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Genomic fingerprints in the tribe *Triticeae* produced by PCR using a tRNA consensus primer

P.V. REDDY* and K.M. SOLIMAN

Department of Plant and Soil Science, Alabama A & M University, Normal, AL 35762, USA
*Department of Plant Pathology, Cook College, Rutgers University, New Brunswick, NJ 08903, USA**

Abstract

Forty-one accessions belonging to ten genera of the tribe *Triticeae* representing both wild and cultivated species were analyzed by polymerase chain reaction (PCR). Of two consensus tRNA primers tested, one primer revealed characteristic amplification products for all of the species. A total of 35 tRNA markers were scored across all accessions. Five genus-specific and three species-specific markers were obtained. Genomic fingerprints were largely conserved within a genus. The phylogram obtained using parsimony has separated most of the accessions into their prevailing taxonomic species and genus groups. The phylogram showed close association among the three genera *Secale*, *Triticum* and *Hordeum* as expected. The *Triticum-Secale* relationship was closer than the *Triticum-Hordeum* or the *Secale-Hordeum* relationships. The tree also reflected the close associations among the forage grass species belonging to *Leymus* and *Elymus*. Thus tDNA–PCR helped to identify species and genera.

Additional key words: parsimony, phylogram, polymerase chain reaction.

Introduction

The tribe *Triticeae*, which contains both rangeland grasses and cereal crops like wheat and barley, is economically the most important of the tribes of the grass family (*Gramineae* syn. *Poaceae*), itself the most economically important family of flowering plants (Kellog 1992). The tribe represents a vast reservoir of genetic variation for the improvement of both grasses and cereal crops. A knowledge of genomic relationships among species in the tribe may help the successful transfer of desirable traits or to create fertile new species through interspecific and intergeneric

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* To whom correspondence should be sent; fax (+908) 932 9377, e-mail veera@rci.rutgers.edu.

hybridization. Many taxa in the tribe are interfertile, and both natural and artificial hybrids are well documented (Jensen and Bickford 1992). This interfertility has been exploited by plant breeders trying to improve the many cultivated species, but it has frustrated attempts to disentangle the relationships in the tribe, so there is still little agreement on the relationships within the *Triticeae*.

Restriction fragment length polymorphisms (RFLPs) (Botstein *et al.* 1980, Tanksley *et al.* 1989) are one type of DNA marker that has been used for determining genetic relationships. These markers are based on variation in the position of restriction endonuclease sites among genotypes. RFLPs have been used to generate extensive genetic maps of several important plant species over periods of several months as opposed to the years required using comparable morphological markers. But the primary disadvantages of using RFLPs in plant genetic resource characterization are the cost of materials, labor, and the processing time.

An alternative method, with the potential to overcome some of the current limitations in fingerprinting of cereals, is based on the Polymerase Chain Reaction (PCR) (Saiki 1989, Mullis and Faloona 1987). With non-random primers, polymorphisms are sought in the distance between two short target sequences, rather than in the presence or absence of restriction endonuclease sites as is the case for standard RFLPs (Skolnick and Wallace 1988). Oligonucleotides that anneal to the target sequences are used to prime the polymerase reactions.

Recently, DNA polymorphisms were detected after amplification using tRNA consensus primers for DNA fingerprinting in bacteria, humans, maize and rice (Welsh and McClelland 1991). tRNA genes occur in multiple copies dispersed throughout the genome in most species (Jinks-Robertson and Nomura 1987, Ohyama *et al.* 1986). The shared sequence motifs of the tRNA genes implies that consensus sequence primers for the polymerase chain reaction are likely to result in a number of characteristic PCR products.

tDNA-PCR, so called to indicate that DNA encoding tRNAs is the target for amplification, is likely to give fingerprints that vary at the species or genus level as the tRNA gene clusters have evolved (Welsh and McClelland 1991). Thus the fingerprints would be a measure of DNA relatedness at this level of classification. Here we describe the use of PCR to fingerprint and to show the relationships in the members of tribe *Triticeae* by using consensus tRNA primers.

