Indian Journal of Agricultural Sciences **84** (12): 1464–9, December 2014/Article
https://doi.org/10.56093/ijas.v84i12.45213

Genetic diversity based on isozymic banding pattern in *Heteropogon contortus* - A perennial tropical forage grass

BILAL AHMAD BHAT[1] and AJOY KUMAR ROY[2]

Indian Grassland and Fodder Research Institute, Jhansi, Uttar Pradesh 284 003

Received: 25 April 2014; Revised accepted: 3 July 2014

ABSTRACT

To ascertain the extent of diversity present among the different accessions of *Heteropogon contortus*-a perennial fodder grass, eighty-nine genotypes collected from different parts of India were compared for four enzymes, viz. peroxidase, superoxidase dismutase (SOD), glutamate oxalo-acetate transaminase (GOT) using starch gel electrophoresis and esterase using Polyacrylamide gel electrophoresis (PAGE). Biochemical markers such as isozymes are useful supplements in identifying the genetic variation in a crop. The study revealed presence of 15 peroxidase bands, 5 SOD bands, 9 esterase bands and 1 GOT band. The GOT band was monomorphic. Polymorphism was observed for all other bands indicating thereby existence of considerable genetic diversity. Frequency of bands varied widely among the genotypes. For peroxidase, band number 3 was found to be most frequent whereas, band number 2 and 14 were least frequent and observed in only 22 and 24 genotypes respectively. None of the bands were found to be species specific. For SOD, bands 1 and 5 were most frequent and observed in 80 and 83 genotypes respectively. While all the bands were represented in 11 genotypes, a single band was observed in 7 genotypes. For esterase, 9 polymorphic bands were distributed in two distinct migration zones. Zone 1 comprised of 9 bands with low mobility (rm value 0.41 to 0.49). Band no. 2, 6 and 7 were present in higher frequency, whereas bands 4 and 5 were present in only 7 and 8 genotypes respectively. For GOT, only one monomorphic band with rm value 0.65 was recorded. Clustering based on UPGMA analysis reveals that genetic diversity could not be correlated to the geographical origin of the accessions. The study indicates that considerable genetic diversity exists in the species and collection from different geographical places will help in identifying suitable species for various agro-ecological situations as suitable forage species for rangeland conditions.

Key words: Fodder, Genetic variability, *Heteropogon*, Isozyme analysis, Perennial grass

Heteropogon contortus, commonly known as black spear grass, a member of family Gramineae (Poaceae), tribe Andropogonae is a perennial fodder grass with wide adaptability being found in tropical, sub-tropical, arid, semi-arid and lower hills. It is known to tolerate various abiotic stress situations such as drought, salinity and nutrient poor habitats. The grass is very palatable in the vegetative stage and is a good source of fodder for rangeland situations. *H. contortus* is drought-tolerant, perennial, C4 bunch grass, apomictic with low germination rate (13%) (Goergen and Daehler 2001). For any genetic improvement programme, knowledge about the genetic variability and similarity among the genotypes is a must. The genetic divergence study helps in identifying the suitable genotypes having desired agronomic attributes for further exploitation. In the present study, different genotypes of *H. contortus* collected from diverse sources were evaluated using isozyme markers and the similarity among them was determined. Despite the use of DNA markers such as RAPDs, AFLPs and RFLPs, isozymes are still widely employed in assessment of genetic variability in species and population (Aradhya *et al*. 1998, Buso *et al*. 1998, Lange and Schiffno-Witmann 2000). Isozymes are especially useful when several taxa and individuals are to be compared as the assumption of homology is more accurate than with some DNA markers (Klaas 1998).

[1]Research Associate, DMR, Pusa Campus, New Delhi (e mail: bhatbilal786@gmail.com); [2]Project Coordinator-Forage Crops (e mail: royak333@rediffmail.com)

MATERIALS AND METHODS

The experimental material comprises of eighty-nine genotypes of *H. contortus* collected from different parts of India (Table 1). Out of different collection places, Jhansi (Uttar Pradesh), Lalitpur (Uttar Pradesh), Datia (Madhya Pradesh), Shivpuri (Madhya Pradesh), Chattarpur (Madhya Pradesh) were of arid to semi-arid climate, whereas Bandipur (Karnataka), Kerala, Mudumulai (Tamil Nadu) belonged to sub humid to humid region. The genotypes were collected in the form of seeds from their natural habitat and raised in IGFRI research farm. The seeds were raised in nursery and transplanted in the field in 50 × 50 cm distance.

