https://doi.org/10.33451/florafauna.v31i1pp69-77
FLORA AND FAUNA ISSN 2456 - 9364 (Online)
2025 Vol. 31 No.1 PP 69-77 ISSN 0971 - 6920 (Print)

Optimizing Callus Induction in *Bryonia laciniosa* : A Combined Approach of Sterilization Protocols and Plant Growth Regulators

***Hemshankar Sahu and Ujjwala Supe**

Department of Biotechnology,
Plant tissue culture laboratory,
St Thomas College, BHILAI-490006, DIST-DURG (CHHATTISGARH) INDIA
*Corresponding Author
E-mail : hemshankarsahu24@gmail.com

Received **:** 32.03.2025; ***Accepted*** : 08.05.2025

How to cite : Sahu H, Supe U. Optimizing Callus Induction in *Bryonia laciniosa :* A Combined Approach of Sterilization Protocols and Plant Growth Regulators. *Flora and Fauna* 2025. 31(1) : 69-77.

ABSTRACT

The usage of traditional drugs by 60% of the global population has heightened concerns regarding the extinction of medicinal plants like *Bryonia laciniosa*, attributed to over harvesting and habitat constraints. This study investigates effective sterilization methods for the *in vitro* culture of *Bryonia laciniosa*, revealing optimal disinfection with 0.2% sodium hypochlorite followed by fungicide (Sofia) treatment. Although 0.3% mercuric chloride yielded high explant survival, it resulted in elevated culture mortality, favoring sodium hypochlorite for successful disinfection. Leaf explants displayed efficient callus induction on MS medium, with the 2,4-D (3.0mg/l) + Kin (0.5mg/l) combination exhibiting the best response, followed by NAA (1.5mg/l) + Kin (1.5mg/l). Maximum callus induction occurred on MS medium supplemented with 2,4-D (3mg/l) + Kin (0.5mg/l). The regenerated callus was transferred to a half-strength MS medium fortified with 2,4-D for elongation. 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.

Figures : 03 References : 24 Tables : 03
KEY WORDS : 2,4-D, *in vitro* callus induction, Kinetin, MS medium, NAA

Introduction

Plants in the Cucurbitaceae family, commonly known as melons, squashes, and gourds,are traditionally used in human diets. It comprises 122 genera and 940 species, with 31 genera and 94 species found in India[14]. *Bryonia laciniosa* synonyms *Bryonopsis laciniosa*, *Diplocyclos palmatus* is a medicinal plant with many therapeutic uses. *Bryonia laciniosa* is well known in India and belongs to the Cucurbitaceae Family. It is locally known as Shivlingi or Garumaru because their seed looks like Shivling. Shivlingi, distributed throughout India, is an annual climber with bright red fruits, and is reported to be highly medicinal[19]. *Bryonia laciniosa* has many therapeutic uses due to its rich phytochemical constituents, including triterpen glycosides, flavonoids, and saponins[4]. Its different parts are used like leaves, fruits, seeds roots, *etc*[5]. Bitter melon, a valuable food and medicinal plant, suffers from germination and variability issues. *In vitro*, regeneration using immature leaf explants offers a solution for mass production and yield improvement [20].

Not much work was done in India and elsewhere on this plant to produce secondary metabolites and tissue culture. The demand for *Bryonia laciniosa* is increasing daily in the Indian and foreign markets.

The seed has poor germination (5-11%), low viability, and a long dormancy period. Due to the large scale and indiscriminate collection of its parts for gainful trade and insufficient attempts either to allow its replenishment or its cultivation. There is a greater need for commercial cultivation of this species [21].

Materials and Methods

Collection of plant samples : *Bryonia laciniosa* plants were collected from the Bhilai region, Durg district, Chhattisgarh, India. These plants were successfully established in the herbal garden of St. Thomas College for future research purposes. Voucher specimens are maintained in the Department of Biotechnology, St. Thomas College. Healthy internodal and leaf explants were collected from two-month-old plants for the initial experiments.

