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RESEARCH ARTICLE

IN VITRO PROPAGATION OF HOLOSTEMMA ADA-KODIEN SCHULT. AN ENDANGERED MEDICINAL PLANT

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ABSTRACT

Holostemma ada-kodien is a medicinal plant belongs to the family Asclepiadaceae. Root tubers contain alpha amyrin, Lupeol, and β- Sitosterol. The present study was to standardize in vitro culture of Holostemma ada-kodien Schult. On Murashige and Skoog (MS) medium supplemented with different combinations of growth regulators to induce shoot, root and callus formation. Among several combinations of BAP, NAA and Kinetin studied, nutrient medium supplemented with 0.1mg/l BAP and 1.0 mg/l Kinetin induced maximum number of shoots. Highest callus proliferation was observed in the medium supplemented with 1.0 mg/l BAP and 1.0 mg/l NAA. Root initiation was also observed in the same medium.

Key words: Multiple shoots, callus proliferation, root initiation, *Holostemma ada-kodien.*

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INTRODUCTION

Holostemma ada-kodien is a medicinal plant belongs to the family Asclepiadaceae. Tuberous roots of the plant are medicinally important and are utilized as a major ingredient of the drug "jivanthi" (Kolammal, 1979). The plant is a twining shrub with opposite leaves, and flowers are developed in axillary umbellate cymes. Roots contain α-amyrin, Lupeol and β- Sitosterol. The roots are used for cough, fever, ophthalmic diseases, stomachache, spermatorrhoea, emaciation, dysentery, tuberculosis, arrested urination, scorpion bite, kidney stones, goiter etc. About 150 metric tons of root tubers per annum are required to major southern Indian pharmacies for Ayurvedic preparations (Nair *et al.* 1992).The destructive and merciless collection of tubers, in recent times, has led to extreme loss of the plant in their natural habitats and consequently it is listed out as vulnerable and rare in FRLHT red list (FRLHT, 1997).Conventional propagation through seeds, stem cutting and root cuttings is too slow to meet the demand of this invaluable therapeutic plant. Hence, *in vitro* tissue culture method might be of great value as an alternate method to achieve rapid multiplication of this species. In the last few decades, plant tissue culture has played an important role for production of large number of plants, independent of seasons, aiming at re-establishment of these plants in their natural habitat. Besides, they also serve as an alternate source of plant raw materials for commercial utilization, which decreases pressure on the plant population in wild habitat (Krishnan *et al*., 2011). Micropropagation through axillary bud multiplication (Sudha *et al.,* 1998) and indirect organogenesis from callus (Sudha *et al.,* 2000) has been reported on this species. An efficient protocol for rapid propagation of *H. ada-kodien* by using shoot tip and nodal explants has been developed in the present study.

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MATERIALS AND METHODS

Mother plants of *Holostemma ada-kodien* were collected from Centre for Medicinal Plants Research (CMPR), AryaVaidya Sala, Changuvetty, Kottakkal. The explants were initially washed in running tap water for about 30 minutes, then with detergent extran for about 5-10 minutes and finally, they were thoroughly washed in distilled water. The explants were surface sterilized in 0.1% mercuric chloride for 10 minutes, followed by thorough washing in distilled water. Then the explants were cut into appropriate size and cultured on MS basal medium. After 30 days, shoots formed were sub-cultured on MS medium containing various combinations of BAP, NAA and Kinetin.

RESULTS AND DISCUSSION

Culture initiation

1-2 shoots were formed from nodal explants when cultured on MS basal medium. After 30 days, shoots formed were sub-cultured on MS media containing various combinations of BAP, NAA and Kinetin Table (1).

