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EFFECT OF PGRS ON ORNAMENTAL PLANT *BRYOPHYLLUM PINNATUM (*LAM.) KURZ

Kamlesh Choure[2], *Shinam Mukhija[1], Chandra Gurnani[1] and Vikram Kumar[1]

[1]Department of Biotechnology, Iase (D) University, Sardarshahar, Churu, Rajasthan.
[2]Department of Biotechnology and Microbiology, Faculty of Life Sciences & Tehnology, Aks University, Satna, Madhya Pradesh.

***Author for Correspondence: Shinam Mukhija**
Department of Biotechnology, Iase (D) University, Sardarshahar, Churu, Rajasthan.

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ABSTRACT
Bryophyllum pinnatum is widely used in ayurvedic system of medicine as astringent, analgesic, carminative and also useful in diarrhoea and vomiting. It is naturalized throughout the hot and moist parts of India. The leaves of *B. pinnatum* have a variety of uses in the traditional system of medicine in India. They are eaten for diabetes, diuresis, dissolving kidney stones, respiratory tract infections, as well as applied to wounds, boils, and insect bites. It is useful for preventing alcoholic, viral and toxic liver damages. The aqueous extract of this plant have shown anti-inflammatory, anti-diabetic, anti-tumor and cutaneous leishmanicidal activities. Best media for Maximum shoot regeneration and shoot length was 0.5mg/l BAP + 1.5mg/l IBA + 1.5mg/l NAA while MS medium while MS Medium with 0.5mg/l BAP + 2.5mg/l IBA + 2.5mg/l NAA in MS media is best for maximum Root regeneration and length.

KEYWORDS: *Bryophyllum pinnatum*, ayurvedic, astringent, carminative.

INTRODUCTION
Plants are important source of medicines and play a key role in world health[1]. In modern medicine, plants are used as sources of direct therapeutic agents, as models for new synthetic compounds and as taxonomic marker for discovery of new compounds. They serve as a raw material base for the elaboration of more complex semisynthetic chemical compounds[2]. The synthesis of bioactive compounds chemically is difficult because of their complex structure and high cost[3]. Herbal preparations from field grown plants are susceptible to infestation by bacteria, fungi and insects that can alter the medicinal content of the preparation[4]. Supply of plants for traditional medicines is failing to satisfy the demand[5]. An efficient and most suited alternative solution to the problems faced by the phyto-pharmaceutical industry is development of *in vitro* systems for the production of medicinal plants and their extracts. The *in vitro* propagated medicinal plants furnish a ready source of uniform, sterile and compatible plant material for biochemical characterization and identification of active constituents[6], [7].

Plant tissue culture techniques have been increasingly applied to many medicinal plants in particular for mass propagation, conservation of germplasm and production of bioactive compounds and for genetic improvement. Large scale plant tissue culture is found to be an attractive approach to the traditional methods of plantations because it offers controlled supply of biochemical independent of plant availability and more consistent product quality[8]. Genus *Bryophyllum* of the family Crassulaceae is a valuable medicinal as well as ornamental plant. These plants are cultivated as ornamental house plants and rock or succulent garden plants[9]. *B. pinnatum* is the air plant, miracle leaf or life plant is a native to Madagascar. It is a popular houseplant and has become naturalized in temperate regions of Asia, The pacific and Caribbean, Australia, New-Zealand, West-Indies, Macaronesia, Mascarenes, Galapagos, Melansia, Polynesia and Hawaii[10].

Bryophyllum pinnatum is used in ethno medicine for treatment of earache, burns, abscesses, ulcer, insect bites, diarrhea and Lithiasis[11]. It has been recorded in Trinidad and Tobago as being used as traditional treatment for hypertension and for the treatment of Kidney stones[12]. In South Eastern Nigeria, this herb is used to facilitate the dropping of the placenta of a newly born baby[13]. It is useful to prevent alcoholic liver damage, viral liver damage, and toxic liver damage and to treat ailments such as infections, rheumatism and inflammation. Alcoholic extracts of *B. pinnatum* showed antimicrobial activity against a number of Gram (+ve) and (-ve)

bacterial strains[14]. Three compounds make *Bryophyllum* their unique medicinal value that is Bryophilin A which shows anti-tumour activity others Bersaldegenin-3-Acetate and Bryophilin C which shows insecticidal properties[15]. It can also lower blood pressure, blood sugar levels showed antioxidant effects which makes it health strengthening agents. Its roots contain glycosides, essential oils and alkaloids.