Materials and methods

Plants: Forty-one accessions belonging to ten genera of the tribe *Triticeae* were used in this study (Table 1). The accessions belonging to the genera *Psathyrostachys, Leymus, Elymus, Agropyron, Elytrigia* and *Hordeum* were obtained as seeds from the USDA-ARS Forage and Range Research Laboratory, Utah State University, Logan, USA. Accessions of *Aegilops, Triticum, Secale* and *Triticale* were obtained as seeds from the National Small Grains Collection, Aberdeen, USA. Plants were grown from seeds in a greenhouse under natural irradiance and at temperature of 20 °C.

DNA Extraction: Five plants were sampled from each accession and total cellular DNA of the pooled sample was prepared as described by Murray and Thompson (1980) and Saghai-Maroof *et al.* (1984). DNA concentration was determined from the absorbance measured at 260 nm with a Beckman spectrophotometer.

Table 1. *Triticeae* accessions used for PCR analysis.

Genus	S. No.	Species	Accession	Origin
Psathyrostachys	1	*Ps. fragilis*	PI-343190	Iran
	2	*Ps. fragilis*	PI-343192	Iran
	3	*Ps. juncea*	PI-499672	China
	4	*Ps. juncea*	PI-499675	China
Leymus	5	*L. cinereus*	PI-236809	Canada
	6	*L. cinereus*	PI-478831	Montana
Elymus	7	*E. trachycaulus*	PI-232156	USA
	8	*E. trachycaulus*	PI-232166	Colorado
	9	*E. caninus*	PI-172364	Turkey
	10	*E. caninus*	PI-251417	Yugoslavia
Agropyron	11	*A. cristatum*	PI-281862	Germany
	12	*A. desertorum*	PI-249143	Portugal
	13	*A. fragile*	PI-314608	USSR
	14	*A. mongolicum*	D-2826	China
Elytrigia	15	*Et. elongatiformis*	PI-229474	Iran
	16	*Et. pungens*	PI-229674	Iran
	17	*Et. pycnantha*	PI-277183	France
Hordeum	18	*H. bulbosum*	PI-318649	Greece
	19	*H. californicum*	PI-531778	California
	20	*H. muticum*	PI-531785	Argentina
	21	*H. procerum*	PI-531787	Argentina
	22	*H. spontaneum*	PI-284744	Israel
	23	*H. spontaneum*	PI-296897	Israel
	24	*H. vulgare*	DL 71	USA
	25	*H. vulgare*	NK 1467	USA
Aegilops	26	*Ae. tauschii*	CIae 3	USSR
	27	*Ae. longissima*	PI-170198	Turkey
	28	*Ae. speltoides*	PI-170203	Turkey
	29	*Ae. biuncialis*	PI-177241	Turkey
Triticum	30	*T. aestivum*	CItr 8399	Argentina
	31	*T. durum*	CItr 3139	Tunisia
	32	*T. spelta*	PI-295064	Hungary
	33	*T. monococcum*	PI-119423	Turkey
	34	*T. vavilovii*	PI-428342	Sweden
Secale	35	*S. cereale*	CIse13	USA
	36	*S. segetale*	CIse 105	Italy
	37	*S. ancestrale*	PI-283971	Algeria
	38	*S. montanum*	PI-205222	Turkey
	39	*S. vavilovii*	PI-253957	Afghanistan
Triticale	40	× *Triticosecale sp.*	CI xt 1	China
	41	× *Triticosecale sp.*	PI-280457	USSR

Primer synthesis: Consensus tRNA primers T5B (5' AATGCTCTACCAACTGAACT3') and T3A (5' GGGGGTTCGAATTCCCGCCGGCCCCA3') were synthesized using the *Pharmacia Gene Assembler Plus*. These primers were developed by Welsh and McClelland (1991) based on over 500 known tRNA sequences. Given that there are one hundred or more divergent tRNA genes in a typical genome, there are likely to be many matches for the primers and these will vary from almost perfect to rather poor matches.

