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Mitochondrial DNA variation in pearl millet and related species*

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Summary. Mitochondrial DNA (mtDNA) restriction endonuclease fragment patterns and patterns of mtDNA hybridized by mitochondrial gene probes were used to study phylogenetic relationships of seven *Pennisetum* species, including five *P. americanum* (pearl millet) ecotypes and a reference species from the distantly related genus, *Panicum*. The restriction patterns of the pearl millet ecotypes were uniform with the exception of the ecotype collected in Ethiopia. The probe hybridization method revealed more variability, with both the Rhodesian and Ethiopian ecotypes differing from the others and from each other. Considerable restriction pattern polymorphism was noted among different species of *Pennisetum*, and *Panicum*. Significant relationships were noted of *Pennisetum polystachyon* to *P. pedicellatum* and of *P. purpureum* to *P. squamulatum* using the restriction pattern method. In addition to those relationships, the hybridization method showed relationships of pearl millet to *P. purpureum* and to *P. squamulatum*. The relationships noted between species by the hybridization method agreed more closely to the cytological data than those indicated by the restriction pattern method. Therefore, the hybridization method appeared to be the preferred method for studying species relationships. The mitochondrial genome size of pearl millet was calculated to be 407 kb and the mitochondrial genome sizes of other *Pennisetum* species ranged from 341 to 486 kb.

Key words: Mitochondrial DNA – Restriction patterns – Mitochondrial gene probes – *Pennisetum* species – Phylogenetic relationship – Mitochondrial genome size

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Introduction

Pearl millet (*Pennisetum americanum* L.) is the most important food cereal in the world's arid tropical and subtropical regions, especially Africa and India (Burton 1983). It is grown on every continent of the world on an estimated 20 million hectares. This crop has the ability to grow rapidly and produce seed in a short season and also can be grown on sandy, rocky soils that are too infertile, dry, acidic or saline to produce maize, sorghum or rice. In the southeastern part of the United States, it is a major forage crop.

Speculations on the progenitor and center of origin of pearl millet have been based on morphological characteristics, cytogenetics, appearance of interspecific hybrids and ease of gene exchange (reviewed by Rachie and Majmudar 1980). However, none of those studies has yielded definitive conclusions. Although it is generally agreed that pearl millet is of African origin (Stapf 1934), there is some controversy as to the specific region of origin. Vavilov (1949) placed the origin of pearl millet in the Ethiopian center based upon the existence of maximum diversity in that center. Based on the occurrence of B chromosomes in pearl millet collections, Pantulu (1960) and Powell and Burton (1966) postulated that Sudan and Nigeria, respectively, were the centers of origin of this crop.

Many investigators have used restriction patterns of organelle DNA as a parameter to resolve the problems relating to the origin or phylogenetic relationships of important plant species. This method was also used to verify the progenitor or center of origin for such species as *Triticum* (Vedel and Quetier 1978; Vedel et al. 1980), *Zea* (Timothy et al. 1979), *Nicotiana* (Rhodes et al. 1981; Kung et al. 1982), *Oenothera* (Gordon et al. 1982), *Pelar-*

gonium (Metzlaff et al. 1981), *Brassica* (Erickson et al. 1986) and *Solanum* (Hosaka 1986).

When different species of higher plants are analyzed, generally chloroplast DNA (ctDNA) sequences are found more conserved compared to their mitochondrial DNA (mtDNA) counterparts. This kind of information is especially useful in making choices for a particular investigation. Although mtDNA restriction pattern variability has been used as a measure of phylogenetic relationship in several plant species, the large size, complexity and rearrangements of the mitochondrial genomes create highly variable patterns that limit interpretation of the data (Sederoff et al. 1981). Those limitations were overcome by comparing the variability of restriction fragments identified by mitochondrial gene probes hybridized to Southern blots of mtDNA such as was done in studying relationships in *Nicotiana* (Bland et al. 1985).

