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Jeffrey F. Pedersen

University of Nebraska-Lincoln, jpedersen1@unl.edu

R. D. Lee

USDA-ARS

D. J. Lee

USDA-ARS

K. P. Vogel

USDA-ARS, kvogel1@unl.edu

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**COMPARISON OF SORGHUM AND INDIANGRASS CHLOROPLAST
GENOMES USING RFLPS**

J. F. Pedersen, R. D. Lee, D. J. Lee, and K. P. Vogel¹

Sorghum and indiangrass are related species based on plant structure and on the presence of a unique secondary metabolite, dhurrin. Both species possess traits that would be of value to agricultural producers if it were possible to transfer traits among the two species. Traditional crossing techniques have been unsuccessful in developing hybrids of the two species. Therefore this study was conducted to investigate the degree of relatedness of the two species using chloroplast DNA. The results indicate that the species may not be as related as previously thought.

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¹ USDA-ARS and the University of Nebraska.

COMPARISON OF SORGHUM AND INDIANGRASS CHLOROPLAST GENOMES USING RFLPS

J. F. Pedersen, R. D. Lee, D. J. Lee, and K. P. Vogel¹

Abstract

Sorghum (*Sorghum bicolor* (L.) Moench) and indiangrass (*Sorghastrum nutans* (L.) Nash) appear closely related based on morphological and chemotaxonomic characters. Each species could potentially provide desirable traits to the other. However, traditional breeding techniques have been unsuccessful in hybridizing these two species. The objective of this study was to determine the relatedness of sorghum and indiangrass chloroplast DNA using RFLPs. Eleven sorghum lines in several cytoplasms, two indiangrass populations, and a corn line were studied using 60 probe-restriction enzyme combinations. Principal component analysis of the results showed sorghum to be as closely related to corn as to indiangrass, with no overlap of clusters among the three groups.

Introduction: Sorghum (*Sorghum bicolor* (L.) Moench) is the fifth most important cereal worldwide and is grown for grain and forage. It is used for both human and livestock consumption. Indiangrass (*Sorghastrum nutans* (L.) Nash) is a warm season, perennial grass species widely distributed in North America and is a common element of hay meadows, pastures and rangelands of the eastern Great Plains. Hybridization attempts between indiangrass ($2n=40$) and diploid ($2n=20$) or tetraploid ($2n=4x=40$) sorghum have been unsuccessful using conventional crossing techniques. Each species could potentially provide desirable traits to the other.

Taxonomically, sorghum and indiangrass are closely linked based on morphological characters (Chase, 1971) and are both members of the tribe Andropogoneae. A chemotaxonomic link has also been established between the two species. Dhurrin [(S)-*p*-hydroxymandilonitrile β -D-glucopranaside], the cyanogenic glucoside found in sorghum, has been detected in indiangrass (Gorz et al., 1979). To determine the prevalence of dhurrin in other species, Haskins et al. (1979) analyzed 72 entries representing 39 species from 14 genera and 2 tribes of grasses. The authors found that dhurrin was only present in indiangrass and one other species, *Sorghastrum pellitum*.

Restriction fragment patterns of chloroplast DNA (cpDNA) have been used to assess genetic relationships among a number of genera including *Bromus* (Pillay and Hilu, 1990), *Hordeum* (Baum and Bailey, 1989), *Triticum-Aegilops* (Terachi et al., 1984), and *Sorghum* (Duvall and Doebley, 1990). Of the three plant genomes, the cpDNA is the simplest to study due to its inherent smaller size in comparison to nuclear and mitochondrial DNA (Palmer, 1987). Other advantages of cpDNA are a highly consistent gene order (Palmer, 1985;), high conservation across the plant

¹USDA-ARS and the University of Nebraska.

to nuclear DNA, cpDNA nucleotide substitutions have been found to occur at a relatively slow rate (Curtis and Clegg, 1984; Palmer, 1985). Across the plant kingdom, chloroplasts have been noted to be very similar, with the majority of chloroplast size variation accounted for by changes in the size of the inverted repeats (Birky, 1988). The objective of this research was to determine the relatedness of sorghum and indiangrass, based on cpDNA RFLPs.

Materials and Methods: Eleven sorghum lines, two indiangrass populations and a maize line were used in this research (Table 1). The A lines KS34-KS39 and the B lines KS40 and CK 60 are a set of near isocyttoplasmic lines possessing a CK60 nucleus, yet different cytoplasms (Ross and Hackerott, 1972). Piper and Greenleaf are two standard commercially available sudangrass cultivars. The line 12-26 represents yet another cytoplasm (but does not contain a CK60 nucleus), and was obtained from Paula Bramal-Cox at Kansas State University. Indiangrass population 2-860489 is relatively early and has been selected for high yield and high IVDMD (*in vitro* dry matter digestibility) while 2-860589 is relatively late and has been selected for three cycles for high yield and one cycle for high IVDMD. Total plant maize DNA, originating from the cold tolerant selected B population, was obtained from the Biometrical Genetics and Maize Breeding Project at the University of Nebraska.

