IJPSR (2018), Volume 9, Issue 3 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 20 June, 2017; received in revised form, 15 December, 2017; accepted, 06 January, 2018; published 01 March, 2018

# *IN VITRO* REGENERATION OF MEDICINAL PLANT *EPHEDRA GERARDIANA*

Indra Rautela [1, 2, 3], Manjul Dhiman [2], Manish Dev Sharma [4] and Pragati Misra [*1]

Department of Molecular and Cellular Engineering [1], Jacob Institute of Biotechnology and Bioengineering, Sam Higginbottom University of Agriculture, Technology and Sciences, Allahabad - 211007, Uttar Pradesh, India.
Department of Botany [2], K. L. DAV (P.G.) College, Roorkee - 247667, Uttarakhand, India.
Department of Biotechnology [3], Uttaranchal College of Applied and Life Sciences, Uttaranchal University, Dehradun - 248001, Uttarakhand, India.
Department of Life Sciences [4], Shri Guru Ram Rai Institute of Technology and Science, Patel Nagar, Dehradun - 248001, Uttarakhand, India.

**Keywords:**

*Ephedra gerardiana*, IBA, BAP, Kn

**Correspondence to Author:**
**Pragati Misra**

Department of Molecular and Cellular Engineering, Jacob Institute of Biotechnology and Bioengineering, Sam Higginbottom University of Agriculture, Technology and Sciences, Allahabad - 211007, Uttar Pradesh, India.

**E-mail:** pragatimisra3@rediffmail.com

**ABSTRACT:** *Ephedra gerardiana* is an important medicinal plant used as Somalata in Indian Ayurvedic system of medicine and as traditional Chinese medicine since several thousand years. Plant is known to contain valuable secondary metabolite such as ephedrine and pseudo ephedrine. These secondary metabolites are utilized for several medicinal purposes. The present study describes the *in vitro* regeneration of *Ephedra gerardiana* using tissue culture technique. Experiments were conducted to study the effect of various combinations of auxins (IBA) and cytokinins (BAP) alone or in different combinations on *in vitro* culture of nodal segments. Shoot induction and elongation from cultured nodal segments were obtained onto MS medium supplemented with various concentrations of BAP and 15 µM Kinetin. MS+IBA media was utilized for *in vitro* root induction. Among different media combinations utilized MS+ 5 µM BAP+ 15 µM kinetin was found best for shooting and ¼ MS+ 20 µM IBA was most appropriate for rooting.

**INTRODUCTION:** Gymnosperms, comprising mostly evergreen trees and shrubs, constitute a highly fascinating group of plants. The elegant habit and wide range of shapes of the conifers bestow on them year round appeal. The timber and other forest products obtained from them enhance their economic value considerably. This very factor has led to the continuous and excessive exploitation of natural stands of gymnosperm forests.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.9(3).1183-88

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.9(3).1183-88

*Ephedra* belongs to class Gnetopsida having three different genera; *Ephedra*, *Gnetum* and *Welwitschia* grouped in monotypic orders and family *viz.* Ephedrales, Gnetales and Welwitschiales respectively [1]. *Ephedra* may be treated as closest relative of the angiosperms however; molecular studies suggest that this is not the ancestor of angiosperms [2, 3, 4, 5, 6].

In India, *Ephedra* is represented by nine species [7] namely *E. foliata*, *E. gerardiana*, *E. intermedia*, *E. nebrodensis*, *E. regeliana*, *E. saxatilis*, *E. pachyclada* and *E. przewalskii*. *Ephedra gerardiana* is found in drier regions of temperate and alpine Himalaya from Kashmir to Sikkim, Chamba, Lahul, Spiti and Ladakh.

This was reported from present day Uttarakhand from dry southern exposure of Deoban Ridge Jaunsar (8000 to 9500 feet) and in Gidikhad below Karamba peak *etc*. It is also found to occur in temperate and alpine Himalayas from Kashmir to Sikkim in Pangi, Lahul, Spiti, Chini and Kilba-Kailash ranges of Kanawar, Shali hills (North of Shimla) Kashmir and Ladakh. The major active ingredients of *Ephedra* are alkaloids that constitute 0.5 to 2.5% of total mass, and are referred to as ephedrine type alkaloids. The six optically active alkaloids that have been isolated from *Ephedra* species are (-) - ephedrine, (+)-psuedoephedrine, (-) -*N*- methylephedrine, (+) - *N*- methylpseudo-ephedrine, (-) - norephedrine, (+) – norpsuedo-ephedrine. Ephedrine is the major isomer comprising of 30 to 90% of total alkaloid fraction accompanied by psuedoephedrine, with trace amount of other ephedrine type alkaloids [8].