Since molecular information on the complexity of the mitochondrial genomes of cultivated pearl millet and its related species is not available, investigations were made to study the interrelationships of pearl millet and other *Pennisetum* species and to identify the probable center of origin of pearl millet. In this paper, we report the phylogenetic relatedness of *P. americanum* ecotypes collected from diverse geographical locations of Africa and six other *Pennisetum* species and one species from a distantly related genus, *Panicum*. We also report the relative usefulness of mtDNA restriction patterns and hybridization patterns of known mitochondrial genes in determining phylogenetic relationships and list the approximate range of mitochondrial genome size in *Pennisetum*.

Materials and methods

The *Pennisetum* species used in this investigation are listed in Table 1. In addition to the species listed, five ecotypes of *P. americanum* were also included in the comparisons. One of those five is Tift 23B, an inbred maintainer line used to maintain the cytoplasmic male sterile female parent of hybrids in wide use in India and southeastern United States. *Panicum maximum* was included as a distantly related reference species. All of the *Pennisetum* germplasm used in this study was obtained from Drs. G.W. Burton and W.W. Hanna, Coastal Plains Experiment Station, Tifton, GA.

Mitochondrial DNA was isolated from soft stem internode tissue from field-grown immature plants according to the procedure described by Smith et al. (1987). In brief, this procedure involved grinding the soft stem tissue in a Waring blender, followed by differential centrifugation to separate cell debris, nuclei and chloroplasts from mitochondria. The mitochondrial pellet was then treated with DNase to remove extramitochondrial DNA, followed by underlayering with shelf buffer, centrifugation and washing with wash buffer. Mitochondria were then lysed with SDS in the presence of proteinase K, and protein and carbohydrate complexes were precipitated with potassium acetate. MtDNA in the supernatant was then precipitated with ammonium acetate and isopropanol. DNA was washed with 70% ethanol and extracted with phenol, phenol/chloroform, and chloroform, then reprecipitated with isopropanol.

Seven maize clones containing known mitochondrial genes: viz. cytochrome *c* oxidase subunits I (*cox*I) (Isaac et al. 1985) and subunit II (*cox*II) (Fox and Leaver 1981); F_1-F_0 ATPase subunit alpha (*atp*A) (Braun and Levings 1985), subunit 6 (*atp*6) (Dewey et al. 1985a) and subunit 9 (*atp*9) (Dewey et al. 1985b); and the 26S (Dale et al. 1984) and 18S–5S (Chao et al. 1984) ribosomal RNA genes; used in this investigation were supplied by C.S. Levings, III, Genetics Departments, North Carolina State University, Raleigh, NC.

Pennisetum mtDNAs were digested with *Bam*HI, *Eco*RV, *Hind*III, *Pst*I, *Sma*I and *Xba*I restriction endonucleases according to the suppliers' instructions. Complete digestion was verified by observation of repeatable restriction patterns in replicated samples. Two µg of digested mtDNA were loaded in each lane. *Hind*III-digested lambda DNA and *Hae*III-digested Phi X 174 DNA were used as molecular markers for DNA fragment size calculations. DNA fragments were separated in 0.8% agarose gel, stained and photgraphed as described by Smith et al. (1987). After photographing, the restriction fragments were transferred from the gels to Nytran membrane (Schleicher and Schuell) as described by Maniatis et al. (1982). Nick translations and DNA hybridizations were carried out as described by Chase and Pring (1986).

Results

Genome size measurements

The size of the *Pennisetum* species mitochondrial genomes were determined by calculating the sum of the sizes of their restriction fragments. Repetitive fragments

Table 1. List of plant species, classification, chromosome number, mode of reproduction and germplasm source used in this investigation

Species	Section	Chromosome no.	Mode of reproduction	Germplasm source[a]
Pennisetum americanum	*Penicillaria*	2x = 14	Sexual	Various (N–75)
P. purpureum	*Penicillaria*	4x = 28	Sexual	
P. polystachyon	*Brevivalvula*	6x = 54	Apomictic	Senegal (PS446)
P. pedicellatum	*Brevivalvula*	6x = 54	Apomictic	Senegal (PS429)
P. ramosum	*Gymnothrix*	2x = 20	Sexual	Ethiopia (PS29)
P. squamulatum	*Heterostachya*	6x = 54	Apomictic	Kenya (PS26)
P. schweinfurthii	*Heterostachya*	2x = 14	Sexual	(PS243)
Panicum maximum	–	4x = 32	Apomictic	

