

IN VITRO MULTIPLICATION OF *HELICTERES ISORA* THROUGH NODAL STEM SEGMENT EXPLANT FROM MATURE PLANT

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ABSTRACT

Helicteres isora is an important medicinal shrub belonging to family Sterculiaceae. It is commonly known as ‘Marorphali’. All parts of the plant are extensively used in indigenous system of medicine. It has cytotoxic, antinociceptive, antifungal, antioxidant, antispasmodic and antibacterial activity and is, used to treat diseases like dyspepsia, colic, scabies, gastropathy, diabetes, diarrhoea and dysentery. It contains high amount of medicinally important secondary metabolites like β -sitosterol, betulinic acid, β -tocopherol, coumarins, ellagic acid etc. In *Helicteres isora* apospory, seed dormancy and low viability of seeds were observed. Therefore, the present work on *Helicteres isora* was

undertaken to develop a highly efficient, reliable and reproducible protocol for their micropropagation. Effect of various basal media viz. MS-medium, B₅-medium and MSB₅-medium on multiple shoot proliferation were studied and it was concluded that MS-medium was best for maximum shoot proliferation. Maximum shoot bud proliferation (14.2 ± 0.545) was observed on MS-medium fortified with BAP (1.5 mg/l) and Kn (1.5 mg/l). Therefore this medium was designated as “shoot bud induction, proliferation and elongation medium”.

KEYWORDS: *Helicteres isora*, Medicinal shrub, BAP, Kn, Multiplication.

INTRODUCTION

Ancient civilisations, India has been known to be a rich repository of medicinal plants. Many plant species that provide medicines have been scientifically evaluated for their possible medical applications. Pharmaceutical industries throughout the world are designing new

drugs and formulations based on indigenous traditional knowledge. Hence demand for raw materials is increasing. Exploitation and collection of plants from their natural habitats is not only insufficient to fulfill the demand but is also a threat to the conservation of forest wealth. Hence, cultivation of medicinal plants has gained popularity in recent times. There are a number of commercially important products that are being extracted from these mass produced field plants. Besides field propagation, plant cell culture technology has been exploited as an efficient and useful tool for conservation, large-scale propagation (micropropagation), production of commercially important metabolites, and biotransformation of intermediates into pharmaceutically important products and genetic enhancement of medicinal plants. All these techniques coupled with comprehensive knowledge of the biosynthetic pathways of targeted compounds will help to enhance and improve the production of secondary metabolites by allowing genetic modifications of medicinal plants.

India has a rich treasure of medicinal plants due to diversity of agro climatic conditions. India is a leading exporter of medicinal plants in the world trade. A large population of both developed and developing countries use plant based raw materials in different formulations of medicine. *Helicteres isora* is an important medicinal shrub belonging to family Sterculiaceae. It is commonly known as 'Marorphali'. All parts of the plant are extensively used in indigenous system of medicine. It has cytotoxic, antinociceptive, antifungal, antioxidant, antispasmodic and antibacterial activity and is, used to treat diseases like dyspepsia, colic, scabies, gastropathy, diabetes, diarrhoea and dysentery. It contains high amount of medicinally important secondary metabolites like β -sitosterol, betulinic acid, β -tocopherol, coumarins, ellagic acid etc. In *Helicteres isora* apospory, seed dormancy and low viability of seeds were observed. Therefore, the present work on *Helicteres isora* was undertaken to develop a highly efficient, reliable and reproducible protocol for their micropropagation.

Thus, to overcome the ruthless exploitation of medicinal plants, there is a great need for the use of modern advanced technologies. Successful *in vitro* regeneration of medicinal plants has been possible through various explants such as leaf tissues, stem segments, shoot buds, apical buds, hypocotyls, cotyledons, roots, anthers etc.^[1-6]

Micropropagation which allows production of clones identical to mother plant can be achieved by using different starting tissues ranging from cotyledons and vegetative meristems to reproductive tissues. Axillary meristem culture is one of the most exciting and important

aspects of *in vitro* cell and tissue culture and has the capability to regenerate and propagate plants from cultured cells and tissues. However, the simplest type of *in vitro* plant propagation is through axillary bud development which exploits the normal ontogenic route for branch development by lateral (axillary) meristems.

