all medium except MS 2, MS 4, MS 8 and MS9 with the highest on MS 1. The internode was not a very responsive explant and did not show very significant response on any medium. For apical bud, the swelling of the explant was observed on all media except MS 5, MS 6 and MS 8. The highest frequency of swelling was observed on MS 2 and MS 3. Swelling was more in medium containing higher concentration of kinetin and less concentration of 2,4-D than the medium containing more amount of 2,4-D and less concentration of kinetin. Because kinetin being a cytokinin promotes cell division, it causes more swelling than 2,4-D. Swelling was more in tubes with leaves and apical bud as explants perhaps because of the presence of meristematic tissue. Similar results were found by Das *et al.* (1999) who showed

multiple shoot induction from cotyledonary node following exposure to 88-8 M benzyl adenine in liquid Murashige and Skoog medium but Kantharajah *et al.*, (1992) found that the shoot tip was the most responsive explant.

The frequency of callogenesis was the least observed response in all the explants with highest frequency being 24% in apical bud. The internode did not showed this response. For callogenesis in leaf, only MS 2, MS 3, MS 8 and MS 9 were found to be effective. All media were not very responsive for callogenesis in node and this response was only observed on the media MS 1, MS 3, MS 5 and MS 6. For apical bud, the callogenesis was observed on MS 1, MS 2 MS 7 and MS 9. The frequency of callogenesis was more on medium containing 2,4-D in higher concentration and low kinetin and also in medium with higher concentration of kinetin and low 2,4-D. This can be due to the fact that perhaps comparable concentration of each hormone is inhibiting each other effect. The node and apical bud cultures showed best response for callus induction perhaps due to the presence of meristematic tissues and auxins.

The observations are quite contradictory to the earlier works in which results were obtained on medium with higher concentration of auxin but not in cytokinins (Kantharajah *et al.*, 1992 and Das *et al.*, 1999). The results of this experiment also suggest that the hormones do not play a very significant effect on the responses. However, Wang *et al.*, 2016 found that hormones play a very significant role in the induction of embryogenic callus from anther explants. They observed that a certain auxin-cytokinin ratios in medium is necessary for callus induction from anther culture of litchi and 2,4-D was the effective hormone in embryogenic callus induction. The

embryogenic callus and haploid cultures were also obtained by Fu and Tang (1983), Fu (1990), Zhou *et al.*, (1996), Yu and Chen (1998), Yu *et al.*, (2000) and Das *et al.*, (2016).

The present study was carried out to develop an efficient micropropagation protocol for litchi. Based on the findings of the experiments, it was found that all medium equally responded for the establishment of cultures. The level of contamination was highest in MS-7 mainly due to mycological associations. Among the explants, leaves showed maximum tendency of establishment followed by apical bud and nodes. Internodes exhibited browning. The medium MS-1 was best for swelling followed by MS-2 and MS-3. MS-7, MS-9 showed equal effect on swelling. The medium MS-4, MS-5 and MS-6 was not very responsive for swelling.

The leaves and apical bud showed maximum swelling followed by nodes. No swelling was observed in internodes even after 20 days of inoculation. The medium with higher concentration of kinetin were best for callogenesis. Medium MS-1, MS-2 and MS-3 showed very good callus formation. The callus formation was also seen on MS-5, MS-6 and MS-9. No callus was obtained on MS-4 and MS-7. The nodes showed best response for callus formation followed by apical bud and leaves. The callus formed in leaves is greenish while that of nodes and apical bud are creamish. The apical bud was found to be the most suitable explant for all responses.

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Author contributions

All author have equally contributed to this article and its publication is approved by all authors.

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