[a] Genotypes used are indicated in the parentheses

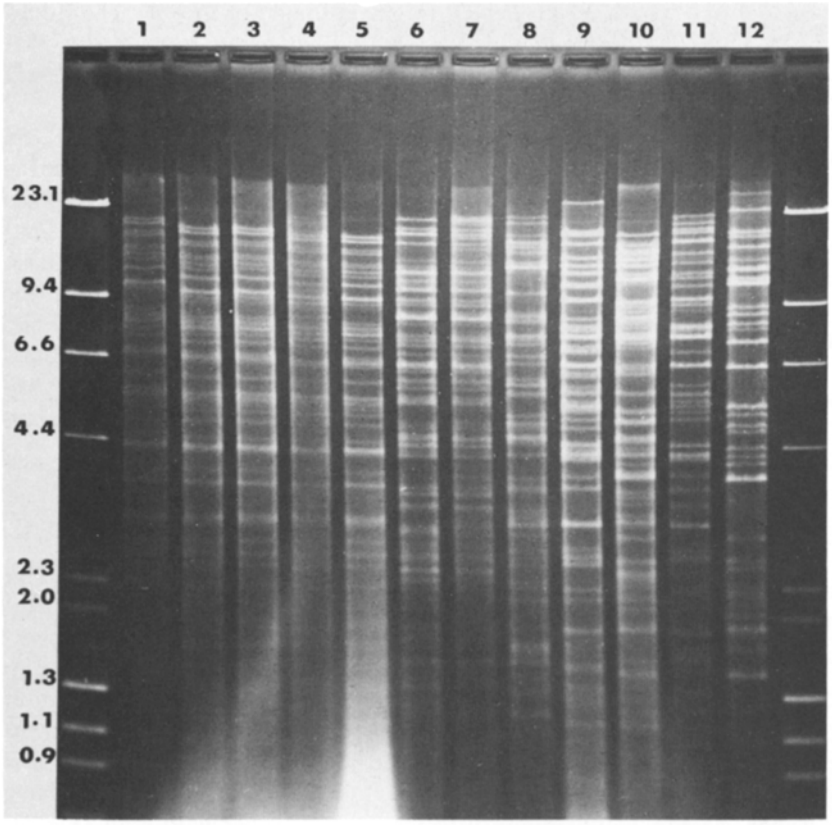

Fig. 1. Restriction fragment patterns of mtDNAs from *Pennisetum americanum* ecotypes, South African, Rhodesian, Nigerian, Ethiopian and 23B (*lanes 1–5*, respectively), *P. polystachyon* (*lane 6*), *P. pedicellatum* (*lane 7*), *P. ramosum* (*lane 8*), *P. squamulatum* (*lane 9*), *P. schweinfurthii* (*lane 10*), *P. purpureum* (*lane 11*), *Panicum maximum* (*lane 12*) generated by digestion with *Xba*I

Table 2. Comparison of total number of restriction fragments produced by different ecotypes of *P. americanum*

Endonuclease	Ecotype					Deviation of Ethiopian[a]
	S. African	Rhodesian	Nigerian	23B	Ethiopian	
*Xba*I	40	40	40	40	42	+3; −1
*Sma*I	48	48	48	48	49	+2; −1
*Eco*RV	42	42	42	42	40	+0; −2
*Pst*I	43	43	43	43	45	+3; −1
*Bam*HI	42	42	42	42	42	+1; −1
*Xho*I	41	41	41	41	40	+0; −1

[a] This column shows the deviation of the Ethiopian ecotype from the standard pearl millet restriction pattern
+ = extra Ethiopian fragments; − = missing Ethiopian fragments

were accounted for by estimating the stoichiometry of the ethidium bromide-stained DNA bands in the gel based on their relative brightness. The estimated mitochondrial genome sizes of *P. americanum, P. polystachyon, P. pedicellatum, P. ramosum, P. squamulatum, P. schweinfurthii, P. purpureum* and *Panicum maximum* were estimated to be 407, 405, 410, 341, 486, 392, 364 and 303 kb, respectively.

