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***In vitro* multiplication of *madhunashini* (*Gymnema sylvestre* Retz.)**

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Abstract

Study was conducted for the *in vitro* multiplication of *madhunashini* by using immature seeds as explants. Immature seeds were cultured directly on to media containing different concentrations and combinations of auxin and cytokinin. Germination of seeds into plantlets was observed in the media containing cytokinins, however, the seeds cultured on media containing auxins at the expense of plantlet formation produced callus. Cent per cent of the explants responded when inoculated on Murashige and Skoog (MS) media containing Benzylaminopurine (BAP - 1.0 mg l⁻¹), Kinetin (Kn - 0.5 mg l⁻¹, 1.0 mg l⁻¹ and 2.0 mg l⁻¹) and BAP + Kn (1.0+1.0 mg l⁻¹). Height of plantlet (19.74 mm) was significantly higher in MS media supplemented with BAP 0.5 mg l⁻¹. Maximum number of leaves (3.12) were produced on MS media supplemented with Kn 2.0 mg l⁻¹. Nodal explants obtained from *in vitro* seedlings when cultured on the media containing cytokinins induced proliferation of shoots. Number of shoots (5.50) produced and length of shoot (18.53 mm) was found to be maximum at lower concentrations of BAP 1.0 mg l⁻¹ and Kn 0.5 mg l⁻¹. Leaves from *in vitro* seedlings when used as explants and cultured on media with cytokinins did not show any response.

Highlights

- This study is the first study in *madhunashini* on the use of immature seeds as explants for *in vitro* culture
- Immature seeds responded well in Murashige and Skoog media
- Rooting was confirmed by using fluorescent microscopy

Keywords: Growth regulators, immature seeds, nodal explants, murashige and skoog media

Madhunashini (*Gymnema sylvestre* Retz.) is a member of the family Asclepiadaceae and indigenous to India. It is called Gudmar in Hindi and *madhunashini* in Kannada and Sanskrit, means sugar or sweetness destroyer. It is used both in the Ayurvedic and Unani medicine. The plant is stomachic, laxative and diuretic. It is used both in the Ayurvedic and Unani

medicine. In India, it has been known from antiquity for the treatment of Diabetes mellitus.

The data on the exact area and production of this crop is not available and due to heavy demand, the plant has become endangered and its commercial cultivation has recently gained importance in



Tamilnadu and other Southern states (Venkatesan *et al.*, 2010). Due to the multifarious applications of *madhunashini*, there is an increasing demand in the developing countries which warrants commercial farming. For large scale commercial cultivation of the crop, a propagation technique which is simple, economical and easy to handle on the commercial scale is necessary. *Madhunashini* multiplies through seeds in its natural habitat. But there are problems like flower shedding, low fruit set and very short span of seed viability (Chandrasekar *et al.*, 2003).

Vegetative propagation of *madhunashini* is also difficult due to poor and delayed rooting of cuttings. To overcome this problem and large scale production of planting material, tissue culture is one of the useful tools. There are studies regarding tissue culture of *madhunashini* using different explants. However, no study yet on *in vitro* seed germination using immature seeds as explants.

Materials and Methods

Surface sterilization of pods

Immature pods were collected and first washed in running tap water for one hour, then washed in Tween 20 (4-5 drops in 100 ml) for 20 minutes and washed with tap water thoroughly. Then, the pods were immersed in Bavistin (2 g l^{-1}) for 30 minutes and rinsed with double distilled water 2-3 times. The final surface sterilization was done in a laminar air flow cabinet with 0.2% HgCl_2 for 10 minutes and then rinsed with sterile water 4-5 times to remove the traces of HgCl_2 .

