DoI: https://doi.org/10.58537/jorangrau.2025.53.1.01

J. Res. ANGRAU 53 (1) 1-10, 2025

IN VITRO CALLUS INDUCTION AND PLANT REGENERATION IN *BRYONIA LACINIOSA* FROM LEAF

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Date of Receipt : 25-01-2025 Date of Acceptance : 18-03-2025

ABSTRACT

This study investigates effective sterilization methods for the *in vitro* culture of *Bryonia laciniosa* (Linn.) in 2022-23, revealing optimal disinfection with 0.2 per cent sodium hypochlorite followed by fungicide (Sofia) treatment. Although 0.3 per cent mercuric chloride yielded high explant survival, it elevated culture mortality, favoring sodium hypochlorite for successful disinfection. An efficient protocol for callusing and regeneration via direct and indirect means was established using leaf segments. The different forms of callus such as compact, nodular and green callus were produced only multiple shoots. Better quality of pure callus was obtained in the medium fortified with MS nutrients, 3.0 mg/L 2,4-D. The induced callus produced multiple shoots on the same mother medium after 3-4 weeks of culture. The induced shoots were also elongated in the same mother medium without the addition of any other plant growth regulator. 7 shoots were regenerated in leaf explants in MS medium with KIN (4 mg/L) and IAA (1 mg/L).Elongated shoots took roots supplemented with 4.0 mg/L KIN and 1.0 mg/L IBA. The regenerated plants were successfully hardened into pots after proper acclimatization. These findings underscore the potential of explants for callus induction, morphological analysis, and indirect plantlet regeneration, emphasizing the importance of *in vitro* techniques in preserving endangered medicinal plants.

Keywords: *Bryonialaciniosa*, leaf, rooting, Regeneration, Callus and MS medium.

INTRODUCTION

Bryonialaciniosa (Linn.), also known by its synonym *Diplocyclospalmatus*, is a medicinal plant from the family Cucurbitaceae, widely recognized in India for its diverse therapeutic applications. Locally called “Shivlingi” due to the resemblance of its seeds to a Shivling, it is also referred to as the Lollipop Climber or Striped Cucumber Plant. The plant is known by multiple botanical synonyms, including *Bryonopsislaciniosa* and *Diplocyclo-spalmatus*, and has numerous Sanskrit names such as Linguini, Bahupatra, Ishwari, Shaivamallika, Swayambhu, Lingi, Chitraphala, Amruta, Pandoli, Lingaja, and Devi. Various parts of the plant’s leaves, fruits, seeds, and roots are traditionally used for medicinal purposes (Chavhan *et al.,* 2019).

Corresponding Author Email: ujsupe@gmail.com; Part of phd thesis submitted to Hemchand Yadav University, Durg, Chattisgarh, Nagaland, University, Lumami.

alkaloids, phenols, flavonoids, tannins, sterols, anthraquinones, cardiac glycosides, saponins, and volatile oils, with aqueous extracts showing the highest concentration of these secondary metabolites (Patel and Kazi, 2023). Literature reports suggest that species from this family are used in the treatment of malaria, epilepsy, diarrhea, leprosy, diabetes, boils, asthma, and as antioxidants (Rolnik and Olas, 2020). The seeds, in particular, are known for improving sexual health and are used in the treatment of male and female infertility, impaired spermato-genesis, asthenozoospermia, teratospermia, constipation, obesity, weight loss, hyperglycemia, and diabetes (Sud and Sud, 2017).

Additionally, *in vitro* adventitious root culture systems have been established as effective platforms for producing plant secondary metabolites and for investigating metabolic pathways (Moradi *et al.,* 2019). Such root cultures offer advantages for clonal propagation and germplasm conservation in medicinal plant species (Srivastava *et al.,* 2019).

MATERIAL AND METHODS

Leaf materials

The leaf of *BryoniaLaciniosa* was obtained from the Botanical Garden, St. Thomas College. Bhilai. Good and healthy explants were selected from the raised plants which weregrown on a 1:1 mixture of sand and soil in earthen pots. Leaves (0.5-0.9 cm) were selected from the first node of 10-15-day-old plantlets. All the explants were used for direct and indirect organogenesis. Explants were washed with tap waterthen sterilized with distilled water and taken in a sterilized glass plate.

