

BY MICHAEL P. ANDERSON

## The fingerprinting of Bermudagrass DNA

Genetic relationships are key to efficient production of high-quality varieties

The fingerprinting of plant, animal and human DNA has been practiced by researchers and forensic scientist for many years, especially garnering widespread attention from notorious cases involving DNA evidence. The DNA fingerprint analysis is so powerful it's capable of distinguishing one individual from another. Each of us has a unique DNA pattern as do plant species and plant varieties.

All organisms have identifiable characteristics, which make an organism unique from all others. Physical characteristics in Bermudagrass,

such as: leaf thickness or colors are obvious and readily discernable. However, some characters require detailed measurements, while others are more qualitative in nature. Some distinguishing features can be observed with little or no training, while others need close inspection by trained and experienced personnel. Many subtle differences among closely related Bermudagrasses can't be readily distinguished visually (See photo on opposite page). Another method is necessary to differentiate these Bermudas - DNA fingerprinting.

Differences among organisms are coded by their DNA. DNA is a long linear molecule consisting of a specific sequence of four distinct chemicals called nucleotides in a linear order. If the human genome were represented by letters standing for each distinct nucleotide (A, T, G, C) on a blank page, the length of the alphabetic sequence would run at least to one million pages, enough to fill 1,000 large volumes. The information in the DNA is carried in the linear sequence of the nucleotides. The DNA sequence dictates the look of an organism and how it responds to the immediate environment. This is different for every organism. Consequently, the DNA sequence can be used to distinguish one organism from another.

DNA fingerprinting is nothing more than a sophisticated technique to sample an organisms DNA sequence, projecting the differences as a kind of bar code for ready identification and comparison. Most DNA fingerprinting depends on a technique known as PCR or polymerase chain reaction. PCR was developed in the mid-1980s

to efficiently amplify specific segments of DNA many, many-fold. The PCR technique uses short DNA segments composed of anywhere from six to 20 nucleotides known as primers that are complementary to segments of the target DNA. The primers figuratively scan for matches in the target DNA sequences. Once a match is found then amplification of that segment begins. If there are many matches, many segments will be amplified.

This mixture of amplified segments known as amplicons can be separated on an electrophoretic gel system which effectively sieves fragments based on size, with the largest slower moving amplicons appearing on top of the gel, and the smaller on the bottom. The gel is stained with fluorescent dyes to reveal what looks like a banding pattern or a bar code. Multiple primers can be used to scan different portions or the total genomic DNA revealing additional bar coding.

Fingerprinting with many primers is capable of differentiating even the most closely related of all organisms. Thus while two Bermudagrasses might be physically indistinguishable from each other, the DNA fingerprinting can highlight the intrinsic differences in their DNA using the PCR-based techniques.

All organisms can be finger printed and their DNA patterns stored and analyzed. Analysis of the banding pattern is performed using a variety of statistical techniques known as cluster analysis. The data is inputted in the form of presence or absence of a particular PCR amplicon or electrophoretic band and cluster analysis analyzes the data and connects those organisms that show

### Summary

- Researchers at OSU use DNA fingerprinting to evaluate the genetic background of Bermudagrass varieties from a worldwide collection.
- Understanding genetic relationships is fundamental to the efficient production of high-quality Bermudagrass varieties.
- DNA fingerprinting coupled to cluster analysis is able to distinguish and infer genetic relationships among even the most closely related organisms from each other.
- DNA fingerprinting can be used in basic and applied research, genetics, plant breeding, marker assisted selection, agricultural forensics and patenting, and ecological genetics.



similar patterns (Figure 3b). However, to be effective there must be enough similarities and differences in the pattern to reveal relationships among all tested organisms.

A number of fingerprinting techniques exist. These techniques differ in the ability to differentiate organisms, the amount of labor required, the extent of automation available, the expense of use, and nature of the specific targeted DNA segments. AFLP, DAF, SSR, RAPD are a few of the more commonly used techniques used to fingerprint DNA. All of these use PCR to amplify segments of DNA based on the DNA sequence.