Pearl millet restriction patterns

No differences were observed in the endonuclease restriction patterns between the pearl millet ecotypes, except for the ecotype collected in Ethiopia. The photograph of the ethidium bromide-stained gel in Fig. 1 (lanes 1–5) shows the restriction fragment similarities when those mtDNAs were digested with *Xba*I. Since the *Xba*I results are representative of the other five endonucleases, only the *Xba*I gel is shown. The numbers of restriction fragments generated by the five ecotypes of *P. americanum* when digested with the six endonucleases are summarized in Table 2. On the average, *Sma*I produced the maximum number of fragments (49) and *Xba*I produced the minimum number (40). Table 2 also summarizes how the Ethiopian ecotype restriction patterns differ from the patterns of the other ecotypes.

Pennisetum species restriction patterns

In contrast to the uniformity of the *Pennisetum americanum* data, the restriction patterns of the *Pennisetum* species were highly variable. The variability of *Xba*I restriction patterns is shown in Fig. 1. Table 3 summarizes the total number of fragments produced when mtDNA from each species was digested with *Xba*I (shown on the diagonal), as well as the number of common fragments shared by each pair of species. The number of fragments generated by different species after digestion with *Xba*I ranged from 39 in the distantly related reference species, *Panicum maximum*, to 57 in *Pennisetum schweinfurthii*.

An index of relatedness of the *Pennisetum* species was calculated by determining the percentage of similar sized fragments shared by the various species pairs using the larger number of total restriction fragments possessed by the pair. Based on those calculations, *P. polystachyon* and *P. pedicellatum* share 89% of their fragments, followed by *P. squamulatum* and *P. purpureum* with 64%. The index of relatedness is listed in parentheses in Table 3.

Pearl millet hybridization patterns

Hybridization of pearl millet mtDNA Southern blots with the maize gene probes gave identical patterns for three of the five pearl millet ecotypes, i.e., South African, Nigerian and 23B, and those patterns will be considered standard pearl millet patterns in this report. Both the Rhodesian and Ethiopian ecotypes differed from the others and had unique patterns. The Rhodesian ecotype had an extra 4.3 kb *Eco*RV fragment when hybridized to the *cox*II probe, and in all ecotypes that probe hybridized to a 2.7 kb *Eco*RV fragment (data not shown). The Ethiopian ecotype also hybridized to an extra 5.1 kb *Pst*I fragment with the *atp*9 probe (Fig. 3), and to an extra 5.0 kb *Pst*I fragment with the *atp*6 probe (Fig. 5).

The Ethiopian ecotype hybridized with the 18S–5S probe had extra 10.2 and 7.4 kb *Pst*I fragments, while the 7.1 kb *Pst*I fragment possessed by the other ecotypes was absent (Fig. 4). With the *atp*6 probe, the 10.8 kb *Pst*I fragment of the other four ecotypes was replaced by a 4.8 kb *Pst* I fragment (Fig. 5), and with the *cox*I probe, the 5.9 kb *Bam*HI fragment possessed by the other ecotypes was missing (Fig. 6). The 26S rRNA and *atp*A probes did not detect differences between the ecotypes and hybridized to 23.0 kb *Sma*I and 14.0 kb *Xho*I fragments, respectively (data not shown).