Now it becomes endangered plant which needs to be conserved as well as explored for its significant green chemistry[16].Thus the aim of the present study was to propagate endangered plant *Bryophyllum pinnatum* by tissue culture for conservation & sustainable development in present scenario.

MATERIALS AND METHODS

Study site

The study was conducted in the Department of Biotechnology, IASE Deemed University, Sardarshahar.

Collection of Explant

In present study, leaves were used as explant. Leaves of *B. pinnatum* were collected from the garden of Maharshi Dayanand college, Sriganganagar under the supervision of Dr. Sudesh Dhingra, Head of Department Biotechnology and transported to Biotechnology lab for the further processing.

Surface Sterilization

Leaves were washed under tap water to remove sand and dust particles and then thoroughly washed with Teepol for about 10 minutes then leaves were rinsed with tap water to make it free from detergent. It was followed by dipping of the leaves in 0.1% mercuric chloride for 5-6 minutes. The leaves were then washed with autoclaved water for at least three times to remove the smell of bleaching solution[17].

Inoculation of Explant

Sections of Equal sizes (1x1 cm^2) were cut from these leaves and were grown on MS (Murashige & Skoog 1962) media supplemented with various concentrations of BAP (0.5-2.5mgl^{-1}), IBA (0.5-2.5mgl^{-1}) and NAA (0.5-2.5mgl^{-1}).

Shoot & Root induction

Surface sterilized nodal explants were cut as described by Spripaoraya et al[18]. The nodal explants of *Bryophyllum pinnatum* were cultured on Murashige and Skoog medium supplemented with 3% w/v Sucrose, different concentrations of BAP, NAA, and IBA which is solidified with 0.8% Agar. The regenerated shoots were separated individually and transferred on MS media containing different concentrations of NAA (0.5-2.5mg/l) or IBA (0.5-2.5mg/l) for proliferation of roots. The pH of media adjusted to 5.8 with 1N NaOH, 1N HCl and autoclaved at121˚C temperature with 15 lbs pressure for 20 minutes. Inoculated explants were kept under control environment with 2500 lux light intensity at temperature of 25±2˚C for 16 hours photoperiod[19]. Data were collected after two weeks including response of shoot and root regeneration (Table 1).

Hardening & Acclimatization

Rooted plantlets were carefully removed from the culture tubes and their roots were thoroughly washed under running tap water and cleaned with fine brush to remove adhered agar. After that the plantlets were covered with sterilized cotton wetted with half strength MS medium for 24 hours in culture room followed by the treatment of Bavistin (0.2% for 10 minutes) to prevent fungal contamination. Finally plantlets were transferred to pots containing sterilized soil, vermiculite and perlite in equal proportion. Pots were kept in greenhouse with 90% humidity and temperature 26±2˚C[20].

Statistical Analysis

Data were collected on shoot and root regeneration. The experiments were laid out in completely randomized design (CRD). Each treatment was replicated thrice and 10 test tubes were used per replication. The data collected was analyzed by SPSS software (Version 13.00) and the means were compared by one way ANOVA.

RESULTS AND DISCUSSION

The investigation has been carried out to infer the influence of different growth regulators on *Bryophyllum pinnatum*. The exploration was conducted on 17 sets which were imparted with various concentrations of BAP (0.5-2.5mg/l), NAA (0.5-2.5mg/l) and IBA (0.5-2.5mg/l) growth regulators. Besides, the range of various growth regulators was maintained according to the given table. These experimental sets were further tested for pattern of growth occurred in shoot regeneration as well as root regeneration. As shoot proliferation and multiplication was perceived within 8-10 days. Hence the outcome which was interpreted from the above consideration reveals that maximum percent shoot and root regeneration took place in A3 and A16 set respectively which was 80±1.45 % for the former and 75±1.84 % for the latter.

Further on the statistical analysis Mean±S.E. and ANOVA was procured at .05 probability level of significance which lead to our understanding showed remarkable difference of Significance, Highly significance and insignificant. This further leads to a conclusion that the shoot regeneration which was acquired in set A3 was statistically highly significant while regeneration in A15 was more and statistically highly significant. The level of significance and highly significance is indicated in the table which emphasize on the efficiency of supplied growth regulators. To enlightened the topic more precisely results are also presented graphically.

