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An in vitro propagation protocol of two submerged macrophytes for lake revegetation in east China

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Abstract

Thirty-six programs have been set up to revegetate the degraded lake wetlands in east China since 2002. Most projects however faced deficiency of submerged macrophyte propagules. To solve the problem, alternative seedling sources must be found besides traditional field collection. This paper deals with an in vitro propagation protocol for two popularly used submerged macrophytes, *Myriophyllum spicatum* L. and *Potamogeton crispus* L. Full strength Murashige and Skoog-based liquid media (MS) plus 3% sucrose in addition to 0–2.0 mg l^{-1} 6-benzylaminopurine (BA) and 0–1.0 mg l^{-1} indoleacetic acid (IAA) were tried for shoot regeneration. Meanwhile, full, half or quarter strength MS in addition to 0, 0.1 or 0.2 mg l^{-1} naphthaleneacetic acid (NAA) were tested for root induction, respectively. Results indicated that both species had the ability of regeneration from stem fragments in MS without further regulators. However, the addition of 2.0 mg l^{-1} BA with 0.2 or 1.0 mg l^{-1} IAA in MS drastically stimulated the regeneration efficiency of *M. spicatum*, while the addition of 2.0 mg l^{-1} BA with 0.2 or 0.5 mg l^{-1} IAA in MS significantly stimulated that of *P. crispus*. For root induction, full strength MS in combination with 0.1or 0.2 mg l^{-1} NAA was preferred by *M. spicatum*, and the same MS without or with 0.1 mg l^{-1} NAA was preferred by *P. crispus*. Seedlings of each species produced from tissue culture room had a 100% survival rate on clay, sandy loam or their mixture (1:1) in an artificial pond, and phenotypic plasticity was exhibited when the nutrient levels varied among the three types of sediments. This acclimation of seedlings helped develop the shoot and root systems, which ensured seedling quality and facilitated the transplantation. Our study has established an effective protocol to produce high quality seedlings for lake revegetation programs at a larger scale. Since the two species we tested represent different regeneration performances in nature but shared similar in vitro propagation conditions, this study has indicated a potentially wide use of the common media for preparing seedlings of other submerged macrophytes.

Keywords: In vitro propagation; Lake; *Myriophyllum spicatum*; *Potamogeton crispus*; Revegetation

1. Introduction

Most urban and suburban shallow lakes in China have undergone accelerated eutrophication in recent years (Jin, 2001). Polluted by agricultural runoff, industrial and domestic wastewater, and unsustained fishery, all the five largest lakes in China have excessive nitrogen (N) and phosphorus (P) levels (Jin, 2001; Qiu et al., 2001). The ensuing deterioration of water quality has caused not only environmental and economic problems, but also serious public health risks (Qiu et al., 2001).

Since 2002, nearly 60 programs have been developed to restore wetlands in China, among which 36 programs are focused on eutrophied lakes. As aquatic macrophytes play a central role in sustaining aquatic ecosystems (Spencer et al., 1997; McCann et al., 2000; Havens et al., 2004), vegetation recovery work has taken place in most lake restoration projects. Although natural restoration of local species and colonization of species from nearby sites have been reported elsewhere (Mauchamp et al., 2002; Price et al., 2002), direct seeding and transplanting has been practiced frequently in China. Such strategies depend on the propagule bank present, including

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seeds, rhizomes and seedlings (Acosta et al., 1999; Combroux et al., 2002). Where the propagule banks have been depleted, the probabilities of developing extensive vegetation naturally are limited, and artificially seeding and transplanting may be a logical alternative for establishing plant communities (Zhang et al., 2001; Mckinstry and Anderson, 2003).

However, the proportion of healthy ecosystems in China is too small to support so large a demand of vegetative propagules, also, sudden large-scale harvests of biomass may put the original fragile donor ecosystems at risk of new imbalances. Submerged macrophytes are even more difficult to be harvested than terrestrial plants. As aquatic grasses are usually too tender to endure the mechanical collection force, harvested materials are mostly fragments of shoots, rhizomes and roots. It is reported that such fragments can regenerate and colonize in nature (Barrat-Segretain and Bornette, 2000; Capers, 2003). However, this capacity varies among species, as well as fragment types and season of fragmentation (Barrat-Segretain et al., 1998; Barrat-Segretain and Bornette, 2000). To solve the issue of propagule shortage, alternative propagule sources must be found to supplement field collections. Also, propagule quality, especially well developed root systems are important considerations.