Ephedra has been used in China for the treatment of cold, typhoid, bronchial asthma, flu edema and various pains from many years. It has also been used as many dietary supplements, weight loss, energy increment and improved athletics performance [9]. Due to a heavy demand, consumption and over-exploitation of Ephedra for medicinal values the demand of Ephedra plant increasing day-by- day so few of the species of this plant are becoming endangered [10]. For future prospect plant tissue culture techniques was found to be one of the best methods in order to fulfill the required demand of this medicinal plant.

## MATERIAL AND METHODS:

**Plant Material:** Plants materials of *Ephedra gerardiana* were collected from natural habitat of Chakrata forest division in the Uttarakhand state and were grown in pots in Botanical Garden of the K.L.DAV (PG) College, Roorkee, Uttarakhand.

**Establishment and *in vitro* Shoot Initiation:** Physiologically fresh nodal segment is generally much more responsive *in vitro*, therefore fresh nodal segment about 1.0 - 2.0 cm long was used. Initially the explant were washed thoroughly with tap water followed by a wash with 1% (v/v) Labolene detergent for 15 minutes and then in running tap water for 30 minutes. Nodal segment were surface sterilized with 70 - 90% ethyl alcohol for 40 seconds, followed by 0.1% (w/v) $HgCl_2$ for 3 minutes. The explants were then rinsed several times with sterile double distilled water to remove all traces of $HgCl_2$ and dried using sterile filter paper discs. The sterilized explants were inoculated on the MS medium fortified with different concentration and combination of cytokinins (BAP & Kn). All the cultures were incubated at 24º ± 2ºC with 16:8 hrs light: dark photoperiod controlled by clock timer and 60% relative humidity.

**Shoot Multiplication:** The shoots obtained from nodal segment were transferred in multiplication medium fortified with cytokinin BA and Kn. The multiple shoots thus excised were placed in rooting media.

**Root Induction:** *In vitro* regenerated shoots were transferred to rooting medium supplemented with MS medium different concentration and varying range of auxin(s) Indole Butyric acid (IBA). The rooted plantlets thus obtained were successfully transferred to the pots containing 1:1:1 ratio of sand, soil and compost for hardening.

**RESULT AND DISCUSSION:** When nodal segment obtained from mother plant were cultured on to MS medium supplemented with different concentration of BAP and Kn. It was found that MS medium supplemented with various concentration of BAP with 15 µM Kn was the most appropriate medium combination for induction and multiplication of *in vitro* shoot.

All culture (100%) exhibited *in vitro* shoot induction on to MS+BAP (2-15 µM) +Kn (15 µM). When nodal segments were cultured on to MS+2 µM BAP+15 µM Kn an average of 2.50 ± 0.03 shoot per culture were obtained with a maximum of 3 shoot. On increasing the concentration of BAP from 2 µM to 5 µM average number of shoot was increased to 6.00 ± 1.7 per culture with a maximum of 15 shoots.

On further increasing the concentration of BAP decrease in average as well a maximum number of shoots produced was observed. Onto MS+8 µM BAP+15 µM Kn average number of shoots was 2.50 ± 1.22 with a maximum of shoots, the average number of shoots obtained further decreased to 2.30 ± 0.91 when concentration of BAP was increased to 10 µM keeping concentration of kinetin same. There was marginal increase in

average number of shoots when concentration of BAP was increased from 10 µM to 15 µM. Onto MS+15 µM BAP+15 µM Kn average number of shoot was found to be 2.87 ± 1.6 with a maximum of 6 shoots **Table 1, Fig. 1, 3**.

Effectiveness of MS medium supplemented with BAP or Kn in regeneration of shoots from cultured nodal segment of *E. gerardiana* has also been reported in earlier study [11]. However in the study the regeneration from nodal segment have been reported when medium was supplemented with Kn or BAP alone whereas in the present study multiple shooting was achieved when both the hormones were utilized in combination.