Nodal explants have been considered as competent explant for plant regeneration *in vitro*.^[7] Quiescent or active meristems are present in axillary buds of nodal stem segments. These buds are capable of producing complete plantlets.^[8] Now by this method of multiplication through nodal stem segment from mature plant, a large number of medicinal plants have been produced.^[9-11]

The main advantage of this method is that it mostly skips the intermediate stage of callusing. Thus this method has the capacity to produce 'true-to-type' plants and is generally described as "Conservative". It also offers a suitable material for biochemical, physiological and genetical studies.^[12]

MATERIALS AND METHODS

Nodal shoot segments bearing axillary buds, collected from mature plant were used as explants for the present set of experiments. They were subsequently cut into smaller segments with one or two nodes per explant. These nodal segments were washed thoroughly with 2% Extran (commercial detergent) and then rinsed thoroughly in sterile distilled water. Subsequently, the explants were surface sterilized with 0.1% mercuric chloride for 1-2 minutes. At times of heavy infection of fungus, 70% ethyl alcohol treatment was also given to the stem explants for 30 seconds followed by several rinses in sterile distilled water. The sterile nodal stem segments were finally cultured on MS-medium supplemented with different concentrations and combinations of phytohormones to obtain multiple shoots.

All experimental manipulations were carried out under aseptic conditions. Cultures were incubated at 26±2°C under 16 hours photoperiod and 50-60% relative humidity for upto four weeks. Five replicates per treatment were taken and experiments were repeated thrice for confirmation of results. Periodic observations were recorded and the results were subjected to statistical analysis.

OBSERVATIONS AND RESULTS

A series of experiments was set up to obtain regeneration from nodal stem segment of *Helicteres isora*. Shoot buds emerged after 2-3 weeks of incubation under controlled environmental conditions.

For obtaining multiple shoots, nodal stem segments were inoculated on various media viz., MS-medium, B₅-medium and MSB₅-medium (Modified MS-medium) supplemented with phytohormones. Besides media and phytohormones various critical factors such as evaluation of seasonal influence on bud proliferation, contamination factor, effect of various concentrations of sucrose, effect of various levels of inorganic nutrient, inorganic nitrogen, vitamin level and effect of sulphates on shoot bud proliferation were also evaluated in this context.

(a) Effect of various basal media on shoot bud proliferation

Various synthetic media viz., MS (Murashige and Skoog, 1962)^[13]; B₅ (Gamborg *et al.*, 1968)^[14] and MSB₅ (Modified MS-medium) were tried to evaluate their regenerative potential, with the objective to find the medium which could support sprouting of optimal number of shoot buds from a single nodal segment.

In these media, sucrose (3.0%), BAP (1.5 mg/l) and Kn (1.5 mg/l) were incorporated. Surface sterilized nodal segments were inoculated on various media and after four weeks it was observed that maximum number of shoot buds proliferated on MS-medium (14.2±0.660) without much callus formation and B₅ medium supported the production of about 6-7 shoot buds with large amount of callus while on MSB₅ medium 8-9 shoot buds were produced. So further experiments were set up with MS-medium.

(b) Effect of phytohormones on shoot bud proliferation

Various phytohormones i.e. cytokinins (BAP, Kn) and auxins (NAA, IAA, IBA) alone or in combination were tried for multiple shoot formation. The optimal levels of phytohormones required for maximum number of healthy shoots was determined.

(i) Effect of 6-benzyl aminopurine (BAP)

MS-medium supplemented with various concentrations of BAP (0.5-4.0 mg/l) was tried for axillary bud proliferation from nodal stem segments keeping basal medium as control (Table-1).

Number of shoot buds increased with concentration of BAP (0.5-1.5 mg/l) [Figs.1A-B]. At 1.5 mg/l a maximum of 9.3 ± 0.895 shoot buds were observed [Fig.1B]. At higher concentration of BAP (3.0-4.0 mg/l) the number of shoots declined with large leaves. These shoot buds could not develop further into healthy and sturdy shoots on the same medium.

