Distinguishing off-types in Tifway and Tifdwarf bermudagrass

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Objective and Brief Summary

The research will determine the feasibility of distinguishing off-types in Tifway and Tifdwarf bermudagrasses.

We have found that DNA banding patterns are powerful in distinguishing off-types from Tifway. Samples of fairway off-types from different golf courses can be matched by their RAPD patterns. Therefore, the off-types appear not to have originated on the various golf courses, but were carried in as planting stock.

For the greens, in contrast, few DNA bands distinguish Tifdwarf from its apparent off-types. We are retesting recollections of one interesting off-type, T-74, which appears to have several distinctive DNA banding pattern differences from Tifdwarf. If we can show again that off-types did not originate on, but were carried to, a golf course, this would minimize the role of recurring mutation as a source of off-types. Morphology data are complementing DNA banding pattern data.

Background

The main idea of the proposal is that DNA banding patterns (i.e., RAPD mark

Fig. 1. *Image of PCR amplification products from 26 bermudagrasses, based on primer AK18. ("Standard" refers to a molecular size reference, and is not grass DNA.) At the top, the banding patterns for 18 greens bermudagrasses (Tifgreen, Tifdwarf, SFG2. . .Tifdwarf) were indistinguishable. In striking contrast, the banding patterns for 8 fairway grasses at the bottom of the image (BRGC3, BRGC2. . .PCC1) varied.*
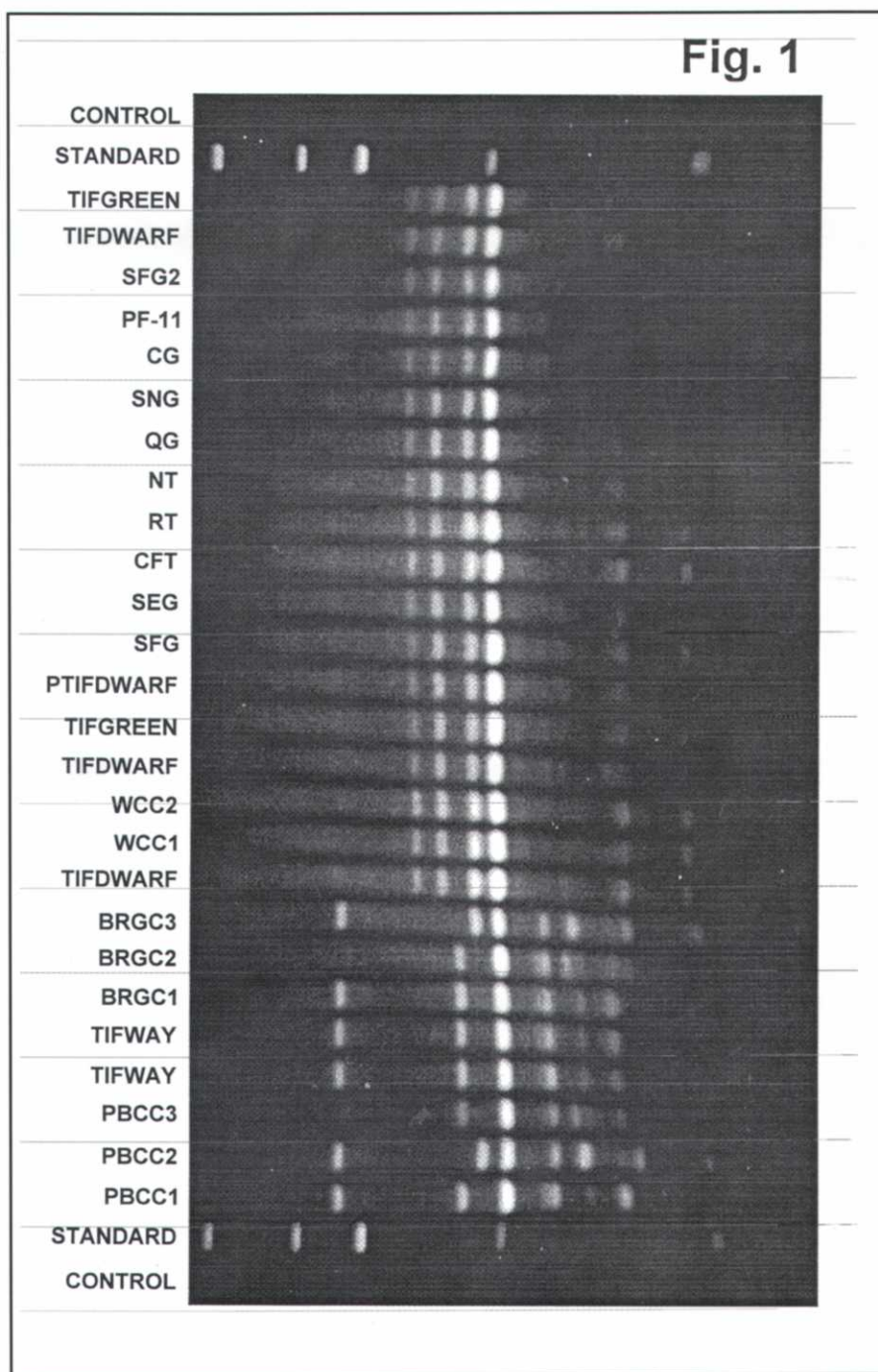
ers) might distinguish bermudagrasses, thereby helping in quality control.