Greenhouse grown seedlings of the sorghum lines were harvested at 6-8 inches in height. Indiangrass samples were harvested when regrowth from greenhouse grown propagules of each population reached 6-8 inches, and were bulked for each population.

Total plant DNA was obtained from lyophilized leaf tissue as outlined by Saghai-Marroof et al. (1984) and digested to completion with *Bam*HI, *Eco*RI, or *Hind*III. Approximately 10 μ g of digested total DNA per lane were loaded onto a gel containing 8 g L⁻¹ agarose and electrophoresis was carried out on a horizontal apparatus in TBE gel buffer. Gels were stained with ethidium bromide and photographed under ultraviolet light. Mobilities of the *Hind*III-digested Lambda DNA markers were measured from the photograph. Restriction fragments were transferred from agarose gels to nylon membranes according to the protocol of Reed and Mann (1985). After transfer was completed, filters were baked at 80 C for 2 h.

A library of sorghum cpDNA *Bam*HI restriction fragments served as hybridization probes for the filter-bound DNA fragments. This library nearly covers the entire sorghum chloroplast genome (Dang and Pring, 1986). Prehybridization, hybridization, filter washing, and detection of hybridized filter-bound fragments were performed in accordance with the instructions provided with a commercially available nonradioactive labeling and detection kit. Sizes of labeled filter-bound probes were determined by regression analysis using *Hind*III-digested Lambda virus DNA as molecular weight markers (Schaffer and Sederoff, 1981).

Presence of a particular band mobility for a certain probe-restriction enzyme combination were scored as a 1 while genotypes lacking that band were scored as 0. All probes for which polymorphisms were

revealed which uniquely differentiated the genotypes were scored in this manner. Principle component analysis was conducted using PROC PRINCOMP in SAS.

Results and Discussion: Of the 60 probe-restriction enzyme combinations surveyed, polymorphisms were revealed 16 times which uniquely differentiated the genotypes. The A lines KS34 through KS39 did not differ for any of the probe-enzyme combinations which agrees with the previously reported results (Chen et al., 1990). Of the sorghum lines examined, zero to five total band differences were noted in comparison to other sorghum genotypes. Comparison between the two indiagrass populations showed five band differences.

The first and second principal components accounted for 56% and 34% of the variation which collectively accounted for greater than 90% of the variation in this data set. The plot of the first and second principal components grouped the sorghum genotypes together in their own cluster (Figure 1). Interestingly, the sorghum line possessing the *Saccharum* cytoplasm also was within this group. Previous research based on rDNA suggested also that *Saccharum* is more closely related to sorghum than maize (Springer et al., 1989), however, their results did not indicate as close of a relationship as revealed in this research. The two indiagrass populations clustered together but not as tightly as the sorghum genotypes. The maize genotype was not associated with the sorghum or indiagrass clusters.

Based on chloroplast DNA restriction length polymorphisms, it appears that cultivated sorghum is as closely related to this maize line as to indiagrass in spite of the close morphological and chemotaxonomic link between *Sorghum* and *Sorghastrum*. As previously stated by Havey (1991), "although there may be no inherent relationship between diversity in the chloroplast genome and crossability, species possessing a similar chloroplast genome are phylogenetically closer and may have accumulated fewer structural changes between the chromosomes...". Although this research was too narrow in scope to address crossability, genetic distances between sorghum and indiagrass based on cpDNA RFLPs would suggest the two are not as closely related as previously thought. Based on a history of unsuccessful hybridization attempts and the results of this study, transfer of genes from sorghum to indiagrass may be unrealistic using conventional breeding methodology.

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Table 1. Sorghum and indianguass lines surveyed for chloroplast DNA polymorphisms.

Line	Cytoplasm
A KS34	<i>Sorghum arundinaceum</i>
A KS35	<i>Sorghum arundinaceum</i>
A KS36	<i>Sorghum verticilliflorum</i>
A KS37	<i>Sorghum bicolor</i> *
A KS38	<i>Sorghum conspicuum</i>
A KS39	<i>Sorghum niloticum</i>
B CK60	<i>Sorghum bicolor</i>
B KS40	<i>Saccharum officinarum</i>
Piper	<i>Sorghum bicolor</i> *
Greenleaf	<i>Sorghum bicolor</i> *
12-26	<i>Sorghum virgatum</i>
2-860489	<i>Sorghastrum nutans</i>
2-860589	<i>Sorghastrum nutans</i>
B Population, S5 line	<i>Zea mays</i>

*formerly *Sorghum sudanese*

Figure 1. Plot of first and second principle components.