Pennisetum species hybridization patterns

The fragment patterns of the *Pennisetum* species mtDNA hybridized to the maize gene probes are complex, therefore, species similarities will be listed here. Species comparisons can be seen in Figs. 2–6. *P. polystachyon* and *P. pedicellatum* have identical single-fragment hybridization patterns when probed with *cox*II, 26S (data not shown) and *atp*9 (Fig. 3), and identical five-fragment patterns when probed with *cox*I (Fig. 6). Those two species have only minor differences when probed with *atp*A (Fig. 2), 18S–5S (Fig. 4) and *atp*6 (Fig. 5). With *atp*A they had four fragments in common, but *P. pedicellatum* had an extra 8.5 kb fragment. With 18S–5S, both species possessed three fragments, but *P. pedicellatum* had an 11.0 kb fragment as well. With *atp*6, both species posses-

Table 3. The number of common *Xba*I-digested mtDNA restriction fragments between each species pair and the index of relationship (in parentheses in percentage form) of each pair. The lower diagonal lists the total number of restriction fragments in each species

	Pam[a]	*Ppol*	*Pped*	*Pram*	*Psqu*	*Psch*	*Ppur*	*Pmax*
Pam	40	21 (40)	22 (39)	14 (33)	15 (33)	13 (23)	14 (34)	10 (25)
Ppol		53	50 (89)	20 (38)	16 (30)	15 (26)	13 (25)	13 (25)
Pped			56	20 (36)	21 (38)	16 (28)	17 (30)	13 (23)
Pram				43	18 (40)	12 (21)	10 (23)	9 (21)
Psqu					45	14 (25)	29 (64)	9 (20)
Psch						57	15 (26)	10 (18)
Ppur							41	8 (20)
Pmax								39

[a] Pam = *Pennisetum americanum*; Ppol = *P. polystachyon*; Pped = *P. pedicellatum*; Pram = *P. ramosum*; Psqu = *P. squamulatum*; Psch = *P. schweinfurthii*; Ppur = *P. purpureum* and Pmax = *Panicum maximum*

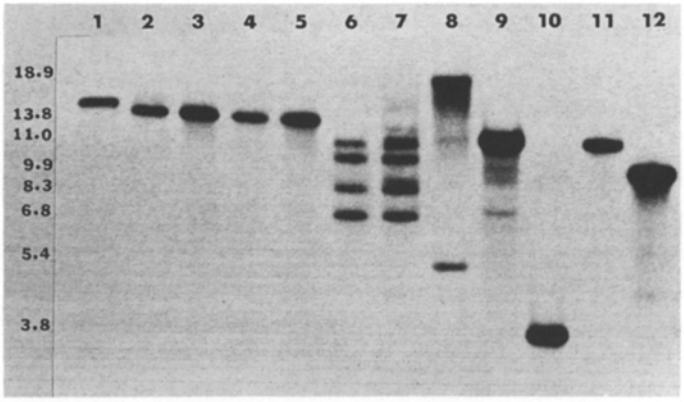

Fig. 2. Hybridization patterns of Southern blots containing *Xho*I-digested *Pennisetum* and *Panicum* mtDNA hybridized to the ^{32}P labeled maize probe containing the subunit alpha of ATPase gene. Sizes of the fragments with homology to that probe are indicated. Lane designations are the same as in Fig. 1

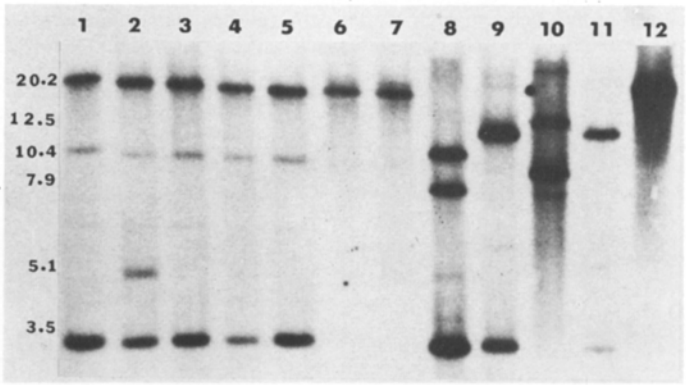

Fig. 3. Hybridization patterns of Southern blots containing *Pst*I-digested *Pennisetum* and *Panicum* mtDNA hybridized to the ^{32}P labeled maize probe containing the subunit 9 of ATPase gene. Sizes of the fragments with homology to that probe are indicated. Lane designations are the same as in Fig. 1