**TABLE 1: EFFECT OF VARIOUS CONCENTRATIONS OF BAP WITH 15 µM KINETIN ONTO NODAL SEGMENT CULTURE OF *EPHEDRA GERARDIANA***

| BAP conc. (µM) | % of culture showing shoot buds | Average no. of shoot | Max. no. of shoot |
|---|---|---|---|
| 2 | 100 | 2.50 ± 0.63$^{e}$ | 3$^{e}$ |
| 5 | 100 | 6.00 ± 1.07$^{a}$ | 15$^{a}$ |
| 8 | 100 | 2.50 ± 1.22$^{bc}$ | 7$^{b}$ |
| 10 | 100 | 2.30 ± 0.91$^{d}$ | 5$^{d}$ |
| 15 | 100 | 2.87 ± 1.16$^{b}$ | 6$^{c,d}$ |

Values are mean of three replicates. Mean values followed by same letters are not significantly different at p ≥ 0.05 DMRT

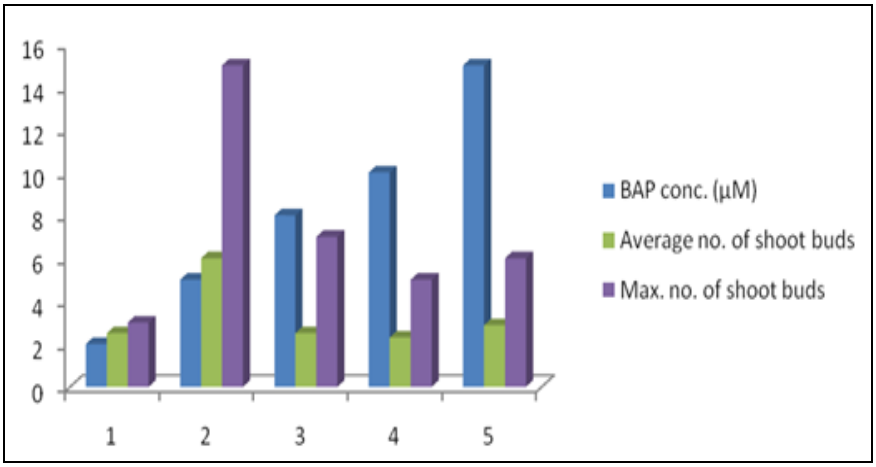


**FIG. 1: VARIOUS CONCENTRATIONS OF BAP WITH 15 µM KINETIN ONTO NODAL SEGMENT CULTURE OF *EPHEDRA GERARDIANA***

The regenerated shoots were aseptically excised and transfer on to basal MS medium for further elongation **Fig. 4**. Well elongated shoots were transferred onto rooting medium for induction of *in vitro* roots. Different strength of MS medium (full, 1/2, 1/4) fortified with different when regenerated shoots were cultured onto full strength MS medium with varying concentration of IBA none the culture exhibited root induction.

When the strength of medium was reduced to half induction of *in vitro* rooting was obtained with 8.54%, 17.66% and 24.0% cultures exhibiting root induction onto media ½ MS+10 µM IBA, ½ MS +15 µM IBA and ½ MS+20 µM IBA respectively. Although induction of root was observed on to half strength MS medium (with different concentration of IBA) but no elongation and growth from induced roots was obtained. Onto ½ MS medium the cultured shoots exhibited swelling towards their base also the basal end turn brown preventing elongation of roots.

When the strength of medium was further reduced to ¼, about 68.66% cultures exhibited development of *in vitro* roots onto ¼ MS+ 10 µM IBA. Average number of roots on the medium was 2.5 with maximum number of 6 roots. On further increasing the concentration of IBA to 10 - 20 µM keeping the strength of medium same 100% cultures developed roots. The average number of roots formed on ¼ MS+ 20 µM IBA was 3 with a maximum of 10 roots obtained onto this medium **Table 2, Fig. 2** and **Fig. 5**.

In concordance with the results obtained in present study, IBA (with reduced strength of based MS medium) have been reported to be an effective

plant growth regulator for induction of *in vitro* rooting [12], ¼ MS + 20 µM IBA have been reported to be most appropriate medium composition for development of *in vitro* roots. In the study two hormones IBA and IAA were evaluated to study their effectiveness to induce roots in culture of *E. gerardiana* and rooting was obtained only onto medium supplemented with IBA and no rooting was obtained onto medium supplemented with IAA. It has also been reported that IAA cannot be used as rooting hormone in Ephedra due to the presence of IAA oxidase in tissue which destroys IAA of the medium [13].