Seed culture

Sterilized pods were cut open, seeds were taken out (8-9 seeds per pod) and inoculated in the glass jars containing media. MS (Murashige and Skoog) basal medium supplemented with 3% Sucrose and 0.5% agar was used in the study. The medium was supplemented

Table 1. Effect of cytokinins on shoot elongation and number of leaves of madhunashini explants at 2 and 4 weeks after culture

Growth regulators (mg l^{-1})	Height of plantlet (mm)		Number of leaves	
	2 weeks	4 weeks	2 weeks	4 weeks
Control	1.60c	3.30d	1.50b	3.50
BAP 0.5	19.74a	20.46a	2.00b	2.25
BAP 1.0	11.80ab	12.45abc	2.00b	2.50
BAP 1.5	11.10b	11.45bc	2.37ab	2.25
BAP 2.0	18.42ab	19.09ab	3.00a	3.00
Kn 0.5	10.82b	11.24bcd	1.62b	2.00
Kn 1.0	16.32ab	17.32abc	1.75b	2.25
Kn 1.5	13.80ab	14.48abc	1.83b	3.00
Kn 2.0	16.95ab	16.00abc	3.12a	3.87
BAP + Kn (1 + 1)	10.43b	10.95cd	1.75b	2.00
Grand mean	13.09	13.67	2.09	2.66
F – test	*	*	*	NS
CD @ 5%	8.40	8.06	0.95	-
SEm±	2.90	2.78	0.33	0.45

* Significant at 5%

NS- Non significant

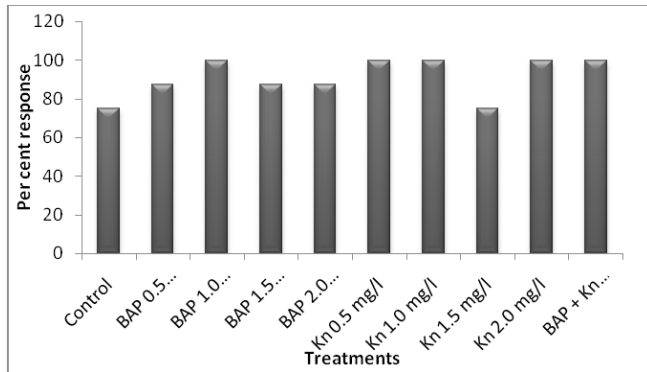


Figure 1(a). Per cent response of immature *madhunashini* seeds in MS media supplemented with different concentrations and combination of BAP and Kn

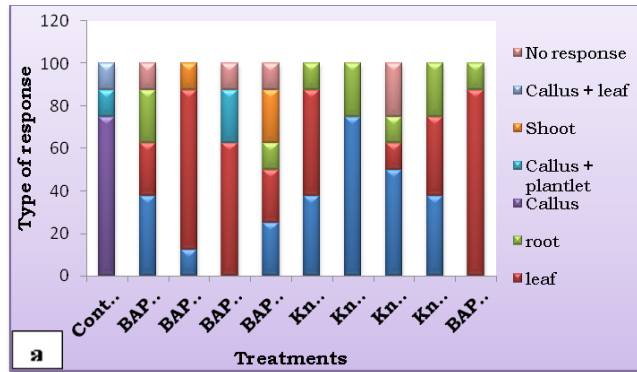
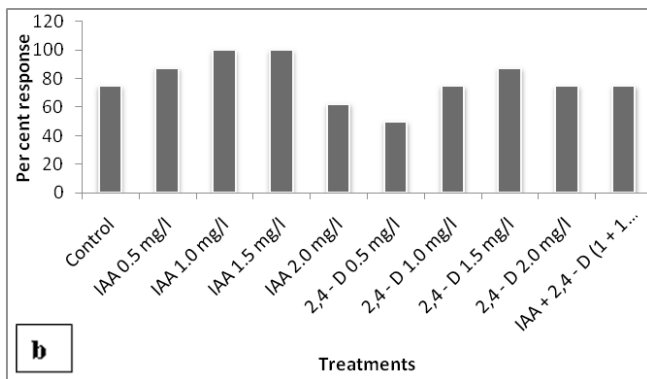
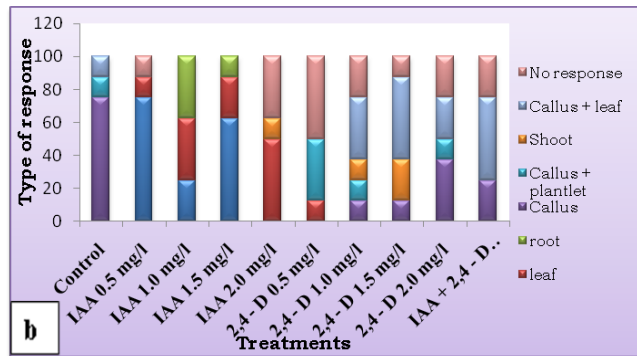