TABLE-1 : Evaluation of the most effective surface sterilization for *Bryonia laciniosa* Explants: A Comparative Study of $HgCl_2$, NaOCl, $CaOCl_2$, and H_2O_2

Sterilizing Agent	Leaf explants			Internode explants		
	% CON	% SUR	% MOR	% CON	% SUR	% MOR
$HgCl_2$(0.1%) (2min)	77.33±2.73	15.67±1.20	6.67±1.2	96.00±1.53	3.33±1.86	4.33±0.88
$HgCl_2$(0.2%) (2min)	47.00±1.15	50.33±0.88	5.33±0.88	56.33±2.19	42.33±1.45	2.00±0.58
$HgCl_2$(0.3%) (2min)	3.33±0.88	90.33±2.60	6.67±0.88	10.00±1.15	84.67±0.33	5.00±1.15
$HgCl_2$(0.4%) (2min)	0.00	0.00±	100.67±1.2	4.00±1	3.67±1.2	97.00±1.15
NaOCl(0.1%) (15min)	60.33±1.45	37.00±0.58	5.67±0.88	32.33±1.45	61.33±1.86	12.00±1.15
NaOCl(0.2%) (15min)	3.33±0.88	96.67±0.88	2.33±0.88	12.33±1.45	85.33±1.2	5.00±0.58
NaOCl(0.3%) (15min)	3.67±0.88	3.33±0.33	96.33±0.88	0.00	97.00±0.88	3.67±1.86
NaOCl(0.4%) (15min)	0.00	0.00	101.33±1.86	0.00	2.33±0.58	98.00±0.88
$CaOCl_2$(0.1%) (15min)	95.33±0.88	6.00±0.58	6.67±1.53	103.00±1.15	0.00	0.00
$CaOCl_2$(0.2%) (15min)	67.00±0.58	26.00±0.58	10.00±1.2	75.67±1.45	22.33±1.2	6.67±1.32
$CaOCl_2$(0.3%) (15min)	48.00±1.33	52.33±1.56	5.00±0.67	57.00±1.33	45.00±3.33	5.00±0.67
$CaOCl_2$(0.4%) (15min)	37.00±1.33	64.00±2.67	6.00±0.67	45.67±2.44	54.00±2.67	9.67±1.56
H_2O_2(0.1%) (15min)	97.67±1.11	4.67±1.11	1.33±0.44	102.33±1.78	0.00	0.00
H_2O_2(0.2%) (15min)	86.67±1.11	14.33±2.89	7.33±1.56	95.67±2.44	6.33±1.11	4.67±1.11
H_2O_2(0.3%) (15min)	53.67±2.44	44.00±2.67	12.67±2.22	73.67±2.44	22.00±1.33	11.00±0.67
H_2O_2(0.4%) (15min)	44.00±2.67	53.67±2.44	12.33±1.78	64.00±2.67	27.00±1.33	16.67±1.11

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

Data presented as means ± SE from 20 explants for treated and repeated three times. This means following the same letters within a column are not significantly different according to the Duncan Multiple Range Test (DMRT) at $P<0.05$ level.SE= standard error.

Selection and Isolation of explants

Good and healthy explants (leaves, internode, *etc*) were selected from the raised plants which we have grown on a 1:1 mixture of sand and soil in earthen pots. Axillary 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 for callus induction. Explants were washed with tap water,then sterilized with distilled water, and taken in a sterilized glass plate.

Surface Sterilization of explants

Experiment 1: 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 a locally available mercuric chloride ($HgCl_2$) was tested at concentrations of 0.1%, 0.2%, 0.3%, and 0.4% for 2 minutes (Hi Media).

Experiment 2: Leaves and Internode were treated as in Experiment 1 up to the pre-sterilization step, where explants were soaked with 0.2% Sofia (Fungicide) for 15 minutesFollowed by surface sterilization in sodium hypochlorite (at concentrations of 0.1%, 0.2%, 0.3%, or 0.4%) for 15 minutes.

Experiment 3: Plant materials treated with 0.2% Sofia (fungicide) for 15 minutes were sterilized in Calcium Hypochlorite (0.1, 0.2, 0.3, or 0.4%) for 15 minutes for the leaves and internode. Afterward, they were rinsed 3-4 times in SDW.