DNA amplification: Each of the two tRNA consensus primers were used singly for DNA amplification following the protocol reported by Williams *et al.* (1990) with minor modifications. The reaction components were 1X reaction buffer [50 mM KCl, 10 mM Tris-HCl (pH 9.0), and 0.01 % Triton X-100], 200 µM of dNTP, 0.2 µM of primer, 2.5 units of *Taq* polymerase, 50 ng of genomic DNA and 1.0 mM $MgCl_2$ in a 0.1 cm^3 reaction mix. The *Taq* polymerase, dNTPs and reaction buffer were purchased from *Promega* (Madison, USA). For the DNA amplification a *Perkin Elmer Cetus* (Foster City, USA) DNA thermal cycler was programmed for 40 cycles at 94 °C for 30 s to denature, 50 °C for 30 s for primer annealing and 72 °C for 2 min for extension. The reaction mixture was overlaid with mineral oil. After all the cycles were completed, 0.03 cm^3 of each sample were loaded in 2.0 % agarose gels in 1X TBE buffer and run at 4 V cm^{-1}. A 100 bp DNA ladder (BRL) was used as a molecular standard. The gels were stained in ethidium bromide solution for 20 min, destained with tap water for 20 min and photographed under UV radiation with *Polaroid Type 55* film. The pictures were scanned for amplification products with a *Pharmacia* (Piscataway, USA) laser densitometer. Each amplification product was identified by its size in base pairs following the primer used in the reaction. In order to confirm accession-specific markers, each amplification was repeated at least twice. Only reproducible bands from multiple runs, regardless of their intensity, were considered in the study.

Data analysis: Bands on gels were scored as present (+) or absent (–) for all accessions studied. The sizes of the fragments were determined both visually and using a *Pharmacia* laser densitometer equipped with gel scanning (*GSXL*) software. The microcomputer package *PAUP* (Phylogenetic Analysis Using Parsimony), *Version 3.1*, developed by Swofford in 1993 (Illinois Natural History Survey, Champaign, USA) was used to calculate a pairwise difference matrix and to construct a phylogenetic tree.

Results

Identification of markers: The sizes of amplified DNA fragments ranged from 500 to 2450 base pairs (Fig. 1). The number of bands in the profiles varied, depending on the primer and accession tested. No amplification was observed when the primer T3A was used. With the primer T5B the fragments ranged from 2 (*Triticosecale* sp.) to 12 (*Psathyrostachys fragilis*). From the amplification products of 41 accessions with T5B primer (Fig. 1*A,B,C*) 35 products that could be used as potential genetic

markers were disclosed by the T5B tRNA primer. The profiles of the amplified products were compared for identification of species-specific and genus-specific markers (Table 2).

