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Direct Organogenesis in *Leptadenia pyrotechnica* (Forssk.) from nodal segment - An important Medicinal Plant

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ABSTRACT

Micropropagation is a scientific technique which is used grow number of plantlets in a short time duration. In order to enhance the efficiency of plant multiplication via direct organogenesis, the influence of plant growth regulators on Leptadenia pyrotechnica was studied. The best results were recorded with MS medium supplemented with 2.5 mg/l BAP.

Key words: *Organogenesis, plant growth regulatorsclonal propagation.*

INTRODUCTION

Leptadenia pyrotechnica (Forssk.) Decne (Synonym-L.Spartinum Wight) belonging to family Asclepiadaceae is one of such medicinal plants, locally known as Khimp or Khip (Rajasthan), Khimparlo, Thahawar, Ranser (Gujarat), Broom bush (English) is an erect ,ascending ,shrub up to 1.5m-3m high with green stem and pale green alternating bushy branches with watery sap. *Leptadenia pyrotechnica* (forsk) decne,is a valuable desert plant which is commonly used in traditional system of medicine for relieving pain and inflammation, as well as in a number of metabolic disorders such as diabetes and obesity. It is common throughout the state of Rajasthan and found in dry habitats particularly in desert zones. In India it is commonly found in Banswara, Palod, Dungarpur and Bikaner.

Leptadenia pyrotechnica is a straight, broomlike shrub, 1.0 m-3.0 m high with green stem and pale green alternating bushy branches. It contains watery sap. The leaves are rarely found and if found, they are small deciduous and linear-lanceolate. The flowers are clustered in short auxillary chymes and are greenish yellow. The sepals of each flower are joined at base only and flower is bisexual pentamerous acting morphic. The seeds are 57mm long, numerous, comose (hairy) with tufted hairs of about 2.5-3.5 cm long flowering and fruiting of *Leptadenia pyrotechnica* occurs from August to January.

The application of tissue culture as a conservation tool of the threatened plants has gained huge thrust in the last two decades as one of the process of ex situ conservation strategy. Regeneration of in vitro derived plants may follow either somatic embryogenesis or organogenesis path of differentiation. Organogenesis is characterized by the production of unipolar bud primordial with subsequent development of shoot and roots while in somatic embryogenesis, the bipolar structure resembles a zygote embryo that develops from somatic cell. Somatic embryos may develop directly on somatic cell or indirectly from callus aggregates during the culture of plant cell, tissue or organ.

Adventitious shoot regeneration form suitable explants is one of the desired in vitro techniques applied for large scale propagation of target plant species, which can be achieved through two different processes direct and indirect. Indirect shoot regeneration implies regeneration of shoots via callus formation while direct shoot regeneration can occur directly from the explants without intermediate proliferation of undifferentiated tissues. In addition, indirect process for adventitious shoot regeneration may produces variation in next generation, which is the major drawback for clonal propagation. That is why direct shoot organogenesis is preferred over indirect process in case of several plant species.

MATERIAL AND METHOD

Explants Collection

The branches (about 5-6 cm) of shoots of *Leptadenia pyrotechnica* (Forssk.) plants were collected from germinated seeds in M Bridge mehthos.

Procurement of Explants

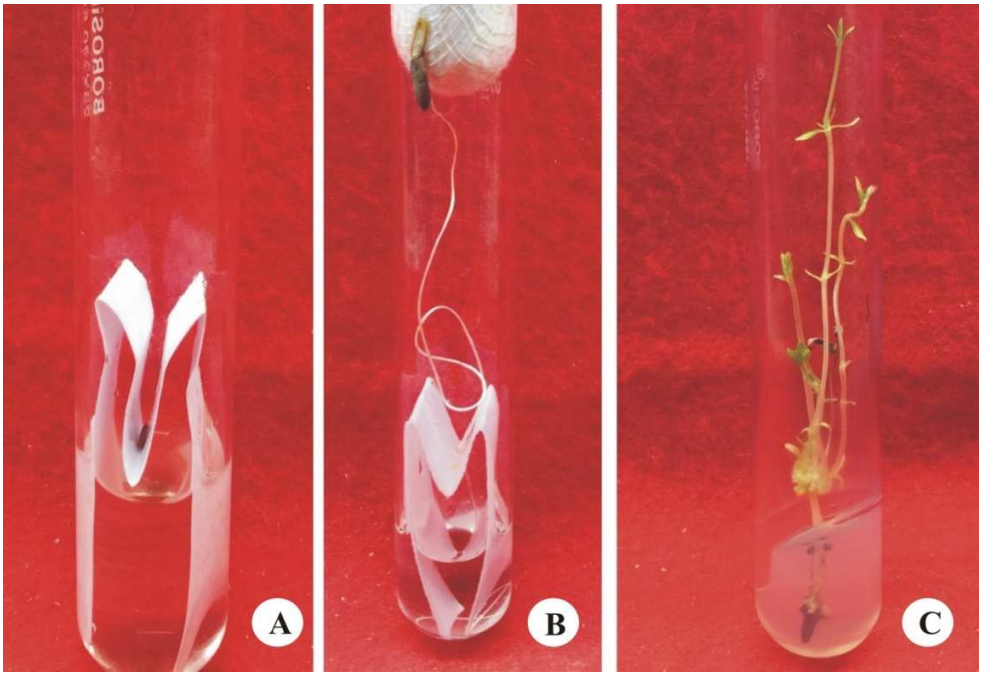
The branches with node explants were washed in running tap water and then washed again thoroughly by adding a few drops of Tween-20 to remove the superficial dust particles as well as fungal and bacterial spores. They were surface sterilized with 0.1% $HgCl_2$ for 5 min followed by rinsing them five times with double distilled water inside the Laminar Air flow chamber.

