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. *Examine cryopreservation of the four species using vitrification methodologies.*

A micropropagation system has been developed for the establishment and proliferation of both buffalograss and zyosiagrass. Buffalograss proliferates to greater than 10 shoots in about 6 weeks in an MS basal medium with 3/10 mg/l of BA. Zoysiagrass proliferates from 3-6 shoots in about 6 weeks in an MS medium with 1 or O.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 has been accomplished 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 min exposure to 2M glycerol + 0.6 M sucrose in combination with a 30 min exposure to PVS2 930% 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 form 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 as well to further improve success.

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TISSUE CULTURE: Micropropagation procedures have been developed for the establishment and proliferation of buffalograss. The use of 1.5-2.5 ml/l of PPM, an antimicrobial commercial preparation, is generally needed to initiate cultures from plants grown in the greenhouse. An MS basal medium with 3 or 10 mg/l of BA results in reasonable proliferation rates, greater than 10 shoots in approximately 6 weeks. These are sufficient proliferation rates for cryopreservation studies.

The micropropagation system for zoysiagrass results in 3-6 shoots in approximately 6 weeks. Several different hormonal combinations appear to give similar results. These include the use of kinetin at 1 mg/l levels or 0.2 mg/l of kinetin in combination with o.5 mg/l of BA or 50 mg/l of adenine sulfate plus 0.5 mg/l of BA. Although the proliferation rate is somewhat lover that that for buffalograss it is sufficient to begin cryopreservation studies similar to that of buffalograss.

We able to establish clean cultures of both bermudagrass and saltgrass. However, we have not achieved sufficient proliferation. We do get 2 shoots per culture although the majority of cultures have only one shoot. These shoots are capable of growing vigorously in vitro. We have initiated saltgrass cultures directly from scarified seed since the clean cultures established via shoot tip from greenhouse plants are few in number, i.e. many cultures exhibit contamination once the PPM is removed from culture.

CRYOPRESERVATION:In vitro plants of buffalograss have been used for cryopreservation experiments using 3 different strategies, namely two-step cooling, vitrification and encapsulation/dehydration. Intial tests with two step cooling gave very low levels of survival after low temperature exposure. There are a number of different variables that could be examined in two-step cooling; we utilized a cryoprotectant solution of polyethylene glycol, glucose and dimethylsulfoxide and cooling rates of 0.3 and 0.5C/min (similar to that used in other shoot tip studies). We decided to emphasize vitrification systems for cryopreservation because of some success in initial tests and ease of application should others wish to adopt methods developed. Encapsulation/dehydration tests were also briefly examined. Although some survival after liquid nitrogen (LN) exposure occurred, dehydration itself was quite injurious.

Vitrification refers to application of cryoprotectant solutions followed by rapid cooling with the aim of solidifying the system without ice crystal formation. Note that both pretreatment of the plant in some manner and preculture of the isolated shoot tips are used prior to the vitrification step. We examined preculture systems using several treatments. Application of 2M glycerol and 0.4 M (or 0.6 M) sucrose for different durations were examined in combination with different durations of application of 30% glycerol, 15% DMSO and 15% ethylene glycol (ca. 0.2M sucrose) ("PVS2"). In several experiments survival, after LN exposure, was obtained. Shoot tips were derived from either cold hardened in vitro plants or in vitro plants held under usual growth conditions.

We examined several combinations of the treatment solutions listed above. Keeping in mine that the comparisons are somewhat qualitative, we find 20 min exposure to 2M glycerol + 0.6 M sucrose adequate when using a 30 min exposure to PVS2, modified by elevating the sucrose level to 0.4 M. Preculture was in 0.3M sucrose. Two other vitrification solutions were tested without obvious improvement in viability.

From these tests, several points became obvious. The main issue is that the quality of shoot tip obtained from the in vitro plant needs to be improved. Untreated, but excised, small shoot tips show varying levels of growth. Only 25 to 50% develop into plants. Preculture treatments appear not to be overly detrimental since a similar range of viability was observed. But this low level of control survival makes statistical comparisons of subsequent treatments difficult, unless much larger shoot tip numbers can be obtained. Size of excised shoot tips and excision from either cold hardened or noncold hardened in vitro plants did not noticeably improve survival. In both control and LN treated shoot tips, the subsequent growth developed not from the apical dome but from the basal region, probably from axillary meristems. Through culture techniques we are trying to improve the frequency of shoot tips in in vitro plants which have obvious axillary meristems.

Cooling rate was important, more rapidly cooled shoot tips showed higher levels of survival. This suggests that the cell contents have not been adequately adjusted by preculture or by a loading solution (2 M glycerol + 0.6 M sucrose) to achieve high levels of vitrification. The implication is that benefits might be gained by examining preculture conditions, particularly with regard to sugar application and exposure duration.

Zoysiagrass in vitro plants are now being tested using vitrification methods described for buffalograss. The multiplication rate for micropropagation of in vitro plants is not as high as for buffalograss and the plants are somewhat larger. Initial tests for survival and methods for improvement are being planned before extensive cryotests are initiated.

Proposed Research for 2000-2001

TISSUE CULTURE: We will focus on germination of saltgrass seed in vitro to provide us with sufficient numbers of plants for the cryopreservation studies. We will continue to evaluate various growth regulator combinations for proliferation studies with a focus on small amounts of 2,4-D in combination with BA and/or NAA.

CRYOPRESERVATION: We will focus on selection of shoot tips which have obvious axillary meristems in continuing the cryopreservation studies. Furthermore, we will continue to look at both preculture and loading solution on increased survival. Specifically we will try growing plants in a media supplemented with cryoprotectants to facilitate uptake. Initially we will focus on sucrose but will include PEG and others as well. The loading solutions will be reexamined to determine more optimum cryoprotectant combinations. We will take the optimum conditions for increasing survival in buffalograss and evaluate these with the other three species.