RAPD markers provide a relatively inexpensive genetic identification tool, but the method is prone to possible errors. To generate diagnostic banding patterns, varying-size fragments of sample DNA must be multiplied or "amplified" using "primers," short pieces of DNA that recognize a specific DNA sequences from the unknown grass. Because amplification is a sensitive step in the RAPD process, we have attempted to control error by selecting primers that are consistent, and we have exchanged primers and procedures between our two laboratories, at Gainesville and Fort Lauderdale. Morphological traits, including chromosome number, are being developed as a potentially faster and cheaper method of prescreening for genetic off-types.

Work Completed

We have completed the screening of 130 primers ("Stage 1" in the proposal), have tested their repeatability ("Stage 2"), have exchanged 11 primers between locations (beginning of "Stage 3"), and have completed the application of primers to a population of 26 grasses ("Stage 4," which was planned for the second year). We have further retested selected primers on six additional fairway samples. We have initiated a study of morphology ("Supplement"), including a replicated grow-out, and preliminary work on roottip chromosomes. (For an explanation, see the original proposal.)

Work Remaining

We will complete the retesting of exchanged primers on bermudagrass which can be distinguished ("Stage 3") and complete the morphological study of all 26 bermudagrasses, including chromosome numbers, and complete a final report by July 1997.