Phytochemical studies have identified bioactive constituents including terpenoids,

Surface Sterilization of explants

After washing the explants were then transferred to laminar air flow. After washing, the explants were dipped in 100ml sterilized distilled water for 15 minutes followed by washing in Tween-20 (1-2 drops in 100ml sterile distilled water) for 1 minute and then were rinsed 3 times with sterile distilled water (SDW) in the laminar flow cabinet. For the pre-sterilization step, the fungicide Sofia (Bhilai Market sector-10) was tested at concentrations of 0.2%. The plant materials were then surface sterilized using mercuric chloride ($HgCl_2$), Sodium hypochlorite, Calcium Hypochlorite, and Hydrogen peroxide (H_2O_2) (HiMedia) with a concentration of 0.1%, 0.2%, 0.3%, and 0.4% for 2 -20 minutes as shown in Table 1.

Organogenic callus induction

Surface sterilized explants were inoculated on MS medium supplemented with different concentrations of growth hormones. In addition to nutrients, it is generally necessary to add growth hormones, so as to get good growth of tissues and organs of the major phytohormones cytokinin in combination with auxins were here used for callus induction and regeneration studies Murashige and Skoog (1962). Leaf explants were cultured on MS basal medium containing 3% (w/v) sucrose, 0.7% (w/v) agar with various concentrations of 2,4-D,NAA and IAA (1.0 – 4.0 mg/l) for callus induction. The effect of hormones on callus induction response was studied and effort was made to determine the appropriate hormone combination for optimal callus growth. Callus induction was observed from 7-15 days. All the cultures were incubated at 25 ± 2^0C under 16h light (2,500 lux) condition.

Plantlet regeneration

Well-developed calli were transferred to regeneration medium containing MS basal salts, 3%(w/v) sucrose, 0.7% (w/v) agar, Different concentrations of IAA (0.5- 1.5 mg/L) in combination with cytokinin (KIN 1.0 – 5.0 mg/L) for shoot bud regeneration were used. Shoot bud differentiation was observed from 10-15 days. These calli were maintained on the same medium and regenerated shoot buds were developed in plantlets. The influence of the auxins (IAA) and cytokinin (KIN) on plantlet development was studied.

Root induction and acclimatization

The plantlets were excised 3cm in length and were transferred to MS basal medium containing 3% (w/v) sucrose, 0.7% (w/v) agar, different concentrations of KIN (1.0- 5.0 mg/L)and IBA (0.5- 1.5 mg/L) for root initiation. Rooting was observed from 7-15 days plantlets with well-developed roots in running tap water, they were grown in red soil, sand, and farm yard (manure) mixture (1:1:1) in the plastic cubs for 15 days and subsequently transferred to pots. All the tissue culture-raised plantlets need gradual acclimatization for their survival in the field condition after growing in the controlled environment Instead of transferring directly to the pots, plantlets were left for a week in the plastic cups at a controlled temperature 24 ± 2^0 C) with 60% relative humidity.

RESULTS AND DISCUSSION

Effect of different sterilizing agents and their different concentrations on the level of microbial and fungal contamination

Sodium hypochlorite at a different concentration ranging from 0.1%-0.4% for 20 minutes with 0.2% Saaf (fungicide) for 15 minutes, the highest average survival rate (90.5%) was achieved. At this optimal concentration, the average contamination rate was a low 6% and the average mortality rate remained minimal at 3%. Leaf explants treated with 0.2% sodium hypochlorite exhibited an even higher survival rate of 95% with just 2% contamination, while internode explants showed a slightly lower, yet still respectable, survival rate of 86% with 10% contamination. Notably, increasing the sodium hypochlorite concentration beyond 0.2% led to a decrease in both survival and contamination, suggesting between effectiveness and plant health. Mercuric chloride (0.3%) for 2 minutes and Sofia (fungicide) (0.2%) for 15 minutes yielded a survival mean value of 87.5%, contamination of 6.5%, and a mortality rate of 6%. Leaf explants exhibited a survival rate of 90%, contamination of 10%, and a mortality rate of 5%. Increasing the mercuric chloride concentration reduced the survival rate and increased mortality. Similarly treating explants with a calcium hypochlorite (0.4%) for 2 minutes, followed by Sofia (fungicide) (0.2%) for 15 minutes, resulted in an average resulted in the average survival rate of 55% an average contamination rate of 38.5% and an average mortality rate of 6.5%.