In our research, we've used DAF primarily for its simplicity, low cost, ease of use and high resolution (Yerramsetty et al., 2005). Others

have used more sophisticated technology to meet similar objectives (Wu et al., 2005) (Zhang et al., 1999). Sophisticated and expensive commercial packages and instrumentation exists to automate and increase the resolution of the fingerprinting procedure. Access to DNA sequencing instrumentation provides a tremendous boost in fingerprinting performance and throughput, but at a significant cost.

#### HOW IT'S USED

How has this technology been used in the past, and how might it be used in the future? We'll focus on what we and others have learned about Bermudagrasses or other species using the DNA fingerprinting techniques.

Oklahoma State University is home to a worldwide collection of Bermudagrass varieties, much to the credit of Charles Talliaferro, Ph.D. Photo: Michael Anderson

DNA fingerprinting has been used initially to look at the genetic relationship among a wide range of Bermudagrasses. Some of the first work highlighted the differences among high quality commercial cultivars and select bermudagrasses found in germplasm collections. In 1995, Caetano Anolles and other researchers surveyed 13 Bermudagrass cultivars including African, Common Bermudagrass and several interspecific hybrids for genetic relatedness using DAF. Results showed that DNA fingerprints were easily distinguishable, and the analysis showed clear genetic relationships among all bermudagrass varieties. To probe the limits of the ability to distinguish Bermudagrasses the authors fingerprinted Tifway and its irradiation induced mutant Tifway II, which presumably differed in one or a few nucleotide changes in the DNA sequence. To differentiate these very closely related varieties, the authors found it necessary to use 81 distinct primer combinations to find a one band difference among all 81 fingerprints (Caetanoanollés et al., 1995). From this early work, it was clear investigators can differentiate and draw genetic relationships even among the most closely related Bermudagrasses.

Breeders often collect from throughout the world a wide range of plant introductions in the hope of finding specific genetic traits that might be put to productive use. The genus *Cynodon* is comprised of nine species (Taliaferro, 1995).

Oklahoma State University is home to a world-wide collection of Bermudagrass varieties and plant introductions that was initiated by the celebrated geneticist Jack Harlan. Charles Taliaferro and more recently Yanqi Wu, two Bermudagrass breeders at OSU, have added significantly to this collection, making it one of the most comprehensive collections of *Cynodon* germplasm in the world. In a survey of this world-wide collection using DAF fingerprinting techniques Assefa et al. 1997 (S. Assefa, 1999) examined 42 bermudas for genetic relatedness and found generally that the fingerprinting supported the taxonomic classification based on morphology by Harlan (Taliaferro, 1995). Understanding the genetic relatedness among *Cynodon* sp. and varieties gave us a better understanding the genetic make up of the *Cynodon* genus.

At times, doubts about the genetic identity of a particular cultivar surfaces. To field personnel, the cultivar doesn't look like what it's supposed to be. In previous work, our laboratory responded to the need to evaluate the widely used variety U3 for genetic fidelity (Anderson et al., 2001). U3 was an early success made up of Bermudas collected from golf courses in the Southern U.S. in the 1930s. U3 showed moderate cold tolerance and fine textured leaves and was a general improvement when compared to previous cultivars. Since then, U3 has been sold and marketed throughout the region.

DNA fingerprinting was employed to distinguish the current labeled U3 from presumably authentic U3 collections assembled from throughout the country. Results showed the currently labeled U3 varieties differed substantially from the presumably authentic U3 varieties (Figure 4). How these differences came about couldn't be addressed by the fingerprinting technique, but the research underscored the need for evaluating current varieties for genetic stability and purity. Additionally, our research (unpublished) as well as others (Zhang et al., 1999) has discovered a few other discrepancies between the historical pedigree claims of several varieties and their actual genetic relationships using fingerprinting techniques.

Often times when researchers conduct experiments with particular varieties or germplasm, it's important to understand the genetic background of the Bermudas involved. When constructing genetic mapping populations it's essential to document the genetic background

of the potential parents beforehand. The parents should differ substantially in the targeted trait while showing significant similarity in genetic background. A preliminary DNA fingerprinting survey of potential parents is the best way to do this reliably. The same can be said when selecting Bermudagrass varieties for basic research analysis. Understanding the genetic background and relationships improves experimental analysis and interpretation significantly.