In vitro tissue culture has been identified as an effective technique for plant propagation. It has received wide success in the propagation of important crop strains (Agrawal and Ram, 1995; Isutsa, 2004), medically or economically valuable trees and grasses (Kane et al., 1999; Rout et al., 2000), and endangered plant species (Sudherson et al., 2003). The interest in using in vitro techniques to help solve the problem of propagule deficiency in revegetation programs is growing. Successful examples include Sarma and Rogers (2000), who developed a regeneration procedure for *Juncus effusus* L., and Wang et al. (2004), who developed a tissue culture protocol for *Scirpus robustus* Pursh. In our previous work, we applied tissue culture techniques in preparing seedlings of *Spartina patens* (Ait.) Muhl, which showed success during vegetating coastal areas in east China (Zhou et al., 2003). We hope that the tissue culture technique will also take function in lake recovery programs in China.

Myriophyllum spicatum L. and *Potamogeton crispus* L. are two submerged macrophytes popularly used in lake revegetation programs (Zhu et al., 2002; Keskinkan et al., 2003; Lauridsen et al., 2003; Sivaci et al., 2004). The two species have been considered effective in reducing eutrophication (especially for N and P; Song et al., 1997; Zhou et al., 2000) and inhibiting blue–green algae (Nakai et al., 2000; He et al., 2002). A distinguishing characteristic of *P. crispus* is its life history, as its turions germinate in autumn and display early rapid growth during April–May, when most other submerged macrophytes are yet dormant (Jian et al., 2003). *M. spicatum* and *P. crispus* are usually planted together in east China.

This paper describes an in vitro propagation study on *M. spicatum* and *P. crispus*. We wanted to identify efficient propagation procedures that could provide (1) adequate seedling quantities; (2) improved seedling quality, especially in well-developed root systems; and (3) consistently healthy and larger seedlings to facilitate transplantation.

2. Material and methods

2.1. Material and disinfection

M spicatum reproduces naturally through seeds, stolons and fragments, while fragments are the primary mechanism responsible for rapid dispersal of the species (Smith et al., 2002). *P. crispus* can produce seeds and turions, with turions serving as the primary dispersal mechanism (Jian et al., 2003). The two macrophytes were once widely distributed in east China, but have been seriously reduced in recent years, due mainly to excessive collection and destruction of their habitats.

Parent material used for in vitro propagation in our experiments was collected from east Taihu Lake (30°55′N, 119°52′E). Fresh shoots of the two species were taken into the laboratory, incubated in soap solution for 2 days and in tap water for another 1 day. The shoots then cut into fragments of 2–3 cm in length.

The disinfection process involved in the study was based on common procedures with ethanol and sodium hypochlorite (Kane et al., 1999; Wang et al., 2004). According to our previous experience, the process for the two submerged species was modified into a submersion in 70% ethanol for 30 s and subsequently 10% commercial bleach (5.75% sodium hypochlorite by weight) for 3 min. All fragments used in the experiment were disinfected with the above procedure, followed by rinsing in sterile distilled water for five times before culture.

2.2. Propagule regeneration

Murashige and Skoog-based liquid media (MS) plus 3% sucrose were used through the regeneration process. The media were adjusted to pH 5.7, divided into 40 ml in each flask and autoclaved at 121 °C for 20 min (Zhou et al., 2003). The disinfected shoots were first cultured in MS media without growth regulators for the first 30 days, then regenerated shoots were collected for medium selection.

To determine the proper medium for shoot regeneration, MS was added with 0.2–2.0 mg l^{-1} 6-benzylaminopurine (BA) and 0.1–1.0 mg l^{-1} indoleacetic acid (IAA). We have found that the concentration of BA should be higher than that of IAA when shoots are expected. Therefore 10 combinations of growth regulators were used in the experiment (Table 1), with five replicates for each combination. MS without regulators (S_0) served as control solution. New stems of plants collected from the first growing cycle were cut into 3 cm in length, with each containing four nodes. The stem fragments were cultured in MS media with different regulators for 20 days before analysis. The number of regenerated shoots from each stem fragment was first counted. The length of each new fresh shoot was also recorded. Finally, the shoots were dried at 80 °C for 60 h and weighed. As each new shoot would develop into an individual, the shoot number indicated quantity of products from different media, length and weight of each new shoot were used to describe the quality of products.

For root induction, new shoots 2–3 cm in length were harvested from shoot-regeneration medium of MS with