Experiment 4: Plant materials like leaf and internode treated with 0.2% Sofia (fungicide) for 15 minutes were sterilized in Hydrogen peroxide (H_2O_2) at four different concentrations of 0.1%, 0.2%, 0.3%, and 0.4% for 20 minutes and then rinsed 3-4 times with sterile distilled water and then dried on sterile filter paper before culture on the Murashige and Skoog (MS) basal medium in the test tube.

Inoculation and Maintenance : After cutting, explants were inoculated in culture vessels containing MS medium with different concentrations of sucrose. Various concentrations and combinations of auxins andcytokinin were used. The pH of the medium was adjusted to 5.6 ± 0.2 with 1N NaOH orHCl before the addition of 0.8% (W/V) agar (Hi media, India) and different concentrations of sucrose, and enriched with different kinds and different concentrations of auxins (including 2,4-D, NAA,) and cytokinin (including kinetin), alone or in combination, as follows: 2,4-D (0.5-3mg/l) Kin (0.5-1.5mg/l), NAA (0.5-3mg/l). Molten culture media were distributed into test tubes (Borosil, India) (25x150mm; 10ml) and closed with non-absorbent cotton plugs, and media were autoclaved at 104kpa and 121^0C for 20 min.Twenty explants were taken per treatment and each treatment was replicated thrice. The cultures were maintained at 25±2 ^{0}C under a 16-hour photoperiod of 35µmol m^{-2} s^{-1} irradiance provided by cool white fluorescent light with 55-65% relative light humidity.

Statistical analysis: Data from three independent experiments represented by 20 replicates from each experiment were subjected to statistical analysis (Mean±SE) and Duncan's Multiple Range Test (DMRT) using SPSS software, version 21.

Results

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

The application of sodium hypochlorite at varying concentrations (0.1%-0.4%) for 20 minutes with 0.2% Saaf (fungicide) for 15 minutes resulted in a leaf explant survival rate of 96.67±0.88%, with 3.33±0.88% contamination and 2.33±0.88% mortality.In contrast, using 0.3% sodium hypochlorite for 20 minutes with 0.2% Saaf yielded a 97.00±0.88 survival rate for internode explants, with no contamination observed and a mortality rate of 3.67±1.86%. Interestingly, increasing the sodium hypochlorite concentration beyond 0.2% for both leaf and internode explants led to a decrease in survival rate and an increase in contamination, suggesting a trade-off between effectiveness and plant health.

Mercuric chloride (0.3%) for 2 minutes and Sofia (fungicide) (0.2%) for 15 minutes resulted in a survival rate of 90.33±2.60%, contamination of 3.33±0.88%, and mortality rate of 6.67±0.88% for leaf explants.

In the case of internode explants, the survival rate was 84.67±0.33, contamination was 10.00±1.15, and mortality rate was 5.00±1.15%. Increasing the mercuric chloride concentration reduced the survival rate and increased mortality.

Similarly treating explants with calcium hypochlorite (0.4%) for 2 minutes, followed by Sofia (fungicide) (0.2%) for 15 minutes, resulted in a survival rate of 64.00±2.67%, contamination 37.00±1.33%, and mortality 6.00±0.67%. When treated with hydrogen peroxide (0.4%) for 20 minutes and Sofia (fungicide) (0.2%) for 15 minutes, leaf explants exhibited a survival value of 53.67±2.44%, a contamination value of 44.00±2.67% and a mortality value of 12.33±1.78%. However, Internode explants experienced lower survival

TABLE-2 : Callus induction in *Bryonia laciniosa* in MS medium with different combinations of 2, 4 D, and Kinetin (mg/l)

2,4-D+ Kinetin (mg/l)	Callus from leaves	Remark on Callus	No of Days	Callus induction % Mean ±SE
0.5+0.5	+	Friable callus	19	50.67±1.45
1+0.5	+	Friable callus	19	51.67±2.60
1.5+0.5	+	Friable callus	19	53.67±0.88
2+0.5	++	Whitish yellow friable	20	54.00±1
2.5+0.5	++	Whitish yellow friable	20	55.00±1.53
3+0.5	+++	Brownish callus	19	65.00±3.21
0.5+1	+	Friable callus	20	52.00±0.58
1+1	+	Friable callus	20	49.33±0.33
1.5+1	++	Friable callus	19	51.67±0.88
2+1	++	White friable	18	54.33±2.03
2.5+1	+++	Compact green	18	63.00±0.58
3+1	+++	Compact green	18	62.67±1.2
0.5+1.5	+	White green	20	62.33±1.45
1+1.5	+	White green	20	62.33±1.2
1.5+1.5	++	White green	20	63.33±2.03
2+1.5	++	Compact green	20	63.00±2.65
2.5+1.5	+++	Compact green	18	62.33±1.45
3+1.5	+++	Compact green	17	60.33±1.45