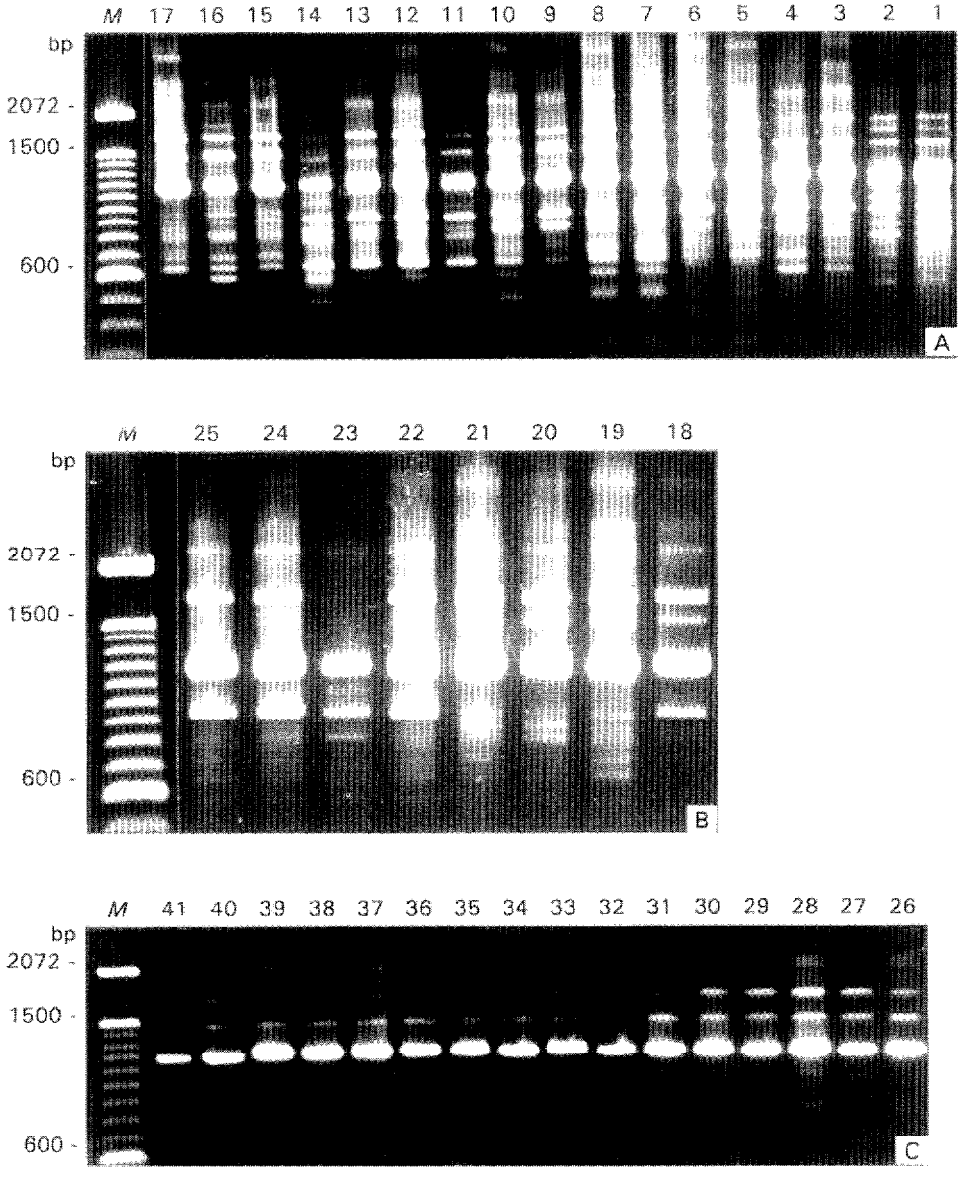

Fig. 1 *A,B,C.* Banding patterns generated by tRNA primer T5B. Numbers on top refers to the accessions listed in Table 1. *M* indicates the 100 bp molecular size standard.

Table 2. Survey of 35 T5B tRNA markers in 41 accessions. "+" means presence, "-" means absence of band.