Plant regeneration

Nodal segments (with a single axillary bud) about 0.5-0.8 cm were prepared aseptically and were implanted vertically on MS medium prepared with specific concentrations of BAP, (1.0-5.0 mg/l) singly for shoot proliferation. Same experiments were repeated for shoot multiplication.

The medium containing 3% sucrose was solidified with 0.8% agar (Qualigens). The pH of the media was adjusted to 5.9±0.02 with 1 N NaOH or 1 N HCl solutions prior to autoclaving. Media poured in culture vessels were steam sterilized by autoclaving at 121°C and 15 psi for 15-20 min. The cultures were incubated under controlled conditions of temperature (25±2°C), light (2000- 2500 lux for 16 h/d provided by fluorescent tubes) and 60-70% humidity. For each experiment a minimum of 7 replicates were taken and experiments were repeated thrice. Observations were recorded after an interval of 3 wk. Once culture conditions for shoot induction from explants were established, the shoots produced *in vitro* were subcultured on fresh medium every 3 wk. The nodal and shoot tip explants were inoculated in various concentrations of BAP.

Fig. 1 (A-C) Micropropagation of *Leptadenia pyrotechnica* (Forssk.)from nodal shoot explants



A. Seed germination in M bridge within a week, **B.** Shoot elongation in M bridge by germinating seed within a week **C.** Shoot multiplication on MS medium supplemented with 2.5 mg/l BAP.

RESULTS AND DISCUSSION

The nodal explants, when inoculated on MS medium containing BAP in the range 1.0-5.0 mg/l showed enhanced shoot proliferation. BAP at its 2.5 mg/l concentration evoked best response. Shoots after their initial proliferation on medium containing 2.5 mg/l BAP were sub-cultured on same fresh medium after every 21 days. Incorporation of BAP or Kn into MS medium supported multiplication of shoots in culture, BAP proved to be a better choice than Kn and the maximum number of shoots were obtained on its 2.5 mg/l concentration (Table 1, Fig. 1- A, B, C.

Among these, the maximum number of shoots (3.28±0.31) and shoot lengt (8.43±0.34) was developed on MS media fortified with 2.5 BAP.

Table 1: Effect of cytokine (BAP) on shoot proliferation from nodal shoot explant of *Leptadenia pyrotechnica* (Forssk.)

Hormone Concentration (mg/L) BAP	Response (%)	Number of Shoot/explants (mean±SE)	Shoot length (in cm) (mean±SE)
1.0	82	4.45±0.61	5.38±0.43
1.5	85	3.22±0.36	5.35±0.63
2.0	81	4.52±0.48	6.29±0.86
2.5	85	5.28±0.31	8.43±0.34
3.0	60	3.85±0.42	6.11±0.43

Medium: MS+ additives; mean± SE, n= 7 replicates

Means having the same letter in each Column dose not differentiate significantly at P< 0.05 (Tukey's test)

CONCLUSION

The seedlings derived from seed germinating in M bridge method. Being juvenile, are frequently used for micropropagation, as they are easy to establish in culture. In *Leptadenia pyrotechnica* (Forssk.), MS medium supplemented with 2.5mg/l BAP concentration proved best. On this medium maximum shoot length (8.43±0.34 cm) and number of shoots (5.28±0.31) was obtained.

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