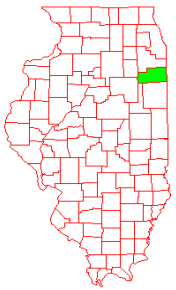
Induction of Callus Tissue from Mature Stem Explants of the Endangered Kankakee Mallow, *Iliamna remota*



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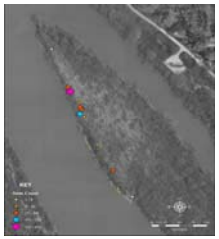

Langham Island, Kankakee, Illinois

ABSTRACT - The Kankakee mallow (*Iliamna remota*) is an herbaceous, perennial of the Malvaceae family and is endangered in Illinois. The purpose of this study was to determine an efficient disinfestation procedure and the most effective levels of auxin and cytokinin needed to regenerate callus tissue from mature stem explants. The disinfestation process involved immersions in 95% EtOH, 0.1% $HgCl_2$, and 10-20% NaOCl, followed by three sterile water rinses. Culture media were composed of Murashige & Skoog (MS) medium containing 0.5 mg/l indolebutyric acid (IBA) with 0.1 mg/l thidiazuron (TDZ)(#1), 0.2 mg/l TDZ(#2), or 0.3 mg/l TDZ(#3); or MS medium containing 0.5 mg/l naphthaleneacetic acid (NAA) with 0.1 mg/l TDZ(#4), 0.2 mg/l TDZ(#5), or 0.3 mg/l TDZ(#6). Explants were cultured for three weeks and then transferred to media without TDZ for an additional 3 weeks. Out of 216 explants cultured, 64% were lost to contamination (58% for the control explants). Culture medium 1, 2, 3, and 5 produced similarly high rates of callus production. These results indicate that the concentrations and ratios of the auxin and cytokinin have a relatively wide range in their effectiveness for calligenesis and IBA was found to have a more positive influence than NAA.

INTRODUCTION

The Kankakee mallow (*Iliamna remota*) has an endemic range limited to Langham (Altorf) Island in the Kankakee River, Kankakee Co., Illinois (McDonnell *et al.*, 2006). While more traditional cultivation methods have been successful in propagating *I. remota*, plant tissue culture has been used to micropropagate species that have become rare or endangered (Fay, 1992) and has the potential to expedite cultivation and use less starting material.

In order to determine the optimal tissue culture medium composition, the influence of varying concentrations and ratios of thidiazuron (TDZ), indole-3-butyric acid (IBA), and α-naphthaleneacetic acid (NAA) on *I. remota* stem explants was examined. TDZ is a cytokinin which has been shown in some studies to proliferate callus at high rates (Murthy *et al.*, 1998). In combination with auxins, such as NAA, TDZ has been found to induce shoot formation (D'Onofrio & Morini, 2005). IBA, the other auxin examined in this study, is known to induce leaf formation and, in conjunction with TDZ, shoot regeneration (Landi & Mezzetti, 2006).

The purpose of this study was to identify an efficient disinfestation procedure for mature stem tissue, as well as to determine the most effective levels of TDZ, IBA, and NAA to produce callus and later caulogenesis (shoot initiation). It is hoped through this study that a greater understanding of how to preserve and propagate this plant will be reached.