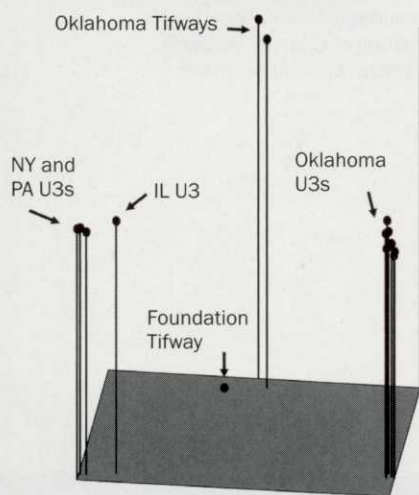
### GAINING DIVERSITY

New Bermudagrass germplasm has been and is now being collected and assembled into world-wide collections from many sources. There are areas where collections have only recently been assembled from specific geographic locations, such as Southern Asia and Southeastern Asia. Recently, Yanqi Wu brought a number of Bermudas from China adding to the OSU germplasm collection. DNA fingerprinting using AFLP technique was used to evaluate the diversity within this germplasm.

The Chinese collection seemed surprisingly diverse (Wu et al., 2006) and distinct from other bermudas from other geographic locations around the world (Wu et al., 2004). Additional work in our laboratory easily separated the Chinese collection from all U.S. varieties tested (unpublished). Over all, the work indicated a source of significant variation in the new Chinese collection which may contain valuable genes for Bermudagrass development. Additional diversity assessments needs to be done on collections from India and other areas not surveyed previously.

The same techniques used for DNA fingerprinting such as AFLP or SSR also are used for molecular genetic analysis of specific traits. The goal here isn't so much an analysis of diversity or genetic relatedness but for locating specific genetic elements or genes that contribute substantially to those traits. This is performed by constructing populations with significant variation in a particular trait of interest and then performing the DNA fingerprinting technique on members of the population to identify specific genetic elements that correlated with the phenotypic expression of that trait. These genetic elements are visualized as unique bands on electrophoretic gels that appear to correlate with traits of interest. The bands are valuable because they can serve as genetic markers, markers that are based on the DNA sequence rather than

**Figure 4. PCA component Analysis**



some physical characteristic of the plant.

Sophisticated computer software analysis can gauge the contribution of the DNA element associated with the marker to the genetic makeup of the phenotype. These markers can be used to increase the efficiency of selection in a process known as marker assisted selection. Marker assisted selection has been shown to be effective in enhancing germplasm improvement in a variety of cropping systems (Mackay and Powell, 2007; Tuberosa and Salvi, 2006; Yamaguchi and Blumwald, 2005). Constructions and evaluation of mapping populations and usage of molecular genetic analysis are major goals of the OSU Bermudagrass team.

Bermudagrass is an outcrossing species indicating an expected level of genetic heterogeneity within Bermudagrass populations. Typically, seeded populations consist of a range of individuals that differ genetically. The genetic diversity within the population may be wide or narrow depending on the way the population was constructed originally. A wide genetic base consists of many individuals that differ substantially from each other. When we characterize genetic populations we must evaluate the entire population, sampling a representative number of individuals. So far, this has rarely if ever been performed on seeded Bermudagrasses.

DNA fingerprinting of individuals within a population provides information concerning the genetic make-up of that population. The individual makeup of the population might change with time depending on natural selection and genetic inflow from neighboring Bermudas. To observe these shifts, DNA fingerprinting can be used to document and track alterations in population make-up of seeded Bermudagrasses under a variety of environmental conditions throughout time. So far, little is known concerning this aspect of Bermudagrass culture, which needs more investigation, especially considering the emergence and use of fertile seeded populations in the Bermudagrass industry.