These genotypes were compared for the four-enzyme

Table 1 Source of *H. contortus* genotypes used in present study

Accession No.	Source	Accession No.	Source	Accession No.	Source
IG 95-374	Lalitpur-UP	IG 95-287	Jhansi-UP	IG 95-24	Lalitpur-UP
IG 02-183	Jhansi-UP	IG 95-286	Datia-MP	IG 95-24	Lalitpur-UP
IG 02-184	Jhansi-UP	IG 95-258	Datia-MP	IG 95-18	Lalitpur-UP
IG 02-185	Jhansi-UP	IG 95-274	Shivpuri- MP	IG 95-19	Lalitpur-UP
IG 02-186	Jhansi-UP	IG 95-277	Shivpuri-MP	IG 95-105	Shivpuri-MP
IG 02-187	Jhansi-UP	IG 95-279	Jhansi-UP	IG 95-07	Jhansi-UP
IG 02-188	Jhansi-UP	IG 95-280	Datia-MP	IG02-193	Lalitpur-UP
IG 02-189	Jhansi-UP	IG 95-283	Datia-MP	IG 95-103	Shivpuri-MP
IG 02-190	Jhansi-UP	IG 95-284	Datia-MP	IG 95-105	Shivpuri-MP
IG 95-371	Lalitpur-UP	IG 95-108	Shivpuri-MP	IG 95-104c	Shivpuri-MP
IG 95-369	Lalitpur-UP	IG 95-109	Shivpuri-MP	IG 97-163	Chattarpur-MP
IG 95-368	Lalitpur-UP	IG 95-110	Shivpuri-MP	IG 97-165	Chattarpur-MP
IG 95-367	Lalitpur-UP	IG 95-111	Shivpuri-MP	IG 97-182	Chattarpur-MP
IG 95-366	Lalitpur-UP	IG 95-271	Datia-MP	IG 97-209	Chattarpur-MP
IG 95-364	Lalitpur-UP	IG 95-273	Shivpuri-MP	IG 97-183	Chattarpur-MP
IG 95-363	Lalitpur-UP	IG02-191	Jhansi-UP	IG 95-242	Chattarpur-MP
IG 95-352	Jhansi-UP	IG02-192	Jhansi-UP	IG 96-190	Bandipur-Karnataka
IG 95-350	Jhansi-UP	IG 95-106	Shivpuri-MP	IG 96-21	Kerala
IG 95-349	Jhansi-UP	IG 95-26	Lalitpur-UP	IG 96-133	Mudumulai-TN
IG 95-348	Jhansi-UP	IG 95-25	Lalitpur-UP	IG 96-97	Kerala
IG 95-347	Jhansi-UP	IG 95-21	Lalitpur-UP	IG 96-129	Mudumulai-TN
IG 95-346	Jhansi-UP	IG 95-20	Lalitpur-UP	IG 96-167	Bandipur-Karnataka
IG 95-345	Jhansi-UP	IG 95-17	Lalitpur-UP	IG 96-164	Bandipur-Karnataka
IG 95-344	Jhansi-UP	IG 95-15	Lalitpur-UP	IG 96-166	Bandipur-Karnataka
IG 95-343	Jhansi-UP	IG 95-13	Lalitpur-UP	IG 95-103	Shivpuri-MP
IG 95-341	Jhansi-UP	IG 95-22	Lalitpur-UP	IG 97-165	Lalitpur-UP
IG 95-293	Lalitpur-UP	IG 95-23	Lalitpur-UP	IG 95-104d	Shivpuri-MP
IG 95-292	Lalitpur-UP	IG 95-23	Lalitpur-UP	SS-1	Jhansi-UP
IG 95-290	Lalitpur-UP	IG 95-104a	Shivpuri-MP	SS-2	Jhansi-UP
IG 95-289	Lalitpur-UP	IG 95-104b	Shivpuri-MP		

