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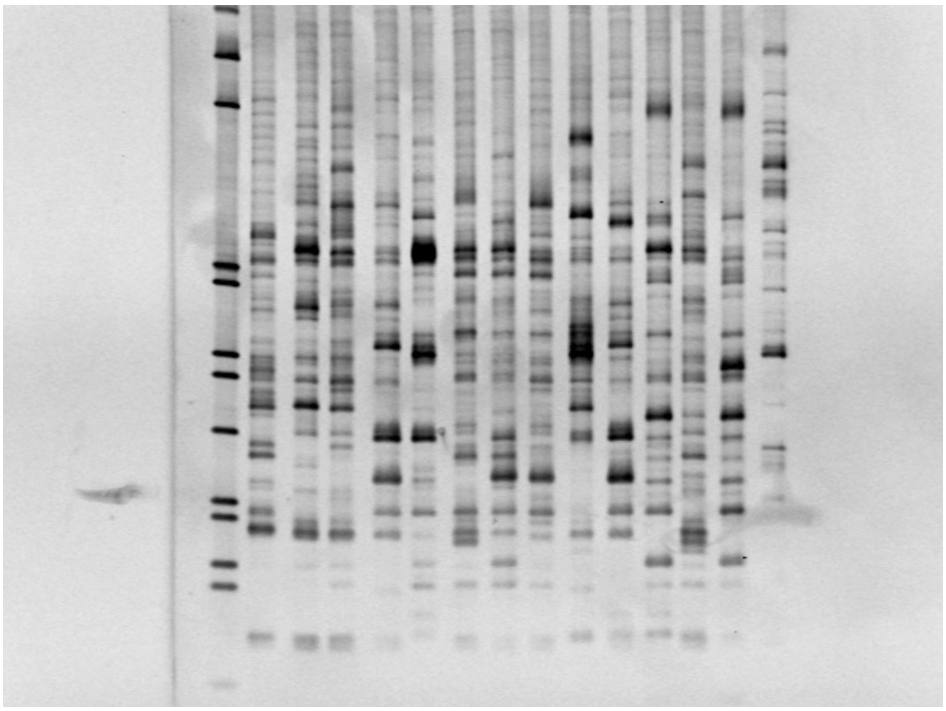

Differences among organisms are coded for by their DNA. Researchers at Oklahoma State University are using "DNA fingerprinting" techniques to distinguish bermudagrass cultivars and selections, and they explain how this powerful tool can be used to distinguish genetic differences that are important in establishing genetic relatedness and protecting plant patents.

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PURPOSE

The purpose of *USGA Turfgrass and Environmental Research Online* is to effectively communicate the results of research projects funded under USGA's Turfgrass and Environmental Research Program to all who can benefit from such knowledge. Since 1983, the USGA has funded more than 290 projects at a cost of $25 million. The private, non-profit research program provides funding opportunities to university faculty interested in working on environmental and turf management problems affecting golf courses. The outstanding playing conditions of today's golf courses are a direct result of ***using science to benefit golf***.

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Fingerprinting of Bermudagrass DNA

Michael P. Anderson and Yanqi Wu

SUMMARY

Differences among organisms are coded for by their DNA. Researchers at Oklahoma State University are using "DNA fingerprinting" techniques to distinguish bermudagrass cultivars and selections, and they explain how this powerful tool can be used to distinguish genetic differences that are important in establishing genetic relatedness and protecting plant patents. This paper's points include:

- Researchers at OSU uses DNA fingerprinting to evaluate genetic background of bermudagrass varieties among bermudagrasses from a world-wide 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.

The fingerprinting of plant, animal, and human DNA (deoxyribonucleic acid) has been practiced among researchers and forensic scientist for many years, especially garnering widespread attention from notorious criminal cases, such as the O.J. Simpson murder case, involving DNA evidence. DNA fingerprint analysis is powerful and capable of distinguishing one individual from another--you from me. Each of us has a unique DNA pattern, as do plant species and plant varieties.

