

Determining the Genetic Stability of Triploid Bermudagrass

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Objectives:

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

Start Date: 1998

Project Duration: 3 years

Total Funding: \$72,790

Application of dinitroaniline class of pre-emergence herbicides is an integral part of triploid bermudagrass sod production. The active ingredients are known to cause chromosome lagging at mitosis, which can give rise to aneuploids. Aneuploids are plants with higher or lower chromosome numbers than expected. Aneuploidy can cause changes in leaf blade width and length. Similar changes are often observed in naturally occurring off-types.

Another alternative which might explain off-types is that long-term, chronic dinitroaniline usage is mutagenic to DNA of perennially grown bermudagrass. Therefore, dinitroaniline herbicides may be a causal agent in off-type formation. A third possibility is that herbicide usage may favor spontaneous (naturally occurring) mutations that cause off-types.

Our goal is to learn more about off-type formation using a two phased experiment. The first phase has been an "off-type induction" experiment. Six selected triploid bermudagrass varieties ('Champion', 'Floradwarf', 'MS-Supreme', 'Tifdwarf', 'Tifeagle', and 'Tifgreen') are subjected to acute (2X) doses of oryzalin and pendimethalin to determine if off-type plants can be produced.

The second phase of the experiment is an "off-type detection" experiment. We are using three separate routes to classify any off-types that might be induced by the off-type induction experiment. These are morphological trait classification, AFLP profiling (a method of DNA fingerprinting), and chromosome counting.

Morphological traits (leaf blade length and width, internode length and diameter) showed no significant variation among



At Mississippi State University, researchers are evaluating the genetic stability of Tifdwarf bermudagrass treated with high rates of dinitroaniline pre-emergence herbicides.

cycles or among herbicide treatments, indicating that no morphological off-types were found by our random sampling. The only significant differences traced to pre-existing intervarietal variation.

Our molecular results to date show that the majority of missing bands occurred in the pendamethalin treatment. However, since missing AFLP bands occurred in the untreated control, we cannot make a firm conclusion regarding what caused their disappearance. Analysis of further cycles will help form a clearer picture. All varieties have shown at least one band loss. Our cytology data has only been completed through Cycle 2. To date, no aneuploids were detected. All samples showing the expected 27 chromosomes.

We have conducted an experiment to determine if bermudagrass contains active transposable elements. These naturally-occurring elements are responsible for many of the spontaneous morphological mutations. To date, we have performed PCR-based experiments to determine if these elements are present in bermudagrass.

We have found four elements from maize which are also in triploid bermudagrass DNA. We hope to document the presence

and activity of these elements as a possible explanation for bermudagrass off-types.

We have assembled a small collection of off-types from golf courses and sod farms in Mississippi and Louisiana. These will be analyzed for differences in AFLP profile and chromosome number from their progenitor varieties.

This grant has facilitated the introduction of two new methods for turfgrass genetic analysis: capillary electrophoresis for rapid production of AFLP profiles. This facilitates genetic analysis in three ways. First, it is not gel-based, so it is simpler to perform. Second, it produces more accurate estimates of DNA band size than previously possible (resolution is accurate to within one base pair). Finally, it makes data output simpler by automatically exporting DNA band data into spreadsheet form.

Summary Points

- No differences in chromosome number or distinguishing polymorphism were found using 44 RFLP markers.
- AFLP markers will be used to discriminate between highly related bermudagrass genotypes.