systems-Glutamate oxalo-acetate transaminase (GOT or AAT, E.C. 6.1.1), Peroxidase (PRX, E.C. 1.11.1.7), Super oxidase desmutase (SOD, E.C. 1.15.1.1) and Esterase (EST. E.C. 3.1.1.2). The study of peroxidase, SOD and GOT was based on horizontal starch gel electrophoresis technique (Smithies 1955) with discontinuous buffer system of Poulik (1957), while esterase was analyzed using vertical slab polyacrylamide gel electrophoresis (PAGE). For starch gel electrophoresis, the crude extract of fresh, young, green and healthy leaves was prepared by homogenizing 1g sample in 1 ml of chilled tris buffer (pH 8.65) and stored in the freezer and thawed just before use. For PAGE 500mg leaf sample was homogenized in 1 ml of chilled grinding buffer (pH 6.75). The homogenates were centrifuged at 10000 rpm for 15 minutes. The supernatant was collected and used for electrophoresis. The gels were stained following standard procedure (Veech 1969) for PRX and for (Wendel and Weeden 1989) EST, GOT and SOD.

The unambiguous bands were scored and numbered on the basis of their relative mobility (rm). The binary data generated on the basis of presence or absence of bands was analyzed for genetic similarity among the genotypes based on Jacquards genetic distance. Dendrogram was formed by Sequential Agglomerative Hierachical and Nested (SAHN) clustering using the Unweighted Pair Group Method with arithmetic mean (UPGMA) algorithm. Analysis was carried out using NTSYS-pc software.

RESULTS AND DISCUSSION

The present study was conducted on 89 genotypes of *H. contortus.* The banding pattern observed in different enzymes after gel electrophoresis is as follows (Table 2, 3).

Banding pattern and frequency distribution

Glutamate oxalo-acetate transaminase (GOT)

Only one GOT band with rm value 0.65 was recorded and it was present in all the genotypes studied. The genotypes however differed for the intensity of bands, which indicates quantitative variation for this enzyme.

Peroxidase (PRX)

A total of 15 peroxidase bands were observed. The rm value ranged from 0.09 to 0.66. All the 15 bands were found to be polymorphic. The frequency of different bands varied among the genotypes. Band number 3 was found to

Table 2 RM values for electrophoretic bands of various enzymes in *H. contortus*

Enzyme	E.C. No.	Band numbers														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
PRX	1.11.1.7	0.09	0.16	0.19	0.23	0.26	0.3	0.33	0.37	0.4	0.44	0.47	0.53	0.56	0.61	0.66
EST	3.1.1.2	0.17	0.19	0.21	0.23	0.41	0.44	0.46	0.47	0.49						
SOD	1.15.1.1	0.54	0.63	0.7	0.78	0.84										
GOT	2.6.1.1	0.65														

be most frequent and was represented in 88 out of total 89 genotypes. Band number 2 and 14 were least frequent and were observed in only 22 and 24 genotypes respectively. The intensity of different bands also varied among the genotypes showing their differential activity. None of the genotypes showed presence of all the 15 bands.

Super oxidase desmutase (SOD)

SOD isozyme pattern showed the presence of 5 electrophoretic bands. The rm value for these bands varied from 0.54 to 0.84. Polymorphism was recorded for all 5 bands. Band 1 and 5 were most frequent and observed in 80 and 83 genotypes respectively. Bands 3 and 4 were found in only 24 and 27 genotypes respectively, whereas band 2 was represented in 62 genotypes. All 5 bands were found in 11 genotypes only, while 7 genotypes out of total 89 showed the presence of a single band.

Esterase (EST)

Esterase isozyme banding pattern revealed the presence of 2 migration zones with a total of 9 bands. The slowest zone comprised of 4 bands with rm value ranging from 0.17 to 0.23, whereas the fastest zone comprised of 5 bands with rm value ranging from 0.41 to 0.49. Polymorphism was observed for all the 9 bands. Band number 2, 6 and 7 were present in higher frequency being recorded in 53, 62 and 63 genotypes respectively, whereas bands 4 and 5 were present in only 7 and 8 genotypes respectively.

