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BRIEF COMMUNICATION

Callus induction and plant regeneration in *Lemna minor* L.

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Abstract

Callus induction was obtained on Murashige and Skoog agar medium with 45 μM 2,4-dichlorophenoxyacetic acid under dark at 25 °C. Among the four explant types investigated, the best callus induction was obtained from two-week old fronds to which a surgical incision was applied in the basal (meristematic) region. This treatment resulted in 89.11 % of fronds producing callus which continued to proliferate for another 24 months. To obtain plant regeneration pieces of calluses were transferred onto Murashige and Skoog agar medium containing 22 μM indole-3-acetic acid and 4.6 μM kinetin and maintained under 16-h photoperiod (irradiance of 30 μmol m^{-2} s^{-1}) at 23 °C. Green fronds formed on all callus pieces. The regenerated fronds were later transferred onto Wang medium where they formed roots. The regenerated *Lemna minor* L. plants obtained through indirect organogenesis did not differ morphologically from individuals forming the stock collection.

Additional key words: *Lemnaceae*, duckweed, frond, organogenesis.

Lemna minor L. is a monocotyledonous angiosperm, floating freely on the surface of stagnant water reservoirs. Its size is small and the shoot system highly reduced. Each individual plant consists of a flattened, lens-like stem-shoot unit and an adventitious root (Landolt 1986). Thanks to their small sizes and large coefficients of asexual reproduction, plants from the family *Lemnaceae* are frequently employed as study systems, both in the field and in laboratory (Severi and Fornasiero 1983, Moon and Stomp 1997, Samardakiewicz and Woźny 2000). Tissue culture techniques within this family, however, have received only a limited attention. Callus induction and plant regeneration have been described only for *Lemna gibba* (Chang and Chiu 1978, Moon and Stomp 1997). Some characteristics of the metabolism of *L. minor* callus have also been investigated (Frick 1991, Frick and Morley 1995). Its morphogenic capacity still remains unknown, and treatments allowing complete regeneration of *L. minor* plants have not been worked out.

The aim of this work was to develop an efficient and reproducible system for callus induction and organogenesis, followed by regeneration of *Lemna minor* plants.

The plants of *Lemna minor* L. used in our experiments had been maintained vegetatively for several years on the Wang liquid medium (Wang 1990). Explants for callus induction were obtained from a two-week old stock culture. The following explants were used: *1*) whole intact plants (fronds with roots), *2*) surgically injured fronds (with an incision in the meristematic area), *3*) frond halves, *4*) basal root segments (approximately 1 cm long). The induction medium contained Murashige and Skoog (1962; MS) basal salts and vitamins supplemented with 45 μM 2,4-dichlorophenoxyacetic (2,4-D), 3 % sucrose and 0.8 % agar. The pH of the medium was adjusted to 5.7 before autoclaving. Petri dishes, each containing nine explants were incubated in darkness at 25 °C. Calluses were maintained by regular subculturing every four weeks.

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Abbreviations: BA - benzyladenine, 2,4-D - 2,4-dichlorophenoxyacetic acid, 2iP - 6-γ,γ-dimethylallylaminopurine, IAA - indole-3-acetic acid, KIN - kinetin, MS - Murashige and Skoog medium (1962).

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For regeneration purposes, the calluses were transferred onto MS medium containing 22 μM indole-3-acetic acid (IAA), 4.6 μM kinetin (KIN), 3 % sucrose and 0.8 % agar. The pH of the medium was 5.7. Cultures were kept in Petri dishes at temperaure of 23 °C and 16-h photoperiod with irradiance of 30 μmol m^{-2} s^{-1} provided by white fluorescent tubes.

For rapid multiplication, and to obtain mature plants, green fronds regenerated through indirect organogenesis were transferred to agar (0.8 %) solidified Wang (1990) medium without sucrose. The pH of the medium was 5.7. The cultures were incubated in Erlenmeyer flasks at 23 °C under continuous light (30 μmol m^{-2} s^{-1}).

Among the four explant types tested, callus formed only on the injured fronds (on 89.11 % individual explants in this group - Table 1). Callus induction was preceded by the appearance of swellings in the basal (meristematic) regions of the fronds observed after five to six weeks of culture. Callus formed in the swollen areas after 8 weeks from the initiation of cultures. After 9 weeks, the quantity of callus exceeded the surface area of the explant. Initially, the callus was cream-yellow, friable and soft, with a granular texture (Fig. 1). After several transfers, we observed a sporadic occurrence of clearly delimited, white, hard and compact spots on the surface of the cream-yellow callus. The callus proliferated relatively fast, doubling its volume at about four week intervals. Such high proliferation rate persisted for over 24 months of culture.

Table 1. The percentage of explants producing callus.

