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Research Article

IN VITRO PROPAGATION OF A DASAMULA MEDICINAL PLANT-PRITHAKPARNI

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ABSTRACT
Large scale multiplication of Prithakparni, *Uraria picta* (Jacq.) Desv. ex DC was achieved from nodal explants on MS medium supplemented with BA alone. The maximum number (15-20 shoots) of adventitious shoots was regenerated on MS medium containing BA (1mg/l). Root induction on *in vitro* generated shoots was achieved on half-strength MS medium supplemented with IBA (1.5 mg/l). Through this procedure about 196 plantlets were generated from single nodal explant over two subcultures. Micropropagation would be useful to generate large number of clonal propagules and biodiversity conservation.

Key Words: *Dasamula Plant, Medicinal Herb, Micropropagation, Uraria Picta*

INTRODUCTION
A valuable medicinal plant, *Uraria picta* (Jacq.) Desv. ex DC. known as Prithakparni in Sanskrit belongs to the family fabaceae. It is suffruticose herb, up to 1.5 m tall and found in dry grasslands, waste places and open deciduous forests and in all plains of India, extending from Himalayas to Ceylon, Malaysia to Philippines (Okusanya, 1991). It is beneficial in catarrhs, bleeding piles and for scorpion sting (Hamid *et al.,* 2004). Roots are aphrodisiac in nature. They are used for fracture healing properties which is essentially credited to its property of accumulation of phosphorous and deposition of calcium (Anonymous, 1976). Its decoction is useful in cough, chills and fevers. The pulverized leaves of this plant are used medicinally in Southern Nigeria as a remedy for gonorrhea. In Ghana, the plant is used in heart troubles (Chauhan, 1999). In India, plant is used as an antidote against the bites of certain vipers (Allen and Allen, 1981). Its use in Dasamula, an ayurvedic medicine has shown significant improvement in patients of primary neurological disorder (Garg *et al.,* 2012). It is also used in the preparation of Abana, an ayurvedic drug remedy useful in the treatment of hypertension, tachycardia and angina (Khanna *et al.,* 1991). It contains flavonoid, exhibiting a range of biological activities like anti-inflammatory, anti-thrombotic, hepatoprotection properties due to free radical scavenging ability (Patwardan, 2005).
The existence of *Uraria picta* in natural population is highly threatened. It is naturally propagated by seeds. However, the seed set is poor and seed viability and percentage of germination is low (Gurav *et al.,* 2008). However, large-scale production is a pre-requisite to meet the pharmaceutical needs and also for the effective conservation of this valuable medicinal plant. Tissue culture techniques, like micropropagation can be applied to generate large number of propagules and biodiversity conservation especially for those species in which either the roots/rhizome or the whole plant is used in drug preparation (Bajaj, 1986).

MATERIALS AND METHODS
Plants of *Uraria picta* (Jacq.) Desv. ex DC. growing in the Botanical Garden of the Department of Botany, Gujarat University, Ahmedabad, Gujarat were used. Seeds were collected from the healthy plants. The seed coat was removed by H_2SO_4 treatment. Seeds were soaked in concentrated H_2SO_4 (30 min.). Seeds were thoroughly washed with water and finally, seeds were surface sterilized with 0.1% mercuric chloride (3 min) followed by extensive (3-4 times) rinse with distilled water (Gurav *et al.,* 2008).

The seeds were germinated on MS basal medium (Murashige and Skoog, 1962). Single-node explants, isolated from 5 weeks old seedlings were used for shoot multiplication. The nodal explants (2-3 cm long) were inoculated on MS medium containing 3% sucrose, 0.8% agar-agar and supplemented with combinations of cytokinin (BA) and auxin (NAA). For induction of roots, *in vitro* shoots were treated with auxin (IBA and NAA) at various concentrations in half-strength MS medium. The pH of media was adjusted to 5.8 prior to sterilization in autoclave. The cultures were incubated in a culture room with 25°C temperature and 16 hr photoperiod provided by white fluorescent tubes (55 µmol/m^2/sec).
The developed plantlets were treated with 1% (w/v) Bavistin (10 min). The treated plantlets were then planted in plastic cups containing a mixture of soil, sand and compost (1:1:1) in the plastic tunnel (Jasrai *et al*., 1999). Humidity was maintained around transplanted sapling in the tunnel by regular spray with water (2-4 weeks). Hardened plantlets were transferred to a shade-area (covered with jute-sheet). The humidity was maintained by keeping the jute-sheet wet with water.

