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RESEARCH ARTICLE

MICROPROPAGATION AND CONSERVATION OF ENDANGERED MEDICINAL PLANT-LEPTADAENIA RETICULATA (RETZ.) WIGHT &ARN.THROUGH NODAL EXPLANT

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A B S T R A C T

Experiments were conducted for micropropagation of endangered medicinal plant Leptadaenia reticulata (Retz.) Wight &Arn using Nodal explants by modified MS medium. The explants of *Leptadaenia reticulata (Retz.)*Wight.&Arn started growing in MS medium supplemented with NAA in combination with BAP within three weeks. The highest percentage and maximum number of shoot induction i.e6 shoots per explantfrom nodal segment was observed on MS medium supplemented with NAA (2.00mg/l) with BAP (4.0mg/). The pre3sent investigation can be very useful for conservation of this endangered medicinal plant.

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INTRODUCTION

Leptadaenia reticulate (Retz) wight&Arn belongs to family Asclepiadaceae is an important medicinal plant. This plant is commonly known as Jivanti, Dori or Swarn. Indian synonyms: Bengali: Bhadjivai, English: Leptadenia, Gujarati: Methidodi or Dodi, Hindi: Dori. Jivanti is jeevana tonic that boosts energy level of the body as per according to ayurveda. The basic healthcare needs of more than 80% ofworld's population depend primarily on herbalmedicine as estimated by the World HealthOrganization[1].This plant is considered to be a *Rasayana* (tonic) drug and is thus used to vitalize, nourish and rejuvenate the body [2] and included among the 10 drugs constituting the jvaniyagana or vitalising group [3]. Rasayana is one of the classes of Ayurveda that improve the general health of the body .Rasayana nourishes and rejuvenates the body and increases longevity, memory enhancement, immunomodulation and adaoption[4.] .

Leptadaenia reticulate (Jivanti) is distributed in tropical and sub-tropical parts of Asia and Africa. In India, it is found in Gujarat, sub-Himalayan tracts from Punjab to Sikkim and Khasi hills and throughout peninsular India, ascending up to an altitude of 900 metres[5]. .Herbs are a natural path to good health such as JIVANTI in Sanskrit literature, the name (jiv =life) indicates that the plant is considered to have the ability to bestow health and vigour. It is an Indian medicinal plant used enormously due to its great value in general debility, involuntary seminal discharge, as a stimulate tonic [6] as a bactericidal, anti fabrifuge, wound healer and in mouth ulcer [7]. Roots are used in many ayurvedic and herbal formulations [8] as a cure for ear, nose, and skin infection [9]. It is also used for increasing milk-yielding capacity in cattle [10,11] and to increase the egg laying capacity of hen in poultry industry. Flowers are good for eye sight. The flowers and tender leaves are used as vegetable and to make bread [12].It is also found to be effective in diseases, wounds andinflammation related to skin, fever, cough (with phlegm),dehydration, tuberculosis, colitis, chicken pox, dysentry,respiratory disorders, eye diseases and night blindness.

Further it can also be applied in treating various bodyailments like bleeding disorders, burning sensation of the body. The extracts of roots and leaves of the plant are found to be possessing antibacterial and antifungalactivities. It also promotes gametogenic and androgenicfunctions of the testes of animal [5].

A cooling, mucilaginous, demulcent with light strengthening and tonic properties of this plant is traditionally used in the treatment of seminal discharges and snake bite [13].Leptadaenia reticulata is such an economically important medicinal plant species of Indian Thar desert [14,15].

Huge demand and multipurpose uses of these plants in pharmaceutical industries, population bloom, urbanization, over-exploitation and recurring drought and famine in this region make these plants species endangered .The increasing demand for this plant material and loss of habitat will put this medicinal species under more pressure which may endanger human health .To date, there are only few reports on the micro propagation of *Leptadaenia reticulata*[16,17,18,19,12].Along with it the low viability of seeds and poor capacity of germination rate restricts the propagation of *Leptadaenia reticulata* through seeds. Plant tissue culture as a technology for ex-situ multiplication is fast and uses small amount of shoots and may succeed when other methods fail [20].Plant tissue and cell culture has an important role to play in solving the problems related to plant improvement. Cell and tissue culture technology if suitably developed may help improve system productivity [17.18].The present study is aimed to develop an

efficient in–vitro method for conservation and micropropagation of *Leptadaenia reticulata* through the nodal segments culture followed by successful regeneration micro-propagated plant in the field condition.