Type of explants	Number of explants	Explants producing callus [%]
Whole intact plants	150	0
Surgically injured fronds	101	89.11
Frond halves	120	0
Basal root segments	100	0

At one week after transfer to the regeneration medium and exposure to photoperiod, numerous, small-sized, green centers appeared within the callus (Fig. 2). Such centers were present on all individual calluses (giving a 100 % regeneration rate) and gradually expanded to a larger tissue area. After another week, differentiation of numerous frond initials was observed in the green callus (Fig. 3). Completely regenerated green fronds were apparent after three to four weeks of culture on the regeneration medium.

Regenerated fronds were separated from the callus and transferred onto agar solidified Wang (1990) medium for rapid multiplication. After about three to four weeks young plants (fronds with well-formed, long roots) were obtained (Fig. 4). Plants obtained through indirect organogenesis did not differ in morphological features (such as colour and habit) from individuals maintained in the stock collection.

The use of the auxin (2,4-D) at 45 μM concentration in combination with the standard MS basal medium formulation in our experiments proved remarkably effective for callus tissue induction in *Lemna minor*. A similar, high concentration of 2,4-D as well as a similar medium type were used for *L. gibba* by Moon and Stomp (1997). Also Chang and Chiu (1978) induced *L. gibba* callus on the MS medium, although in their study the optimal plant growth regulators combination involved both 2iP and 2,4-D. Further, Frick (1991) reported callus induction in *L. minor* at 2,4-D concentration as low as 9 μM, but only on a specially formulated medium and using a specific explant preparation protocol. In this study, we introduced surgical incision of the fronds as an important step in the callus induction procedure in *L. minor*, which led to a very high rate (89.11 %) of callus formation. We also found that elevated temperature (25 °C) and darkness had a stimulatory effect on the callus induction process. Frick (1991) obtained callus in *L. minor* under continuous illumination. More, Moon and Stomp (1997) declared that light is a necessary factor in callus induction in *L. gibba*, even though only 10 % of their explants formed callus. A beneficial effect of elevated temperature (25 - 28 °C) for callus induction in *L. gibba* was demonstrated by Chang and Chiu (1978).

We suggest that a high concentration of 2,4-D, the MS medium, frond incision, elevated temperature and darkness are effective and efficient factors in inducing callus tissue production in *Lemna minor* L. This is the first report of regeneration of *L. minor* plants. Regeneration was obtained in the presence of cytokinin (KIN) and auxin (IAA). The transfer of the callus tissue induced in the dark onto the regeneration medium and applying photoperiod caused morphogenesis and a rapid (taking only 3 - 4 weeks of culture) regeneration. Green fronds formed on all individual calluses (100 % efficiency). A similar, fast regeneration in *L. gibba* was described by Chang and Chiu (1978) who also used a combination of cytokinin and auxin in the regeneration medium, and exposed their cultures to photoperiod. Compared to our findings, the results of Moon and Stomp (1997) who obtained regeneration of *L. gibba* plants on SH medium (Schenk and Hildebrandt 1972) without any plant growth regulators, seem surprising. In our cultures, after transfer to the multiplication medium fronds of the *L. minor*, regenerants reached natural dimensions, roots were produced, and the resulting plants became exact copies of individuals forming the stock collection.