DNA Differences

All organisms have identifiable characteristics. These characteristics make an organism unique from all others. Physical characteristics in bermudagrass, such as leaf thickness or leaf color, are obvious and readily discernable (Figure 1). 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 cannot be readily distinguished visually. Another method is necessary to differentiate these bermudagrasses: DNA fingerprinting.

Differences among organisms are coded for by their DNA. DNA is a very long linear molecule made up of a specific sequence of four distinct chemicals called nucleotides in a linear order. If human DNA were represented by single letters standing for each distinct nucleotide (adenine, cytosine, guanine, and thymine) 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

Figure 1. Oklahoma State University is home to a world-wide collection of bermudagrass varieties much to the credit of Dr. Charles Taliaferro (shown above).

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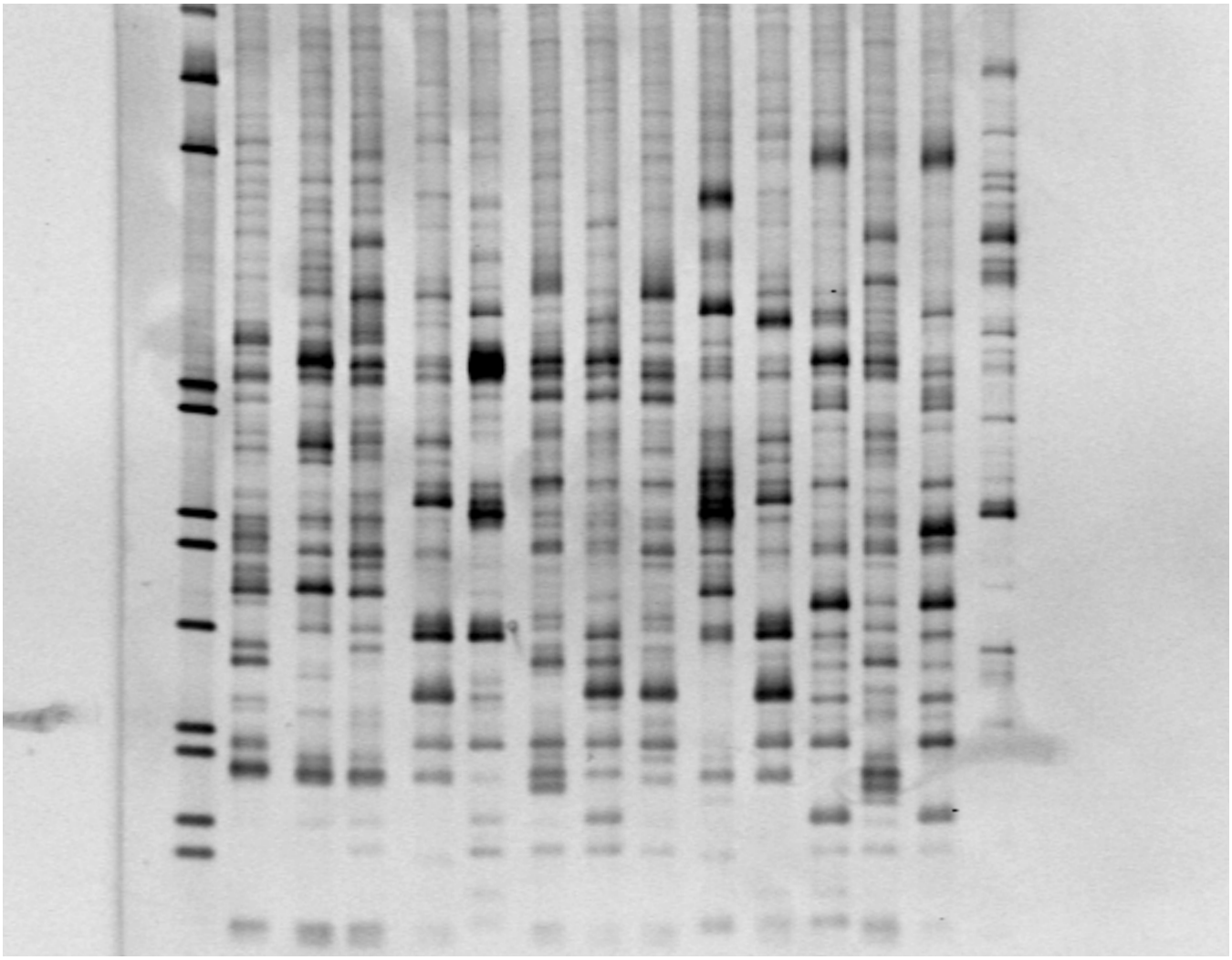