The Means with different Letters as superscripts are significant ($P<0.05$). The means with the same letters or having common letters(s) are not significantly different.

TABLE-3 : Callus induction in *Bryonia laciniosa* in MS medium with different combinations of NAA and Kinetin (mg/l)

NAA+ Kinetin (mg/l)	Callus from leaves	Remarks on callus	No of days	Callus induction % Mean ±SE
0.5+0.5	+	Friable callus	19	50.00±1.15
1+0.5	++	Friable callus	19	51.67±1.45
1.5+0.5	++	Greenish white	18	50.33±0.88
2+0.5	++	Greenish white	18	42.67±1.76
2.5+0.5	++	Greenish white	18	53.67±1.76
3+0.5	++	Greenish white	18	53.00±0.58
0.5+1	+	Friable callus	20	54.00±1.73
1+1	+	Friable callus	20	53.67±1.2
1.5+1	++	White Friable	19	55.33±2.19
2+1	++	Greenish white	20	52.33±1.2
2.5+1	+	Whitish yellow Friable	19	53.00±2.65
3+1	+	Whitish yellow Friable	19	52.00±1.53
0.5+1.5	++	Whitish yellow callus	19	50.33±1.67
1+1.5	++	Greenish white	20	52.00±3.06
1.5+1.5	+++	Compact green	21	55.33±2.19
2+1.5	+++	Compact green	22	53.33±0.88
2.5+1.5	+++	Compact green	23	55.67±2.03
3+1.5	++	Greenish white	21	51.33±2.67

The Means with different Letters as superscripts are significant (P<0.05). The means with the same letters or having common letters(s) are not significantly different.