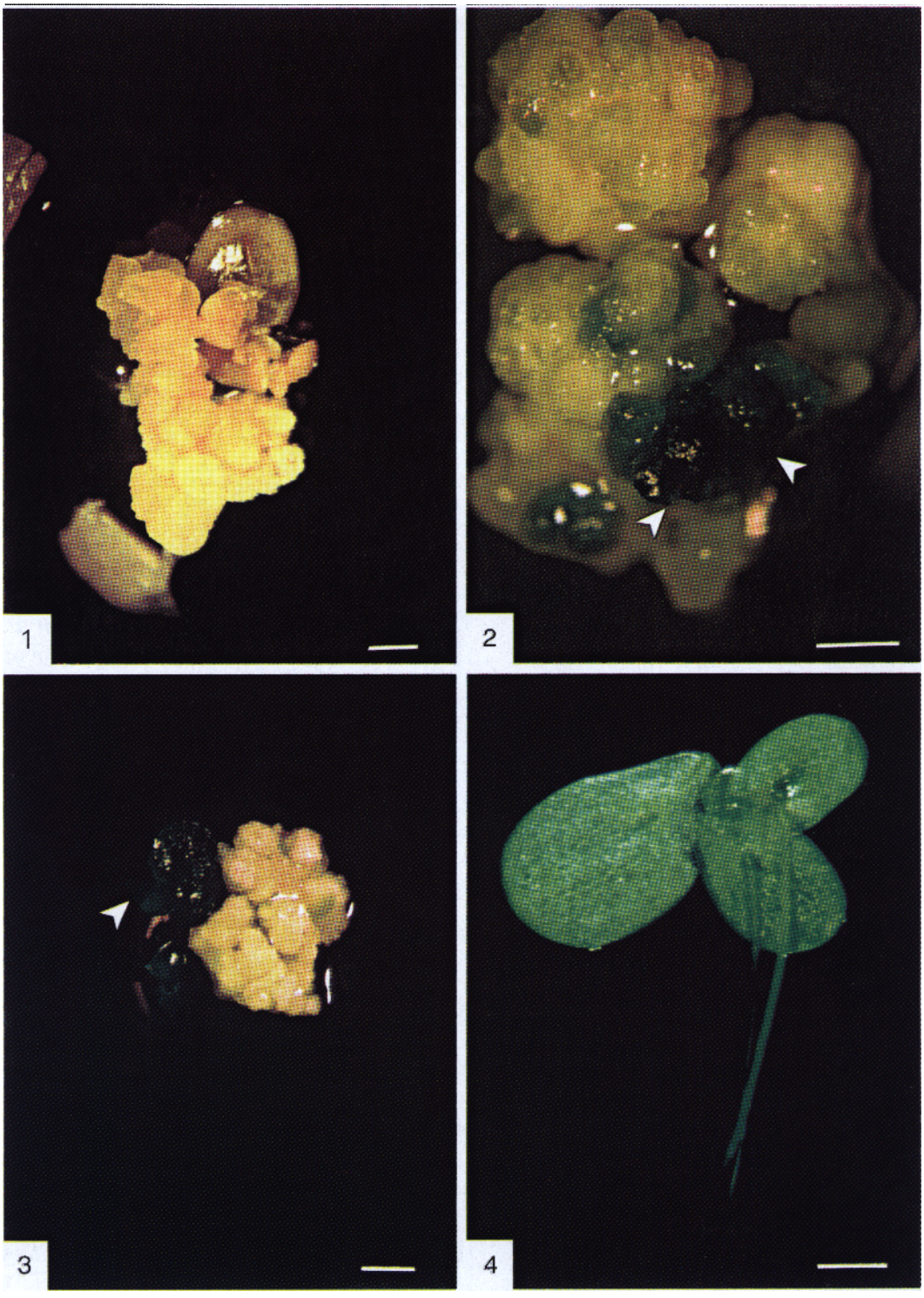

Fig. 1. Cream-yellow, friable and soft callus of *Lemna minor* L. - 9th week on callus induction medium (MS + 45 μM 2,4-D). *Scale bar* = 1 mm.
Fig. 2. Green centers (*arrowhead*) on the surface of cream-yellow callus -1 week on regeneration medium (MS + 22 μM IAA and 4.6 μM KIN). *Scale bar* = 1 mm.
Fig. 3. New fronds (*arrowhead*) formed on the callus - 2nd week on regeneration medium. *Scale bar* = 1 mm.
Fig. 4. Well-developed plants after four weeks on multiplication medium. *Scale bar* = 2 mm.

References

Chang, W.C., Chiu, P. L.: Regeneration of *Lemna gibba* G3 through callus culture. - Z. Pflanzenphysiol. **89**: 91-94, 1978.

Frick, H.: Callogenesis and carbohydrate utilization in *Lemna minor*. - J. Plant Physiol. **137**: 397-401, 1991.

Frick, H., Morley, K.: Metabolism of lactose by *Lemna minor* L. (duckweed) callus. - Process Biochem. **30**: 57-62, 1995.

Landolt, E.: The Family of *Lemnaceae* - a Monographic Study. Vol. 1. Zurich, Switzerland. - Veröff. geobot. Inst. E.T.H., Stiftung Rübel 1986.

Moon, H.K., Stomp, A.M.: Effects of medium components and light on callus induction, growth, and frond regeneration in *Lemna gibba* (duckweed). - *In Vitro* cell. dev. Biol. Plant **33**: 20-25, 1997.

Murashige, T., Skoog, F.: A revised medium for rapid growth and bioassays with tobacoo tissue cultures. - Physiol. Plant. **15**: 473-496, 1962.

Samardakiewicz, S., Woźny, A.: The distribution of lead in duckweed (*Lemna minor* L.) root tip. - Plant Soil **226**: 107-111, 2000.

Schenk, R.U., Hildebrandt, A.C.: Medium and techniques for induction and growth of monocotyledonous and dicotyledonous plant cell cultures. - Can. J. Bot. **50**: 199-204, 1972.

Severi, A., Fornasiero, B.: Morphological variations in *Lemna minor* L. and possible relationship with abscisic acid. - Caryologia **36**: 57-64, 1983.

Wang, W.: Literature review on duckweed toxiticy testing. - Environ. Res. **52**: 7-22, 1990.