MATERIALS AND METHODS

Healthy and young shoot cuttings of *Leptadaenia reticulata* bearing 6 to 8 nodes were collected from mature plants growing in AFRI Nursery.After removing the leaves, the nodal segments (1- 1.5cm) were thoroughly washed under running tap water (10 min) and washed with Tween 20 for 10 minutes, followed by treatment with bavistin (15 min) then washed with distilled water 4-5 times. Under aseptic condition, the explants were surface sterilized with 70% ethanol then washed with autoclaved distilled water 2-3 times , followed by treatment with 0.1% of mercuric chloride for 4 minutes and finally washed six times with autoclaved distilled water.These explants were kept in chilled antioxidants (ascorbic acid 100 mg/L,citric acid 50 mg/L) for 20 minutes to prevent browning of explants. The surface sterilized explants were cultured on various media such as M.S[21]and B5[22]. MS medium used, had the concentration of 3%sucrose and 0.8% agar.

The surface sterilized explants were cultured on MS media fortified with various concentrations of plant growth regulators (NAA/BAP) at varied concentration (1.0mg/l – 4.0 mg/l of BAP) and (0.5 mg/L - 2 mg/L of NAA)for bud sprouting and multiple shoot induction. The in vitro developed shoots from nodal segments were excised and transferred to MS medium with various auxins (IAA/ NAA/IBA) at varied concentration (0.5-4.0 mg/l) for their root induction. The pH of the medium was adjusted to 5.8 prior autoclaving at 121°C for 15 min. The cultures were kept under dark for 2 days initially and then transferred to culture room which were maintained at 25±2°C with 16 hrs photoperiod under fluorescent light. Each and every experiment were performed with 24replicates and repeated twice and the pH of the media were adjusted to 5.8 with 1N NaOH or HCl were dispensed in to jam bottles. The media were autoclaved at 121ºC at 15 psi pressure for 15 min.. In vitro derived shoots from the explants were excised after 4 weeks and sub cultured on to a fresh medium with the same concentrations of growth regulators. For rooting, 6 -8 cm long regenerated shoots were excised and cultured on half strength or full strength MS medium supplemented with different concentrations of activated charcoal (200 mg/l), IBA (0.5, 0.1,1.5 and 2.0 mg/l) for rooting. Plantlets with well developed root and shoot system were removed and transferred to to small plastic pots having a sterile 'soilrite' (soilless compost and soil conditioner) and acclimatize.

RESULTS AND DISCUSSION

The explants of *Leptadaenia reticulata (Retz.)* Wight.&Arn started growing in MS medium supplemented with NAA in combination with BAP within three weeks. The highest percentage and maximum number of shoot induction i.e6 shoots per explantfrom nodal segment was observed on MS medium supplemented with NAA (2.00mg/l) with BAP (4.0mg/) (Table-1) (Plate-1). For the shoot regeneration, cytokinin is effective when used in combination with an auxin [23]. The combination of NAA and BAP are also well known to induce multiple shootlets in different plant species [24,25,26,12]. Multiplication of in vitro raised plants were done on M.S medium supplemented with NAA (2.00mg/l) with BAP (4.0mg/) .For induction of roots, *in vitro* grown shoots of size (6- 8cm) were transferred to half and full strength MS medium along with different concentration of IAA, IBA and activated charcoal. The best rooting was evidenced with the use of half strength MS medium.

Table 1 Effect of various conc. Of PGR's on shoot proliferation from stem nodal explants of *Leptadaenia reticulate*

MS + conc Of (mg/L) BAP	PGR'S NAA	Percentage of explant sprouted	Mean no. of shoots ±SD	Mean length of shoots±(cm)
1.0	0.5	30	1.14 ±0.76	3.64±0.26
2.0	1.0	50	3.16 ±1.06	4.62 ±0.54
3.0	1.5	75	4.13 ±2.68	4.34 ±0.36
4.0	2.0	85	6.12 ±0.82	3.82±0.26

Data from 24 replicates in two experirnents (Mean±SD)