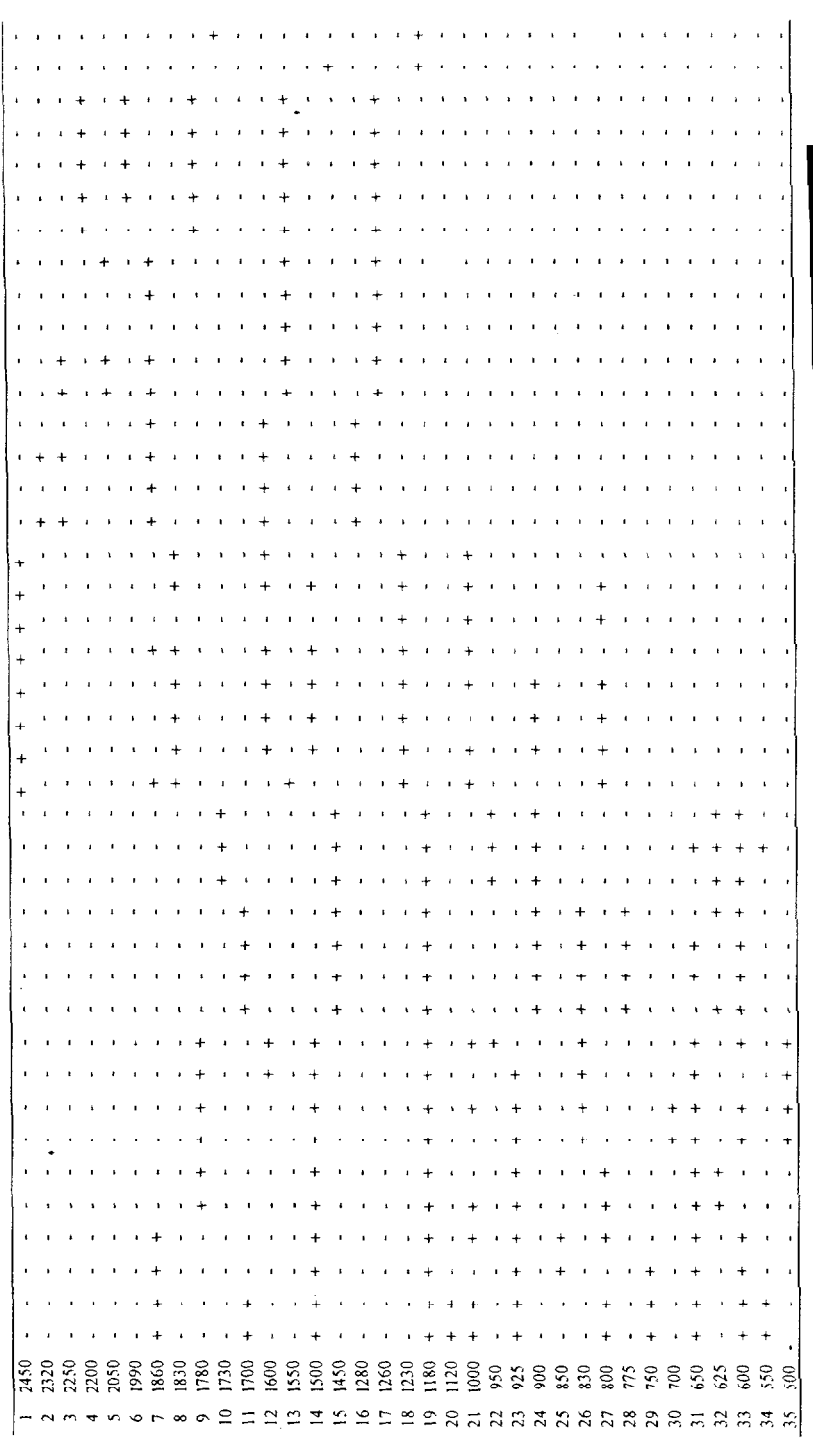

Table 3. Pairwise fragment difference among 41 accessions belongs to tribe *Triticeae*.