(ii) Effect of kinetin (Kn)

In order to find out the effect of Kn on shoot proliferation, MS-medium containing sucrose (3.0%) was supplemented with Kn (0.5-4.0 mg/l), keeping the basal medium as control (Table-1).

Nodal stem segment explant from mature plant, when cultured on basal MS-medium without kinetin (control), turned brown within one week of culture. The overall response of the explant to kinetin at all levels was also not significant. At low concentrations (1.5 mg/l) of Kn, 5.8 ± 0.764 shoot buds were obtained [Fig.1C]. Maximum 7.8 ± 0.722 shoot buds were obtained at 2.0 mg/l Kn [Fig.1D]. The concentration of kinetin higher than 2.0 mg/l was neither effective in increasing the rate of growth of axillary bud nor in inducing bud sprouting. It was concluded that kinetin alone was not very effective in inducing the sprouting of the axillary bud.

(iii) Combined effect of BAP and kinetin

BAP (0.5-3.0 mg/l) was incorporated with various concentrations of Kn (0.5-3.0 mg/l) on MS-medium to study their combined effect on axillary bud proliferation. After four weeks of incubation it was observed that combined effect of BAP and Kn showed synergistic effect on shoot bud proliferation (Table-1). At lower concentration of BAP (1.0 mg/l) and Kn (1.0 mg/l), 8.7 ± 0.465 shoot buds were obtained [Fig.1E]. At concentration of BAP (1.5 mg/l) and Kn (1.5 mg/l), a maximum of 14.2 ± 0.545 shoot buds were obtained [Fig.1F]. With increase in the concentration of BAP (2.0-3.0 mg/l) and Kn (2.0-3.0 mg/l), the number of shoot buds declined and further growth of shoot buds remained arrested on this medium.

Table 1: Effect of cytokinin/s on shoot bud proliferation through nodal stem segment explant from mature plant.

<i>Medium</i>	:	MS + Sucrose (3.0%) + BAP/Kn (0.5-4.0 mg/l); BAP (0.5-3.0 mg/l) + Kn (0.5-3.0 mg/l)	
<i>Explant</i>	:	Nodal stem segment from mature plant	
<i>Incubation</i>	:	At 26±2°C under 16 hours photoperiod (2000-3000 lux) upto 4 weeks	
S. No.	Cytokinin concentration (mg/l)		No. of shoot buds per explant *Mean ± S.E.
	<i>Control: MS basal medium</i>		Nil
	BAP	Kn	
1.	0.5	-	1.4 ± 0.957
2.	1.0	-	2.7 ± 0.791
3.	1.5	-	9.3 ± 0.895
4.	2.0	-	5.6 ± 0.457
5.	2.5	-	4.8 ± 0.339
6.	3.0	-	4.1 ± 0.629
7.	4.0	-	3.2 ± 0.385
8.	-	0.5	1.2 ± 0.564
9.	-	1.0	2.4 ± 0.436
10.	-	1.5	5.8 ± 0.764
11.	-	2.0	7.8 ± 0.722
12.	-	2.5	3.1 ± 0.577
13.	-	3.0	2.6 ± 0.284
14.	-	4.0	1.0 ± 0.679
15.	0.5	0.5	7.7 ± 0.325
16.	1.0	1.0	8.7 ± 0.465
17.	1.5	1.5	14.2 ± 0.545
18.	2.0	2.0	11.4 ± 0.221
19.	2.5	2.5	10.8 ± 0.670
20.	3.0	3.0	8.4 ± 0.730

