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Micropropagation of *Marsdenia brunoniana* Wight & Arn. - A rare antidiabetic plant

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Abstract

Shoot multiplication of *M. brunoniana* Wight & Arn. was achieved 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 medium fortified with 1.0 mg/l BAP. The highest degree of shoot proliferation 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%.

Introduction

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). *Marsdenia* contains many chemical compounds like two polyoxypregnanes, designated marstenacigenins A and B (Sheng-Xiang et al. 1996) and polyhydroxy pregnane ester named tenasogenin in *M. tenacissima* (Singhal et al. 1980). Marsdenin, is a glycoside isolated from *M. erecta* R. Br. (Baytop et al. 1959). Most of them have medicinal value. *M. brunoniana* 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 habitat 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*

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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.

Materials and Methods

Shoots of two-month-old plants of *Marsdenia 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 air flow cabinet. 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. MS was used with 3% (w/v) sucrose and gelled with 0.8% (w/v) agar-agar. The pH of all media was adjusted to 5.8 and sterilized by autoclave at 121°C for 15 min. The cultures were incubated at 25 ± 1°C under a 16 hr 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 at an interval of four weeks. For root induction, *in vitro* microshoots 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 was poured to the plastic cups at five-day regular intervals 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 a two-day 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 ten replicates for each treatment.

Results and Discussion

Nodal explants were cultured on MS fortified with different concentrations of BAP and Kn individually and also in combinations for multiple shoot bud induction and data have been presented in Table 1. Nodal buds when cultured on MS with different concentrations of BAP (0.5 - 5.0 mg/l), produced maximum number of shoots on the medium containing 1 mg/l of BAP within six weeks of incubation, with an average length of 3 cm (Fig. 1A). Increase or decrease in the concentration of BAP beyond the optimum level, a smaller number of shoot buds. These results are in agreement with earlier findings of Ramasubbu et al. (2009) in *Pedalium murex* and *Physalis angulata,* however in the present findings 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* (Komavalli 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 do not enhance shoot proliferation.

Table 1. Effect of concentrations of BAP and Kn on bud breaking and multiple shoot induction from nodal explants of *M. 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 represent an average of three triplicates with ten explants in each M ± S.E.

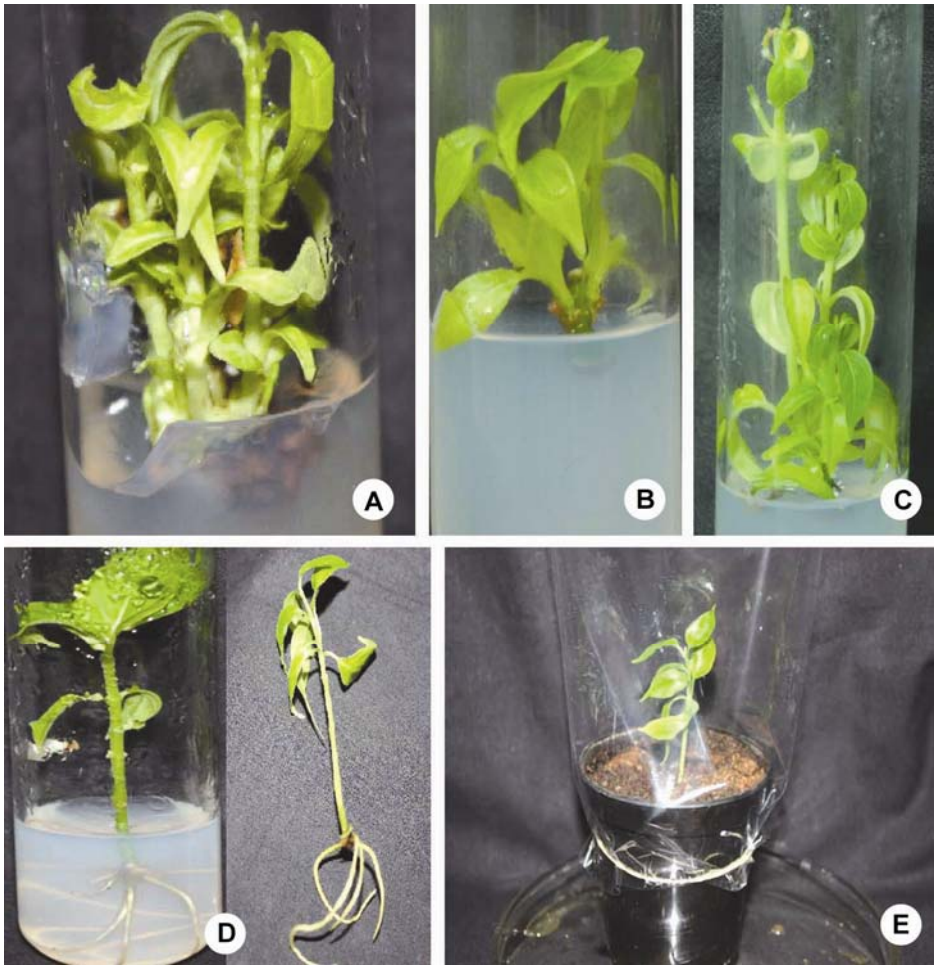

Fig. 1. A. Maximum number of shoot multiplication on MS + BAP 1.0 mg/l. B. Shoot multiplication on MS + Kn 1.0 mg/l. C. Shoot multiplication on MS + BAP + Kn 1.0 mg/l each. D. Root induction on half MS + NAA 0.5 mg/l. E. Plant under acclimation.

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

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 mg/l). Whereas, increase in the concentrations of BAP and Kn decreased the number of shoot buds, this results corroborate 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 indicates that, among different concentrations and combinations of cytokinins (BAP and Kn), BAP alone particularly at 1mg/l induced maximum number of shoot buds when compared to either Kn alone or combined with Kn in different concentrations.

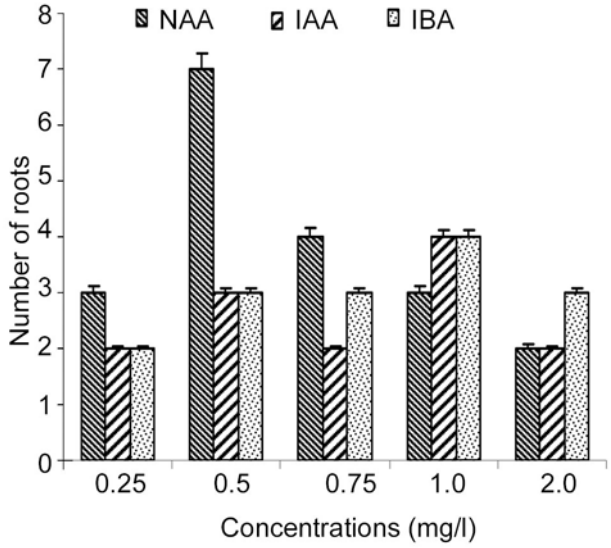

Fig. 2. Effect of different concentrations of auxins on root induction.

In vitro induced shoots were successfully rooted in MS medium supplemented with NAA at strength of 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 the medium containing different concentrations of IBA and IAA (0.25 - 2.0 mg/l) produced not only a smaller number of roots but also weak shoots.

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