- Short communication

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Micropropagation of *Marsdenia brunoniana* Wight & Arn. - A Rare Antidiabetic Plant

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Shoot multiplication of *Marsdenia brunoniana* was obtained from the nodal explants of mature plants using MS with different concentrations and combinations of growth regulators. Maximum explant response and highest number of shoots per explant was obtained on MS fortified with 1.0 mg/l BAP. The highest degree of shoot profilieration was found to be 90%. The combination of BAP and Kn was also found to be effective for regeneration. The regenerated shoots were successfully rooted on MS supplemented with 0.5 mg/l NAA, after sequential hardening, survival rate was 90%.

The genus *Marsdenia* of the family Asclepiadaceae consists of 100 species distributed throughout tropical countries. In India it is represented by 13 species as reported by Jagtap and Singh (1999). The genus *Marsdenia* contains many chemical compounds like two polyoxypregnanes, designated marstenacigenins A and B, in *Marsdenia tenacissima* (Sheng-Xiang Qiu et al. 1996), polyhydroxy pregnane ester named tenasogenin in *Marsdenia tenacissima* (Singhal et al. 1980) and Marsdenin, is a glycoside isolated from *Marsdenia erecta* R. Br. (Baytop et al. 1959). Most of them have medicinal value. *Marsdenia brunoniana* Wight & Arn. is one such rare medicinal twining shrub found in Tamilnadu and Karnataka states of Peninsular India (Natarajan 2004). The leaves of the plant have been extensively used for the treatment of diabetes (Kottaimuthu 2008). Conventionally this plant is propagated through the seeds. Natural population of the plant species is decreasing due to habit destruction, overexploitation along with poor seed setting and poor seed germination. There have been no reports on *in vitro* propagation of *M. brunoniana*. Hence the *in vitro* propagation of this medicinally important species was undertaken. The present study describes the maximization of shoot multiplication through *in vitro* propagation of *M. brunoniana* by using standard culture medium fortified with different growth regulators.

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Shoots of two-month-old plants of *M. brunoniana* grown in the Botanical garden of Sri Krishnadevaraya University were selected as explants. The leaves and roots were discarded and shoots were washed thoroughly under running tap water (20 min). Nodal portion was used as an explant. They were then treated (15 min) with two drops of aqueous surfactant - Tween 20 (5% v/v) for 5 min, followed by repeated rinsing with distilled water. Further, sterilization was done under aseptic conditions in Laminar Flow. Explants were surface sterilized with 50% (v/v) ethyl alcohol (1 min) followed by 0.1% (w/v) $HgCl_2$ (3 min). Finally, the explants were washed thoroughly (five times) with sterilized distilled water and cut into appropriate size (1 cm) and inoculated on sterilized medium. The culture medium used was MS basal medium with 3% (w/v) sucrose and gelled with 0.8% (w/v) agar. The pH of all media was adjusted to 5.8 and sterilized by autoclave at 121°C (15 min). The cultures were incubated at 25 ± 1°C under a 16 hrs photoperiod (50 $\mu E^{-2}/s$ irradiance) provided by cool white fluorescent tubes. Various plant growth regulators *viz.,* BAP (0.5 - 5 mg/l), Kn (0.5 - 5 mg/l) and NAA, IAA, IBA (0.25 - 2 mg/l) were tried individually or in combination to obtain the multiple shoot bud induction. Observations were recorded after an interval of four weeks. For root induction, *in vitro* micro-shoots with six fully expanded leaves were excised and transferred to half strength MS semisolid medium supplemented with NAA (0.5 mg/l). Roots were initiated after the fifth day of inoculation in the medium containing 0.5 mg/l NAA and fully profuse roots developed after three weeks. Rooted micro-shoots were thoroughly washed to remove the adhering gel and planted in 5 cm plastic cups containing a mixture of peat moss and organic manure (1 : 1). Plastic cups were covered with polythene bags to maintain humidity. Plants were kept in culture room for ten days. Half strength MS macro-salts were poured to the plastic cups at five days regular interval until the new leaves developed. Plants were transferred to pots containing organic manure, garden soil and forest humus (1 : 1 : 1). The pots were watered at two days interval and were maintained in greenhouse. The survival rate was recorded one month after transfer to pots. All experiments were repeated at least three times with 10 replicates for each treatment.