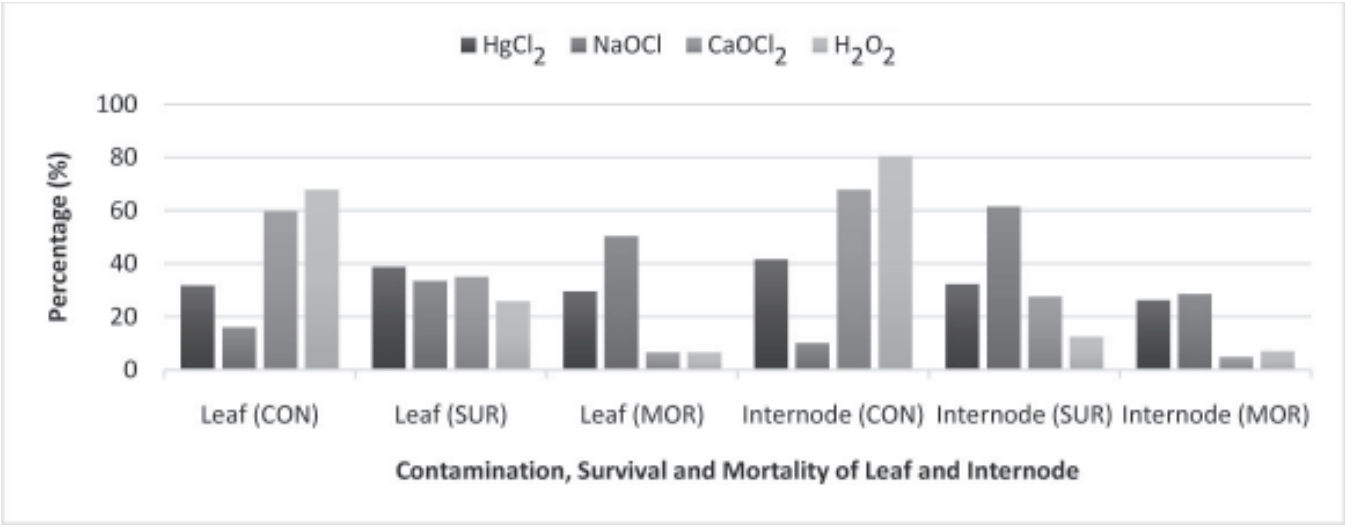

Fig. 1 : Results obtained from different surface sterilizing agents like $HgCl_2$, $NaOCl_2$, $CaOCl_2$, and H_2O_2on Leaf and Internode explants in *Bryonia laciniosa* Bars with different letters indicate a significant difference (Pd"0.5%) according to Duncan's Multiple- range Tests (DMRTs)

(less than 50%) and higher contamination (greater than 40%) compared to leaf explants (Table-1).

Effect of growth regulator on callus induction

The investigation was carried out on the various concentrations 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 call us 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 was repeated three times. The observations were recorded at the end. The data were analyzed and putin 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 (65.00±3.21%) 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 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 55.33±2.19% on a combination of 1.5 mg/l of NAA and 1.5mg/l of kinetin (Table-3).

Discussion

The successful establishment of micropropagation culture relies heavily on effective sterilization procedures. While the importance of this step is well recognized,optimal sterilization protocols can vary depending on the explant tissue culture type and the genotype of the source plant. This research investigates the influence of these factors on sterilization requirements, aiming to balance the elimination of these factors on sterilization requirements, aiming to balance the elimination of contaminated microorganisms with the preservation of explant viability and culture ability[6]. Sodium hypochlorite and Mercuric Chloride were tested on the contamination-free establishment of Gerbera leaf under *in-vitro* conditions. The best sterilization agent was found to be a combination of mercuric chloride ($HgCl_2$) 0.1% + Sodium hypochlorite 4 % for 3 minutes[13]. They found that the ideal conditions were submerging the plant tissue in a sodium hypochlorite (NaOCl) solutionof 1.62% for 13.96 minutes. This method can potentially revolutionize how we approach plant tissue culture through computational modeling[9]. An effective disinfection method for strawberry micropropagation using runner tips and nodal segments as explants was developed. The explants were surface sterilized with different sterilants for different durations. Surface sterilization with mercuric chloride (0.1%) for 4 min was the optimum duration[11].This study explores the impact of various serialization protocols and plant growth regulators on explant disinfection efficiency, callus induction, and secondary metabolite production in *Melia azedarach* L. callus cultures. The findings revealed that the lowest rates of explant contamination and browning were achieved through pretreatment with benomyl and sterilization using 7% H_2O_2 and NaOCl 2%. Adjusting the pH of NaOCl reduced microbial contamination but harmed explant viability and callus induction[1].Calcium hypochlorite (Ca (ClO_2) effectively sterilizes coconut embryos, with a0.5% (w/v) solution minimizing

