Determining the Genetic Stability of Triploid Bermudagrasses

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Objectives:

Determine the origin of the off-types that arise in hybrid (triploid) bermudagrass golf greens through a combination of cytological and molecular analyses.

The goal of the turfgrass breeding and management group at Mississippi State is to determine the source of off-type sectors that occur in bermudagrass *(Cynodon dactylon* x *C. transvaalensis* Burtt-Davy) putting green varieties.
Off-types which have been studied in other grass species are frequently due to absence of one or more chromosomes, a condition known as aneuploidy. This chromosome loss may occur spontaneously, or be induced by application of mitotic inhibitor compounds, such as dinitroanilines. For example, oryzalin is frequently used in place of colchicine to induce chromosome doubling in lab experiments. When a seedling receives a dinitroaniline dose that is inadequate for suppression of cell division, aneuploidy may sometimes be the result. If aneuploidy occurs in bermudagrass greens varieties which have been chronically treated with dinitroanline, we wish to correlate it with the formation of off-types.
Our multiple-cycle experiment integrates results from laboratory, greenhouse, and field tests. Each cycle begins with a greenhouse phase. The varieties Tifgreen, Tifdwarf, TifEagle, MS-Supreme, Champion, and Floradwarf are established from small stolon pieces (2 nodes in length) in horticultural flats containing a methyl bromide-fumigated 3:1 masonry sand:peat moss mix, and grass is maintained at about 1/4" mowing height to encourage lateral growth in the flats. When the grass reaches 75 percent coverage of the flat, they are subjected to a single drench application equivalent to a 2X rate of oryzalin or pendimethalin (controls receive no herbicide). At the conclusion of the herbicide application, the flats are left unmowed so they can recover from the stringent treatment. After the treated grasses recover, sod samples are collected and shredded into sprigs. The sprigs are used for two purposes, first of which is to establish new flats for the next cycle of treatment. The second is for our field experiment phase; the samples will be planted in a fumigated field plot which has been set aside for long-term "archiving" of all grass samples taken from the treated flats during the experiment. This plot was planted with tall fescue for 6 seasons prior to the fumigation and has no observable bermudagrass contamination. The purpose of archiving these samples is to give any potential variant sectors a chance to grow out and express their altered phenotypes. The plots are 4' x 4' and are replicated within each variety x treatment x cycle combination. The plots are allowed to reach full coverage without mowing, to lower the chance of contaminating the hybrid bermudas in our test. As of November 1999, three cycles of herbicide treatment

have been completed and a fourth is underway. We intend to complete a total of six to seven cycles.

The laboratory phase of our experiment involves screening of AFLP primer pairs to establish a control DNA fingerprint for each of the 6 varieties. The technique allows us to visualize radio-labeled DNA bands which have been separated on a 5percent polyacrylamide gel. To date 38 out of 64 commercially available AFLP primer pairs (from Keygene, Inc, distributed by Gibco/BRL) have been screened on the controls. All six of our triploid bermudagrass genotypes can now be clearly distinguished from one another. Furthermore, this technique produces so many potentially informative DNA fingerprint bands, that it should allow monitoring of changes in fingerprint within each variety, whether they occur in response to treatment or as a result of spontaneous mutation. As our treated material becomes available, it will be tested against these primer pairs to detect fluctuations from the control samples.

A new technique which greatly simplifies root-tip cytology in bermudagrass has been adapted from a technique used in maize cytology. Chromosome counts are made from bermudagrass root-tip cells which have been subjected to 150 psi nitrous oxide gas for 2 hours. These are fixed in ice-cold 90 percent acetic acid for 30 minutes, digested in 1% cellulase, 0.5% pectinase and then suspended in 1: 1 methanol:acetic acid. The root-tips are then smeared on a regular microscope slide and air-dried for 10 minutes before applying 2% acetocarmine for visual examination of chromosomes. This technique routinely yields several dozen clearly countable mitotic figures per slide. Our cytological examinations of the six varieties to date have only revealed the expected triploid number of 27 chromosomes.