S.No	Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41
1	*Ps. fragilis*	-																																								
2	*Ps. fragilis*	0	-																																							
3	*Ps. juncea*	6	6	-																																						
4	*Ps. juncea*	5	5	3	-																																					
5	*L. cinereus*	8	8	8	5	-																																				
6	*L. cinereus*	9	9	7	6	1	-																																			
7	*E. trachycaulus*	11	11	7	8	7	6	-																																		
8	*E. trachycaulus*	10	10	8	7	6	7	1	-																																	
9	*E. caninus*	12	12	8	9	6	5	3	4	-																																
10	*E. caninus*	12	12	10	9	8	9	5	4	4	-																															
11	*A. cristatum*	14	14	12	13	12	11	11	12	12	12	-																														
12	*A. desertorum*	12	12	10	11	12	11	9	10	10	10	2	-																													
13	*A. fragile*	12	12	10	11	12	11	9	10	10	10	2	0	-																												
14	*A. mongolicum*	14	14	12	13	12	11	11	12	12	12	0	2	2	-																											
15	*Et. elongitiformis*	15	15	11	12	11	10	12	13	13	11	5	7	7	5	-																										
16	*Et. pungens*	13	13	11	12	11	10	12	13	13	11	7	7	7	7	2	-																									
17	*Et. pycnantha*	15	15	11	12	11	10	12	13	13	11	5	7	7	5	0	2	-																								
18	*H. bulbosum*	13	13	13	10	11	12	16	15	15	15	15	15	15	15	14	16	14	-																							
19	*H. californicum*	14	14	14	11	10	11	15	14	12	12	14	14	14	14	13	15	13	5	-																						
20	*H. muticum*	15	15	13	12	11	10	14	15	11	13	13	13	13	13	12	14	12	6	1	-																					
21	*H. procerum*	14	14	14	11	10	11	15	14	12	12	14	14	14	14	13	15	13	5	0	1	-																				
22	*H. spontaneum*	13	13	11	10	11	12	14	13	11	11	15	15	15	15	14	16	14	4	3	4	3	-																			
23	*H. spontaneum*	12	12	12	9	8	9	13	12	12	12	12	12	12	12	11	13	11	3	4	5	4	5	-																		
24	*H. vulgare*	13	13	13	10	9	10	14	13	11	11	15	15	15	15	14	16	14	4	1	2	1	2	3	-																	
25	*H. vulgare*	15	15	13	12	11	12	14	13	11	11	13	13	13	13	12	14	12	4	3	4	3	2	3	2	-																
26	*Ae. tauschi*	15	15	11	12	13	12	14	15	11	13	13	13	13	13	12	14	12	10	11	10	11	8	9	10	8	-															
27	*Ae. longissima*	13	13	9	10	11	10	12	13	9	11	11	11	11	11	10	12	10	8	9	8	9	6	7	8	6	2	-														
28	*Ae. speltoides*	15	15	11	12	13	12	14	15	11	13	13	13	13	13	12	14	12	10	11	10	11	8	9	10	8	0	2	-													
29	*Ae. bicornis*	13	13	9	10	11	10	12	13	9	11	11	11	11	11	10	12	10	8	9	8	9	6	7	8	6	2	0	2	-												
30	*T. aestivum*	15	15	11	12	13	12	14	15	13	15	13	13	13	13	12	14	12	8	13	12	13	10	9	12	10	6	6	6	6	-											
31	*T. durum*	15	15	11	12	13	12	14	15	13	15	13	13	13	13	12	14	12	8	13	12	13	10	9	12	10	6	6	6	6	0	-										
32	*T. spelta*	14	14	10	11	10	9	11	12	10	12	10	10	10	10	9	11	9	7	10	9	10	9	6	9	7	7	5	7	5	3	3	-									
33	*T. monococcum*	13	13	9	10	11	10	12	13	11	13	11	11	11	11	0	12	10	6	11	10	11	8	7	10	8	6	4	6	4	2	2	1	-								
34	*T. vavilovii*	14	14	10	11	12	11	13	14	12	14	12	12	12	12	1	13	11	7	12	11	12	9	8	11	9	7	5	7	5	1	1	2	1	-							
35	*S. cereale*	16	16	12	13	10	9	11	12	10	12	12	12	12	12	1	13	11	9	12	11	12	11	8	11	9	9	7	9	7	5	5	2	3	4	-						
36	*S. segetale*	17	17	13	14	11	10	12	13	11	13	13	13	13	13	2	14	12	10	13	12	13	12	9	12	10	10	8	10	8	6	6	3	4	5	1	-					
37	*S. ancestrale*	17	17	13	14	11	10	12	13	11	13	13	13	13	13	2	14	12	10	13	12	13	12	9	12	10	10	8	10	8	6	6	3	4	5	1	0	-				
38	*S. montanum*	17	17	13	14	11	10	12	13	11	13	13	13	13	13	12	14	12	10	13	12	13	12	9	12	10	10	8	10	8	6	6	3	4	5	1	0	0	-			
39	*S. vavilovii*	17	17	13	14	11	10	12	13	11	13	13	13	13	13	12	14	12	10	13	12	13	12	9	12	10	10	8	10	8	6	6	3	4	5	1	0	0	0	-		
40	x *Triticosecale*	12	12	8	9	8	7	9	10	8	10	6	6	6	6	5	7	5	9	10	9	10	9	6	9	7	7	5	7	5	7	7	4	5	6	6	7	7	7	7	-	
41	x *Triticosecale*	12	12	8	9	8	7	9	10	8	10	8	8	8	8	5	7	5	9	10	9	10	9	6	9	7	7	5	7	5	7	7	4	5	6	6	7	7	7	7	2	-

Accession relationships: All pairwise fragment differences among accessions were calculated with the *PAUP* program (Table 3). These data indicate the number of