Similarity among genotypes

The similarity among genotypes was estimated using the Jacquard similarity matrix. Dendrogram was formed by SAHN agglomerative clustering using the UPGMA algorithm. The dendrogram based on 30 bands of four enzyme systems (GOT, PRX, SOD and EST) of 89 genotypes showed the presence of 11 main clusters which were further divided into sub-clusters (Table 4, Fig 1).

Cluster 1 comprised of 5 genotypes with intra-cluster similarity ranging from 77 to 100%. This cluster was further sub-divided into 2 sub-clusters. Sub-cluster 1-1 comprised of 3 genotypes with intra sub-cluster similarity of 81 to 88%. Sub-cluster 1-1 was 77% similar to sub-cluster 1-2, which comprised of 2 genotypes with 81% similarity amongst them. Cluster 2 comprised of 24 genotypes. The intra-cluster similarity ranged from 73 to 100%. It was further divided into 5 sub-clusters. Sub-cluster 2-1 comprised of 12 genotypes with 82 to 95% intra sub-cluster similarity. Sub-cluster 2-2 was represented by a single genotype, which appeared to be a bridge between sub-clusters 2-1 and 2-3. The genotypes representing sub-cluster 2-2 were 79% similar to both the sub-clusters 2-1 and 2-3. Sub-cluster 2-4 comprised of 4 genotypes with 79 to 84% intra sub-cluster similarity. Sub-cluster 2-5 represented by a single genotype was 74% similar to sub-cluster 2-4.

Cluster-3 was represented by 6 genotypes with intra-cluster similarity ranging from 77 to 100%. It was further

Table 3 Frequency of occurrence (%) of isozyme bands in different populations

Enzyme	Isozyme bands	Frequency of occurrence (%) South India	North India	Total
PRX				
	1	12.5	37.0	34.8
	2	50.0	22.2	24.7
	3	100.0	98.8	100.0
	4	50.0	61.7	60.7
	5	0.0	54.3	49.4
	6	87.5	79.0	79.8
	7	75.0	79.0	78.7
	8	37.5	66.7	64.0
	9	62.5	53.1	53.9
	10	75.0	84.0	83.1
	11	62.5	64.2	64.0
	12	75.0	65.4	66.3
	13	37.5	64.2	61.8
	14	0.0	29.6	27.0
	15	100.0	85.2	86.5
EST				
	1	12.5	34.6	32.6
	2	50.0	60.5	59.6
	3	0.0	43.2	39.3
	4	0.0	8.6	7.9
	5	50.0	4.9	9.0
	6	37.5	72.8	69.7
	7	50.0	72.8	70.8
	8	25.0	49.4	47.2
	9	12.5	14.8	14.6
SOD				
	1	100.0	90.1	91.0
	2	100.0	65.4	68.5
	3	0.0	29.6	27.0
	4	25.0	30.9	30.3
	5	87.5	93.8	93.3
GOT				
	1	100.0	100.0	100.0

Table 4 Grouping of genotypes into clusters based on isozyme banding pattern

Cluster no.	Sub-cluster no.	Accession number
1	1-1	IG 95-374, IG 97-182, IG 95-242
	1-2	IG 95-271, IG 96-190
2	2-1	IG 02-187, IG 02-188, IG 95-24, IG 95-19, IG 02-190,IG 95-17, IG 95-104c, IG 95-18, IG 95-279, IG 95-279,IG 95-105, IG 95-23
	2-2	IG 95-104b
	2-3	IG 95-371, IG 95-106, IG 95-21, IG 95-367, IG 95-26, IG 96-133
	2-4	IG 95-25, IG 95-20, IG 95-22, IG 96-21
	2-5	IG 95-366
3	3-1	IG 95-15, IG 95-13, IG 96-164, SS-1
	3-2	IG 96-129, IG 96-167
4	4-1	IG 02-183, IG 95-349, IG 95-345, IG 95-344, IG 95-350, IG 95-290, IG02-191, IG 95-293,
	4-2	IG 95-111, IG 95-363,IG 95-343, IG 95-348, IG 97-163, IG 97-165, IG 97-209
	4-3	IG 95-286, IG 95-280, IG 95-283
	4-4	IG 95-346, IG 95-258, IG 95-103
	4-5	IG 02-189, SS-2, IG 97-165
5	5-1	IG 02-184
	5-2	IG 02-185, IG 02-186, IG 95-292, IG 95-364, IG 95-352
6	6-1	IG 95-369, IG 95-368, IG 95-341
	6-2	IG 95-287, IG 95-110, IG 95-109, IG02-192, IG 95-284
	6-3	IG 95-347, IG 95-274, IG 95-23, IG 95-108
7	7-1	IG 95-277, IG 95-103, IG 95-104a
	7-2	IG 95-105
8		IG 95-289, IG 02-193
9		IG 96-97, IG 96-166, IG 95-104d
10		IG 95-273
11		IG 95-07, IG 97-183

