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In vitro Multiplication of *Terminalia Bellerica* (Gaertn.) Roxb.

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Abstract: ***The present investigation aimed to develop standard protocol for in vitro propagation of Terminalia Bellerica (Gaertn.) Roxb. It is one of the important medicinal plants belongs to family combretaceae. Node segment explants were tried on MS medium along with various concentration of growth hormone. The regeneration of multiple shooting was achieved on MS medium along with 0.5 mg/L of IAA in combination of various concentrations of BAP and KIN. The maximum percentage of regeneration and maximum length of shoot were recorded on 0.5 mg/L of IAA in combination of 4.0 mg/L of BAP and KIN using nodal segment explant however 4.5 mg/L using nodal segment explant respectively.***

Keywords: *in vitro*, *Terminalia Bellerica*, IAA, BAP

1. Introduction

Terminalia bellirica Roxb. belongs to family Combretaceae is the secondary host plant of the tasar silkworm, Antheraea myllita Drury. It is a tall deciduous tree found throughout large parts of India except in arid regions. It also occurs in Nepal, Sri Lanka, Southeast Asia, and Malaysia. Apart from serving as food for silkworms, this plant has several pharmaceutical values. Its fruit is a laxative and is used as a major constituent of 'triphala', a traditional ayurvedic herbal formulation consisting of the dried fruits of Terminalia chebula, *Terminalia* bellirica, and Phyllanthus emblica. In addition, the fruit rind pericarp of *Terminalia bellirica* constitutes the ayurvedic drug 'vibhitaki' (Ramesh M. *et al*., 2005).

Nature serves as primary source for the cure of ailments. It is estimated that, in many developing countries, two third of the population is dependent on medicinal plants to meet primary healthcare needs. The use of herbal medicine is increasing due to its safety, efficacy and therapeutic potential as compared to synthetic pharmaceutical products. However, the potential of higher plants as a source of herbal medicine is unexplored. Therefore, some species to update the current state of knowledge and one such plant is *Terminalia bellerica* (Kumari *et al*., 2017).

The commercial exploitation of *Terminalia* by various industries along with losses due to indiscriminate felling of trees, conversion of forest land into agriculture land and human settlements have all led to rapid depletion of genetic resources of *Terminalia. bellerica*. There is an increasing need for the preservation of these plants through systematic cultivation for germplasm conservation, selection of desired genotypes and mass propagation of superior clones. Conventional methods of *Terminalia bellerica* propagation are insufficient because of poor seed germination and low survival rates of stem cuttings. Therefore, alternative methods for propagation of selected trees are needed. Tissue culture has proved to be a promising technique for conservation and large - scale multiplication of several woody species. Earlier reports on micropropagation of *Terminalia bellerica* used juvenile cotyledonary node as explant (Dangi *et al*., 2014).

The protocol by taking explants from 10 - year - old plants. In spite of several protocols for regeneration being reported in *Terminaliabellerica* the regeneration efficiency has been shown to be compromised by phenolic exudation, basal callusing, vitrification and shoot tip necrosis. Also, clonal fidelity of regenerated plants is a major consideration in commercial micropropagation of trees. Plant cell culture results in high frequency of variation in regenerated plants. Owing to this variation, the resulting plant may not possess the same properties as that of the parent plant (Phulwaria *et al*., 2012)

Description and distribution of plant:
*Terminaliabellerica*is grows wild at an elevation of up to 2000m in wide variety of ecologies. It is native to Sri Lanka, India, Bangladesh, Bhutan, Thailand, China, Indonesia, Pakistan, Malaysia, Nepal, Cambodia and Vietnam. In India, it is commonly found in Madhya Pradesh, Uttar Pradesh, Punjab and Maharashtra. Ecologically It is mostly found in monsoon forests, mixed deciduous forests or dry deciduous dipterocarp forests, associated with teak. It flowers in the month of October - November and fruits in November - December. The tree sheds leave in November with young ones appearing together with flowers.

Medicinal and chemical properties:
Terminaliabellerica plant is growing widely throughout the Indian subcontinent, Sri Lanka and SouthEast Asia. In the Traditional system of medicine like Ayurveda, Siddha and Unani, its medicinal uses have been described in several diseases and the action in almost all system. Glucoside, Tannins, Gallic acid, Ellagic acid, Ethylgalate, Gallylglucose, Chebulanic acid is mainly believed to be responsible for its various therapeutic actions. It is used as antioxidant, antimicrobial, anti - diarrheal, anticancer, anti - diabetic, antihypertensive, hepatic protective and immune stimulatory agent. The gums of the plant have been used for the formulation of microencapsulated drug delivery system (Das *et al*., 2014).

