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Induction of callus from lesser duckweed (*Lemna minor* L.) on Basal media fortified with PGRs.

Sudipta Kumar Sil[1] and Santanu Gupta*

[1]Department of Botany, University of Gour Banga, Malda-732103 (India)
*Department of Botany, Malda College, Malda-732101 (India)
*Corresponding Author: Email : santanubotanist@gmail.com

Abstract

Lemna minor L. is gregariously growing aquatic macrophyte which can be propagated *in vitro* in liquid basal media. The current paper focuses on callus induction from aseptic fronds of *Lemna minor* L. in three different types of basal culture media namely Murashige & Skoog basal medium; Gamborg B5 basal medium; SH- Schenk & Hildebrand basal medium supplemented with three PGRs namely IBA- (Indole butyric acid); 2, 4- D (2,4-dichlorophenoxy acetic acid); BAP- (6-benzyl aminopurine). MS medium fortified with PGRs was least responsive in callus initiation whereas Gamborg B5 medium and SH media supplemented with IBA and BA were most effective in callus induction from fronds of *Lemna minor* L.

Key words : Callus, Plant Growth Regulators, MS media, SH media, Days to Callus Induction (DCI).

Duckweeds are smallest angiosperms which propagate vigorously in eutrophic water bodies of tropical countries like India. *Lemna minor* L., commonly known as lesser duckweeds, is present on the surface of nutrient rich water bodies in tropical parts. Due to their extreme small size it is very difficult to identify them in naked eyes[1]. As the name suggests (Lemna =water, minor= small)., these plants bears a single succulent thallus which is oval in outline ranging less than 5mm in length and 2.5 mm in width. The lower side of the thallus is bears a single root ranging from 2mm-3 mm in length with an obtuse tip. They perpetuate mainly through vegetative means producing genetically identical daughter fronds . Offset connects the daughter fronds with the mother fronds establishing random supply of photosynthates from one frond to the other[10].

These aquatic macrophytes are store house of a hand full of secondary metabolites which shows anti oxidative, anti microbial and anti cancerous property[4]. Such metabolites concentration may vary when grown under *in vitro* conditions. *L.minor* L. is known to possess a rich source of phenols and flavonoids that are extremely important for their in aquatic environment. *L. minor* L. are rich source of oxalic acid which leads to their unpalatiblity.

Like other duckweeds, *L. minor* L. are rich source of plant protein that contain all the different amount of amino acids in high proportions which may be related with their high accumulation of nitrogen and phosphorus from the water bodies[5]. They can sustain the growth of poultry birds and fishes as protein supplement[3]. The growth of invasive algal species are reduced at an alarming rate when *L. minor* L. infiltrate those aquatic water bodies. Duckweeds like *L. minor* L. are also store house of beta carotene and lycopene, whose concentrations increase drastically when cultured in nutrient rich medium[6].

Different aseptic liquid culture media contains a variety of macro and micro elements which can proliferate *in vitro* growth of *L.minor* L. like MS basal medium, Hoagland media and SH media (Schenk and Hildebrandt, 1972)[8]. These media supports frond proliferation in case of *L. minor* L. However Schenk and Hildebrandt[8] supported favourable frond regeneration at a faster rate as compared to other two media. However previous reports suggests Schenk and Hildebrandt[8] media, do not support callus induction among duckweed species along with *L.minor* L. whereas Murashige and Skoog media (1972) supports favourable callus induction with greater biomass[6]. Hoagland culture media mixed with trace amount of EDTA is reported to supports the *in vitro* flowering of *L. minor* L. It has been reported that Hoagland Culture media augmented with *Acinetobacter calcoaceticus* P23 strain of PGPB doubled the biomass of *L. minor* L. under liquid growth conditions. Very few reports were there regarding *in vitro* callus regeneration of *L. minor* L. from West Bengal. using basal media like Murashige and Skoog basal medium[7], Hoagland media and Schenk and Hildebrandt[8]. The present paper focuses on the callus induction of lesser duckweed, *L.minor* L. in basal culture media fortified with different concentrations of PGRs.