#### AGRICULTURAL FORENSICS AND PATENTING

DNA fingerprinting also can be used in areas of agricultural forensics. One case illustrates this use. Years ago, a farmer was concerned about theft of Bermudagrass hay bales from his farm. The farmer had several suspected culprits in mind and contacted us to determine if DNA could be used to support a claim prior to legal action. To prove the claim, samples would have

to be taken from the farms of the suspect and victim, and DNA fingerprint analysis performed and evaluated. DNA fingerprinting could never prove complete identity between the collected materials but could provide evidence to support a forensic conclusion based on a certain level of probability.

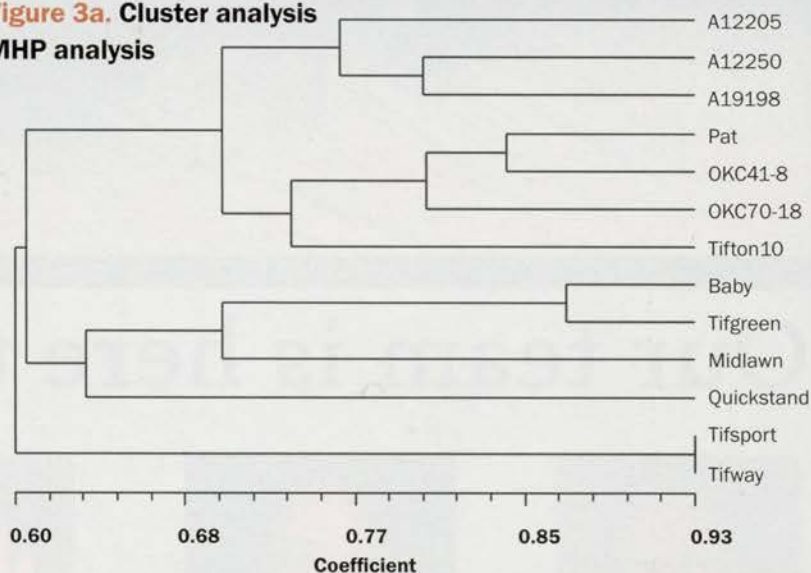
Further supporting evidence including cultural histories and practices among the im-

plicated parties would have to be provided – a significant and costly undertaking. The evidence would have to be evaluated by an expert using quantitative and statistical models before a legal opinion could be constructed. In this case, the effort appeared too costly in terms of time and money; however, there might be cases where the expense and effort is justifiable.