Table 2 Effect of MS medium supplemented with different concentrations of IBA on in vitro rooting of *Leptadaenia reticulate*

MS + IBA (mg/L) IBA	Percentage of rooting	Mean no. of roots ±SD	Mean length of roots±(cm)
0.5	30	4.0±0.14	0.8±0.18
1	80	5.8±0.26	3.4±0.43
2	70	3.6±0.42	2.6±0.16
3	50	2.8±0.44	2.4±0.54
4	40	2.2±0.12	1.3±0.46

Data from 24 replicates in two experirnents (Mean±SD)

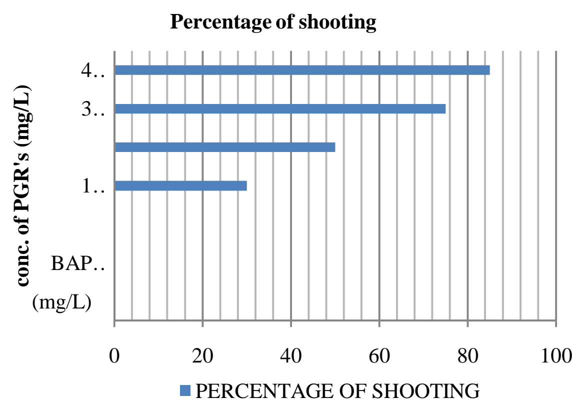

Figure 1 Growth period 25 days

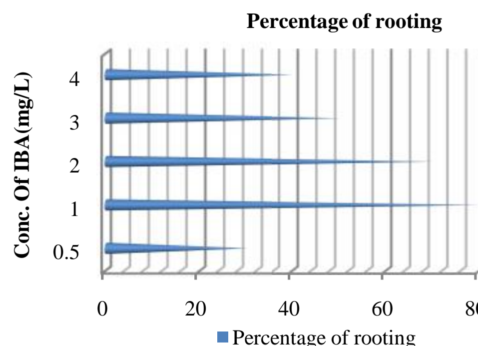

Figure 2 Growth period 30 days

Of the different concentration hormone tested, best response was obtained with 1.0 mg/l IBA (Table 2), where as IAA to

produce satisfactory result. Though the roots were observed in case of 0.5mg/l IBA but are short and weak as compare to the roots developed in case of 1.0mg/l IBA. Effect of IBA in root induction was also reported in many plant [27,28,29,30,31,12]. The use of activated charcoal forrooting produced the better results, so the same can be used further as the substitute for hormone and also to minimize cost of media. Similar results are also been reported in [32].After 25 days plantlets with well-developed shoot and root system were transferred to cups containing sterile 'soilrite' (soilless compost and soil conditioner) .The plants were kept in a culture room for 20 Days. 60% of plants were successfully established in polycups. After 20 days the polycups hardened plants were transferred to pots and kept in green house, then sequential hardening process was carried in the field. High relative humidity was maintained by covering the plants with transparent polythene bags under controlled condition. After 4 weeks, the plants were transferred to the soil and 82% survival was recorded.

A -Bud breaking from ex plant. B-Multiple shoots production.

C-*In vitro* rooted plantlet. D-Acclimatization of *In vitro* raised plant.

Figure 3 Different Stages In Micropropagation Of *Leptadaenia reticulata.*

CONCLUSION

The natural strand of this species is first disappearing due to its restricted distribution and indiscriminate exploitation for medicinal use by pharmaceutical industry. As a result, it is now listed as an endangered species by the international union for conservation of nature and natural resources. The plant is the one of the ingredient of many formulations which has been used to recover from physiological, bacterial diseases or even form cancer.Plant tissue culture as a technology is alternate method for its propagation and has been increasingly exploited to achieve success. Thus the present study reveals the usefulness of *Leptadaenia reticulate* (Retz.) Wight &Arn.for several medicinal purposes indicates the potential of this plant as a source of potent drugs. Hence, there is a need for developing alternative method for quick and efficient method for conservation and utilisation of germplasm of *Leptadaenia reticulate* (Retz.) Wight &Arn.Our present investigation offers a potential and reliable protocol for large scale production of *Leptadaenia reticulata* (Retz.) Wight & Arnthrough nodal segmentsfor conservation and mass propagation of *Leptadaenia reticulata.*

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