**TABLE 2: ROOTING RESPONSE OF REGENERATED SHOOTS OF *E. GERARDIANA***

| Rooting medium | % Cultures showing induction of roots | Average no. of roots | Max. no. of roots |
|---|---|---|---|
| ½ MS + 10µM IBA | 8.54 | 0.0 | 0.0 |
| ½ MS + 15µM IBA | 17.66 | 0.0 | 0.0 |
| ½ MS + 20µM IBA | 24.0 | 0.0 | 0.0 |
| ¼ MS + 10µM IBA | 68.66 | 2.5 [b] | 6 [c] |
| ¼ MS + 15µM IBA | 100 | 2.2 [c] | 5 [b] |
| ¼ MS + 20µM IBA | 100 | 3.0 [a] | 10 [a] |

Values are mean of three replicates. Mean values followed by same letters are not significantly different at p ≥ 0.05 DMRT

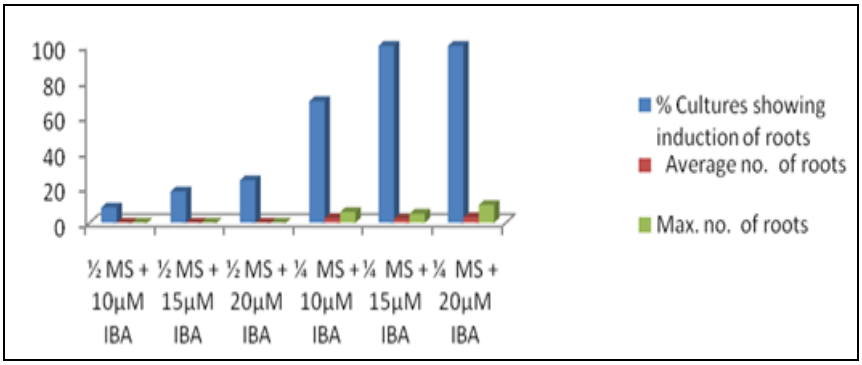


**FIG. 2: ROOTING RESPONSE OF REGENERATED SHOOTS OF *E. GERARDIANA* AT DIFFERENT STRENGTH MEDIUM AND IBA CONCENTRATION**

In concordance with the results obtained in present study, IBA (with reduced strength of based MS medium) have been reported to be an effective plant growth regulator for induction of *in vitro* rooting [12], ¼ MS + 20 µM IBA have been reported to be most appropriate medium composition for development of *in vitro* roots. In the study two hormones IBA and IAA were evaluated to study their effectiveness to induce roots in culture of *E. gerardiana* and rooting was obtained only onto medium supplemented with IBA and no rooting was obtained onto medium supplemented with IAA. It has also been reported that IAA cannot be used as rooting hormone in *Ephedra* due to the presence of IAA oxidase in tissue which destroys IAA of the medium [13].

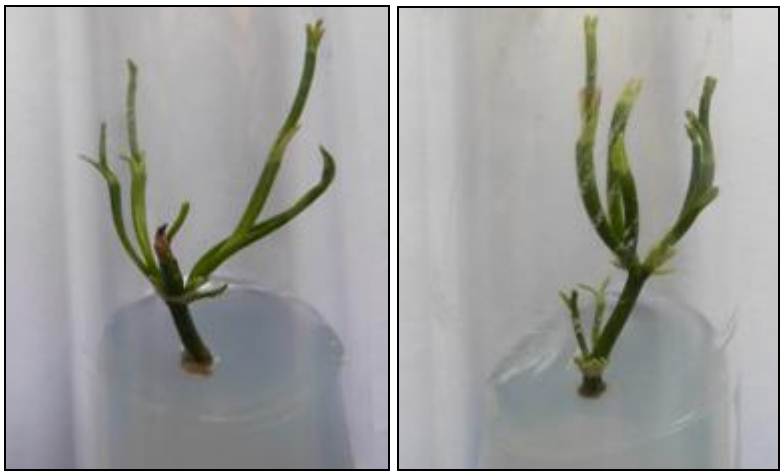

**FIG. 3: MULTIPLE SHOOTS OF *EPHEDRA GERARDIANA* COMBINATION OF 5 µM BAP WITH 15 µM Kn**

**FIG. 4: ELONGATION WITH BASAL MEDIUM**

Beside *Ephedra* effectiveness of IBA (with full as well as ½ strength medium) in induction of *in vitro* rooting has also been reported in other medicinal plants also. Formation of *in vitro* roots from regenerated shoots of *R. serpentina* onto IBA supplemented medium [14]. In a similar way development of *in vitro* roots from cultured shoots of *W. somnifera* onto ½ MS+IBA medium has also been reported [15].

**FIG. 5: ROOT INITIATION AND ELONGATION WITH ¼ MS + 20 µM IBA**

Plants with well developed roots were removed from culture vessel under aseptic conditions, after removing all traces of medium plants were transferred to pots **Fig. 6** containing soil: sand: composite (1:1:1). Pots were transferred to mist chamber and then to green house after 4 - 5 days. After the onset of emergence of new shoots plants were transferred to natural condition. About 60.4% plants survived during the process of acclimatization.