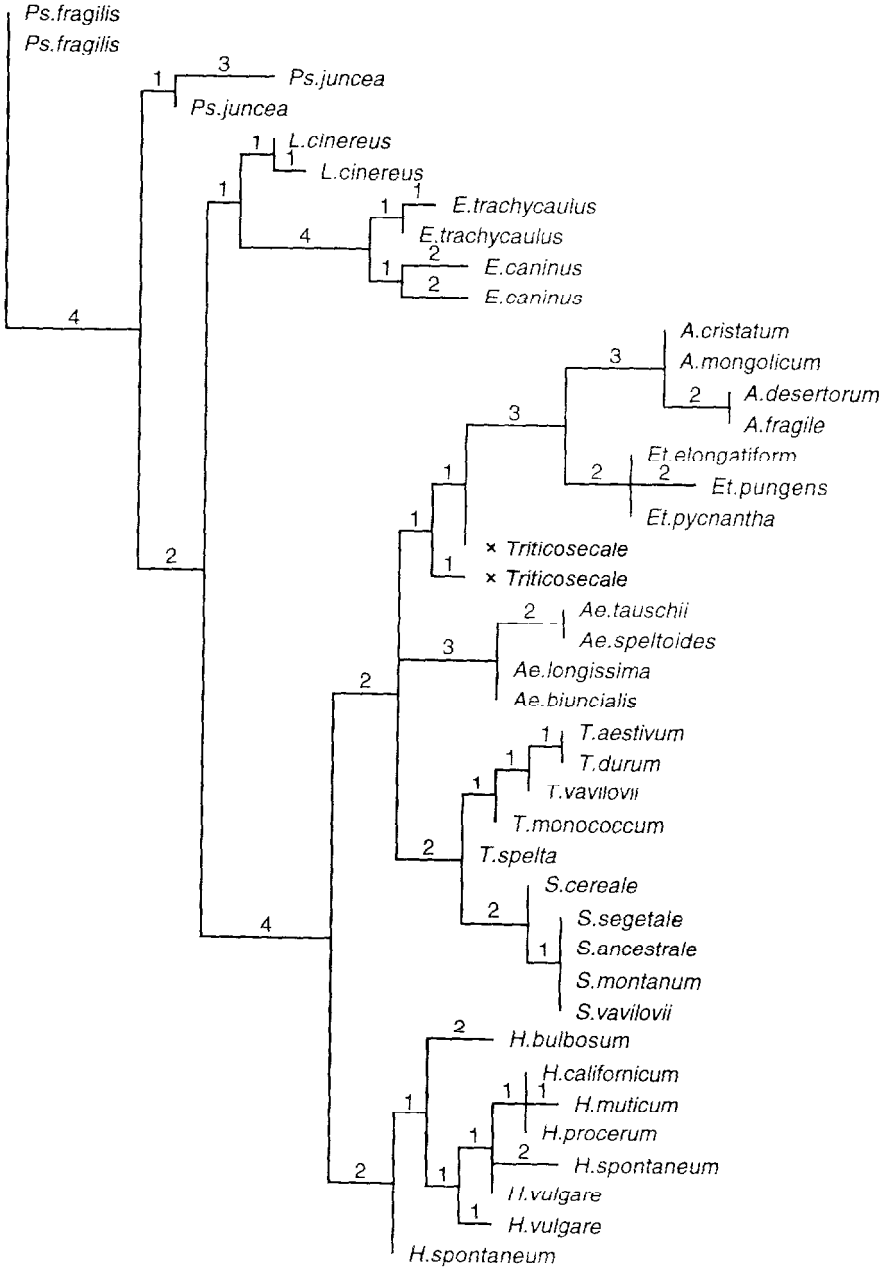

Fig. 2. The phyogenetic tree generated by the *PAUP* program. Numbers on the branches are the length of branches. Tree length = 70, Consistency Index (CI) = 0.5, Homoplasy Index (HI) = 0.5, Retention Index = 0.847, and the Rescale Consistency Index (RC) = 0.424.

markers that were different between the accessions of each pair. Inspite of a wide range in average differences among genera (Table 4), a smaller trend was observed within genera. Thus the DNA bands amplified by the tRNA primer T5B clearly resolved most genera within the tribe *Triticeae*.

Table 4. Average pairwise fragment differences among 10 genera belonging to the tribe *Triticeae*.