Different concentrations of BAP, IBA and NAA had highly significant effect on the shoot and root

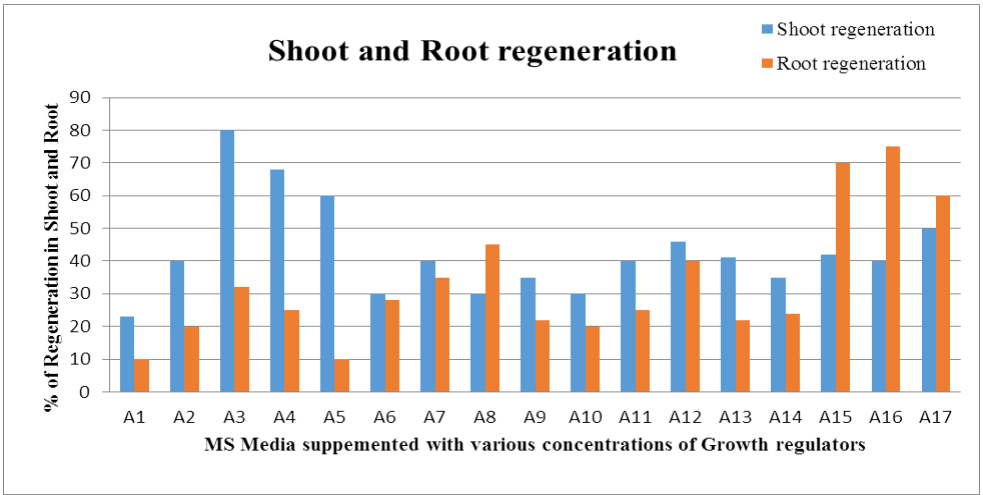
regeneration. Barik et al had obtained direct shoot regeneration in combination of BAP and NAA in *Lathyrus sativus*[21]. A cytokinin supplement to MS was essential to induce shoot proliferation. A combination of cytokinin and auxin improves the percentage of shoot regeneration as well as the shoot number and shoot length. Similar type of response was observed in medicinal plants like *W. somnifera*[22], [23], *Abutilon indicum*[24], *Bryophyllum pinnatum*, *Bryophyllum daigremontianum*[25]. Growth regulators added to the growth media during the shoot proliferation stage played a definitive role in growth enhancement of *Bryophyllum* cultured *In vitro*.

Bryophyllum pinnatum produced 2.8 times times more shoots in 10^{-6}M BAP as compared to water[26]. Karapoff reported that bud initiation was stimulated by BAP and he obtained average of 1.18±0.82 roots in 10^{-6} M BAP[27]. IBA has been reported to have a stimulatory effect on root induction in many medicinal plant species including *Withania somnifera*[28], *Centella asiatica*[29] and Ginger[30]. The rooted plants were acclimatized ex vitro in the green house. The In vitro grown plants performed well under field conditions and they were morphologically identical to the mother plants. Assays of acclimatization carried out by other authors working with different species of *Bryophyllum* showed that plantlets obtained *in vitro* are easily acclimatized[31], [32]. Micro-propagation is more rapid, continuous and efficient than propagation *via* conventional cutting because it can supply uniform and consistent plant material for investigations of important secondary metabolites produced by this species, as well as its use as an ornamental plant. The procedure reported in this paper suggests that tissue culture could be a commercially feasible method for *Bryophyllum* propagation and will also considerably facilitate large-scale propagation and conservation of this medicinally important plant species.

Table 1 showing shoot and root regeneration

Code	Concentration of Growth Regulators in mg/l			Shoot regeneration (%)	Root regeneration (%)
	BAP	NAA	IBA		
A1	0.5	0.5	0.5	23±1.52a	10±0.66a
A2	1.0	0.5	0.5	40±0.88a	20±0.33a
A3	1.5	0.5	0.5	80±1.45b	32±1.52b
A4	2.0	0.5	0.5	68±0.88b	25±2.33b
A5	2.5	0.5	0.5	60±1.45b	10±0.57b
A6	0.5	1.0	0.5	30±0.88a	28±1.45a
A7	0.5	1.5	0.5	40±2.33c	35±1.52c
A8	0.5	2.0	0.5	30±0.33b	45±0.0b
A9	0.5	2.5	0.5	35±3.17a	22±1.52a
A10	0.5	0.5	1.0	30±1.45a	20±1.76a
A11	0.5	0.5	1.5	40±0.0b	25±0.33b
A12	0.5	0.5	2.0	46±1.52c	40±4.04c
A13	0.5	0.5	2.5	41±0.88a	22±1.45a
A14	0.5	1.0	1.0	35±2.72a	24±0.57a
A15	0.5	1.5	1.5	42±1.66a	70±1.52a
A16	0.5	2.0	2.0	40±1.20c	75±1.84c
A17	0.5	2.5	2.5	50±0.57a	60±2.33a

Values shown are Mean±S.E. Values with different letters within columns differ significantly at p=0.05 Significant; b- Highly significant; c-Non significant