Figure 2. DAF DNA fingerprint gel of bermudagrass varieties (12).

the linear sequence of nucleotides. The DNA sequence dictates the look of an organism and how it responds to the immediate environment and 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 organism's DNA sequence, projecting the differences as a kind of "bar code" for ready identification and comparison (Figure 2).

Most DNA fingerprinting depends on a technique known as PCR or "polymerase chain reaction". PCR was developed in the mid-80s to efficiently amplify specific segments of DNA many, many-fold. The PCR technique uses short DNA segments composed of anywhere from 6 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 the amplicons 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 (Figure 2). 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 may be physically indistinguish-

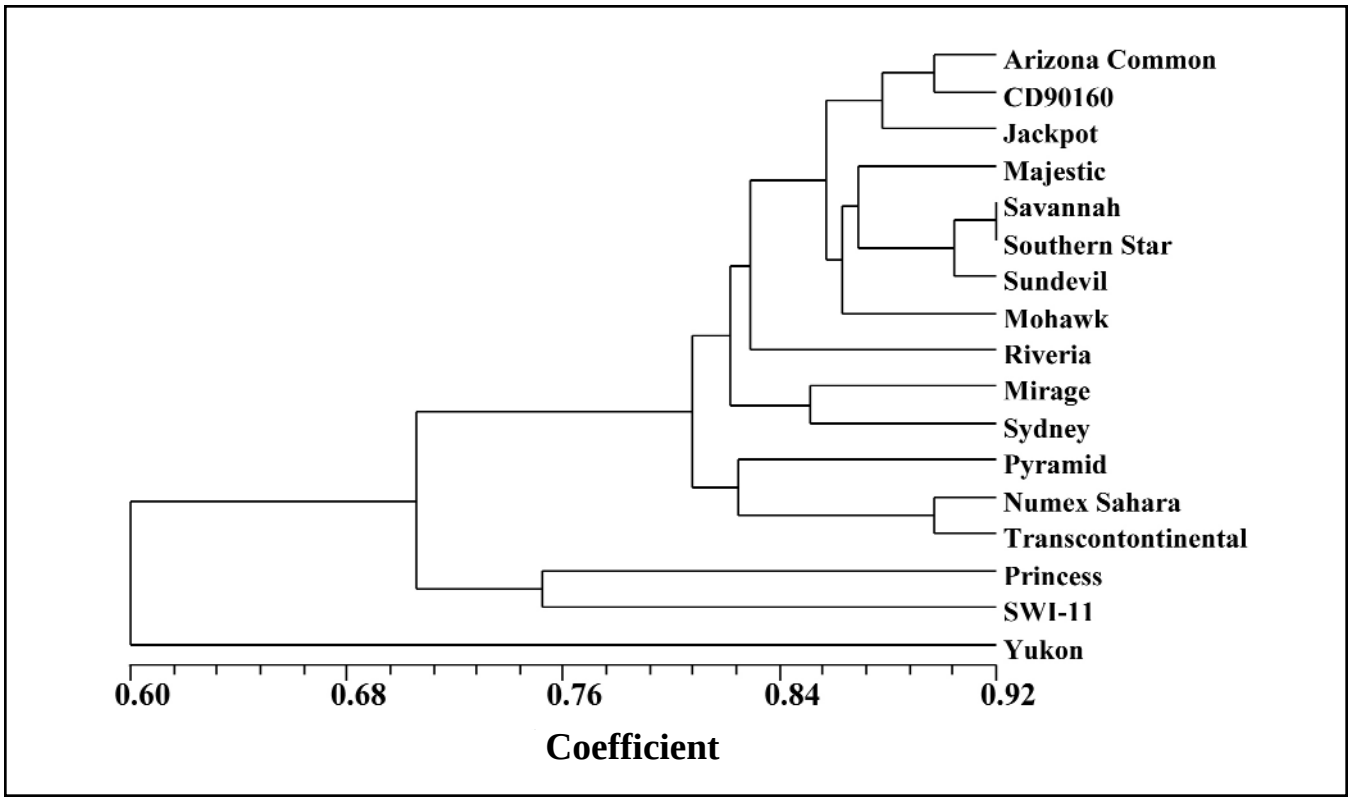

Figure 3. Cluster analysis of bermudagrass seeded varieties

able from each other, DNA fingerprinting can highlight the intrinsic differences in their DNA using PCR-based techniques.

All organisms can be fingerprinted 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 similar patterns (Figure 3). However, to be effective, there must be enough similarities as well as 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 (Amplified Fragment Length Polymorphism), DAF (DNA Amplification Fingerprinting), SSR (Simple Sequence Repeats), and RAPD (Random Amplication of Polymorphic DNA) are a few of the more commonly used techniques to fingerprint DNA. All of these utilize PCR to amplify segments of DNA based on the DNA sequence. In our research we have used primarily DAF for its simplicity, low cost, ease of use, and high resolution (12). Others have used more sophisticated technology to meet similar objectives (8, 13). 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 is DNA Fingerprinting Used?

How has this technology been used in the past, and how might it be used in the future? In the remainder of the article we will focus on what we and others have learned about bermudagrasses or other species using the DNA fingerprinting

