Long-Term Preservation of Clonally Propagated Turfgrass Species

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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 methodolgies.

Start Date: 1998
Project Duration: 3 years
Total Funding: $49,701

The production of improved turfgrass lines requires suitable germplasm. Preservation of this is essential for breeding programs using conventional methods and the molecular techniques now available. Although seeds are the main form in which germplasm is preserved for many turfgrass species, the ability to generate hybrids and the increased use of vegetatively-propagated lines necessitates preservation methods for clones.

Vegetatively-held lines can be preserved by normal greenhouse, screenhouse or field methods, but the ability to multiply, manipulate and hold these lines *in vitro* is important in modern technology. Holding lines under field conditions exposes them to events that can lead to loss of the lines and considerable effort is often needed where a number of lines are being held.

Cryogenic storage systems are now used extensively for seeds and is being applied to clonal propagules such as buds or shoot tips. Cryogenic storage requires very low maintenance and is fairly inexpensive. The existence of a backup also can allow some minimization of the usual form of holding, such as space in the field.

The development of cryopreservation methods for a wide array of species has been considerable in the past few years. Recent advances and understanding of the physical and chemical events that occur during the process have aided in this development.

A micropropagation system has been developed for the establishment and proliferation of both buffalograss and zoysiagrass. Buffalograss produces greater than ten shoots in about six weeks in an MS basal medium with 3-10 mg/L of benzyl adenine (BA). Zoysiagrass proliferates from three to six shoots in about six weeks in an MS medium with 1 or 0.2 mg/L of kinetin in combination with 0.5 mg/L of BA or with 50 mg/L of adenine sulfate plus 0.5 mg/L of BA.

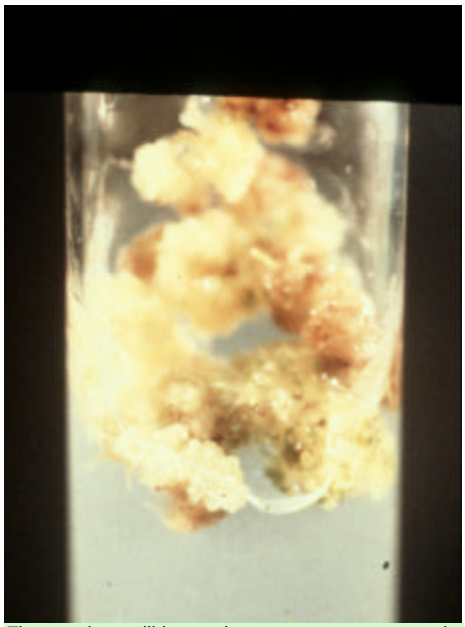
Although clean cultures have been established with both saltgrass and bermudagrass the proliferation rates are minimal. Work is continuing with both species.

Buffalograss has been cryopreserved with the vitrification system. This system employs the application of cryoprotectant solutions followed by rapid cooling with the aim of solidifying the system without ice crystal formation. Both pretreatment of the plant in some manner and preculture of the isolated shoot tips are used prior to the vitrification step.

Several different combinations of cryoprotectants were evaluated, but the use of a 20-minute exposure to 2M glycerol + 0.6 M sucrose in combination with a 30-minute exposure to PVS2, 30% glycol, 15 % DMSO and 15% ethylene glycol. Preculture was in 0.3 M sucrose. Low levels of survival were noted.

This plus the low levels of survival of the controls, nonfrozed shoot tips, indicated that our problems lie in the excision of appropriate shoot tips. It appears that the growth from the excised tips developed from axillary buds and not from the apical dome from the basal region. Therefore we continue to evaluate how we might better select those shoot tips that have the likelihood of axillary buds.

Furthermore, since a rapid cooling rate appears to be critical it suggests that we are not getting adequate adjustment of the cell contents during preculture or by a loading solution. We will therefore concentrate on these aspects to further improve success.



Tissue culture will be used to recover cryo-preserved tissues of important vegetative, warm-season cultivars.

Summary Points

- Three cryopreservation methods are being tested: two-step cooling, vitrification, and encapsulation/dehydration.
- Cooling rate was important - more rapidly cooled shoot tips showed higher levels of survival.
- Attention to quality of shoot tips is important - need axillary meristems.
- For buffalograss, vitrification has worked the best.
- Progress is being made to cyropreserve bermudagrass and saltgrass.
- Micropropagation systems for buffalograss and zoysiagrass are needed that will allow sufficient proliferation rates for cryopreservation studies.