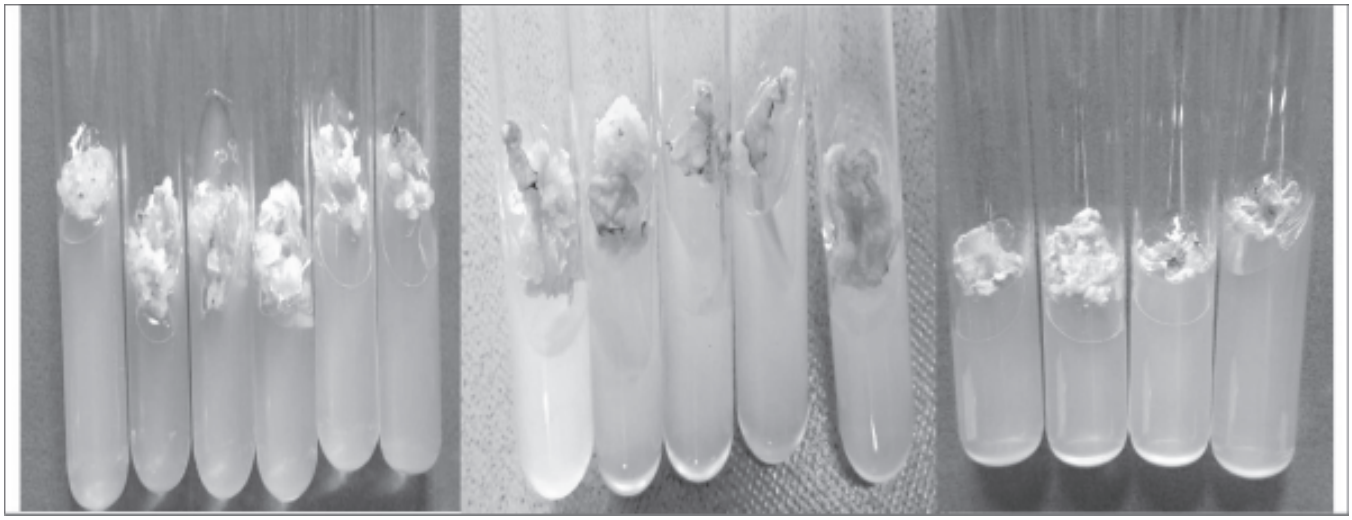

Fig.2 : Leaf explant conditions after sterilization treatment for *in vitro* culture establishment in *Bryonia laciniosa* (A) leaf explant by treating with Sodium hypochlorite (0.2%) (20 minutes) (B) internode explant treated with Mercuric chloride (0.3%) (2minutes) (C) leaf explant by treated with Calcium hypochlorite (0.4%) (2 minutes).

contamination and achieving a 93.33% germination rate[15].

A new regeneration protocol for sterilizing, producing multiple shoots, and rooting *Lagenaria siceraria* has been developed, using sodium hypochlorite for effective sterilization, BAP for efficient multiple shoot production, and NAA for optimal root induction and root production[12]. Nodal explants from a *Diplocyclos palmatus* mother plant were cultured on MS basal medium supplemented with various cytokinins and auxins to evaluate their morphogenic response.The optimal results were achieved with a combination of 5.0 µM BAP and 2.0 µM NAA, yielding an average of 8.3 shoots per explant and a shoot length of 7.2 cm after six weeks. Following a four-week transfer, shoots were successfully rooted in a low-nutrient medium (½ MS + 1.0 µM IBA), producing an average of 11.0 roots per shoot and a root length of 7.4 cm. The regenerated plantlets achieved an 80% survival rate upon acclimatization to natural conditions[24].Young leaves from shoots of *G. lotoides* L. were used as explants for callus induction and shoot regeneration. Maximum callus induction (100%) was achieved on media containing 2,4-D (0.5, 2.0, 3.5 mg/L) or NAA (2.0, 2.5 mg/L) in combination with 0.5 mg/L BAP. Although all these combinations induced maximum callus, 2,4-D proved to be overall most effective. The highest regeneration frequency (20%) was observed on a medium containing 0.5 mg/L BAP. The greatest number (2.83 ± 1.22) and length (2.16 ± 0.87 cm) of shoots per explant were obtained on medium supplemented with 0.25 mg/L BAP and 0.5 mg/L Kinetin[23]. The highest rate of Callus induction was observed from the leaf explants on MS medium with 2,4-D (3 mg/l)+ Kin (1 mg/l)[22].Leaf explants

Fig.3 : Callus induction from leaf explants of *Bryonia laciniosa* cultured on Murashige and Skoog (MS) media supplemented with different concentrations of plant growth regulators (A) Brownish callus 2,4-D 3mg/l+ Kinetin 0.5mg/l (B) Compact green callus- NAA 1.5mg/l + kinetin 1.5mg/l(C) White yellow callus 2,4-D 2.0mg/l + 0.5 kinetin (D) Friable callus NAA 0.5mg/l + Kinetin 0.5mg/l.

from *Echinopskebericho* were cultured on MS medium containing different concentrations of BAP or kinetin (KIN) and NAA for shoot multiplication. Shoots were excised from these explants and further cultured on MS medium with varying BAP and KIN concentrations along with NAA. The highest mean number of initiated shoots (4.00 ± 0.57) with 100% shoot induction was achieved on a medium containing 1.0 mg/L BAP and 0.2 mg/L NAA[7].