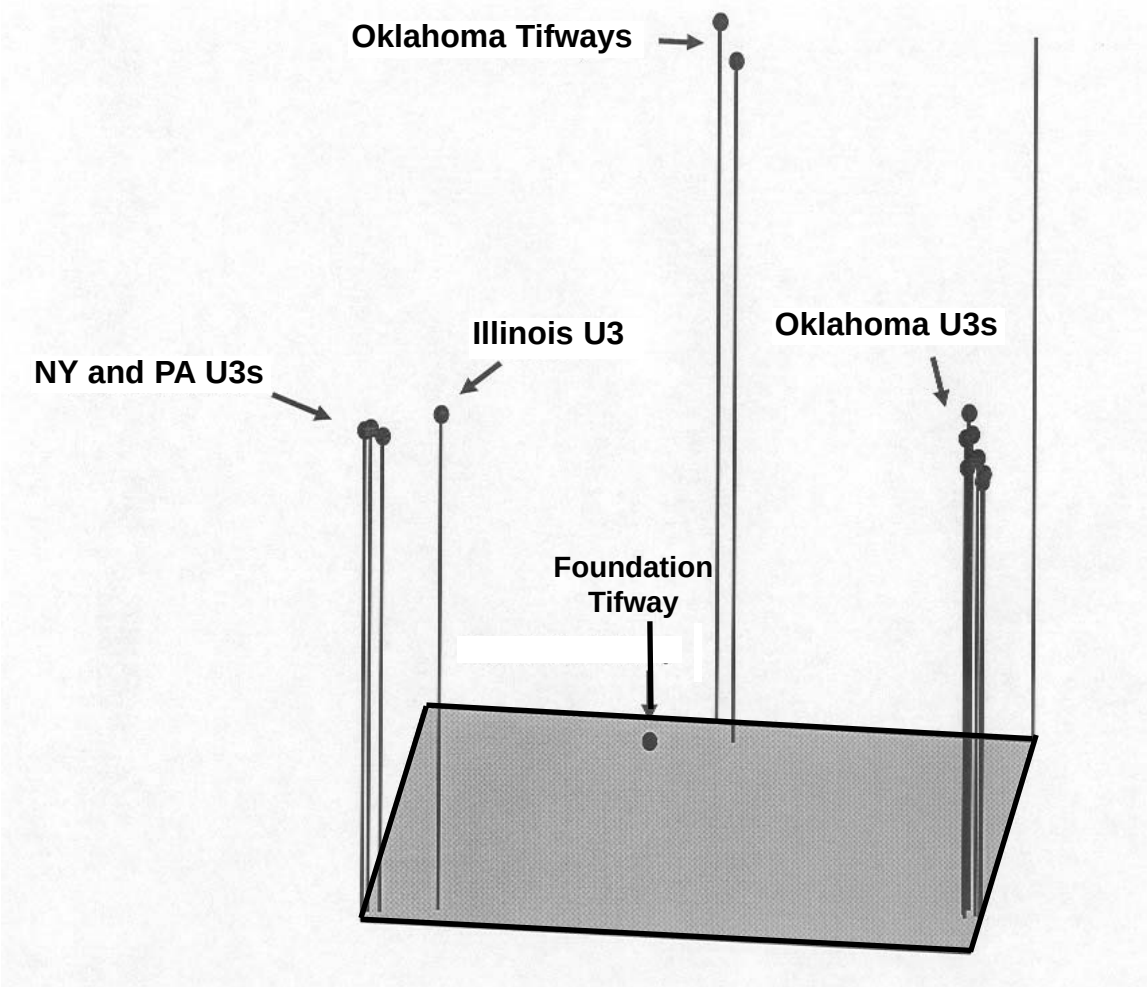

Figure 4. Principal Component Analysis of DNA fingerprinting pattern of commercial U3 bermudagrass and the putative standards. The wide separation between the commercial 'U3' and standard 'U3' indicated substantial genetic differences among these varieties.

techniques.

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. Caetano-Anolles et al. (3) 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, we fingerprinted 'Tifway' and its irradiation-induced mutant,'Tifway II', which presumably differed in one or a few nucleotide changes in the DNA sequence. In order 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 (3). From this early work, it was clear that investigators can differentiate and draw genetic relationships even among the most closely related bermudagrasses.

Breeders often collect from around the world a wide range of plant introductions in the hope of finding specific genetic traits that may be put to productive use. The genus *Cynodon*

(bermudagrasses) is comprised of 9 species (6). 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. (2) examined 42 bermudagrassess for genetic relatedness and found generally that the fingerprinting supported the taxonomic classification based on morphology by Harlan (6). Understanding the genetic relatedness among *Cynodon spp.* and varieties gave us a better understanding of the genetic make up of the *Cynodon* genus.

At times doubts about the genetic identity of a particular variety surface. To field personnel, the variety does not look like what it is supposed to be. In previous work our laboratory responded to the need to evaluate the widely used variety 'U3' for genetic fidelity (1). 'U3' was an early success made up of bermudagrasses collected from golf courses in the Southern USA 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 around the country. Results showed that the currently labeled 'U3' varieties differed substantially from the presumably authentic 'U3' varieties (Figure 4). How these differences came about could not be addressed by the fingerprinting technique, but the research underscored the need for evaluating current varieties for genetic stability and purity. In addition, our research (unpublished), as well as others (13), 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 is important to understand the genetic background of the bermudagrasses involved. When constructing genetic mapping populations it is 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 Bermudagrass Diversity World-wide

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 and southeastern Asia. Recently, a number of bermudagrasses from China was added 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 (10) and distinct from other bermudagrasses from other geographic locations around the world (9). Further work in our laboratory easily separated the Chinese collection from all US 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 previously surveyed.

The same techniques used for DNA fingerprinting such as AFLP or SSR are also used for molecular genetic analysis of specific traits. The goal here is not so much an analysis of diversity or genetic relatedness but for locating specific genet-

ic elements or genes that contribute substantially to those traits. This is performed by first 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 in that they can serve as genetic markers, markers that are based on the DNA sequence rather than some physical characteristic of the plant.

Sophisticated computer software analysis can guage 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 very effective in enhancing germplasm improvement in a variety of cropping systems (5, 7, 11). Constructions and evaluation of mapping populations and utilization 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 originally constructed. 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 populations provides information concerning the genetic make-up of that population. The individual makeup of the population may change with time depending on natural selection and genetic inflow from neighboring bermudagrasses. 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 over time. So far, very 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 can also be utilized in areas of agricultural forensics. One case illustrates this use. A number of years ago a farmer was concerned about the 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. In order 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 implicated 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 may be cases where the expense and effort is justifiable.

