EFFECTS OF MEDIUM COMPONENTS AND LIGHT ON CALLUS INDUCTION, GROWTH, AND FROND REGENERATION IN LEMNA GIBBA (DUCKWEED)

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SUMMARY

Basal media, plant growth regulator type and concentration, sucrose, and light were examined for their effects on duckweed (Lemna gibba) frond proliferation, callus induction and growth, and frond regeneration. Murashige and Skoog medium proved best for callus induction and growth, while Schenk and Hildebrandt medium proved best for frond proliferation. The ability of auxin to induce callus was associated with the relative strength of the four auxins tested, with 20 or 50 μM 2,4-dichlorophenoxyacetic acid giving the highest frequency (10%) of fronds producing callus. Auxin combinations did not improve callus induction frequency. Auxin in combination with other plant growth regulators was needed for long-term callus growth; the two superior plant growth regulator combinations were 10 μM naphthaleneacetic acid, 10 μM gibberellic acid, and 2 μM benzyladenine with either 1 or 20 μM 2,4-dichlorophenoxyacetic acid. Three percent sucrose was best for callus induction and growth. Callus induction and growth required light. Callus that proliferated from each frond's meristic zone contained a mixture of dedifferentiated and somewhat organized cell masses. Continual callus selection was required to produce mostly dedifferentiated, slow-growing callus cell lines. Frond regeneration occurred on Schenk and Hildebrandt medium without plant growth regulators but was promoted by 1 μM benzyladenine. Callus maintained its ability to regenerate fronds for at least 10 mo. Regenerated fronds showed a slower growth rate than normal fronds and a low percentage of abnormal morphologies that reverted to normal after one or two subcultures.

Key words: tissue culture; plant growth regulators; media; light; sucrose.

INTRODUCTION

Duckweeds are the sole members of the Lemnaceae. All 32 species within the family’s 4 genera are free-floating, aquatic monocots, found on still or slow-moving fresh water. The duckweeds have long been an object of botanical fascination in the field, in the laboratory, and on still or slow-moving fresh water. The duckweeds have long been most recently for their potential in wastewater remediation (Landolt laboratory on simple mineral nutrient solutions, proliferating asexually, and Kandeler, 1987). They can be conveniently grown in the laboratory across genera, species, and geographical isolates within species (strains). The ease of propagation, clonal growth habit, morphological organization, and small genome size make the duckweeds excellent subjects for developmental and molecular studies with the laboratory convenience of yeast. The ease of asexual propagation and long-term stability of clones has allowed the establishment of an extensive collection of over 900 strains across all species of Lemnaceae by Landolt (1986). Individual strains within this collection have been used for a variety of duckweed studies by a number of researchers over the last several decades. The collection has recently been transferred to our laboratory.

Commercial use of duckweeds in a variety of applications is increasing. A recent patent search has identified 78 patents. Applications include the use of duckweed and its proteins for livestock feed and food additives (Dewanji, 1993; Rokonuddin et al., 1993; Haustein et al., 1994), the use of duckweed for wastewater cleanup and for integrated wastewater-aquaculture systems (Oron et al., 1988; Cui et al., 1994), and isolation of useful secondary metabolites synthesized by duckweeds, including a steroid that induces flowering (Kaihara and Takimoto, 1991), an algicide (Crombie and Heavers, 1992), and compounds with pharmacological activity (Mesmar and Abusauda, 1991). Duckweeds are quite sensitive to environmental pollutants and are used as bioindicator species (Holst and Elbgwanger, 1982; ASTM, 1991; Jenner and Janssen-Mommen, 1993). Fresh and processed duckweed are marketed for human consumption.

Further development of commercial uses of duckweeds could be done by exploiting the genetic diversity within the Lemnaceae or by enhancing the genetic diversity through genetic engineering. Despite increasing interest in both basic science and technological applications, cell and molecular technology for duckweed has not been developed to any great extent. Methods for mutagenesis are available (Slavin and Cohen, 1988), and limited gene cloning has been done

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Duckweed plants used in these experiments were produced from a *L. gibba* G3 culture provided by J. Slovin, USDA (Beltsville, MD). Frond stock cultures were maintained on modified Hoagland's solution containing 0.08 mg/l 1(16 mM) CaSO4·2H2O and 10 g/l sucrose. All media components were combined, the medium pH adjusted to 5.8, and then autoclaved at 121 °C for 20 min, with the exception of indoleacetic acid (IAA) and gibberellic acid 3 (GA3), which were filter-sterilized and then added to cooled, autoclaved media. Unless stated otherwise, incubation of stock fronds and experimental cultures was at 23 °C, under a 16-h light photoperiod of approximately 40 μmol m⁻² s⁻¹ illumination provided by Gro-Lux fluorescent lights. Stock fronds taken from 2-wk-old cultures were used to test the effects of (a) basal media, (b) plant growth regulator type and concentration, and (c) sucrose on frond proliferation, frond survival, and callus induction. Callus proliferated in these experiments was then used to test the effects of plant growth regulator type and concentration, sucrose concentration, and light on callus proliferation and frond regeneration.