S. No	Genus	1	2	3	4	5	6	7	8	9	10
1	*Psathyrostachys*	4.2									
2	*Leymus*	7.5	1.0								
3	*Elymus*	9.8	6.8	3.5							
4	*Agropyron*	12.3	11.5	10.8	1.3						
5	*Elytrigia*	12.9	10.5	12.3	6.3	2					
6	*Hordeum*	12.7	10.5	13	13.9	13.5	3.1				
7	*Aegilops*	12.3	11.5	12.3	12	11.7	8.6	1.3			
8	*Triticum*	12.5	11.3	13.1	11.8	11.5	9.8	5.8	1.6		
9	*Secale*	15.1	10.3	12.1	12.8	12.5	11.2	8.8	4.6	0.4	
10	*Triticale*	10.3	7.5	9.3	7.0	5.7	8.6	6.0	11.6	6.8	2.0

Table 5. Genus specific markers

S. No.	Genus	Marker
7, 8, 9, 10	*Elymus*	500
11, 12, 13, 14	*Agropyron*	775
18, 19, 20, 21, 22, 23, 24, 25	*Hordeum*	1230, 2450
26, 27, 28, 29	*Aegilops*	1280, 2320
35, 36, 37, 38, 39	*Secale*	2200

Table 6. Species specific markers

S. No.	Name of the species	Marker
1	*Psathyrostachys fragilis*	1120
2	*Psathyrostachys fragilis*	1120
3	*Psathyrostachys juncea*	850
4	*Psathyrostachys juncea*	850
7	*Elymua trachycaulus*	700
8	*Elymua trachycaulus*	700

Discussion

The experiments conducted by Welsh and McClelland (1991) indicate that consensus tRNA gene primers that amplify the region between the tRNA genes can be used to generate PCR fingerprints that are generally invariant among strains of the same

species and are often substantially conserved among related species. This property made the method applicable to the identification of species by a genome-based method that is independent of other criteria such as morphology. Because this method can be performed easily, and it is independent of genome size, it may be a useful method for rapidly examining large numbers of different species.

Most of the polymorphisms disclosed by the primer were useful in the differentiation of the tribe *Triticeae* into different genera and species. As expected, more monomorphic bands than polymorphic bands were observed within the genus. For example marker 1280 was present only in the four species of genus *Aegilops*. This suggests a high degree of evolutionary relationship among these accessions.

The phylogenetic tree obtained using parsimony separated most of the accessions into their corresponding species and genera (Fig. 2). *Psathyrostachys* was used as an out group because that genus is considered relatively primitive in the tribe (Baum *et al.* 1987). The phylogram based on tRNA primers shows a close association among *Psathyrostachys*, *Leymus* and *Elymus* species. Several crosses have been successfully made betwen *Psathyrostachys* and *Leymus* species (Dewey 1984), which is consistent with the associations shown here. The phylogenetic tree also clustered *Agropyron* and *Elytrigia* species together, suggesting a close relationship between these two genera. The phylogram showed a close association among the three genera *Secale, Triticum*, and *Hordeum*. The *Triticum-Secale* relationship was closer than the *Triticum-Hordeum* and *Secale-Hordeum* relationship. This supports the fact that *Triticum* and *Secale* are placed in the same subtribe, *Triticinae*, whereas *Hordeum* is placed in another subtribe *Hordeinae*.

The association between *Triticum* and *Aegilops* was also intense in the phylogram. This agrees with chloroplast DNA study of Ogihara and Tsunewaki (1988) where variation within the *Triticum/Aegilops* group was generally found to be low. Thirteen restriction enzymes were needed to detect enough variation to distinguish the various genomic groups. At the genera level, Bowman *et al.* (1983) found no strict demarcation in chloroplast DNA banding patterns between *Aegilops* and *Triticum* and thus they supported the merger of the two genera as proposed earlier by Bowden (1959). We too support the merger of two genera *Aegilops* and *Triticum* based on our tRNA study.

The tRNA primer T5B provided enough polymorphism to construct a phylogenetic tree between species. Single tRNA primers can be used to compare genomes of organisms at the species/genus level. tRNA primer also revealed that *Agropyron* and *Elytrigia* are more closely related to the *Triticum-Aegilops* cluster compared to other forage grasses. The taxonomic relationships showed by this method agrees with the previous morphological and molecular studies. Thus this simplest method can be used to compare genomes of different organisms at the species/genus level.

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