Nodal explants were cultured on MS fortified with different concentrations of cytokinins individually and also in combinations for multiple shoot bud induction and data are represented in the Table 1. Nodal bud when cultured on MS with different concentrations of BAP (0.5 - 5 mg/l), maximum number of shoots was induced on medium containing 1 mg/l BAP within six weeks of incubation, with average length of 3 cm (Fig. 1A). Increase or decrease in the concentration of BAP beyond the optimum level, induced less number of shoot buds. These results are concurrent with earlier findings of *Pedalium murex* and *Physalis angulata* (Ramasubbu et al. 2009), however the length of shoots increased. During subculture, basal axillary buds of the developed axillary buds

also underwent initiation. Enhanced shoot multiplication in subsequent culture is in accordance with published literature on Asclepiadacean medicinal plants like *Gymnema sylvestre* (Komalavalli and Rao 2000), *Hemidesmus indicus* (Sreekumar et al. 2000) and *Holostemma ada-kodien* (Martin 2002). However, in *Hemidesmus indicus* (Patnaik and Debata 1996) repeated subcultures did not enhance shoot proliferation.

Whereas, when nodal explants cultured on MS fortified with different concentrations of Kn (0.5 - 5.0 mg/l), only 2 - 3 shoot buds were induced (Fig. 1B) as reported in *Holostemma ada-kodien* (Martin 2002) and *Curculigo orchioides* (Nagesh et al. 2008).

Table 1. Effect of concentrations of BAP and Kn on bud breaking and multiple shoot induction from nodal explants of *Marsdenia brunoniana*

BAP (mg/l)	Kn (mg/l)	% response	No. of shoots (Mean ± SE)	Shoot length in cm (Mean ± SE)
0.5	--	70	2.3 ± 0.30	5.7 ± 0.26
1.0	--	90	7.2 ± 0.24	2.5 ± 0.34
2.0	--	80	3.1 ± 0.23	3.0 ± 0.33
3.0	--	75	2.8 ± 0.32	5.1 ± 0.23
5.0	--	79	3.0 ± 0.25	5.8 ± 0.24
--	0.5	60	1.8 ± 0.24	5.7 ± 0.21
--	1.0	80	2.8 ± 0.24	1.4 ± 0.16
--	2.0	75	2.1 ± 0.27	3.6 ± 0.26
--	3.0	70	1.7 ± 0.21	5.1 ± 0.27
--	5.0	78	1.9 ± 0.17	3.8 ± 0.24
0.5	0.5	80	3.9 ± 0.37	3.3 ± 0.26
1.0	1.0	85	5.7 ± 0.30	2.7 ± 0.21
2.0	2.0	75	2.8 ± 0.29	4.9 ± 0.31
3.0	3.0	70	1.9 ± 0.23	3.6 ± 0.22
5.0	5.0	72	2.2 ± 0.20	3.5 ± 0.26

Data are the average of three triplicates with 10 explants. Values represent the M ± S.E

However, when nodal explants were cultured on MS containing different concentrations of BAP + Kn in different combinations for multiple shoot induction, maximum number of (5) shoots were induced on medium containing BAP (1.0 mg/l) combined with Kn (1.0 mg/l). Whereas, increase in the concentrations of BAP and Kn decreased the number of shoot buds, this result corroborates with earlier findings in *Pedalium murex* and *Physalis angulata* (Ramasubbu 2009). Average length of the shoot buds increased when compared to medium containing BAP or Kn alone (Fig. 1C).

The present investigation clearly indicated that, among different concentrations and combinations of BAP and Kn, BAP alone particularly at 1.0 mg/l induced maximum number of shoot buds when compared to either Kn alone or combined with Kn in different concentrations.