Results Thus Far

We found several primer-derived markers for distinguishing fairway off-types, but few markers for distinguishing greens off-types. This is apparent in the image for primer AK18 (*Fig. 1*). The banding patterns for 18 greens bermudagrasses (Tifgreen, Tifdwarf, SFG2...Tifdwarf) were indistinguishable. The banding patterns for 8 fairway grasses (BRGC3, BRGC2...PCC1) varied. As another example, primer 719 (*Fig. 2*) showed that the dominant matrix grass on fairways of two golf courses was indistinguishable from the Tifway foundation, but two off-types from fairways of each golf course were not Tifway. Furthermore, the off-types matched across golf courses, indicating that they had been propagated and planted from a common source, possibly as a contaminant, and not through recent mutation or seedling variation. the genetically matched bermudagrasses were also similar morphologically, and the off-types produced abundant pollen, so they must be tetraploids ($2\underline{n} = 36$). We found that a uniform, desirable, fairway grass (T-20) from a third golf course matched the genetic signature of the dark-green
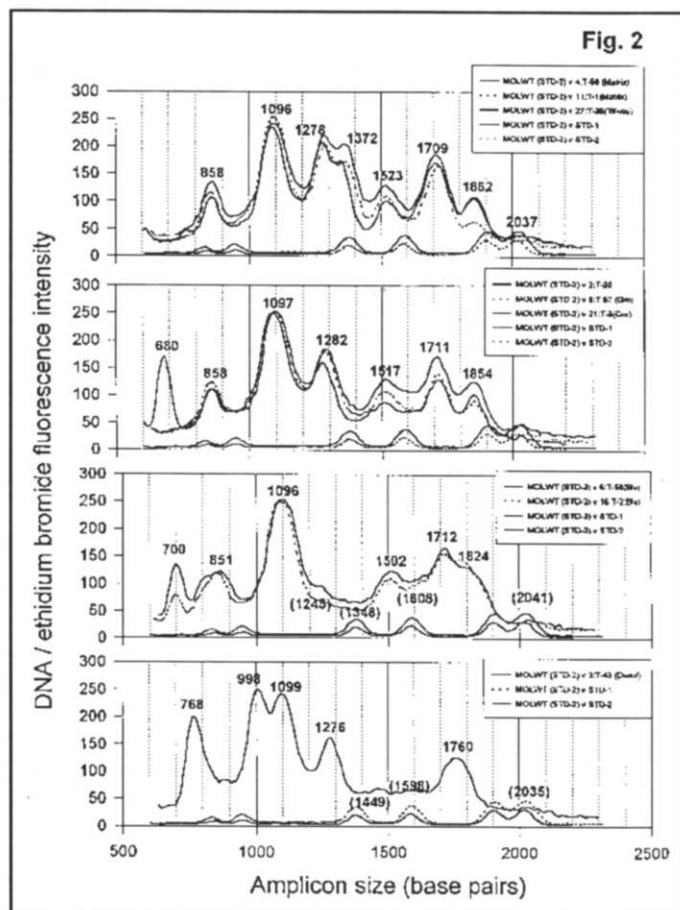
Fig. 2. *Comparison of DNA profiles of nine bermudagrasses from PCR amplification with UBC primer 719. Ethidium bromide fluorescence intensity in an agarose gel was resolved at 75 dpi (dots per inch) in a digitized scan of an enlarged photograph, maximizing digital contrast. This is the second amplification in a series, hence the designation 719-2. The profiles of similar grasses (e.g., Tifway = T-35 and matrix grasses T-1 and T-56) are superimposed. Another 17 grasses were indistinguishable from Tifdwarf, thus they are not represented. Towards the bottom of each panel are the profiles of the two molecular weight standards. Amplicon sizes of the sample bands were estimated as a log-quadratic function of migration distance. The function was derived by lest-squares regression of the known base pair sizes of the standards (831, 947, 1375, 1584, 1904 and 2027) on their respective migration distances. All peaks were fitted iteratively using a Gaussian amplitude curve with a smoothing coefficient of 11 (PeakFit, Jandel Scientific, San Rafael, CA).*

off-type from the other two golf courses. Morphological data (e.g., leaf texture and pollen production) indicated that many other fairway bermudagrass accessions are not Tifway. Clustering based on morphological differences (internode length, stolon thickness, and number of inflorescences) brought unknown fairway bermudagrasses together in plausible groups, consistent with the original field observations (*Fig. 3*). Subsequent DNA profiling of six more fairway variants showed a repetition of similar patterns, which resulted in a clustering of genetic relationships (*Fig. 4*). The results from DNA complemented the morphology.

We found DNA patterns from six primers that may distinguish some greens grasses (including foundation standards, trade types, and off-types). The banding patterns for some primers were unstable among extractions from the same grass,

producing spurious results. Only one primer, CG119, showed banding pattern variation among more than three greens grasses. One dwarf-type grass, T-74, was distinguishable, using any of four primers, from Tifdwarf and all other greens grasses. However, this result awaits confirmation at Fort Lauderdale. Therefore, 36 new samples were recollected from the same golf course, in the expectation that we might be able to show the repeated occurrence of T-74 across different greens. While the RAPD patterns for fairway bermudagrasses are strong and consistent, we need to cautiously retest those on greens bermudagrasses, because of repeatability problems inherent in the RAPD method. Among green bermudagrasses, there were differences in stolon thickness ($P < 0.05$) and internode length ($P < 0.0001$). Surprisingly, the foundation Tifdwarf and foundation Tifgreen clustered together, while several trade types and off-types clustered together (*Fig. 3*). These results are very interesting and encouraging.