When treated with hydrogen peroxide (0.4%) for 2 minutes and Sofia (fungicide) (0.2%) for 15 minutes, explants exhibited an average survival rate of 37.5%, a contamination rate of 50% and a mortality rate of 12.5%. leaf explants fared better, with a 50% survival rate, 40% contamination and 10% mortality. All the results were shown in Table 1.

Effect of growth regulator on callus induction

The investigation was carried out on the various concentration of 2,4-D (0.5-3mg/l) Kinetin (1.5-0.5mg/l) and NAA (0.5-3mg/l), Kinetin (0.5-1.5mg/l) for Establishment of culture and callus induction in Table 2-3. The data were recorded on the number of leaves and callus formation. Three replicates were taken for each treatment, and each experiment

Table 1. Standardization of surface sterilization for various explants in *Bryonialaciniosa* (Linn.) by $HgCl_2$, NaOCl, $CaOCl_2$ and H_2O_2.

SterilizingAgent	Leaf explants			Mean		
	% CON	% SUR	% MOR	% CON	% SUR	% MOR
$HgCl_2$ (0.1%) (2min)	79	15	6	87	8.5	4.5
$HgCl_2$ (0.2%) (2min)	45	50	5	51.5	45	3.5
$HgCl_2$ (0.3%) (2min)	3	90	7	6.5	87.5	6
$HgCl_2$ (0.4%) (2min)	0	0	100	1.5	01	97.5
NaOCl (0.1%) (20min)	60	36	4	45	48	7
NaOCl (0.2%) (20min)	2	95	2	6	90.5	3
NaOCl (0.3%) (20min)	2	3	95	01	50.5	48.5
NaOCl (0.4%) (20min)	0	0	100	00	01	50
$CaOCl_2$ (0.1%) (20min)	93	5	7	96.5	2.5	3.5
$CaOCl_2$ (0.2%) (20min)	65	25	10	69.5	22.5	8
$CaOCl_2$ (0.3%) (20min)	46	50	4	50.5	45	4.5
$CaOCl_2$ (0.4%) (20min)	35	60	5	38.5	55	6.5
H_2O_2 (0.1%) (20min)	96	3	1	98	1.5	0.5
H_2O_2 (0.2%) (20min)	85	10	5	88.5	7.5	4
H_2O_2 (0.3%) (20min)	50	40	10	60	30	10
H_2O_2 (0.4%) (20min)	40	50	10	50	37.5	12.5

% CON- % Contamination, **% SUR**- % Survival,
% MOR- % Mortality (Browning or Blacking of explants).

was repeated three times. The observations were recorded at the end of each. The data were analyzed and put in the table form. However, the best results in the form of an amount of callus obtained were on MS medium augmented with different concentrations and combinations of 2,4-D and kinetin (Table 2). Among all combinations and concentrations tested, the highest callus induction (65%) was achieved for leaf explants cultured on a medium containing 3mg/l 2,4-D and 0.5mg/l kinetin. Increasing or decreasing either the 2,4-D or kinetin concentration resulted in a lower percentage of callus formation. So the concentration of 3mg/l of both 2,4-D and 0.5mg/l kinetin proved to be the threshold concentration. While using the combination of NAA and kinetin maximum amount of callus obtained was 57% on a combination of 1.5 mg/l of NAA and 1.5mg/l of kinetin (Table 3). In Figure 1(A-C) results were shown. In this study organogenic callus induction was achieved from explants by using the above said concentration. But in other cases, the concentration of phytochormones varies for the induction of organogenic callus depending on the explants. These results conform with Ibrahim and Al-Nema (2023).