2. Materials and Methods

Preparation of explant and sterilization:
The explant like nodal segment and mature fruits were collected from young healthy plantlets of *Terminaliabellerica*, from campus of Dr. Babasaheb Ambedkar Marathwada University, Aurangabad. All these explants were washed with running tap water for 5 minutes, followed by 70% ethanol for 1 minute and finally with distilled water for 3 minutes. Surface sterilization of explant was carried out by washing with sterile distilled water for 3 minutes followed by various concentration of mercuric chloride ($Hgcl_2$), nodal segment with 0.3% of $Hgcl_2$. It was followed by two subsequent rinses with sterilized double distill water in laminar airflow. These explants were cuts in to small pieces and inoculated on suitable MS media.

Culture media and Culture condition:
All experiments of investigation were tried on MS media (Murashige and Skoog, 1962) supplemented with various concentration of growth regulators. MS medium was fortified with 3 % sucrose and clerigar for solidification respectively and pH was adjusted to 5.6 - 5.8. The media were steam sterilized in an autoclave under 15 psi and 121°C. After the inoculation culture bottles were transfers to culture room under a 16 h photoperiod supplied by cool white fluorescent cool tubes light and temperature at 25± 2°C. At least 5 replicates raised for each treatment and data were recorded in table.

3. Results and Discussion

Standard protocol for surface sterilization of explant was analyzed by trial - and - error method. Surface sterilization of nodal segment explant were tried with 0.1 - 0.3% of $HgCl_2$ for 3 - and 5 - minutes duration. The maximum microbe's free cultures and high regeneration percentage were recorded at 0.3% $HgCl_2$ for nodal segment explant during the present study. Shoot regeneration was achieved from nodal segment explant from BAP and KIN in combination of 0.5 mg/L of IAA. Higher concentration of KIN was found effective to induced shoot regeneration. The maximum shoot induction percentage along with shoot length was recorded from 0.5 mg/L of IAA in combination of 4.0 mg/L of BAP and 4.5 mg/L of KIN shoot length using nodal segment explant respectively.

Photo pleat:

IAA 0.5 mg/L+BAP 4.0 mg/L

IAA 0.5 mg/L+KIN 4.5 mg/L

Effect of various concentrations of auxins (IAA) in combination with cytokinins (BAP and KIN) for multiple shoot formation from nodal segment as explant.

Explant	Concentration of growth regulators mg/l			No. of shoots/ Explant
	IAA	BAP	KIN	
IAA 0.5 mg/L+ BAP 4.0 mg/L	0.5	0.5	- -	7.54±0.120
	0.5	1.0	- -	7.94±0.143
	0.5	1.5	- -	8.02±0.146
	0.5	2.0	- -	8.00±0.044
	0.5	2.5	- -	7.76±0.067
	0.5	3.0	- -	7.82±0.037
	0.5	3.5	- -	8.12±0.086
	0.5	**4.0**	**- -**	**8.84±0.213**
	0.5	4.5	- -	8.08±0.058
	0.5	5.0	- -	7.78±0.058
Nodal segment	0.5	- -	0.5	7.12±0.052

	0.5	- -	1.0	7.18±0.058
	0.5	- -	1.5	7.48±0.106
	0.5	- -	2.0	7.66±0.050
	0.5	- -	2.5	7.66±0.927
	0.5	- -	3.0	8.16±0.050
	0.5	- -	3.5	8.42±0.185
	0.5	- -	4.0	8.94±0.169
	0.5	**- -**	**4.5**	**9.16±0.172**
	0.5	- -	5.0	8.51±0.104

Values represent the mean ± SE and percentage response on three separate experiments, each based on a minimum of five replicates.

4. Conclusion

Terminalia bellerica is an important medicinal plant with diverse pharmacological spectrum. Terminalia bellerica is widely used in Ayurveda, Siddha, Chinese medicine etc. The vast study done on the plant proved that the plant has many important phyto constituents like Gallo - tannic acid, bellericanin, ellagic acid, gallic acid, termilignan, thanni lignan, flavone and anolignan B, Tannins, ellargic acid, ethyl gallate, galloyl glucose and chebulaginic acid, phenyllemblin, β - sitosterol, mannitol, glucose, fructose and rhamnose. These compounds were found to be responsible for many of the pharmacological activities such as antimicrobial, antioxidant, antidiarrhoeal, antidiabetic, analgesic, immunomodulatory, antihypertensive, antisolmonella, hepatoprotective, antispasmodic and bronchodilatory activities. Further the plant is used in the treatment of gastric ulcer, constipation, general debility, piles. Hence, this plant provides a significant role in the prevention and treatment of a disease. Further evaluation needs to be carried out in order to explore the concealed areas and their practical clinical applications, which can be used for the welfare of the mankind.

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