

Available Online through
www.ijptonline.com

IN VITRO PLANT REGENERATION USING SHOOT TIP CULTURE IN COMMERCIAL CULTIVAR OF TEAK

M.Guru Prasad*¹, D.Sandeep Raja , Shanthi Sri K.V. , M.Srinu Naik, SK.Jaffar²

Dept of Biochemistry, Acharya Nagarjuna University, Guntur-522 510, A.P, India.

Dept of Biochemistry, Acharya Nagarjuna University, Guntur-522 510,A.P, India.

Dept of Food and Nutritional Sciences, Acharya Nagarjuna University, Guntur-522 510, A.P, India.

Dept of Biochemistry, Acharya Nagarjuna University, Guntur-522 510, A.P, India.

Dept of Geology, Acharya Nagarjuna University, Guntur-522 510, A.P, India.

Email: Guru_biotech@rediffmail.com

Received on 14-04-2012

Accepted on 30-04-2012

Abstract

A protocol for direct shoot regeneration without intervening callus phase was developed by shoot tip culture of MS (murashige and skoog) medium for teak. The study reveal that aseptically inoculated shoot tip of teak in established shoot induction and shoot multiplication was also observed at same concentration .At BAP 1mg/l, IAA 1mg/l and kin 1mg/l the shoots were weak ,tiny and non separable ,whereas BAP 3mg/l, IAA 2mg/l and kin 2mg/l concentration the rate of multiplication is high ,well grown ,easily separable and healthy plantlets .The root induction was observed NAA 2mg/l and complete plantlets were hardened and transferred to green house for established with a survival rate of 72 percent .

Key words: shoot tip culture, teak, shoot induction, root induction.

Introduction

Teak is the most important in the world. Tissue culture of teak has received considerable research attention because of it economic importance as a cash crop. Plant regeneration to tissue culture technique would be a viable alternative for improving the quality and production of teak. Therefore, the present investigation has been under taken to establish plant regeneration protocol through shoot tip culture in teak. The present communication thus deal with the studies of direct shoot regeneration from shoottip, multiple shoots and root induction and then establishment of plants in green house.

Materials and Methods

The plants were raised and maintained under field conditions as per the recommended agronomic practices. These plants served as the source of explants for the in vitro studies (Orlikowska Kidder WE.1993). Shoot tips were collected from 5-6 months well established plants stem segment of about 3-4cm together with meristem tip, were excised in a laminar air flow chamber. They were sterile for 30 minutes with fungicide (carbendazion 1%) and bactericide (streptomycin 1%) and then with 80% ethanol for 45 seconds. The segments were then washed 3 times with double distilled water under sterile conditions and then shoot tip was dissected.

Media used for shoot tip culture establishment multiplication of shoots and root induction was containing the ingredients as furnished in table -1 and 2. A half strength medium (Murashige T and Skoog F., 1962) of salts was also used mainly for growth of plantlets.

Micro propagation was conducted in a well defined conditions of the culture room and maintained at $25 \pm 2^{\circ}\text{C}$, uniform light (ca 1000 lux) provide by fluorescent tubes (7200⁰ k) over a light / dark cycles of 16/8 hours. The resulting plantlets were contacted and transplanted into polystyrene well containing peat soil and plants were transferred to green house after 20 days.

Results and Discussions

Shoot tip containing apical meristem of teak were inoculated with different concentrations of BAP for shoot tip initiations and establishment. Small shoots started appearing within 7-10 days in many of the culture tubes. Initially they was problem of tissue turning browning due to release of phenols from the base of shoot tip, which slightly hinder the shoot growth. This phenol released was (Chandra A and Pental D et al., 2003) controlled by 1% citric acid and 1% poly vinyl pyrrolidine. The percent establishment varied from different levels of growth hormones like BAP, IAA and KIN. But with BAP 3mg/l, IAA 2mg/l and Kin 2mg/l has been standardized.