Table 2 Callus induction in *Bryonialaciniosa* (Linn.) in MS medium with different combinations of 2, 4 D and Kinetin (mg/l)

2,4-D + Kinetin(mg/l)	Callus from leaves	Remarks on callus	No. of Day	Callus induction %
0.5+0.5	+	Friable callus	19	51
1.0 + 0.5	+	Friable callus	19	52
1.5+0.5	+	White yellow friable	19	52
2.0+0.5	++	White yellow friable	20	56
2.5+0.5	++	Brownish callus	20	56
3.0 +0.5	+++	Friable callus	19	65
0.5+1.0	+	Friable callus	20	51
1.0 + 1.0	+	Friable callus	20	50
1.5+1.0	++	White Friable	19	50
2.0+1.0	++	Compact green	18	58
2.5+1.0	+++	Compact green	18	63
3.0 +1.0	+++	White green	18	62
0.5+1.5	+	White green	20	60
1.0 + 1.5	+	White green	20	60
1.5+1.5	++	Compact green	20	60
2.0+1.5	++	Compact green	20	58
2.5+1.5	+++	Compact green	18	60
3.0 +1.5	+++	Compact green	17	60

Effect of growth regulator on Plant Regeneration

Greenish compact nodular calli obtained from leaf explants were selected for regeneration studies. These calli were transferred to regeneration medium, before that these calli were cut into 2 or 3 pieces and subcultured. After subculture on the parental medium shoot buds were initiated between 10-15 days. The shoots were maintained on the same medium for 20 to 25 days to get sufficient growth. The regeneration frequency ranged between 20.0 to 68.8%. Higher frequency of shoot regeneration was observed on medium containing 1.0 mg/l IAA (66.6%)(Fig.1). Greenish compact nodular calli were suitable for plant regeneration. These calli were maintained on parental medium for long time (or) subcultured on parental medium where they induced shoot buds between 15 to 25 days (Table 4). After that the shoots were elongated and established into plantlets. There are differences in shoot regeneration frequency among different concentrations and combinations of auxin and cytokinin. The IAA was highly significant than the other treatments. During callus induction there is no significant change in callus morphology and all the obtained calli at KIN (1-5 mg/L and IAA (0.5-1.5 mg/L) showed green, friable and nodular in nature. But in the case of multiple shoot induction significant variation was observed. In leaf explant organogenic callus was derived

Table 3 Callus induction in *Bryonialaciniosa* (Linn.) in MS medium with different combinations of NAA and Kinetin (mg/l)

NAA + Kinetin (mg/l)	Callus from leaves	Remarks on callus	No. of Day	Callus induction %
0.5+0.5	+	Friable callus	19	50
1.0 + 0.5	++	Friable callus	19	52
1.5+0.5	++	Greenish white	18	50
2.0+0.5	++	Greenish white	18	40
2.5+0.5	++	Greenish white	18	51
3.0 +0.5	++	Greenish white	18	54
0.5+1.0	+	Friable callus	20	51
1.0 + 1.0	+	Friable callus	20	53
1.5+1.0	++	White Friable	19	51
2.0+1.0	++	Greenish white	20	50
2.5+1.0	+	Whitish yellow Friable	19	49
3.0 +1.0	+	Whitish yellow Friable	19	49
0.5+1.5	++	Whitish yellow Friable	19	47
1.0 + 1.5	++	Greenish white	20	46
1.5+1.5	+++	Compact green	21	57
2.0+1.5	+++	Compact green	22	55
2.5+1.5	+++	Compact green	23	56
3.0 +1.5	++	Greenish white	21	46

only 7.1 ± 0.5 shoots which were regenerated (Fig. 1 D-H). This variation in the number of shoots may be due to the explant dependent response. The maximum regeneration frequencies are leaf explants respectively. Regeneration frequency was very low at the lower concentration treatments. Higher concentrations of auxin reduced the shoot regeneration frequency. The increase and decrease in regeneration frequency is attributed to the cytokinin concentrations. Teshome and Feyissa (2015) demonstrated that the highest regeneration frequency was achieved on a medium supplemented with 0.5 mg/L BAP, while the maximum number of shoots and the greatest shoot length per explant were obtained with a combination of 0.25mg/L BAP and 0.5mg/L kinetin.