**Frond proliferation and callus induction.** The differing abilities of basal media to support frond proliferation and callus induction were tested using: MS (Murashige and Skoog, 1962), half-strength MS, NN (Nitsch and Nitsch, 1969), SH (Schenk and Hildebrandt, 1972), and B5 (Gamborg et al., 1968). Four fronds per medium treatment were placed ventral side down on 25 ml of medium in a 100 X 15 mm petri dish. The experiment was replicated 5 times (5 plates), giving a total of 20 observations per treatment. After 4 wk in culture, each frond was scored for frond proliferation, frond survival, and callus induction. Callus derived from this initial experiment was transferred to MS medium with either 50 μM 2,4-D and 2 μM benzyladenine (BA) or 1 μM 2,4-D, 10 μM naphthaleneacetic acid (NAA), 10 μM GA3, and 2 μM BA to maintain growth. This callus was used in subsequent experiments to test the effects of plant growth regulators, sucrose concentration, and light on callus proliferation and frond regeneration.

Two experiments were conducted to determine the effects of (a) auxin type and concentration and (b) sucrose concentration on frond proliferation, frond survival, and callus induction. The auxin experiment had 22 auxin treatments: 4 auxins, 2,4-D, IAA, NAA, and indolebutyric acid (IBA) were each tested at 4 concentrations: 0, 2, 20, and 50 μM (16 treatments), and 20 μM IAA, IBA, or NAA were tested in combination with either 20 or 50 μM 2,4-D (6 treatments). MS containing 30 g/l sucrose and 10 g/l agar was used as the basal medium. In the sucrose experiment, concentrations of 0, 10, 30, and 80 g/l sucrose were tested using MS basal medium supplemented with 50 μM 2,4-D. In both experiments, 4 fronds per medium treatment were placed ventral side down on 25 ml of medium in a 100 X 15 mm petri dish, sealed with parafilm. The experiment was replicated 5 times (5 plates), giving a total of 20 observations per treatment. After 4 wk in culture, each frond was scored for frond proliferation, frond survival, and callus induction. Each experiment was replicated 3 times giving a total of 15 observations per treatment. After 4 wk in culture, the ability of each callus piece to regenerate fronds (frequency of frond regeneration) and the number of fronds regenerated per callus piece was determined.

Fronds regenerated in this experiment were used to test the relative growth rates between regenerated fronds and stock fronds maintained on modified Hoagland's solution. Both regenerated and stock fronds were cultured on SH or MS medium without plant growth regulators, or with either BA or 2IP at 1 μM. Each of the 12 treatment combinations, 5 callus pieces per callus, were tested in triplicate giving a total of 30 observations per treatment. After 4 wk of culture, frond proliferation was determined by final frond count and by total frond fresh weight.

All experiments were done using SAS (SAS Institute Inc., Cary, NC) analysis of variance procedures. Duncan's Multiple Range test was used to establish main effects of medium type and auxin type in Table 1 and Figure 2; contrast analyses were performed on the data from experiments with factorial structure. Specific analyses and the results are noted in the appropriate figure and table footnotes and text. All tests for significance were conducted at the α = 0.05 level.

**RESULTS AND DISCUSSION**

Frond proliferation and callus induction. Frond proliferation preceded callus formation on all basal media tested supplemented with 50 μM 2,4-D, beginning 3–5 d after culture establishment and continuing for the first 2 wk of culture. Fronds curled irregularly in all treatments and white or yellow senescent fronds were observed after 2 wk. By Week 4, frond proliferation on MS or half-strength MS media was significantly retarded over that seen on SH, NN, or B5 media (Table 1). Frond senescence was low and uniformly distributed across all media, except SH medium, which showed significantly greater senescence (Table 1).

The frequency of callus induction from fronds varied significantly among basal media. Media that promoted frond proliferation did not favor callus induction (Table 1). Callus induction was best on MS and half-strength MS media but the frequency of fronds giving rise...