Finally, DNA fingerprinting can have an impact in the area of patent protection. Many years and effort 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 in order 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 ecotypes that 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 (4). 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 may be an important element in designing more effective control methods.

In summary, DNA fingerprinting is a valuable technology that is being used to assist producers, breeders, geneticist, and researchers in evaluating 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 world-wide and at protecting plant varieties from infringement. At OSU, our projects have been involved in using DNA fingerprinting to further bermudagrass improvement.

Acknowledgement

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Literature Cited

1. Anderson, M.P., C.M. Taliaferro, D.L. Martin, and C.S. Anderson. 2001. Comparative DNA profiling of U-3 turf bermudagrass strains. *Crop Science* 41:1184-1189. (TGIF Record 74669)

2. Assefa, S., C. M. Taliaferro, M.P. Anderson, B.G. de los Reyes, and R.M. Edwards. 1999. Diversity among *Cynodon* accessions and taxa based on DNA amplification fingerprinting. *Genome* 42:465-474. (TGIF Record 125499)

3. Caetano-Anolles, G., L.M. Callahan, P.E. Williams, K.R. Weaver, and P.M. Gresshoff. 1995. DNA amplification fingerprinting analysis of bermudagrass (*Cynodon*) - Genetic-relationships between species and interspecific crosses. *Theoretical and Applied Genetics* 91:228-235. (TGIF Record 56345)

4. Farris, R.L., D.S. Murray, M.P. Anderson, and Praveen Yerramsetty. 2004. Adaptation of biology of *Sericea lespedeza*. *Proc. South. Weed Sci. Soc.* 57.

5. Mackay, I., and W. Powell. 2007. Methods for linkage disequilibrium mapping in crops. *Trends in Plant Science* 12:57-63.

6. Taliaferro, C.M. 1995. Diversity and vulnerability of bermuda turfgrass species. *Crop Science* 35:327-332. (TGIF Record 34342)

7. Tuberosa, R., and S. Salvi. 2006. Genomics-based approaches to improve drought tolerance of crops. *Trends in Plant Science* 11:405-412. (TGIF Record 125487)

8. Wu, Y.Q., C.M. Taliaferro, G.H. Bai, and M.P. Anderson. 2005. Genetic diversity of *Cynodon transvaalensis* Burtt-Davy and its relatedness to hexaploid *C. dactylon* (L.) Pers. as indicated by AFLP markers. *Crop Science* 45:848-853. (TGIF Record 104608)

9. Wu, Y.Q., C.M. Taliaferro, G.H. Bai, and M.P. Anderson. 2004. AFLP analysis of *Cynodon dactylon* (L.) Pers. var. *dactylon* genetic variation. *Genome* 47:689-696. (TGIF Record 125504)

10. Wu, Y.Q., C.M. Taliaferro, G.H. Bai, D.L. Martin, J.A. Anderson, M.P. Anderson, and R.M. Edwards. 2006. Genetic analyses of Chinese *Cynodon* accessions by flow cytometry and AFLP markers. *Crop Science* 46:917-926. (TGIF Record 110350)

11. Yamaguchi, T., and E. Blumwald. 2005. Developing salt-tolerant crop plants: challenges and opportunities. *Trends in Plant Science* 10:615-620. (TGIF Record 125498)

12. Yerramsetty, P.N., M.P. Anderson, C.M. Taliaferro, and D.L. Martin. 2005. DNA fingerprinting of seeded bermudagrass cultivars. *Crop Science* 45:772-777. (TGIF Record 103020)

13. Zhang, L.H., P. Ozias-Akins, G. Kochert, S. Kresovich, R. Dean, and W. Hanna. 1999. Differentiation of bermudagrass (*Cynodon spp.*) genotypes by AFLP analyses. *Theoretical and Applied Genetics* 98:895-902. (TGIF Record 66690)