# In Vitro Maintenance of Bermudagrass Germplasm

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## Introduction

There are currently 213 accessions of bermudagrass (Cynodon spp.) maintained clonally by the Plant Genetic Resources Conservation Unit located in Griffin, Georgia as part of the National Plant Germplasm System. These accessions have been traditionally maintained as potted plants in the greenhouse. Due to the aggressive nature of many of the bermudagrass accessions, constant trimming and generous spacing between accessions is required in order to prevent cross-contamination of the accessions. Typical problems that arise when growing plants under greenhouse conditions also demand continual monitoring. This high amount of monitoring and required greenhouse space limits the efficiency of maintaining the collection. Additionally, back up of the material by the National Center for Genetic Resources Preservation (NCGRP) in Fort Collins is not possible under these conditions. Effort is being made to transfer these accessions into tissue culture.

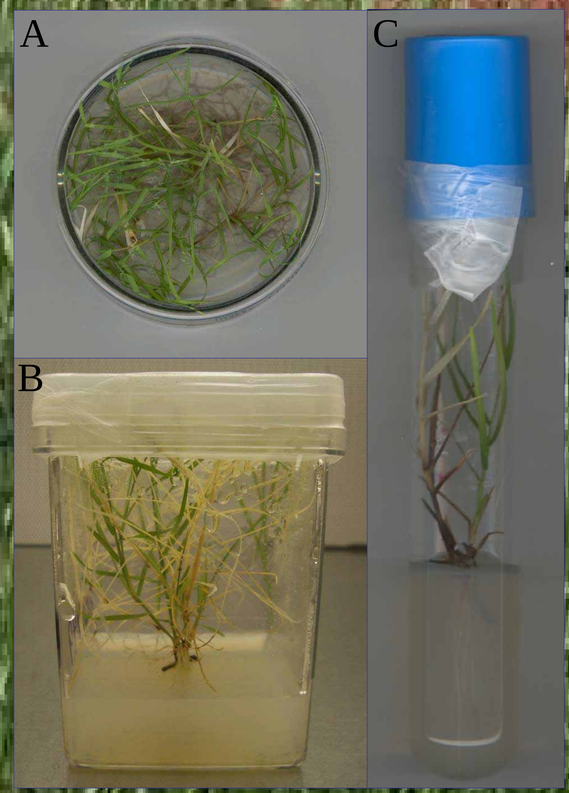
## Techniques

Stolon cuttings from greenhouse plants are surface sterilized using a one minute wash in 70% ethanol followed by a ten minute wash in a 50% sodium hypochorite solution. After three rinses in sterile water, cuttings are cultured onto MS basal media supplemented with 20% (w/v) sucrose and 10% (w/v) agar. Tissue cultures are maintained in germination chambers using a temperature regime of 20°C/27°C (10h night/14h day). Thus far, we have transferred approximately one-third of the Cynodon clonal collection into tissue culture. Frequent sub-culturing of the established accessions has been required, but simple changes to in vitro vessels from standard culture tubes to magenta boxes and petri dishes has extended the time between sub-culturing. Alterations in the temperature regime and media constituents such as nitrogen supplementation, Hoagland's solution, and reduction of sucrose concentration are being explored that may further extend time between subculturing. Significant variation in initial culture establishment and prolonged health of the cultures has been observed among different bermudagrass accessions.

Stolon cuttings are taken from the plants in the greenhouse

Cuttings are sterilized in 70% ethanol (1 min) and then 50% hypochlorite solution (10 min)

Sterilized cuttings are placed on MS media until new shoots and roots form




Rooted cuttings are placed in culture vessels
(A = 100 x 15 mm petri dish; B = magenta box; C = culture tube)

## Conclusions

The establishment of an in vitro collection will allow for more efficient accession maintenance and back up of the material at NCGRP. It will also provide easier distribution of the material due to the fact that plant roots will not have to be washed free from soil before distibution. More efficient germplasm maintenance and distribution will allow for the acquisition of new material into the collection that was not feasible in the past and increase the overall value of the bermudagrass germplasm collection.