Figure 2(a). Kind of response of immature seeds in different concentrations and combination of BAP and Kn



(b) Per cent response of immature *madhunashini* seeds in MS media supplemented with different concentrations and combination of IAA and 2, 4-D



(b) Kind of response of immature *madhunashini* seeds in different concentrations and combination of IAA and 2, 4-D



Plate 1. (a) Plantlet emergence in cytokinin rich media (b) Caulogenesis in auxin rich media

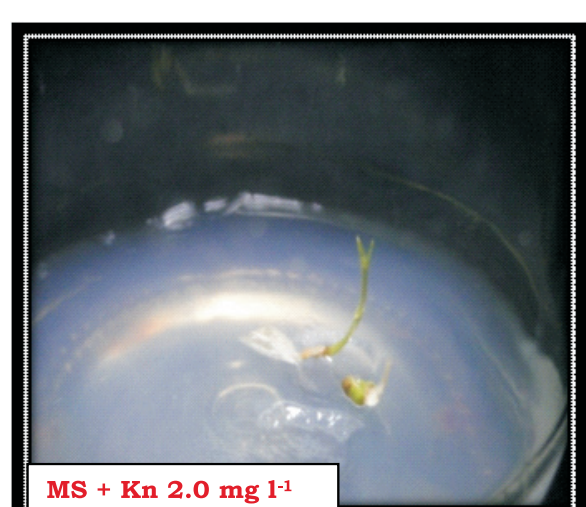
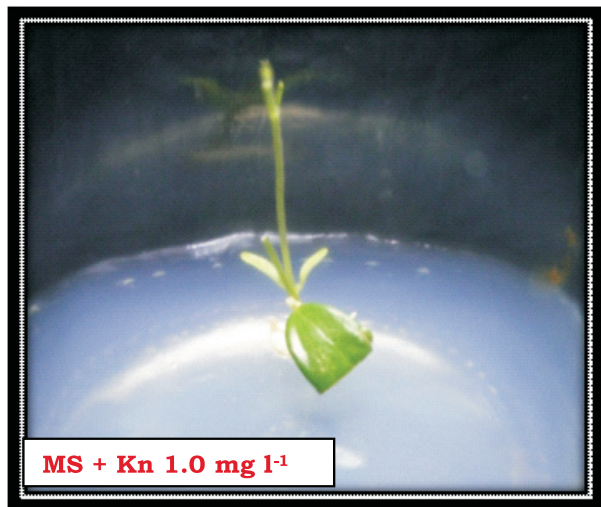
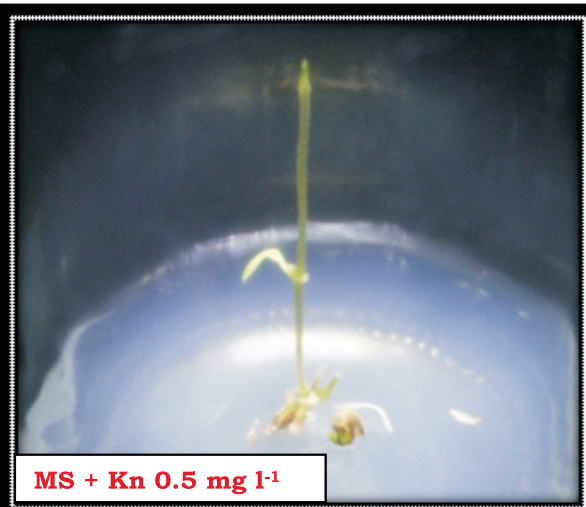
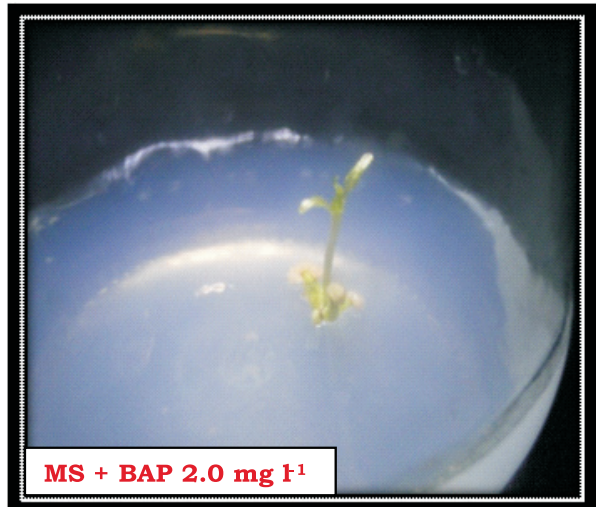
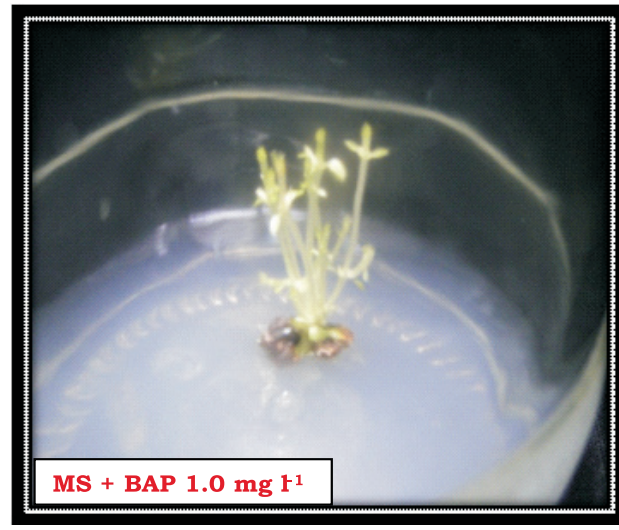
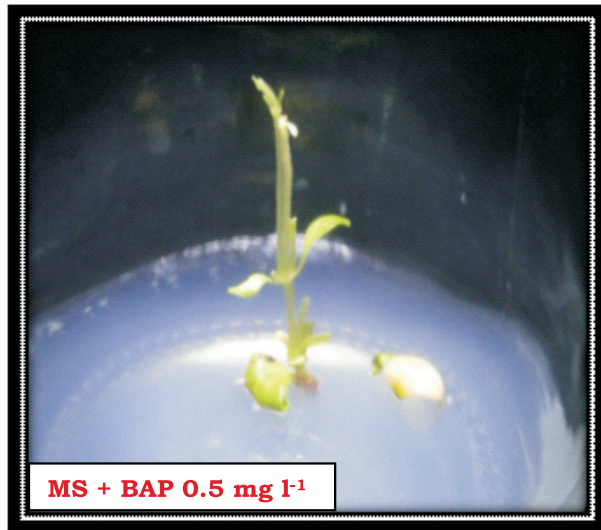


Plate 2. Shoot proliferation in different concentrations of BAP and Kn media

with BAP, Kn, IAA and 2,4-D at 0.5, 1.0, 1.5 and 2.0 mg l⁻¹, BAP + Kn and IAA + 2,4 – D at 1+1 mg l⁻¹. p^H of the medium was maintained at 5.8. Cultures were incubated at 25°C under white fluorescent light with 16:8 hours photoperiod. Cultures were observed daily, and observation were recorded at regular fortnightly intervals.

Sub culturing of leaf and nodal explants

Leaf and nodal explants from the germinated seedlings were subcultured to MS media supplemented with different concentrations of cytokinin media (BAP and Kn at 0.5, 1.0 and 2.0 mg l⁻¹). Cultures were observed daily and observation was recorded at regular fortnightly intervals.