sub-divided into 2 sub-clusters. Sub-cluster 3-1 comprised of 4 genotypes that show 79 to 88% similarity. Sub-cluster 3-2 represented by 2 genotypes with 86% intra sub-cluster similarity showed 74% similarity with sub-cluster 3-1. Cluster-4 was the largest group consisting of 26 genotypes. The genotypes show 70 to 100% similarity. It was further divided into 4 sub-clusters. Sub-cluster 4-1 comprised of 17 genotypes with 75 to 93% similarity among them. This sub-cluster was 75% similar to sub-cluster 4-2, which comprised of 3 genotypes with 82 to 85% intra sub-cluster similarity. Sub-cluster 4-3 consisted of 3 genotypes with 72% similarity. Sub-cluster 4-4 represented by 3 genotypes with 81 to 90% intra sub-cluster similarity showed 70% similarity with sub-cluster 4-3. Cluster-5 comprised of 6 genotypes with 74 to 96 % intra-cluster similarity. It was further subdivided into 2 sub-clusters. Sub-cluster 5-1 comprised of a single genotype. This sub-cluster was 74% similar to sub-cluster 5-2, which consisted of 5 genotypes with 77 to 96% intra sub-cluster similarity.

Cluster 6 comprised of 12 genotypes. The intra-cluster similarity ranged from 69 to 96%. This cluster was further subdivided into 3 sub-clusters. Sub-cluster 6-1 comprised of 3 genotypes with 81 to 88% intra sub-cluster similarity and showed 75% similarity with sub-cluster 6-2, which comprised of 5 genotypes with 81 to 96% similarity among them. Sub-cluster 6-3 comprised of 4 genotypes. The genotypes showed 70 to 83% similarity. Cluster 7 comprised of 4 genotypes with intra-cluster similarity ranging from 68 to 100%. The cluster was further subdivided into 2 sub-clusters. Sub-cluster 7-1 represented by 3 genotypes with 73% intra sub-cluster similarity showed 68% similarity with sub-cluster 7-2, which comprised only one genotype. Cluster 8 consisted of 2 genotypes with 80% similarity among them. Cluster 8 was 58% similar to cluster 9, which comprised of 3 genotypes with intra sub-cluster similarity ranging from 58 to 66%. Cluster 10 represented by a single genotype was 49% similar to cluster 11, which comprised of 2 genotypes with an intra sub-cluster similarity of 68%. On the basis of RAPD markers, in 56 individuals from 6 Hawaiian populations of *Heteropogon contortus* (pili grass), 55 unique genotypes were detected using 33 polymorphic markers (Cairno and Daehler 1999). The lack of uniformity among individuals may indicate frequent sexual reproduction in these populations. Cluster analysis revealed high degree of clustering for most populations, but populations from different islands did not cluster together.