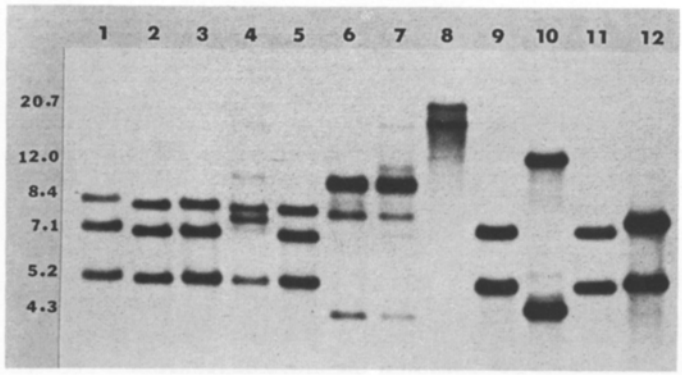

Fig. 4. Hybridization patterns of Southern blots containing *Xba*I-digested *Pennisetum* and *Panicum* mtDNA hybridized to the ^{32}P labeled maize probe containing the 18S-5S rRNA gene. Sizes of the fragments with homology to that probe are indicated. Lane designations are the same as in Fig. 1

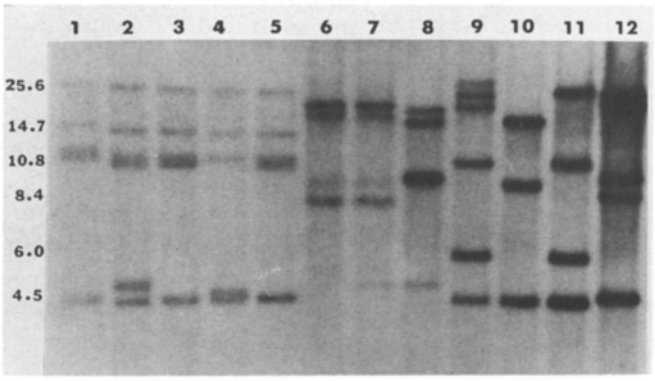

Fig. 5. Hybridization patterns of Southern blots containing *Pst*I-digested *Pennisetum* and *Panicum* mtDNA hybridized to the ^{32}P labeled maize probe containing the subunit 6 of ATPase gene. Sizes of the fragments with homology to that probe are indicated. Lane designations are the same as in Fig. 1

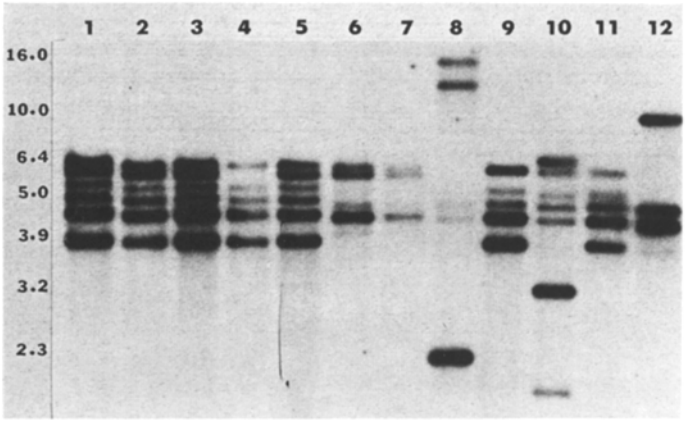

Fig. 6. Hybridization patterns of Southern blots containing *Bam*HI-digested *Pennisetum* and *Panicum* mtDNA hybridized to the ^{32}P labeled maize probe containing the cytochrome oxidase subunit I gene. Sizes of the fragments with homology to that probe are indicated. Lane designations are the same as in Fig. 1

Table 4. The number of common fragments hybridized by all seven probes between each species pair and the index of relationship (in parentheses in percentage form) of each species pair. The total number of hybridized fragments in each species is listed on the lower diagonal