RESULTS AND DISCUSSION

H_2SO_4 treatment was suitable for 100% seed germination. Seeds without such acid treatment failed to demonstrate germination. Earlier, Okusanya *et al.,* (1991) reported significantly both germination response of *Uraria* under light. The nodal explants of 5 weeks old seedlings were cultured on MS medium supplemented with different concentrations of BA (1-4 mg/l) in absence and presence of NAA (0.5 mg/l), The maximum response (15-20 shoots) for the formation of adventitious shoots (Figure 1A-B) was noted with 1 mg/l BA (Table 1). Increasing concentration of BA demonstrated reduced number of multiple shoots on nodal explants.
Shoot regeneration have been reported earlier on MS medium containing BA alone through callus (Anand *et al.,* 1998) and 13.2 µM BA from nodal explants (Gurav *et al.,* 2008) or in combination with IAA and adenine sulfate (Rai *et al.,* 2010). Ahire *et al.,* (2011) reported micropropagation of *Uraria* through leaf derived callus. The present study exemplifies multiple shoot induction from nodal explants on MS medium with low concentration of BA alone. Excision and culture of *in vitro* regenerated shoots facilitated further multiplication of multiple shoots on same MS medium. On an average, 14 multiple shoots were noted in subsequent subcultures (Figure 1C).

Table 1: Effect of growth regulators on multiple-shoot formation in nodal explants of *Uraria*

Growth regulators (mg/l)		Multiple shoots Mean ±S.E.
BA	**NAA**	
1	-	14.4±3.85
2	-	4.6±0.66
3	-	1±0
4	-	1.3±0.32
1	0.5	8±2
2	0.5	8±2
3	0.5	9.4±1.70
4	0.5	7.8±2.72

In vitro regenerated shoots (2-3cm in length) with 2-3 leaves were used for rooting. The root inducing growth regulators, IBA and NAA were employed in half strength MS medium. Result differed depending upon the concentration, nature and strength of auxins employed. Rooting response was lower in full-strength medium. Among the media tried, half- strength MS medium supplemented with 1.5mg/l IBA was the best (Table 2) for number of roots developed and their growth (Figure 1D). Plantlets were successfully acclimatized without greenhouse facilities (Figure 1E). A high humidity was maintained by a regular spray in the tunnel covering with polyethylene sheet.