Fronds of *L.minor* L. were collected from pure liquid culture maintained in our tissue culture laboratory maintained in pH 5.8, relative humidity 100%, photoperiod 16:8, light intensity 22.2 µmol m^{-2} s^{-1} and temperature 28±2°C in SH basal medium (HIMEDIA PT059) were selected as explants. The fronds were collected in mid log phase of their culture and were then surface sterilized using 0.1% mercuric chloride solution for 1 min then were transferred to culture media. Three different types of basal culture media (Murashige & Skoog basal medium; Gamborg B5 basal medium; SH- Schenk & Hildebrand basal medium) along with three PGRs namely IBA- (Indole butyric acid); 2, 4- D (2, 4-dichloro-phenoxy acetic acid); BAP- (6-benzyl aminopurine) were used in combination. pH of the media were kept 5.8-5.9. For the first three days, all the culture tubes containing explants of *Lemna minor* L. were kept in complete darkness. Then all the culture tubes were maintained at 24±2°C temperature, 80% relative humidity, photoperiod of 16:8 and 2200 lux light intensity. Days to callus induction (DCI) were observed for each separate concentrations and percentage of callus induction (%CI) were calculated.

The media composition, PGR along with the Days to callus induction and callus induction percentage were placed in Table-1 and Table-2. Table-3 depicts the biomass of callus obtained from fronds as well as frond discs of *L. minor* L.

Table-1. Induction of callus using explant from *Lemna minor*

Explant	Media compositions	DCI	% CI	Nature of callus
Entire frond	B5+1.0 µM IBA	NA	NA	NA
	B5+1.0 µM IBA+ 1.0 µM BA	NA	NA	NA
	B5+1.0 µM 2,4-D	18	20	small, brown,hard
	B5+1.0 µM IBA+ 1.0 µM BA	NA	NA	NA
	B5+1.0 µM IBA+ 2.0 µM BA	15	30	Small, brown, friable
	B5+2.0 µM IBA+ 2.0 µM BA	NA	NA	NA
	B5+2.0 µM IBA+ 4.0 µM BA	15	60	Medium, brown, hard
Frond disc	B5+1.0 µM IBA+ 1.0 µM BA	NA	NA	NA
	B5+1.0 µM 2,4-D	22	20	small, brown,hard
	B5+1.0 µM IBA+ 2.0 µM BA	18	30	Medium, brown, hard
	B5+2.0 µM IBA+ 4.0 µM BA	15	50	Medium, brown, hard

B5- Murashige & Skoog basal medium; B5- Gamborg's B5 basal medium; SH- Shenk & Hildebrand basal medium; IBA- Indole butyric acid; 2,4-D-2,4-dichlorophenoxy acetic acid; BAP- 6-benzyl aminopurine, DCI- Days to Callus Induction; CI-Callus Induction; %CI calculated on the basis of 10 tubes in triplicates; NA-Not applicable.

Table-2: Comparison of the role of different basal media in callus induction of *Lemna minor*

Explant	Media compositions	DCI	% CI	Nature of callus
Entire frond	MS+1.0 µM 2,4-D	21	25	small, brown, hard
	B5+1.0 µM IBA+ 1.0 µM BA	NA	NA	NA
	B5+1.0 µM 2,4-D	18	20	small, brown, hard
	MS+1.0 µM IBA+ 2.0 µM BA	20	20	small, brown, hard
	B5+1.0 µM IBA+ 2.0 µM BA	17	40	Small, brown, friable
	MS+2.0 µM IBA+ 4.0 µM BA	NA	NA	NA
	B5+2.0 µM IBA+ 4.0 µM BA	18	80	Medium, brown, hard
Frond disc	MS+1.0 µM 2,4-D	NA	NA	NA
	B5+1.0 µM 2,4-D	21	20	small, brown, hard
	MS+2.0 µM IBA+ 4.0 µM BA	18	30	Medium, brown, hard
	B5+2.0 µM IBA+ 4.0 µM BA	18	60	Medium, brown, hard
Both type	B5+2.0 µM IBA+ 4.0 µM BA	18	80	Medium, brown, hard

B5- Murashige & Skoog basal medium; B5- Gamborg's B5basal medium; SH- Shenk & Hildebrand basal medium; IBA- Indole butyric acid; 2,4-D-2,4-dichlorophenoxy acetic acid; BA- 6-benzyl aminopurine, DCI- Days to Callus Induction; CI-Callus Induction; %CI calculated on the basis of 10 tubes in triplicates; NA-Not applicable.