	Pam[a]	*Ppol*	*Pped*	*Pram*	*Psqu*	*Psch*	*Ppur*	*Pmax*
Pam	20	6 (30)	6 (30)	3 (15)	12 (54)	6 (30)	12 (60)	6 (30)
Ppol		19	19 (95)	4 (21)	5 (23)	7 (35)	4 (21)	3 (16)
Pped			20	4 (20)	5 (23)	7 (35)	4 (20)	3 (15)
Pram				19	4 (18)	7 (35)	4 (21)	2 (11)
Psqu					22	8 (36)	18 (82)	6 (27)
Psch						20	6 (30)	4 (20)
Ppur							18	5 (28)
Pmax								14

[a] Pam = *Pennisetum americanum*; Ppol = *P. polystachyon*; Pped = *P. pedicellatum*; Pram = *P. ramosum*; Psqu = *P. squamulatum*; Psch = *P. schweinfurthii*; Ppur = *P. purpureum* and Pmax = *Panicum maximum*

sed five fragments, but *P. pedicellatum* had an additional 5.0 kb fragment.

Hybridization patterns of *P. squamulatum* and *P. purpureum* both exhibited a single major fragment when probed with *atp*A (Fig. 2), two fragments when probed with 18S–5S (Fig. 4) and five fragments when probed with *cox*I (Fig. 6). The *P. americanum* (Ethiopian ecotype) *cox*I pattern was also similar to *P. squamulatum* and *P. purpureum* (Fig. 6). *P. americanum*, *P. squamulatum*, *P. purpureum*, *P. schweinfurthii* and *Panicum maximum* had identical single-fragment patterns when probed with *cox*II (data not shown). When probed with the 26S probe, *P. schweinfurthii* and *Panicum maximum* had identical single-fragment patterns; *P. squamulatum* and *P. purpureum* had three similar fragments, however, their larger fragments were not alike (data not shown). *P. ramosum* did not produce hybridization patterns similar to any other species.

Table 4 summarizes the total number of fragments hybridized by all seven probes for each species (listed on the diagonal), the number of shared fragments between each species pair, and the index of relatedness between each species pair (listed in parentheses below the number of shared fragments). The species with the highest total number of hybridized fragments was *P. squamulatum* with 22 and the species with the fewest fragments was *Panicum maximum* with 14. The number of shared fragments between species pairs ranged from two (*Pennisetum ramosum* and *Panicum maximum*), to 19 (*Pennisetum polystachyon* and *P. pedicellatum*). The two species with the highest index of relatedness of 95 were *P. polystachyon* and *P. pedicellatum*; the next highest were *P. squamulatum* and *P. purpureum* with an index of 82. Both *P. squamulatum* and *P. purpureum* have significant indices of relatedness with *P. americanum* of 54 and 60, respectively.

Discussion

The *Pennisetum* mitochondrial genome sizes ranged from 364 kb for *P. purpureum* to 486b for *P. squamulatum* and are in the range of the known plant mitochondrial genome sizes of 138 kb for *Solanum* (Quetier and Vedel 1977) to 2500 kb for *Cucumis* (Ward et al. 1981). The mitochondrial genome size of *P. americanum* previously reported as 355 kb by Smith et al. (1987) did not account for repetitive DNA fragments. The inclusion of repeated sequences in this report accounts for the larger size of 407 kb reported here. More precise estimates of genome sizes could be obtained by analyzing large overlapping clones which would give better estimates of repeated fragments.

Restriction patterns are excellent tools for studying phylogenetic relationships in plant species, however, they depend only on fragment size. It is possible that similar sized fragments contain different DNA sequences. A more stringent measure of genome variability is the comparison of fragments containing homologous sequences identified by hybridization to specific DNA probes. We compared restriction and hybridization patterns to measure relationships using pearl millet ecotypes with limited mtDNA variability, and *Pennisetum* species having highly variable mtDNA.

