Long Term Preservation of Clonally Propagated Turfgrass Species

Harrison Hughes

Colorado State University

Leigh E. Towill

NSSL, USDA, Fort Collins, CO

Goals:

- *Initiate in vitro cultures of bermudagrass, zoysiagrass, saltgrass and buffalograss in vitro.*
- *Develop suitable shoot tip culture media for proliferation of the 4 species.*
- *Examine cryopreservation protocols for the 4 species.*

Clones of saltgrass (6), buffalograss (3), bermudagrass (1), and zoysiagrass (1), were established in the greenhouse and grown for a source of materials to put into tissue culture. It is important to establish the clones in vitro as the protocol to be used for cryopreservation requires very small growing points which will need to be established in vitro for after freezing. If the tissue contains bacteria or fungal contaminates they will likely overgrow any plant tissue thus obscuring positive results.

Various treatments involving different lengths of time in bleach and the use of or lack thereof of varying concentrations of PPM, a commercially patented compound with antibiotic activity, were used to disinfect tissue samples of buffalograss, bermudagrass, and saltgrass. Basal medium used was ½ strength MS and Nitsch and Nitsch vitamins plus 5mg/l thiamine, 2 mg/l glycine and 30 g/l sucrose. Best results were obtained when small sections (1-2 cm) were used and when either a bleach soak for 20 minutes (for buffalograss) or 10 minute soak in bleach with 5 ppm/l of PPM in the medium (for bermudagrass).

Clean cultures of clones of buffalograss, bermudagrass, and saltgrass have been established and are being proliferated for use in the cryo studies.

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Specific clones of saltgrass (6), buffalograss (3), bermudagrass (1), and zoysiagrass (1) were established in market packs in a nonsoil growing media in late June. Establishment in the greenhouse was necessary to provide a source of explants throughout the winter season. Furthermore, explants taken from the greenhouse, as compared to the field, are more easier freed from bacterial and fungal contamination. These were maintained under natural lighting at 25 + or - 3 C day and 21 +or - 3 C night temperatures. Plants were fertilized once or twice a week and watered as needed. Approximately 6-8 market packs per clone have been established.

Only nodal sections from young, rapidly growing stolons/rhizomes were harvested as explants for initiation of in vitro cultures. Initial experiments evaluated large (5 cm or greater) and small (1-2 cm) nodal sections of buffalograss with varying disinfestation procedures. Sections were given standard soapy water and rinse treatments followed by surface sterilization in 10% bleach plus surfactant for 10 or 20 minutes. Nodal sections were then plated onto a basal medium of ½ strength MS with Nitsch and Nitsch vitamins plus 5 mg/l thiamine, 2 mg/l glycine, and 30 g/l of sucrose. A commercially available patented compund, PPM, which has antibiotic activity was added to some of the media at 2 or 5 mg/l. Approximately 20% of the cultures were free of contamination with slightly more obtained from smaller sections with a 20 minute soak in 10% bleach. The addition of PPM did not increase the number of cultures free from contamination with buffalograss.

Nodal sections of bermudagrass were disinfested as before except that they were surface sterilized in 10% bleach for only 10 minutes. They were subsequently plated onto media as before but containing no IBA or 0.5 mg/l of IBA and at 0, 2 or 5 mg/l of PPM. Those cultures without PPM in the media were overgrown by a fungus as were most of the cultures with only 2 mg/l of PPM. Approximately ½ of the cultures with 5 mg/l of PPM have remained contamination free. Another experiment with bermudagrass involved shaking the nodal sections over a 2 ½ day period in a liquid medium with 5 mg/l of PPM followed by various plating procedures onto varying medium. Only a few cultures remain clean at this time as those plated directly onto medium with low levels of PPM became contaminated.

Nodal sections from saltgrass were given standard disinfestation treatments followed by culture for 5 days in liquid medium containing 1/4 MS, 2% sucrose and 5. 10 or 20 mg/l of PPM. Nodal sections were subsequently transferred into standard medium with no BA or 10 mg/l of BA and 1, 2, or 5 mg/l of PPM. After culture for approximately 2 weeks ½ of the clean cultures from each treatment combination were transferred to similar medium but without PPM. Some cultures which initially were clean when transferred to media without PPM became contaminated while nearly all cultures maintained within media with PPM remained free of contamination. This would indicate that the PPM reduces growth of contaminates but may not necessarily eliminate all bacteria and fungi.

Currently we have approximately 60 cultures of buffalograss, bermudagrass, and saltgrass with no apparent contamination.

In the coming year, we intend to continue our efforts into developing a better protocol for obtaining clean cultures with shake culture of nodal sections in PPM, higher levels of bleach and culture of explants in media with PPM. While doing this, we will also develop media to stimulate proliferation and growth of the grass by testing varying amounts of growth regulators on the clean cultures. Once sufficient clean cultures are available we will initiate cryopreservation studies.