MATERIALS & METHODS

- Treatments each included 36 internodal stem sections, 15mm in length, and split lengthwise.
- Explants went through the following disinfestations treatments: 1) 95% EtOH 30s, 0.1% $HgCl_2$ (mercuric chloride) 60s, 10% $NaOCl_2$ (bleach, + 1 drop of tween) 10 min.; 2) 95% EtOH 30s, 0.1% $HgCl_2$ 60s, 15% bleach 15 min.; 3) 95% EtOH 30s, 0.1% $HgCl_2$ 60s, 20% bleach 20 min.; 4) 95% EtOH 60s, 0.1% $HgCl_2$ 60s, 10% bleach 10 min.; 5) 95% EtOH 60s, 0.1% $HgCl_2$ 60s, 15% bleach 15 min.; 6) 95% EtOH 60s, 0.1% $HgCl_2$ 60s, 20% bleach 20 min.. Each treatment was followed by 3 sterile water rinses. The control treatment contained only MS (Murashige and Skoog) basal medium.
- Explants were cultured horizontally, cut side down, on MS medium containing 0.5 mg/l IBA with 0.1 mg/l TDZ, 0.2 mg/l TDZ, or 0.3 mg/l TDZ; or MS medium containing 0.5 mg/l NAA with 0.1 mg/l TDZ, 0.2 mg/l TDZ, or 0.3 mg/l TDZ. Thirty-six sections were placed in each of the 6 different media, and 36 sections were also placed in MS basal medium containing no growth regulators (control).
- The culture tubes were placed in a growth room at 23°C, 2 klux, and 16 h photoperiod.
- After 3 weeks, the explants were transferred to the same media, but without TDZ, for an additional three weeks.
- The tubes were monitored weekly for contamination, tissue swelling, callus formation, and shoot formation.

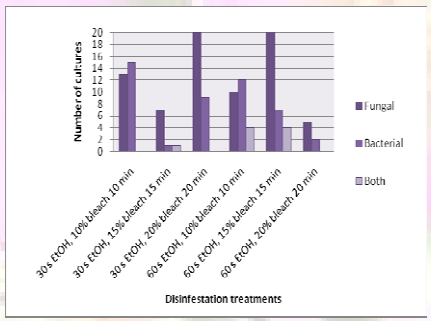

Figure 1. Cultures lost to contamination.

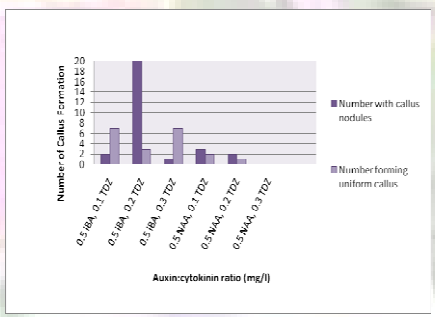

Figure 2. Influence of auxin:cytokinin ratio on callus formation.

RESULTS AND DISCUSSION

Treatment 2 and 6 had the lowest amount of contamination (Figure 1). However, treatment 6 may have been damaged by overexposure to the ethanol and bleach solutions, since it did not show any callus formation (Figure 2).

Hormonal balance is a key factor in the regulation of morphogenesis. The interaction between auxin and cytokinin throughout development is complex and the balance between them controls the formation of roots, shoots and callus tissue *in vitro*. BA has been the most extensively used cytokinin in tissue culture, while recently the important effects of TDZ have come to light (Landi & Mezzetti, 2006). Previous work by McDonnell (2006) showed more callus growth, tissue differentiation, and early shoot structures in media containing TDZ compared to BA. In their work on *Cydonia oblonga*, D'onofrio & Morini (2005) showed that the application of TDZ resulted in a dramatic increase in the production of adventitious shoots, suggesting that the shoot regeneration process required the presence of a cytokinin. TDZ also modulates the endogenous levels of auxin by increasing the levels of the natural auxin indoleacetic acid (IAA). TDZ, when combined with IBA, increased the number of explants with individual calli, confirming the positive effects of auxin on the promotion of caulogenesis.

When compared to IBA, NAA produced fewer explants with callus in our study (Figure 2). Thus, IBA is a more effective auxin when compared to NAA. The greatest tissue response observed was callus development, although the beginning of shoot organogenesis was observed in a small number of cultures.

Disinfestation set-up in sterile transfer hood

Friable callus (0.5 mg/l NAA, 0.2 mg/l TDZ)

Nodular callus (0.5 mg/l IBA, 0.2 mg/l TDZ)

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