At this level of BAP at the end of 25th day, 10-15 shoots originated from a single shoot tip meristem (Lane WD et al., 1979). The rate of multiplication is high in adventitious shoots and somatic embryogenesis but the occurrence of monoclonal variation is a draw back. To overcome buds through meristem /shoot tip culture. The

multiplication rate of 5-10 percent within 25 days is sufficient to produce large number of shoots economically in most species.

Table-1: Effect of growth regulator against shooting.

s.no	Growth regulator			No of explants	No of shoots	Percentage of shoots
	BAP	IAA	KIN			
1	1	0.5	0.5	8	2-3	30
2	2	1.0	1.0	8	3-4	40
3	3	2.0	2.0	8	5-8	80
4	4	3.0	3.0	8	6-7	70
5	5	4.0	4.0	8	3-4	40
6	6	5.0	5.0	8	2-3	20
7	7	6.0	6.0	8	-	-

Table- 2: Effect of NAA against rooting.

s.no	Growth regulator NAA	No of days	Root frequency
1	0.1	20	-
2	0.5	20	40
3	1.0	20	50
4	1.5	20	60
5	2.0	20	80
6	2.5	20	50
7	3.0	20	CALLUS

Among the other treatments, BAP 3mg/l, IAA2mg/l and Kin 2mg/l recorded (T.A.Thorpe et al., 1990) better overall rate of multiplication (%) and another with strong shoot . At this rate of multiplication one can produce enormous number (2x10⁸) of plants from a single shoot tip in a year over a 4-5 weeks micro propagation cycles.

Rooting can be achieved (Jaiwal P K and Gulati et al., 1995) either by transferring the shoots to medium lacking cytokines with or without a rooting hormone .Rooting can be improved by lowering the concentration of macro salts to a half or less. Auxin is essential for root induction in many species. Hardening of plantlet is an important step in tissue culture studies. They used potting mixture of vermiculate ,coconut peat and sandy soil in 1:1:1 ratio proportion .The plantlets with good root system were taken and transplanted into potting mixture by trimming the leaves .The survival rate of micro propagated plants(M.L.Christianson and D.A.Warnick .,1983) was 72% .This is due to the variation (Somers D et al .,2003) in the method of propagation and environmental factors because temperature and humidity are the two key factors ,which controls survival rate in poly house.

Conclusions

Teak is most important cultivar of India. It is useful for maximum production of wood. By using conventional methods we can not regenerate the plants, the above protocol gives better regeneration and yield good quality of products. Regeneration of plant from shoot tip is easy and convenient method to give maximum yield.

References

1. Jaiwal P K and Gulati A, Current status and future strategies of in vitro techniques for genetic improvement of mungbean (*Vigna radiata*).*Euphtica*, 86 (1995) 167-181.
2. Somers D A, Samac D A and Olhoft M. Recent advances in legume transformation. *Plant phsiol*, 131(2003)892-899.
3. Chandra A and Pental D. Regeneration and genetic transformation of grain legumes: An overview.*Curr sci*,84(2003)381-387.
4. Lane WD (1979) .In vitr propagation of spirea bunalda and prunus cistena from shoot apices.*Can.j.Plant.sci*.59, 1025-1029.
5. Murashinge T and Skoog F (1962) a revised medium for rapid growth and bioassay with tobacco tissue cultures.*Phsiol.plant*.15, 473-497.
6. T.A.Thorpe, Organogenesis in vitro: structural, physiological and biochemical aspects .*int. Rev. ctol.* , 11A (1980)71-111.
7. M.L.Christianson and D.A.Warnick, Competence and determination in the process of in vitro shoot organogenesis .*Dev.Biol.* 95(1983)288-293.
8. Orlikowska Kidder WE.1993.In vitro regeneration and multiplication of safflower (Chatham's tinctorius L.) *Plant science* 93:151-157
9. Halamkova, E., Vagera, J., Ohnoutkova,L.:Regeneration capacity of calli derived from immature embryos in spring barley cultivars-*Biol.Plant*.48:313-316,2004

Corresponding Author:

M. Guru Prasad*¹

Email: Guru_biotech@rediffmail.com