**FIG. 6: HARDENING OF *EPHEDRA GERARDIANA* CONTAINING 1:1:1 RATIO OF SAND, SOIL AND COMPOST**

**CONCLUSION:** Considering the present endanderd status of *Ephedra gerardiana* there is requirement of development of protocol for conservation and mass propagation of the species. In this contest the present study represent an easy and effective method for conservation of *Ephedra gerardiana*. The *in vitro* raised plant can further be subjected to studies for enhanced production of medicinally important secondary metabolites.

**ACKNOWLEDGEMENT:** Authors are grateful to Uttarakhand Council of Science and Technology (UCOST) Dehradun, Uttarakhand for providing financial funding in form of major research project.

**CONFLICT OF INTEREST:** The authors declared no conflict of interest.

## REFERENCES:

1. Rautela N, Dhiman M, Sharma MD, Kumar N and Misra P: Assessment of genetic fidelity of mother plant and *in vitro* raised medicinal plant *Ephedra gerardiana* through molecular marker. Journal of Plant Development Sciences 2017; 9(3): 191-200.
2. O'Dowd NA, Mc Cauley PG, Wilson G, Parnell JAN, Kavanaugh TAK and Mc Connel DJ: Ephedra species: *in vitro* culture, micropropagation and the production of ephedrine and other alkaloids. In Y.P.S. Bajaj (ed.). Biotechnology in Agriculture and Forestry. Medicinal and Aromatic Plants X. Springer-Verlag, Berlin, Germany 1998; 41.
3. Martin PG and Dowd JM: A phylogenetic tree for some monocotyledons and gymnosperms derived from protein sequences. Taxon 1986; 35: 469-475.
4. Chase MW, Soltis DE, Olmstead RG, Morgan D, Les DH, Mishler BD and Duvall MR: Phylogenetics of seeds plants: an analysis of nucleotide sequences from the plastid gene rbcL. Annals of the Missouri Botanical Garden 1993; 80: 528-580.
5. Doyle JA, Donoghue MJ and Zimmer EA: Integration of morphological and ribosomal RNA data on the origin of angiosperms. Annals of the Missouri Botanical Garden. 1994; 81: 419-450.
6. Nickrent DL and Soltis DE: A comparison of angiosperm phylogenies from nuclear 18S r DNA and rbcL sequences. Annals of the Missouri Botanical Garden 1995; 82: 208-234.
7. Sahni KC: Gymnosperms of India and Adjacent Countries. Bishen Singh Mahendra Pal Singh, Dehradun, India. 1990.
8. Blumenthal M and King P: Ma Huang: Ancient herb, modern medicine, regulatory dilemma; A review of the botany, chemistry, medicinal uses, safety concerns, and legal status of Ephedra and its alkaloids. Herbal Gram. 1995; 22.
9. Abourashed EA, El-Alfy AT, Khan IA and Walker L: Ephedra in prespective- A current review. Phytother. Res. 2003; 17: 703-712
10. Chavez ML: More evidence supporting the ban of ephedra dietary supplements. The Annals of Pharmacotherapy. 2004; 38: 895-897.
11. Mikage M and Kakiuchi N: The recent situation of the resources of Chinese crude drug Ma-huang, Ephedrae Herba. J Tradit Med. 2005; 22(1): 61–69.
12. Garla M, Pratush A, Kumar S, Singh S and Shivani: *In vitro* callus induction and shoot regeneration in Ephedra-A medicinal plant. Annals of biological research 2011; 2(6): 645-651.
13. Sharma V, Gupta KS and Diman M: Regeneration of plant from nodal and internodal segment cultures of *Ephedra gerardiana* using Thidiazuron. Plant tissue culture and biotechnology 2012; 22(2): 153-161.
14. Straus J and Gerding RK: Auxin oxidase and growth control in tissue cultures of Ephedra. Plant Physiol. 1963; 38: 621-627.
15. Chaudhary V, Singh S, Vaid S and Sharma N: *In vitro* regeneration of *Rauwolfia serpentine* through anther culture studies. Biotechnology International 2015; 8(3): 93-100.
16. Sharma N, Koshy EP and Dhiman M: Indirect regeneration from leaf segments for mass Propagation of *Withania somnifera*: An endangered medicinal plant. International Journal of Pharm and Biological Sciences 2014; 5(3B): 764-772.

**How to cite this article:**
Rautela I, Dhiman M, Sharma MD and Misra P: *In vitro* regeneration of medicinal plant *Ephedra gerardiana*. Int J Pharm Sci & Res 2018; 9(3): 1183-88. doi: 10.13040/IJPSR.0975-8232.9(3).1183-88.