Possible Significance

While this work is ongoing, several possible conclusions are anticipated:

1. The sparseness of RAPD markers which distinguish among greens bermudagrasses, e.g. Tifdwarf and its off-types, is consistent with their possible origin as point mutations. PCR-based DNA profiling, such a RAPDs markers, may not presently be a practical means of
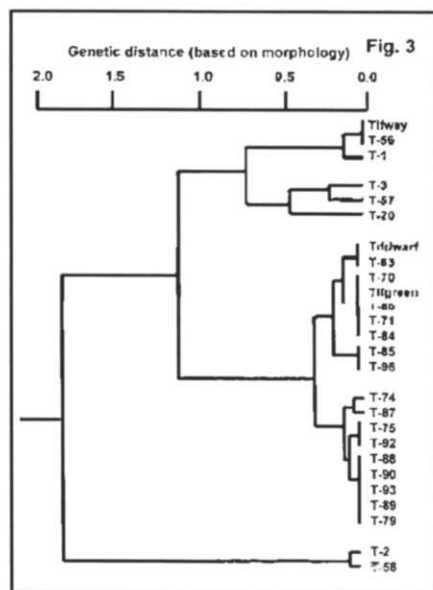

Fig. 3. *Phenogram of genetic relatedness of 26 bermudagrass samples based on three morphologic traits (stolon thickness, internode length, and number of inflorescences per pot). Cluster analysis was performed on the matrix of Euclidean genetic distances by the unweighted pair-group method (METHOD = AVE, the CLUSTER procedure of SAS 6.03, The SAS Institute, Cary, NC). Bermudagrass samples with a genetic distance close to zero (e.g., Tifway T-1 T-56) were not statistically different. Grasses T-2 and T-58 branched close to the main trunk, which reflects heavy weighting for the prolific seedhead production of those two samples.*

identification for greens bermudagrasses.

2. Morphological variations were detectable among greens bermudagrasses, at the 0.01% probability level, thus real genetic differences exist. In other words, based on preliminary data, not all products labelled as Tifdwarf are really Tifdwarf.

3. The abundance of RAPD markers which distinguish among fairway bermudagrasses, their contrasting morphology, and the presence of abundant pollen in the off-types, is consistent with their origin as seedling variations.

4 The recurrence of matching genetic off-types on fairways from different golf courses is consistent with their having been planted, not arising after planting. If we can show the same for greens, then it will support the idea of a need for stepped-up quality control in the expansion of plant material.

5. At this time, an absolute assurance of genetic purity does not exist; rather, the Greens Committee should be aware

Fig. 4

Genetic distance (based on DNA profiles)

2.0   1.5   1.0   0.5   0.0

Tifway
T-1
T-56
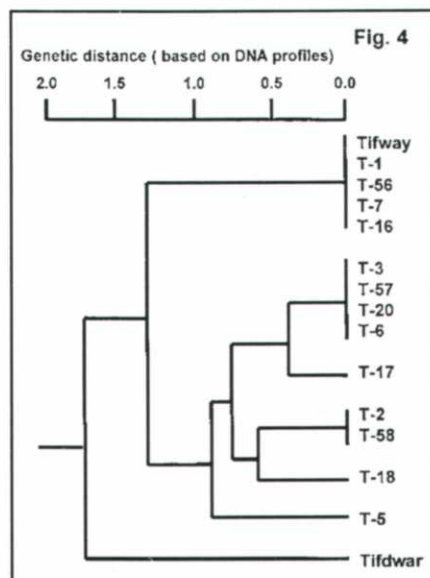T-7
T-16
T-3
T-57
T-20
T-6
T-17
T-2
T-58
T-18
T-5
Tifdwar

Fig. 4. Phenogram of genetic relatedness of 15 bermudagrass samples based on 25 RAPD markers and five common bands derived from seven primers. A pairwise index of genetic distances was obtained (Nei and Li, 1979) from a presence-absence matrix. Cluster analysis was performed on genetic distances by the unweighted pair-group method. Bermudagrass samples with a genetic distance of zero (e.g., Tifway = T-35, T-1, T-56, T-7 and T-16) were identical for the presence or absence of all 25 RAPD markers. T-17 was relatively close to the cluster of T-3, T-57, T-20 and T-6, differing in the presence or absence of only 3 bands. Bermudagrass samples which branched far to the left of the main trunk (e.g., Tifdwarf = T-43, genetic distance = 1.733) had many (between 12 and 18) band differences from all other samples.

that off-type variations typically are noticed several years after bermudagrass areas are planted, even after the most diligent research by those involved in the purchase of planing material. Once noticed in established playing areas, off-

types ten to become more prevalent over time.

6. It may be possible to use this technique to distinguish genetic variants that are superior to Tifway for use on Florida golf courses. ⌐