Conclusion

This study established an effective sterilization protocol for field-grown *Bryonia laciniosa* (Linn.) explants intended for *in vitro* culture. Treatment with 0.2% sodium hypochlorite for 20 minutes achieved the highest percentage of aseptic cultures while maintaining acceptable explants survival, highlighting its suitability for this purpose. Additionally, an efficient protocol for callus induction was developed using a combination of 2,4-D and kinetin in the culture medium. This protocol holds significant potential for mass propagation of *Bryonia laciniosa* (Linn.), a rare medicinal herb. It facilitates the production of valuable secondary metabolites through callus culture, offering a sustainable alternative to harvesting the plants themselves.

References

1. Ahmadpoor F, Zare N, Asghari R. Sterilization protocols and the effect of plant growth regulators on callus induction and secondary metabolites production in *in-vitro* cultures *Melia azedarach* L. *AMB Expr.* 2022; **12** : 3.https://doi.org/10.5897/AJB2013.12918.
2. Azad MAK, Yokota S, Ohkubo T, Andoh Y, Yahara S, Yoshizawa N. *In-vitro* regeneration of the medicinal woody plant *Phellodendron amurense* Rupr through excised leaves, *Plant Cell, Tissueand Organ Culture*. 80 : 43-50.https://doi.org/10.1007/s11240-004-8809-5.
3. Bakar DA, Ahmed BA, Taha R. *In vitro* Callus Induction and Plant Regeneration of*Celosia argentea*-An Important Medicinal Plant, *Brazilian Archives of Biology and Technology an International Journal.* 2014; **57** : 860-86.https://doi.org/10.1590/S1516-8913201402611.
4. Benjamin ED, Ishaku GA, Peingurta FA, Afolabi AS. Callus culture for the production of therapeutic compounds. *American Journal of Plant Biology.* 2019; **4**(4) : 76-84. https://doi.org/10.11648/j.ajpb.20190404.14.
5. Chavhan SA,Wathurkar RN, Shinde SA. PhytopharmacognosticReviewon*Bryonia laciniosa*, *Int J Pharmacogn Chinese Med.* 2019; **3** (3): 000170.https://doi.org/10.23880/ipcm-16000170.
6. Eliwa GI, El-Dengawy EF, Gawish MS, Yamany MM. A comprehensive study on *in vitro* propagation of some imported peach root stocks: *In vitro* explant surface sterilization and bud proliferation. *Scientific reports*. 2024; **14**(1) : 5586. https://doi.org/10.1038/s41598-024-55685-3.
7. Enyew M, Feyissa T. *In vitro* shoot regeneration from leaf explants of *Echinops kebericho*: an endangered endemic medicinal plant. *Plant Biosystems- An international Journal Dealing with All Aspects of Plant Biology*. 2024; **153**(2): 199-204.https://doi.org/10.1080/11263504.2018.1448014.
8. Gupta M, Mazumdar, UK, Shivakumar T, Vamsi ML, Karki SS, Sambath kumar R. Evaluation of the anti-inflammatory activity of chloroform extract of *Bryonia laciniosa* in experimental of chloroform extract of *Bryonia laciniosa* in experimental animal models, *Biol Pharm Bull*. 2003; **26** :1342-1344.https://doi.org/10.1248/bpb.26.1342.
9. Hesami M, Naderi R, Tohidfar M. Modeling and Optimizing *In vitro* Sterilization of *Chrysanthemum via* Multilayer Perceptron- Non-dominated Sorting Genetic Algorithm-II (MLP-NSGAII). *Frontier in plant science*. 2019; **10**: 282. https://doi.org/10.3389/fpls.2019.00282.
10. Jadhav D. Ethnomedicinal Plant used by Bhil tribe of Bibdod, Madhya Pradesh, *Indian Journal of Traditional Knowledge*. 2006; **5**(2) : 263-267.http://nopr.niscpr.res.in/handle/123456789/6849.
11. Jan A, Bhat SJA, Mir MA, Bhat M A, Imtiyaz A, Wani, Rather JA. Surface sterilization method for reducing microbial contamination of field grown strawberry explants intended for *in vitro* culture, *African Journal of Biotechnology*. 2013; **12** (39) : 5749-5753. https://doi.org/10.5897/AJB2013.12918.
12. Kabiru T, Njenga PK. *In vitro* propagation of *Lagenaria siceraria:* A plant of the Cucurbitaceae family with high medicinal value. *Asian Plant Research Journal*. 2013; **11**(5): 42-52.https://doi.org/10.9734/aprj/2023/v11i5224.
13. Kumar M, Prasad Y, Yadav A, Kumar A. Effect of two different surface sterilization (Sodium Hypochlorite and Mercuric Chloride) agents under *in-vitro* leaf explants in Gerbera (*Gerbera jamesonii- Bolus*), *The Pharma*