*values are 95% confidence limits for mean

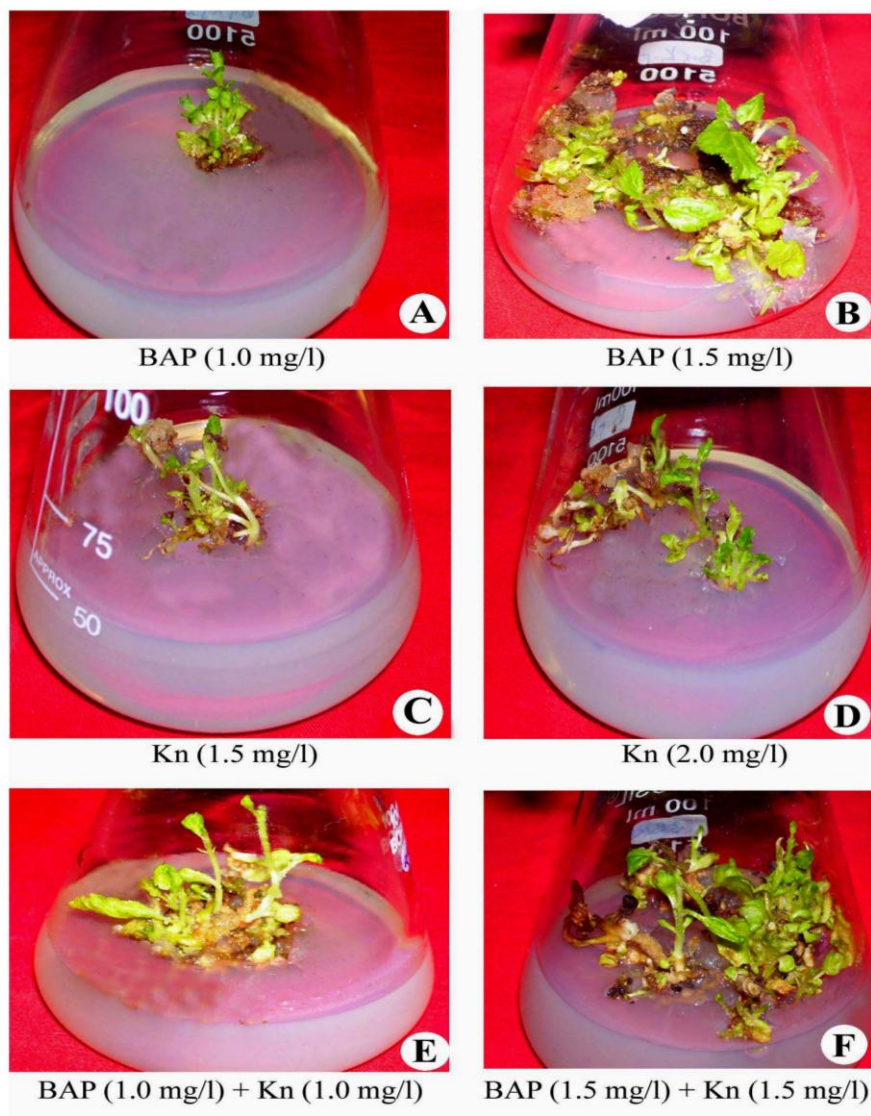


Figure-1

Figs. 1A-F: Effect of Cytokinin (BAP/Kn) alone or in combination on multiplication shoot proliferation from nodal stem segment on MS-medium

DISCUSSIONS

During the present investigation, experiments were carried out, to obtain multiple shoots through nodal segments from mature plant in *Helicteres isora*. Results indicated that neither BAP nor Kn alone proved beneficial for multiple shoot bud formation. BAP in combination with Kn on MS medium was effective in shoot bud production. Maximum shoots (14.2 ± 0.545) were obtained on MS medium fortified with 1.5 mg/l BAP and 1.5 mg/l Kn. Further proliferation and elongation of shoot buds was also observed on the same medium. The interacting influence of BAP and Kn was significant in this investigation as has also been reported by some earlier workers in several medicinal plant species.^[15-16]

When BAP and Kn alone were used in MS medium for shoot multiplication in *Helicteres isora* lesser number of shoots were produced. However, BAP (1.5 mg/l) was found to be superior over Kn (2.0 mg/l) in the present investigation. There are several reasons for the preferred use of BAP as cytokinin. Slow degradation of BAP is one of these reasons and it can be autoclaved without losing its activity.^[17] The superiority of BAP over Kn has also been reported for multiple shoot induction in several plant species.^[18-19]

CONCLUSIONS

From the above observations and results on multiple shoot proliferation through nodal stem segment explant from mature plant of *Helicteres isora*, the Maximum shoot bud induction, proliferation and elongation was obtained on MS-medium supplemented with BAP (1.5 mg/l) and Kn(1.5 mg/l). Therefore this medium was designated as “shoot bud induction, proliferation and elongation medium”.

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