Rooting

Proliferated shoots were sub cultured for rooting into the MS media supplemented with IBA 1.0 mg l⁻¹. Observations were recorded at weekly intervals.

Results and Discussion

Effect of growth regulator supplemented media on culture of immature seeds

Cent per cent of the explants responded when inoculated on MS media containing BAP (1.0 mg l⁻¹), Kn (0.5 mg l⁻¹, 1.0 mg l⁻¹ and 2.0 mg l⁻¹) and BAP + Kn (1.0+1.0 mg l⁻¹) compared to control (Figure 1a

and b). Cytokinins were found efficient for seed germination than auxins (Figure 2a and b). It may be due to; cytokinin is a well known hormone for cell division, emergence of plantlets and their growth. Whereas, auxin is known to promote callus formation by keeping cells in unorganized condition. [Nikolic et al. \(2006\)](#) while working on *Lotus corniculatus* found that the germination percentage was highest in cytokinin rich media.

Height of plantlet was maximum in MS media with BAP (0.5 mg l⁻¹) and it is on par with other concentrations of BAP and Kn (Table 1). It is evident from this experiment that cytokinins help in cell division and shoot formation. Number of leaves was produced in the MS media containing cytokinins than auxins. This may be due to; cytokinin helps in increasing intermodal length and leaf growth, as cytokinin promotes cell expansion in leaves (Taiz and Zeiger, 2003). In stevia also BAP was found to be best in producing multiple shoots, shoot length and number of leaves (Pallavi et al., 2012).

Response of leaf and nodal explants in different concentrations of cytokinin

Number of shoots produced and shoot length were maximum in the MS media supplemented with BAP than Kn. BAP was found superior than Kn (Table 2). Similar results were obtained by Joshi and Dhar

Table 2. Subculturing of leaf and nodal explants of *madhunashini* in different concentrations of cytokinins

Growth regulators (mg l ⁻¹)	Number of days taken for bud break	Number of shoots	Shoot length (mm)	Number of leaves	Response of leaf explants
BAP 0.5	7.00	2.80ab	17.51ab	2.12	0
BAP 1.0	7.75	5.50a	7.44c	3.10	0
BAP 2.0	7.60	2.60b	9.43bc	2.45	0
Kn 0.5	7.60	2.00b	18.53a	2.66	0
Kn 1.0	8.80	1.00b	7.63c	3.33	0
Kn 2.0	7.75	1.25b	11.94abc	2.50	0
F – test	NS	*	*	NS	
CD @ 5%	-	2.86	8.65	-	
SEm±	0.35	0.87	2.96	0.58	

* Significant

NS – Non significant



(2003) in *Saussurea obvallata* and Hwang (2005) in *Zanthoxylum piperitum*. The application of BAP at lower levels (1.0 μ M) has proven extremely beneficial for induction of multiple shoots and subsequent shoot multiplication as also reported earlier in *Picrorhiza kurrooa* (Lal et al. 1988 and Chandhra et al., 2006). In *ocimum maximum* number of shoots (12.75 \pm 0.64) was recorded with MS medium containing BAP alone (Kishor et al., 2013).

In leaf explants there was no response in different concentrations of BAP and Kn, this may be due to, the growth regulator concentration may be not sufficient.

Rooting of *in vitro* grown microshoots

After subculturing the microshoots to rooting media bulging of basal portion of the shoot was observed. But, there were no visual appearance of roots after 20 days of subculture. So, the fluorescent microscope study was carried out to ascertain the rooting.

Acknowledgements

Authors are thankful to all the members of Plant Tissue Culture Laboratory, University of Agricultural Sciences, Bangalore for their help and cooperation.

Abbreviations : 2,4-D - Dichlorophenoxyacetic Acid, BAP – 6 benzylaminopurine, g l⁻¹ -gram per litre, HgCl₂ – mercuric chloride, IAA – indole- 3- acetic acid, IBA – Indole Butyric Acid, Kn – Kinetin, mg l⁻¹ –milligram per litre, MS- Murashige and Skoog

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