Geographical distribution vis a vis genetic distance

Out of 89 genotypes evaluated in the present study, 81 belonged to semi-arid parts of southern UP and northern MP. It included typical semi-arid climate of Jhansi, Lalitpur and Chattarpur as well as comparatively more humid pats

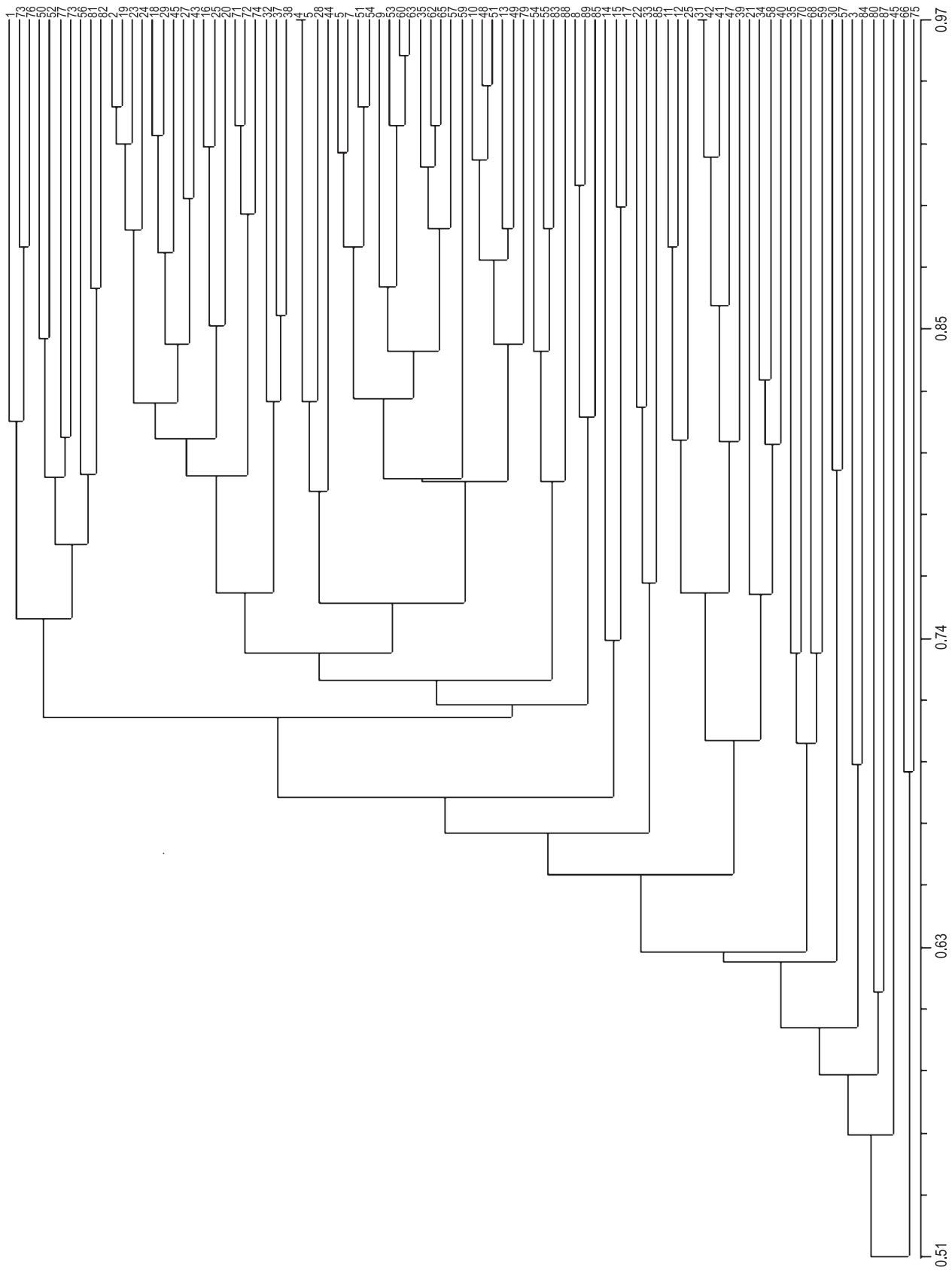

Fig 1 Dendrogram showing similarity among *H. contortus* genotypes

of Shivpuri. Eight genotypes belonged to sub-humid to humid parts of Kerala, Tamil Nadu and Karnataka. The clustering of genotypes was independent of their geographical origin. The eight genotypes from south India were highly divergent and represented in cluster 1 (1 genotype), cluster 2 (1 genotype), cluster 3 (3 genotypes) and cluster 9 (2 genotypes). *Heteropogon contortus* being largely apomictic in nature, the increased variability in sub-humid dry regions of south India might be due to the presence of major grasslands as well as favorable climate for natural crossing. Apomixis does not lead to the reduction in the diversity of grasses (Jain *et al*. 2006). A high level of isozyme banding patterns have been reported in guinea grass (Assienan and Noirot 1995), where authors emphasized evolution of new types through sexual recombination or other means namely hybridizing of indigenous materials with sexual/apomictic exotic lines. Guinea grass (*Panicum maximum* Jacq.) consists largely of apomictic population, although many sexual plants are also available in nature (Assienan *et al*. 1993). *Heteropogon contortus* growing wild in natural habitats exhibits large phenotypic variation. Even diverse forms have been reported from material collected at the same locations and the clustering patterns based on morphological attributes in *Heteropogon contortus* indicated independent groupings with their geographical distribution (Bhat and Roy 2007).

The study resulted in grouping of genotypes based on their genetic distance and can be exploited in the genetic improvement programme. The closely related genotypes are expected to cluster together and representative genotypes from a cluster can be used for evaluation under specific ecological situations and stress conditions and for making any hybridization to transfer the traits from some identified sexual genotypes.

REFERENCES

Aradhya M K, Kee L K, Zee F T and Manshardt R M. 1998. Genetic variability in Macadamia. *Genetic Resources and Crop Evoution* **45:** 18–32.