Innovation Journal. 2021; **10**(7): 1346-1349.

14. Patel SB, Attar UA, Sakate DM, Ghane SG. Efficient extraction of cucurbitacins from *Diplocyclos palmatus* (L.) C. Jeffrey: Optimization using response surface methodology, extraction methods, and study of some important bioactivities. *Scientific Reports.* 2020; **10**(1): 2109.https://doi.org/10.1038/s41598-020-58924-5.
15. Phuong Quynh T, Lan XuanH T, Ai My T, Thanh Thao N, Phuong Thao N, Thien Quang N. Effect of calcium hypochlorite on surface sterilization and seedling growth of Vietnamese coconut varieties. *Vietnam Journal of Biotechnology*. 2022; **20**(4): 663–673. https://doi.org/10.15625/1811-4989/16221.
16. Sagharyan M, Ganjeali A, Cheniany M, Mousavi Kouhi SM. Optimization of Callus Induction with Enhancing Production of Phenolic Compounds Production and Antioxidants Activity in Callus Cultures of *Nepeta binaloudensis* Jamzad (Lamiaceae). *Iranian Journal of biotechnology*. 2020; **18**(4): 2621.https://doi.org/10.30498/IJB.2020.2621.
17. Saito K, Sudo H, Yamazaki M, Yamakawa T, Aimi N. Bioreactor production of camptothecin by hairy root cultures of *Ophiorrhiza pumila., Biotechnology Letters*. 2002; **24**: 359-363.https://doi.org/10.1023/A:1014568904957.
18. Sen MK, Nasrin S, Rahman S, Jamal AHM. *In vitro* callus induction and plant let regeneration of *Achyranthes aspera* L., a high-value medicinal plant, *Asian Pacific Journal of Tropical Biomedicine.* 2014; **4** (1): 40-46.https://doi.org/10.1016/S2221-1691(14)60206-9.
19. Singh V, Malviya T, Tripathi DN, Naraian U. An *Escherichia coli* Antimicrobial Effect of *Arabino glucomann* an from Fruit of *Bryonia lacinosa, Carbohydrate Polimers.* 2009; **75**: 534-537.https://doi.org/10.1016/j.carbpol.2008.08.025.
20. Stevenson Naitchede LH, Nyende AB, Runo S, Borlay AJ. Plant regeneration from embryogenic callus-derived from immature leaves of *Momordica charantia* L. *Heliyon*. 2023; **9**(11) : 22122.https://doi.org/10.1016/j.heliyon.2023.e22122.
21. Supe U. Effect of Growth Regulator on Callus Induction of *Bryonia laciniosa*, *International Journal of Applied Biotechnology and Biochemistry*. 2011; **1**(14): 449-452.
22. Supe U. *In vitro* characteristics of callus induction of *Bryonia laciniosa*, *International Journal of Innovation in Engineering and Science*. 2016; **1** (2):8-10.
23. Teshome S, Feyissa T. *In Vitro* Callus Induction and Shoot Regeneration from Leaf Explants of *Glinus lotoides* (L.)- An Important Medicinal Plant. *American Journal of Plant Sciences.* 2015; 06: 1329-134.https://doi.org/10.4236/ajps.2015.69132.
24. Upadhyay A, Shahzad A, Ahmad Z, Alatar AA, Guerriero G, Faisal M. High-frequency direct organogenesis, genetic homogeneity, chemical characterization, and leaf ultra-structural study of regenerants in *Diplocyclos palmatus* (L.) C. *Jeffrey. Agronomy.* 2021; **11**(11): 2164.https://doi.org/10.3390/agronomy11112164.