Rooting of regenerants and acclimatization

The well-developed shoots were transferred to rooting media containing different concentrations of KIN and IBA (Table 5). The well-developed shoots from leaf derived callus were isolated and transferred to root induction medium.

Table 4. Response of leaf bit explant to various combination of Kinetin and IAA in MS medium on induction of shoots after 30 and 60 days of culturer text here

Concentration of Kinetin + MS (mg/l)	Concent-ration of IAA + MS (mg/l)	Mean no.of shoots per explant after 15 days± S.E.	Frequency of regene-.ration	Amount of callus*	Physical Nature of callus *
0	0.0	0.0	0.0	-	-
1	0.5	2.3 ± 0.2	25	+	B, F, S
2	0.5	3.4 ± 0.6	27	+	B, F, S
3	0.5	3.8 ± 0.2	37	+	B, F, S
4	0.5	4.7 ±0.1	40	+	B, F, S, N
5	0.5	3.9 ± 0.2	42	++	B, F, S, N
1	1.0	2.9 ± 0.8	47	+	B, F, S
2	1.0	3.6 ± 0.4	51	+	B, F, S
3	1.0	5.7 ± 0.9	59	+	B, F.S
4	1.0	7.0 ± 0.4	66	+	B, F, S, N
5	1.0	6.4 ± 0.6	62	++	B, F, S, N
1	1.5	4.9 ± 0.6	62	+	B, F, S
2	1.5	4.2 ± 0.8	54	+	B, F, S
3	1.5	5.0 ± 0.7	35	+	B, F, S
4	1.5	5.8 ± 0.5	26	+	B, F, S, N
5	1.5	4.4 ± 0.2	15	++	B, F, N, S

As the concentration of KIN was gradually increased the response of explant to the medium also increased to a certain extent i.e. up to 5 mg/L, it was also observed that KIN 4 mg/L and IBA 1 m/L showed maximum 4.0 shoots after 30 days and 7 shoots after 60 days of inoculation. A further increased in the level of IBA up to 1.5 mg/l lowered the number of shoots developed per explant. Callus was formed at base of shoots when concentrations of Kinetin were low as well as high. Callus formed is brownish, friable, and soft but also in some combination it was nodular.

Rhizogenesis was observed to a maximum level in these leaf explants i.e., 78% maximum of rhizogenesis was obtained at 4 mg/L KIN and 1.0 mg/l IBA (Table 3). Root induction took place between 15 to 20 days after transfer to rooting media. The rooting frequency was high in all these three explants' regenerants. In general, higher levels of IBA showed a low frequency of root induction.

The well-developed rooted plantlets were first transferred to plastic pots having vermiculture and garden soil (3:1). The pots were kept in covered glass trays for a week in an incubator at 25 ± 2°C under a 16th photoperiod. After 8 to 10 days these plantlets were transferred to the earthen pots and then to the field. The hardened plants showed 78.5 ± 2.0 % survival rates in the field condition (Figure 1I). Based on this protocol we planned to induce the somaclonal variation among the regenerants and this may be lead to the

Table 5. Effect of Kinetin and IBA on root induction in regenerated plantlets.

Concentrationof Kinetin + MS (mg/l)	Concentration of IBA + MS (mg/l)	Mean no. of shoots per explant after 15days ± S.E.	Frequency of rooting
0	0.0	0.0	00
1	0.5	1.1 ± 0.1	17
2	0.5	1.9 ± 0.3	20
3	0.5	2.0 ± 0.4	26
4	0.5	2.8 ± 0.7	37
5	0.5	2.3 ± 0.6	42
1	1.0	1.6 ± 0.2	53
2	1.0	2.2 ± 0.2	64
3	1.0	3.0 ± 0.1	70
4	1.0	4.0 ± 0.2	78
5	1.0	2.6 ± 0.9	66
1	1.5	1.5 ± 0.4	43
2	1.5	2.4 ± 0.7	47
3	1.5	2.7 ± 0.6	35
4	1.5	3.9 ± 0.1	22
5	1.5	2.6 ± 0.3	14

regeneration of a new variety in bryonia. It will also be useful for the improvement of Bryonia germplasm improvement. Dasari and Shasthree (2015) reported that the highest rooting ability was achieved from stem explants cultured on MS medium supplemented with 2.0mg/L IAA and 1.5 mg/L IBA; multiple fibrous roots developed from cotyledon explants on MS medium fortified with 2.0 mg/L 2,4-D, while leaf explants produced clustered roots onMS medium containing 1.5mg/L IBA and 2.0mg/L IAA.