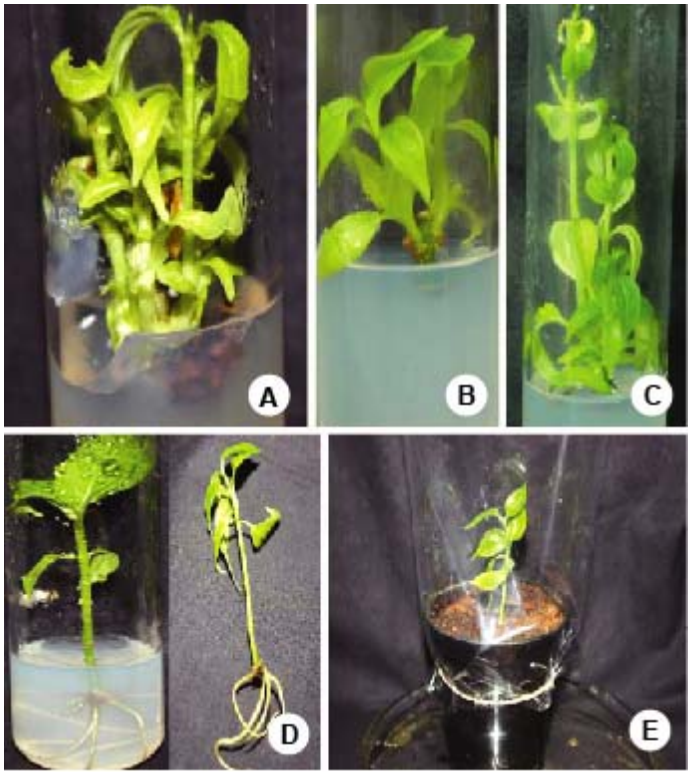

Fig. 1. A. Maximum number of shoot multiplication on MS + BAP 1.0 mg/l. B. Shoot multiplication on MS + Kn 1.0 mg/l. C. Shoot multiplication on MS + BAP + Kn 1.0 mg/l each. D. Root induction on half strength of MS + NAA 0.5 mg/l. E. Plant under acclimatization.

In vitro induced shoots were successfully rooted in MS supplemented with NAA 0.5 mg/l. After sequential hardening, the plantlets were transferred to greenhouse where 90% of them survived. NAA was best for rooting of other Asclepiadaceae members such as three varieties of *Caralluma* (Aruna et al. 2009) and *Ceropegia intermedia* (Karuppusamy et al. 2009). Shoots cultured on medium containing different concentrations of IBA and IAA (0.25 - 2.0 mg/l) produced not only less number of roots but also weak shoots.

References

Aruna V, Kiranmai C, Karuppusamy S, and **Pullaiah, T** (2009) Micropropagation of three varieties of *Caralluma adscendens* via nodal explants. J. Plant Biochem. Biotech. **18**(1): 121-123.

Baytop T, Tanker M, Öner N and **Tekman S** (1959) Sugars of the glycoside of the root of *Marsdenia erecta* R. Br. Nature **184**: 1319.

Jagtap AP and **Singh NP** (1999) Fascicles of Flora of India, Fasclicle 24. Botanical survey of India, Calcutta, India.: 124-125.

Karuppusamy S, Kiranmai C, Aruna V and **Pullaiah T** (2009) *In vitro* conservation of *Ceropegia intermedia* – An endemic plant of south India. African J. Biotech. **8**(17): 4052-4057.

Komalavalli N and **Rao MV** (2000) *In vitro* micropropagation of *Gymnema sylvestre* - A multipurpose medicinal plant. Plant. Cell. Tiss. Org. Cult. **61**: 97-105.

Kottaimuthu R (2008) Ethnobotany of the Valaiyans of Karandamalai, Dindigul District, Tamil Nadu, India. Ethnobotanical Leaflets **12**: 195-203.

Martin KP (2002) Rapid propagation of *Holostemma ada-kodien* Schult., a rare medicinal plant, through axillary bud multiplication and indirect organogenesis. Plant Cell Rep. **21**: 112-117.

Nagesh KS, Nayaka HMA, **Dharmesh SA**., **Shanthamma C** and **Pullaiah T** (2008) *In vitro* propagation and antioxidant activity of *Curculigo orchioides*. J. Trop. Med. Plants **9**(2): 405-410.

Natarajan D (2004) Identification of conservation priority sites using remote sensing and GIS - A case study from Chitteri hills, Eastern Ghat, Tamil nadu. Curr. Sci. **86** (9): 1316-1323.

Patnaik J and **Debata** (1996) Micropropagation of *Hemidesmus indicus* (L.) R. Br. through axillary bud culture. Plant Cell Rep. **15:** 427-430.

Ramasubbu, R. (2009) Micropropagation and estimation of biochemical constituents in *Pedalium murex* L. and *Physalis angulata* L. J. Sci. Trans. Environ. Technov. **2**(4):226-230.

Sheng-Xiang Qiu, Si-Qi Luo, Long-Ze Lin and **Geoffrey A. Cordell** (1996) Further polyoxypregnanes from *Marsdenia tenacissima* (Roxb.) Moon. Phytochemistry **41**(5): 1385-1388.

Singhal S, Maheshwari P, Khare, and **Anakshi Khare** (1980) Tenasogenin, a Pregnane ester from *Marsdenia tenacissima* (Roxb.) Moon. Phytochem. **19**(11): 2431-2433.

Sreekumar S, Seeni S and **Pushpangadan P** (2000) Micropropagation of *Hemidesmus indicus* for cultivation and production of 2-hydroxy 4-methoxy benzaldehyde. Plant. Cell Tiss. Org. Cult. **62**: 211-117.