Table-3. Comparison of callus biomass induced on same combination of PGR fortified with three types of basal media for the duckweeds under study

Explant	Media compositions	Biomass of callus [mg]
Lemna minor Entire frond	MS+1.0 µM 2,4-D	06 ± 0.05
	B5+1.0 µM 2,4-D	09 ± 0.06
	MS+1.0 µM IBA+ 2.0 µM BA	12 .6 ± 0.15
	B5+1.0 µM IBA+ 2.0 µM BA	15.5 ± 0.18
	MS+2.0 µM IBA+ 4.0 µM BA	25 ± 0.25
	B5+2.0 µM IBA+ 4.0 µM BA	35 ± 0.45
	SH+2.0 µM IBA+ 4.0 µM BA	40 ± 0.50
Lemna minor Frond disc	MS+1.0 µM 2,4-D	08 ± 0.12
	B5+1.0 µM 2,4-D	10.50 ± 0.25
	MS+1.0 µM IBA+ 2.0 µM BA	16 ± 0.55
	B5+1.0 µM IBA+ 2.0 µM BA	18 ± 0.25
	MS+2.0 µM IBA+ 4.0 µM BA	42 ± 0.50
	B5+2.0 µM IBA+ 4.0 µM BA	45 ± 0.45
	SH+2.0 µM IBA+ 4.0 µM BA	45 ± 0.45

Biomass of callus expressed as mean ± SD

Among the three basal culture media used, MS media was found to be least responsive for callus induction in *Lemna minor* L. in combination with three different PGRs used both in terms of Days to Callus induction (DCI) and percentage of callus (%CI). B5 media was found to induce callus in *Lemna minor* L. in higher percentage in combination with IBA and BA in the ratio of 2:1. SH media only in combination with IBA and BA in the ratio of 2:1 induced callus growth in *Lemna minor* L., whereas other combination with SH media fortified with different PGR failed to produce callus under *in vitro* condition. Among the PGRs used, 2,4-D in combination with three basal media were found to be less effective as compared to other two PGRs namely IBA and BA. Days to callus induction in B5 media fortified with IBA and BA were found to be lower in *Lemna minor* L. However it was observed that in case of percentage of callus induction, B5 Gamborg media supplemented with IBA and BA in 2:1 ratio showed highest efficacy. Similar PGRs along with B5 Gamborg media in 1:1 ratio failed to induce callus after 21 days. MS media fortified with 2, 4-D found to induce callus at lower percentage as compared to B5 media. B5 media fortified with 2.0 µM IBA+ 4.0 µM BA showed highest percentage of callus induction in *Lemna minor* L. with lower DCI. Similar results were obtained for *Spirodela polyrrhiza* (L.) Schleid.SH media fortified with BA and IBA (2:1) showed lower DCI and highest callus induction[9]. B5 fortified BA and IBA in 2:1 ratio showed lower DCI along with higher percentage of callus induction as compared to the 1:1 combination of both IBA and BA under *in vitro* growth conditions.

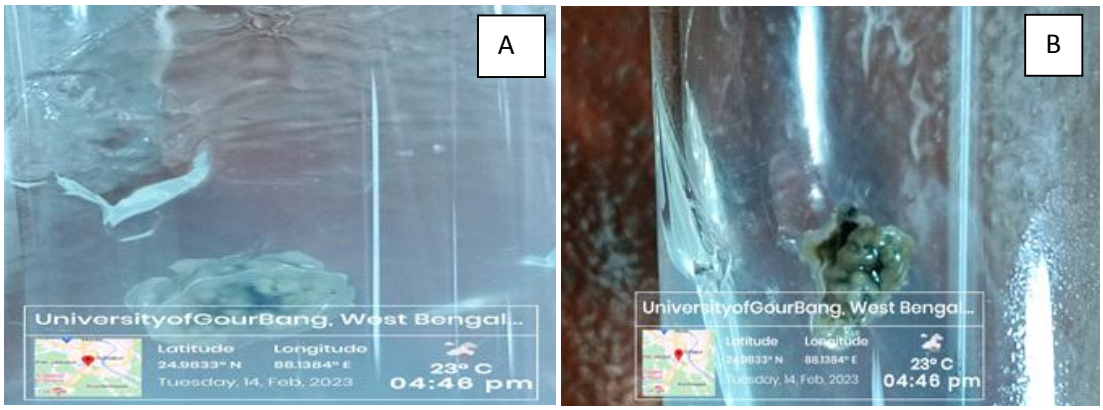

Figure A -Mature callus of *Lemna minor* L. in B5 media supplemented with 2.0 µM IBA+ 4.0 µM BA
Figure B- Mature callus of *Lemna minor* L in SH media supplemented with 2.0 µM IBA+ 4.0 µM BA

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