CONCLUSIONS

In this study, a simple and effective regeneration system was developed for *Bryonialaciniosa* using leaves as an explant source for callus induction and regeneration. Findings revealed that 0.2% NaOCl (20 min) achieved the highest survival rate (95%) and low contamination (2%) for leaf explants.High potential for callus induction in leaf explants was found when grown in MS medium with 3.0 mg/L 2,4-D. The highest plant regeneration capacity was observed for callus when it was developed in MS culture medium with KIN (4 mg/L) and IAA (1 mg/L). Overall the results suggest that for optimal tissue culture in *Bryonialaciniosa*, a combination of 0.2% NaOCl, 3 mg/l 2,4-D, 4 mg/l kinetin, and1.0 mg/l IAA would yield the best outcomes in terms of explant survival, callus induction, plant regeneration, and rooting.The presented plant regeneration system is an important tool for genetic manipulation and improvement of this valuable medicinal plant.

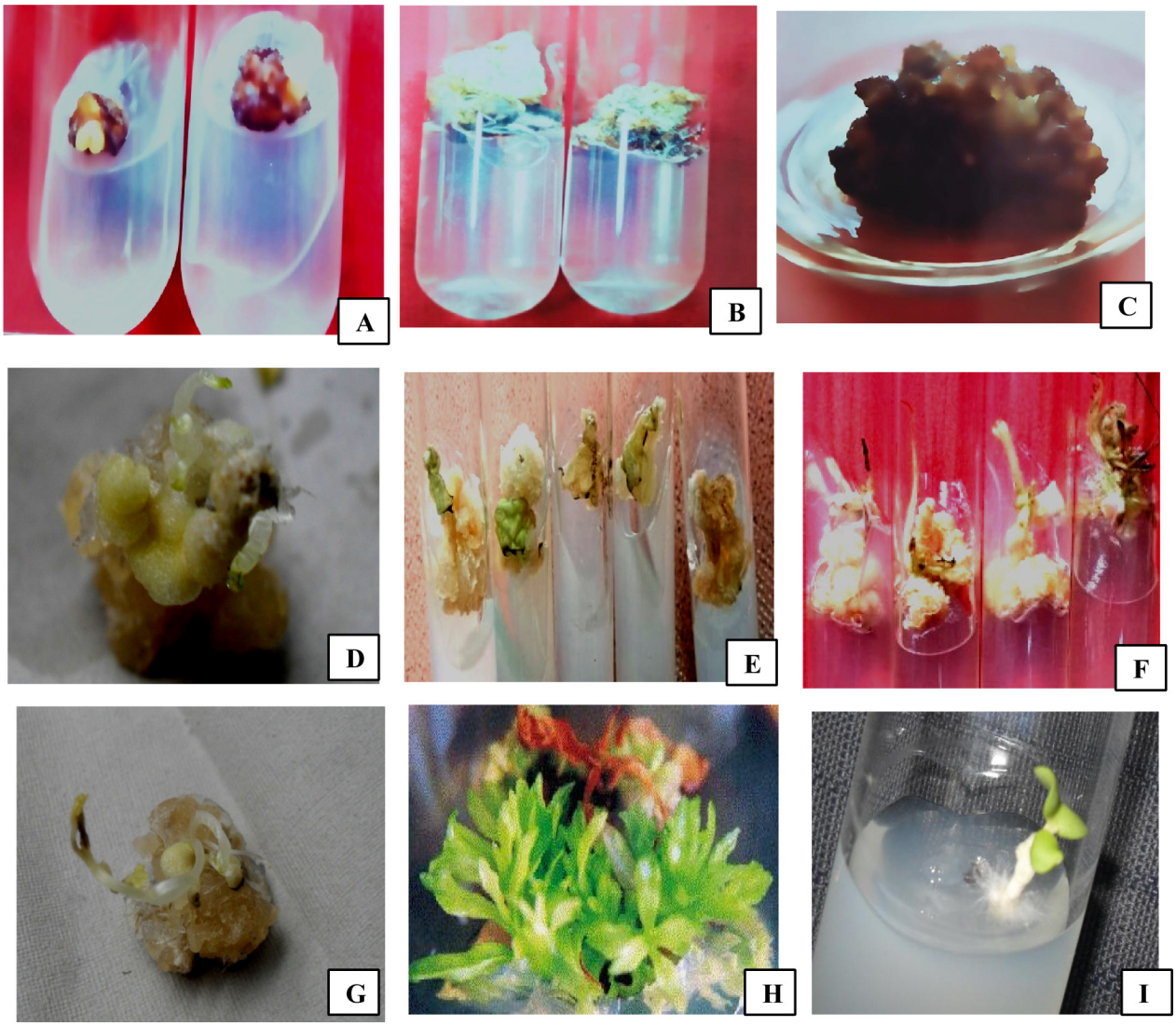

Figure 1.Organogenic callus induction and plant regeneration in *BryoniaLaciniosa*

A) 2 weeks old callus from leaf explant in MS medium with 3 mg/l 2,4-D

B) 2 weeks old callus in MS medium supplemented with Kinetin and IAA

C) Subcultured callus after 2 weeks containing 4 mg/l IBA and Kinetin

D-G) Induction of adventitious bud in callus **A**

H) Multiple shoot proliferation from callus

I) Single Regenerated shoot of *BryoniaLaciniosa* with roots**A**

REFERENCES

Chavhan, S. A., Wathurkar, R. N and Shinde, S. A. 2019. Phytopharmacognostic Review of *Bryonialaciniosa* (Shivlingi Beej). International Journal of Pharmacognosy and Chinese Medicine.3(3): 1-7.

Dasari, R and Shasthree, T. 2015. Adventitious rooting and proliferation from different explants of *Citrullus colocynthis* (L.)