Assienan B and Noirot M. 1995. Isozyme polymorphism and organization of the agamic complex of the maximae (*Panicum maximum* Jacq., *P. infestum* Anders. and *P. trichocladium* K. Schum.) in Tanzania. *Theoretical and Applied Genetics* **91:** 672–80.

Assienan B M, Noirot and Gnagne Y. 1993. Inheritance and genetic diversity of some enzymes in the sexual and diploid pool of the agamic complex of maximae (*Panicum maximum* Jacq., *P. infestum* Anders. and *P. trichocladium* K. Schum.). *Euphytica* **68:** 231–9.

Bhat B A and Roy A K. 2007. Genetic diversity in *Heteropogon contortus*. *Range Mangement and Agroforestry* **28** (2): 394–7.

Buso G S C, Rangel P H and Fereira M E. 1998. Analysis of genetic variability of south American wild rice populations. (*Oryza glumaepatula*) with isozymes and RAPD markers. *Molecular Ecology* **7:** 107–17.

Cairno D A and Daehler C C. 1999. Genetic variation in an apomictic grass, *Heteropogon contortus*, in the Hawaiian Islands. *Molecular Ecology* **8:** 2 127–32.

Goergen E and Daehler C C. 2001. Reproductive ecology of a native Hawaian grass (*Heteropogon contortus*; Poaceae) versus its invasive alien competitor (*Pennisetum setaceum*; Poaceae). *International Journal of Plant Sciences* **162:** 317–26.

Jain A, Roy A K, Kaushal P, Malaviya D R and Zadoo S N. 2006. Isozyme banding pattern and estimation of genetic diversity among guinea grass gemplasm. *Genetic Resources and Crop Evoution* **49:** 339–47.

Klaas M. 1998. Application and impact of molecular markers on evolutionary and diversity studies in *Allium. Plant Breeding* **117:** 297–308.

Lange O and Schiffino-Wittmann M T. 2000. Isozyme variation in wild and cultivated species of the genus *Trifolium* L. (Leguminaceae). *Annual Botany* **86:** 339–45.

Poulik M D. 1957. Starch gel electrophoresis in a discontinuous system of buffers. *Nature* **180:** 1 477–9.

Smithies O. 1955. Zone electrophoresis in starch gel: group variation in the serum proteins of normal human adults. *Biochemistry Journal* **61:** 629–41.

Veech J A. 1969. Localization of peroxidase in infected tobaccos susceptible and resistant to black shank. *Phytopathology* **59:** 556–71.

Wendel J F and Weeden N F. 1989. Visualization and interpretation of plant isozymes. (*In*) *Plant Biology*. Soltis D S and Soltis P S (Eds). Chapman and Hall, London.