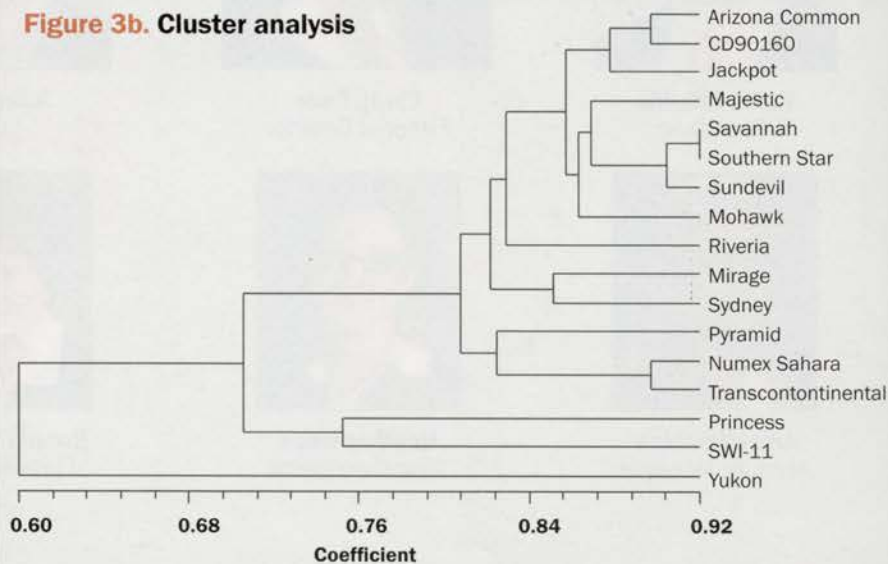
Finally, DNA fingerprinting can have an

**Figure 3a. Cluster analysis**

**MHP analysis**



**Figure 3b. Cluster analysis**



impact in the area of patent protection. Many years and efforts are expended to develop commercial varieties. Institutions have a substantial investment in terms of developmental cost, and are increasingly desirous of recovering some of that cost through plant variety protection, and the collection of royalties from consumers. To support the patent application process, differences in morphology, cultural characteristics and pedigree needs to be presented to distinguish the proposed variety from those that are currently available. DNA fingerprinting is currently being used on a limited basis to document the genetic differences of new varieties in the patent process. Any infringement on the patent would have to use the DNA fingerprints and other characteristics to justify a patent infringement lawsuit. The process may be costly and subject to interpretation by experts, but may be worth the effort when the stakes are large.

#### ECOLOGICAL GENETICS

Ecological studies in the natural environment are often times helpful in distinguishing among eco-

types that might differ in desirable or undesirable characteristics. At OSU, we collaborated with a project seeking to identify various ecotypes of *Sericea lespedeza*, a major introduced invasive species that threatens forage production on natural pasture lands in Oklahoma (Farris, 2004). The idea was to look at genetic background of the different ecotypes and its relationship to the ability to control this problem pest. Understanding the genetic base of the *Sericea lespedeza* populations might be an important element in designing more effective control methods.

DNA fingerprinting is a valuable technology that's being used to assist producers, breeders, geneticist and researchers evaluate Bermudagrass populations and germplasm for genetic diversity and background. Information from DNA fingerprinting techniques allow researchers to make informed decisions concerning progress in developing high quality bermudagrass lines. DNA fingerprinting technology remains a powerful technique in assessing the genetic diversity of Bermudagrasses worldwide and at protecting plant varieties from infringement. Our projects

have been involved in using DNA fingerprinting to improve Bermudagrass more. GCI

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*Editor's note: References for this article can be found at [www.golfcourseindustry.com](http://www.golfcourseindustry.com).*

## IMPACT ON THE BUSINESS

BY CINDY CODE

# Fingerprinting takes the guesswork out of turf identification

**F**rom a crop perspective, genetics plays an important role in purity of product, yield and how inputs such as pesticides and fertilizers will react.

DNA fingerprinting in turfgrass allows superintendents and breeders the ability to distinguish between plant varieties, particularly if a choice needs to be made between two or more varieties.

"Fingerprinting looks at the DNA of a particular variety," says Michael Anderson, Ph.D. "It doesn't really check or tell the health of plant, but can distinguish one from the other."

While many superintendents look at variations of color to determine the purity of a

turfgrass plant, the only way to determine whether it's a pure variety is through fingerprinting, which can determine this without question.

This technology, introduced in the 1980s, aids in the identification rather than the maintenance of turfgrass, although fingerprinting is helpful in determining whether or not a turfgrass variety has been contaminated.

Anderson relates a situation in which superintendents and others were wondering whether or not a variety was really what they said and thought it was.

"We did a bunch of tests on a variety that we thought was U3 Bermudagrass. That's how it was labeled, but it didn't look like the

original U3. To nail it down, we conducted fingerprinting and found it was absolutely different from U3."

Fingerprinting answers academic questions such as which variety is better adapted for local conditions.

Rather than trusting the label or counting on visual gut checks, fingerprinting is the only method that will provide superintendents with an absolute.

While superintendents can make do without fingerprinting, breeders, sod producers and researchers rely on the technology to ascertain what variety they're working with.

"In turf plots, I want to know if a variety has been overgrown by a contaminant," Anderson says.

"Contamination can impact the production of new varieties."

Anderson says he's been using these techniques for the past 10 years in his Bermudagrass research because even he can't always distinguish between varieties. He can't leave it to chance when beginning a research project. He needs to know the genetic background of what he's working with.

Breeders also will use fingerprinting to compare the genetics of Bermudagrass collected worldwide.

Superintendents most often can test Bermudagrass on their courses visually, but if contamination is an issue, fingerprinting will help you get a straight answer. GCI