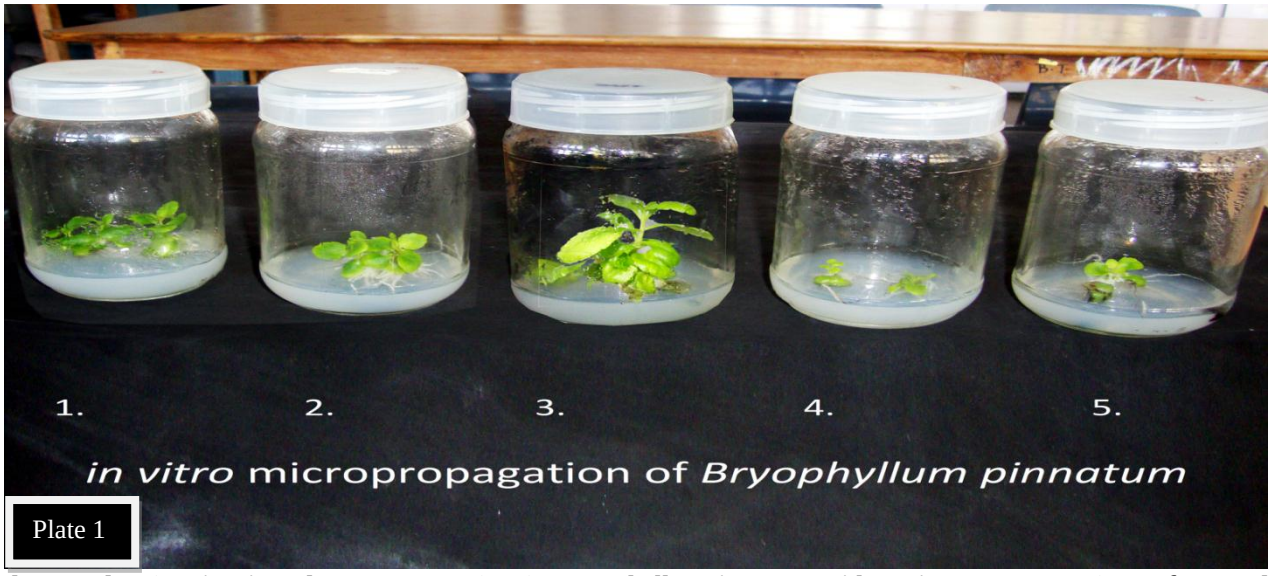


Plate 1 Showing *in vitro* shoot regeneration in *Bryophyllumpinnatum with various* concentrations of Growth regulators

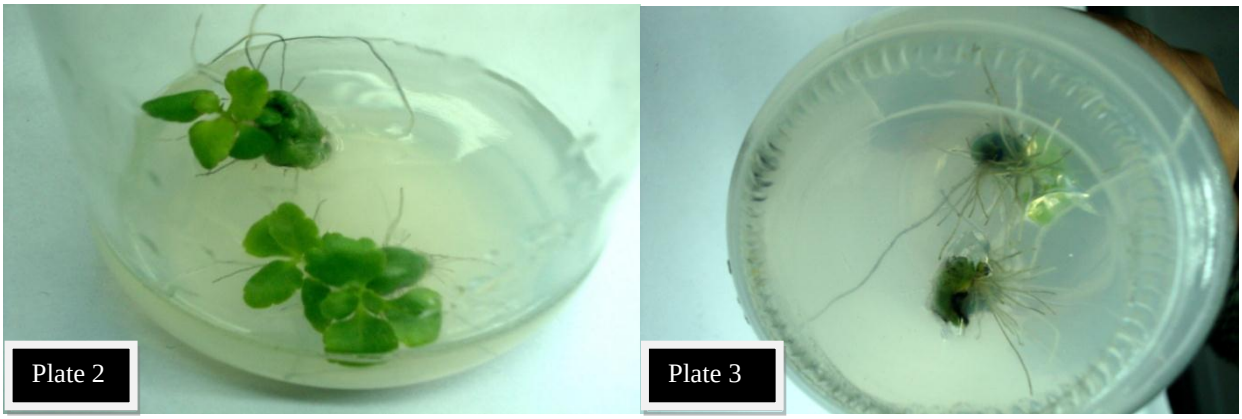


Plate 2 and 3 showing shoot and root regeneration

Plate 4 showing shoot and root length

CONCLUSION

Eventually all in all it can be deduce from the above findings that tissue culturing method employs Micro-propagation has been extensively used in elaborating the utility of plant. *Bryophyllum pinnatum* justify its use for the production of secondary metabolites for research to be carried out efficiently.

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