Schrad: An endangered medicinally important cucurbit. Asian Journal of Biotechnology.7(2): 88–95.

Ibrahim, A, Z and Al-Nema, Q. S. 2023. Efficient callus induction from various explants of *Jatropha curcas*. Nativa, Sinop. 11(4): 572-576.

Moradi, F., Mehrjerdi, M. Z., Vahdati, K and Hasanloo, T. 2019. Effect of different factors on induction of hairy roots in Iranian garlic. Plant Productions. 41(4): 43-54.

Murashige, T. and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol plant 15: 473- 497.

Patel, S. B and Kazi, N. K. 2023. Qualitative analysis and TLC screening of various fruit extracts of medicinally important climber *Diplocyclospalmatus* (L) C. Jeffrey. International Journal of Pharmaceutical Research and Applications. *8*(1): 1329–1337.

Rolnik, A and Olas, B. 2020. Vegetables from Cucurbitaceae family and their products; positive effect on human health. Nutrition. 78: 110788.

Srivastava, M., Singh, G., Sharma, S., Shukla, S and Misra, P. 2019. Elicitation enhanced the yield of glycyrrhizin and antioxidant activities in hairy root cultures of *Glycyrrhizaglabra* L. Journal of Plant Growth Regulation. 38(2): 373-384.

Sud, K and Sud, S. 2017. A scientific review on *Shivlingibeej* (*Bryonialaciniosa*): A mystical ethno-medicine for infertility. European Journal of Biomedical and Pharmaceutical Sciences. *4*(8): 1098–1102.

Teshome, S and Feyissa, T. 2015. In vitro callus induction and shoot regeneration from leaf explants of *Glinuslotoides* (L.): An important medicinal plant. American Journal of Plant Sciences. 6(9): 1468–1479.

Ujjwala Supe, Nikita Mishra and Hemshankar Sahu 2025. In vitro callus induction and plant regeneration in Bryonia laciniosa from leaf. The Journal of Research, ANGRAU. Vol. 53 (1): 1-10