Table 1. Effect of MS media provided with different combinations of growth regulators on shoot and callus multiplication of *H adakodien*. Growth period was 30days

Growth regulators(mg/l)			Number of shoots/node	Callus proliferation
BAP	NAA	Kinetin		
Hormone free			1.5±0.5	Moderate
-	-	0.5	3.6±0.8	Moderate
0.5	-	-	2.6±0.5	Low
0.5	0.5	-	2.5±0.6	Moderate
1.0	1.0	-	5.6±0.3	High
1.0	0.5	-	1.8±0.7	High
1.0	-	0.1	1.4±0.4	High
0.1	-	1.0	9.0±0.8	High
1.0	-	0.5	3.7±0.3	Low
0.5	-	1.0	4.4±0.6	Low
-	0.5	0.5	3.4±0.5	Moderate
-	0.5	1.0	6.4±2.0	Low

***Holostemmaada-kodien* initiated in M S medium containing 0.1mg/l BAP and 1.0mg/l Kinetin. High callus proliferation is also observed**

***Holostemmaada-kodien* initiated in M S medium containing 0.5mg/l NAA and 1.0mg/l Kinetin. Numerous shoots proliferated from the base of the stem after pruning.**

Axillary Bud Multiplication

Axillary bud proliferation was observed from sub-cultured nodal explants when cultured on MS medium containing different combinations of PGRs. About 4 shoots/explant were induced in medium containing Kinetin alone, whereas BAP alone induced about 3 shoots/explant. BAP in combination with Kinetin induced multiple shoots from axillary bud. Medium containing 0.1mg/l BAP and 1.0 mg/l Kinetin produced highest number of shoots (10 shoots/explant) whereas the reverse combination facilitated the least number of axillary buds. About 6 shoots/explant were observed in the medium containing 1.0mg/l kinetin in combination with 0.5mg/l NAA. According to Sudha et al. (1998) 1.0 mg l–1 BAP in combination with 0.1 mg l–1 NAA was effective, and they obtained 3.8 shoots per node. Callus proliferation was low in this combination whereas the medium having 1.0mg/l BAP and 0.5mg/l NAA induced only about 2 shoots/explant with high callus proliferation. The present study provides information about the efficacy of Kinetin in combination with low concentration of BAP in facilitating axillary bud multiplication. This is in accordance with the view that *Holostemma ada-kodien* has high indigenous concentration of auxin. Thus, axillary bud proliferation in *H. ada-kodien* is more depended on the concentration of which cytokinin is used. High proliferation of axillary buds was observed after trimming off the initial axillary shoots from the main shoot. Among the different cultures medium containing 1.0 mg/l Kinetin with 0.5mg/l NAA showed such rapid proliferation of axillary buds after trimming. This also shows the effect of kinetin in the rapid proliferation of *H.ada-kodien*.

The described protocol could be used for the rapid propagation, conservation and genetic transformation studies of this

valuable, endangered medicinal plant through axillary bud multiplication.

REFERENCES

FRLHT, 1997. Medicinal plants of India- Guidelines for national policy and conservation programs (Foundation for Revitalization of Local Health Traditions), Bangalore (India).

Karmarkar S. H., Keshavachandran R. and Augustin A. 2001. Biochemical Evaluation of Root Tubers and *In Vitro* Induced Callus of Adapathiyan *(Holostemma Ada-Kodien* K. Schum.). *Journal of Tropical Agriculture,* 39: 108-110.

Kolammal M. 1979. Pharmacognosy of ayurvedic drugs. University of Kerala, Trivandrum, pp 214. 4.

Martin K. P. 2000. Rapid propagation of *Holostemma ada-kodien* Schult., a rare medicinal plant, through axillary bud multiplication and indirect organogenesis, Plant cell reports, Springer.

Meena Thomas Irimpan, Jolly C.I. and Sheela D. 2011. A Study of the Phytochemical Composition and Antibacterial activity of *Holostemma ada-kodien* Schultes. *International Journal of PharmTech Research.*

Nair K. V, Nair A. R, Nair C. P. R. 1992. Technoeconomic data on cultivation, preservation of some south Indian medicinal plants. *Aryavaidyan*, 5:238–240.

Pushparajan G. and SanoopSurendran, 2014. Studies on medicinal plants. 4. Micropropagation of *Holostemma ada-kodien* Schult.- a rare medicinal plant, *International journal of advanced research.*

Sudha C.G, Krishnan P. N,Pushpangadan P. 1998. In vitro propagation of *Holostemma annulare*(Roxb.) K. Schum. a rare medicinalplant. *In Vitro Cell Dev Biol Plant,* 33:57–63

Sudha C. G, Krishnan P. N, Seeni S, Pushpangadan P. 2000. Regenerationof plant from *in vitro* root segments of *Holostemma annulare*(Roxb.) K. Schum. a rare medicinal plant. *Curr Sci.,* 78:503–506

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