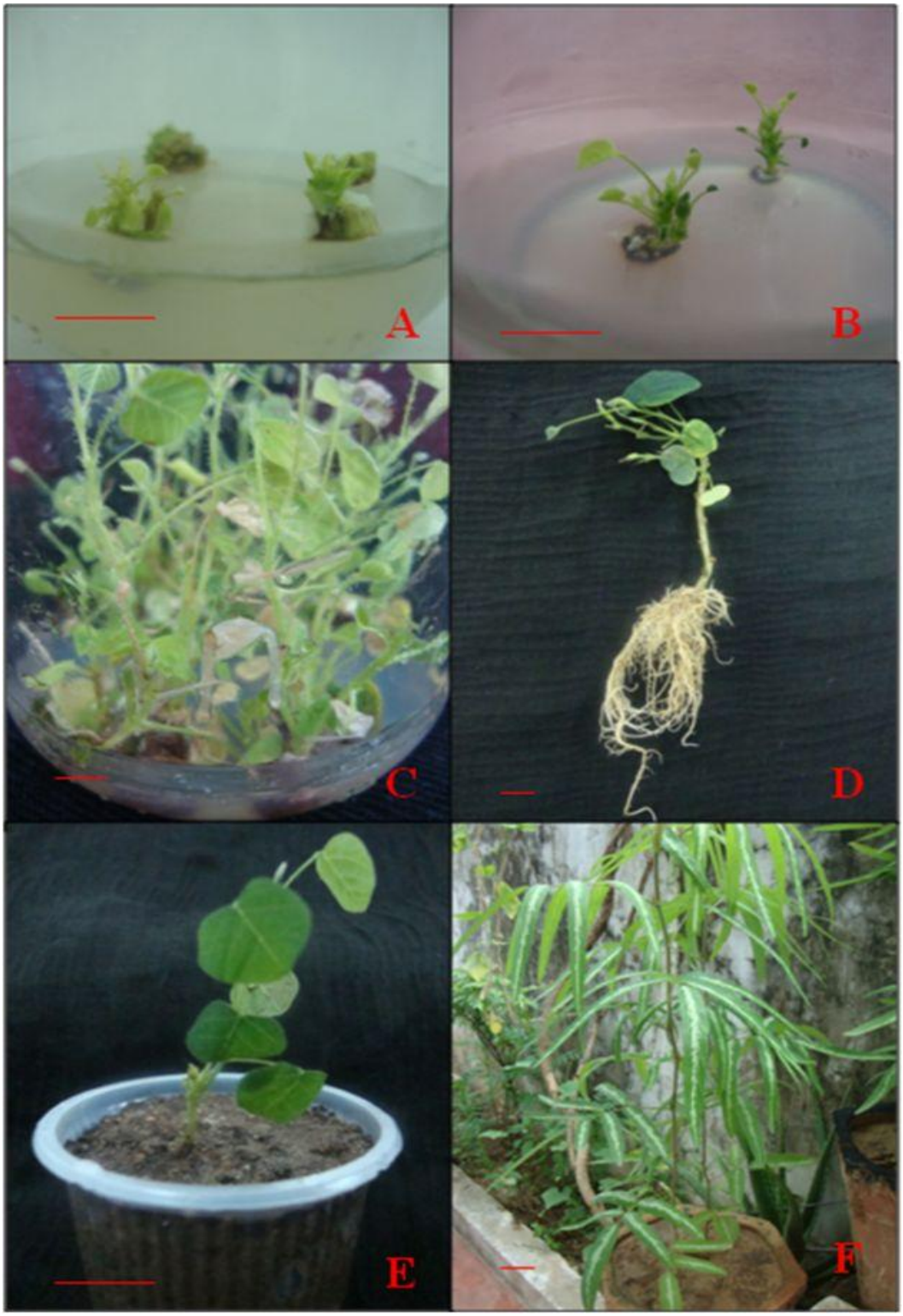

Figure 1: Micropropagation of *Uraria picta* (Jacq.) Desv. ex DC. A) Sprouting of nodal buds; **B)** *In vitro* generated shoots (2 month old); **C)** Multiple shoots in 2nd subculture; **D)** Rooted shoots; **E)** Hardening of *Uraria* plantlets; **F)** Plant in the earthen pot. **Horizontal bar in each photograph is equal to 1 cm.**

Table 2: Induction and root growth on *in vitro* shoots of *Uraria* on half-strength MS medium with various auxins

Growth hormones (mg/l)		No. of roots Mean±S.E.	Root length (cm) Mean±S.E.
IBA	**NAA**		
-	-	1.5±0.49	2.33±1.07
0.5	-	5.66±1.32	2.05±0.71
1	-	4.33±2.7	5.84±1.14
1.5	-	9±1	6.95±2.08
2	-	4±0	3.62±0.55
2.5	-	8±0	6.33±2.22
-	0.5	4±0.57	0.78±0.17
-	1	5.8±1.37	1±0.37
-	1.5	7±3	0.42±0.14
-	2	7±1.29	0.92±0.35

After 4 weeks of hardening, in the shed (jute-sheet), plantlets were transferred to the field. The generated plants exhibited normal features without any morphological variation (Figure 1F). Through this procedure about 196 plantlets were generated from a single explant over two subsequent subcultures. The results described here, demonstrate successful and reproducible clonal propagation of a valuable medicinal plant *Uraria picta*.

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