Long-Term Preservation of Clonally Propagated Turfgrass Species

Colorado State University

Harrison G. Hughes

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Objectives:

1. *Develop suitable micropropagation procedures for selected genotypes of bermudagrass, zoysiagrass, saltgrass and buffalograss.*
2. *Develop suitable shoot tip culture media (STCM) for the four species.*
3. *Examine cryopreservation of the four species using vitrification methodologies.*

Clones of all 4 species (bermudagrass, buffalograss, saltgrass, and zyosiagrass) have been established *in vitro* using a ½ strength MS medium with Nitsch & Nitsch vitamins, 5 mg/l of thiamine, 2 mg/l of glycine, 30 g of sucrose with the pH adjusted to 5.7 and 7 g of agar and varying amounts of growth regulators. We now can initiate cultures from the greenhouse with reduced contamination following a disinfestation procedure that includes a pre-wash in the greenhouse followed by a 15-30 minute wash in soapy water in the laboratory. After 30-60 minutes in a running deionized water rinse, the nodal sections are surface disinfested in 10% bleach with Tween 20 for 15 minutes followed by 3 sterile water rinses. This procedure, sometimes with PPM (a proprietary anti-microbial compound), results in 50% or greater clean cultures. Rapidly growing nodal sections work best and preferably those not established in soil.

We have succeeded in proliferating both buffalograss and saltgrass using the aforementioned media with high levels of BA, 10 mg/l. However, proliferation remains relatively slow requiring 8-12 weeks to develop sufficiently for subculture. We continue to evaluate various levels of BA for subcultured shoots in combination with various other growth substances, primarily low levels of 2,4-D and NAA. We are proliferating these two species in sufficient quantities to provide for cryopreservation studies in the coming year.

Although we have approximately 50 cultures of both bermudagrass and zoysiagrass *in vitro,* proliferation is minimal and requires further research. BA levels of 10 mg/l often cause yellowing and eventual death of cultures of these two species, especially bermudagrass.

Due to the slow progress in developing rapid proliferation of tissue cultures of the species, we started evaluating procedures other than regrowth for determination of viability of frozen buds. We initially looked at browning as an indication of cell/tissue death. Buds subjected to immersion into liquid nitrogen (-196C) without protective treatments and thawed remained green for at 48 hours after freezing. Tissues normally brown, an indication of cell death, after freezing to these temperatures and subsequent thawing. We also looked at the vital stain tetrazolium, TDZ, as an indication of live/dead cells. Again the success was somewhat limited.

We now believe that with increased numbers of cultures of both buffalograss as well as saltgrass that regrowth of frozen buds is once again the preferred procedure. We therefore will begin more detailed freezing studies using regrowth *in vitro* to evaluate survival.