Pearl millet comparisons

Identical restriction patterns were observed among the different *P. americanum* ecotypes, except for the Ethiopian ecotype that differed from the standard type with each of the endonucleases used. The uniformity of the pearl millet restriction patterns was not unexpected. Clegg et al. (1984) were unable to detect variation between chloroplast DNAs of 12 *P. americanum* ecotypes. Similarly, no mtDNA variation was observed among populations within subspecies of *Hedysarum spinisissimum* from different geographical origins (Baatout et al. 1985). In contrast, variation between different races of annual teosinte was reported by Timothy et al. (1979). It is generally observed that ctDNAs are more conserved than the mtDNAs (DeBonte et al. 1984; Baatout et al. 1985). In the case of *Hedysarum*, no variation was observed between different populations based on either ct- or mtDNAs which may indicate that those populations diverged relatively recently. On the other hand, in the case of teosinte, different races may have diverged more distantly allowing the mitochondrial genome to undergo numerous rearrangements.

With the hybridization data, both the Rhodesian and Ethiopian ecotypes deviated from the standard pearl millet patterns. This data shows that the hybridization methodology is more effective in revealing the variability that exists. The restriction pattern data supports the Vavilov (1949) hypothesis that Ethiopia is the center of diversity and the probable center of origin for pearl millet. However, the hybridization pattern variability expressed in both the Ethiopian and Rhodesian ecotypes confounded that conclusion. Analysis of a greater number of pearl millet lines from those regions will be needed before more definitive conclusions can be made.

Pennisetum species comparisons

A great deal of restriction fragment polymorphism was present among the different species of *Pennisetum* that complicated comparisons, therefore, we analyzed that data by comparing the percentage of similar fragment sizes shared between species pairs in a similar way to that described by DeBonte et al. (1984) who compared carrot species. Based on that data, *P. polystachyon* and *P. pedicellatum* were the most closely related species in the study, followed by *P. squamulatum* and *P. purpureum*. Distantly related *Panicum maximum* had an index of relatedness range of 18 to 25 with the *Pennisetum* species, therefore indices in that range show little relationship. Several species had indices significantly above that level indicating some relation to one another (Table 3).

Analyses of the fragments that hybridized to the seven maize probes also showed close relationships between *P. polystachyon* and *P. pedicellatum* (Table 4). That result is also supported with morphological similarities. Both were collected from Senegal, are apomictic hexaploids, have a chromosome number of 54, show similar chromosome pairing and are conventionally classified under the same section. *Brevivalvula* (Table 1). The mtDNA data suggest that both species originated from the same cytoplasmic stock and may even be considered one species rather than two.

All 18 hybridized fragments of *P. purpureum* were similar in size to the hybridized fragments of *P. squamulatum* (Table 4), however, since the highest fragment number of the species pair was used to calculate the percentage of shared fragments, the index of relatedness of that pair is 89. The indicated close relationship of the cytoplasms of those two species suggests that they may have common origin. However, those two species are grouped under different taxonomic sections and have different modes of reproduction and basic chromosome numbers (Table 1).

The hybridization data also indicated that *P. squamulatum* and *P. purpureum* were both related to *P. americanum* with indices of 54 and 60, respectively. Those data suggest that *P. squamulatum* and *P. purpureum* may have originated from *P. americanum* or that all three may have a common origin. Cytogenetic studies of interspecific single and double cross hybrids by Dujardin and Hanna (1984) also indicated some relationship among the nuclear genomes of those three species. The restriction pattern data showed indices of relatedness of only 33 and 34 between pearl millet and those two species.

The distant relationship of *P. ramosum* to the other *Pennisetums* (Table 4) was expected because it was the only *Pennisetum* used in this investigation classified to the *Gymnothrix* section and was supported by cytogenetic data (W.W. Hanna, personal communication). Several other indices of relatedness calculated from the hybridization data indicated that several *Pennisetum* species were not much more closely related to one another than to the distantly related, *Panicum maximum* (Table 4).

A comparison of the two methods of studying phylogenetic relationships shows that more differences were revealed by the hybridization data. With that method sequence homology and similar fragment size were necessary to demonstrate similarity. In the *Pennisetum* species comparisons, the hybridization data showed significant relationships of pearl millet to *P. squamulatum* and to *P. purpureum*, and also of *P. polystachyon* to *P. pedicellatum* and of *P. squamulatum* to *P. purpureum*, that seem to better fit the supporting information. Overall, it appears that the more stringent measurement of similarity of the hybridization patterns would be the preferred method.

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