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Studies on genetic diversity in napier germplasm through D^2 and isozyme analysis

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Abstract : Fifty three genotypes of napier grass *(Pennisetum purpureum.* (K) Schum) germplasm assembled from different countries were evaluated for their genetic diversity through Mahalanobis D^2 analysis and isozyme pattern studies for esterase and polyphenol oxidase. The D^2 analysis grouped the genotypes in six clusters which did not indicate any relationship between genetic divergence and geographic distribution. Cluster 11 had a maximum number of 35 genotypes and four clusters had only one genotype each. The inter cluster distance was the maximum between cluster V and VI. The isozyme analysis grouped the genotypes into two major groups A and B and group A consisted of two sub groups Al and A2. It was observed that the isozyme approach is a more reliable method for studying the genetic diversity between different genotypes. *(**Key words** : Napier grass, Genetic diversity, Isozyme analysis).*

The progress of improvement of forage crops in India is slow compared to other food and cash crops. In India there is a deficit of more than 50 per cent of green fodder and 25 per cent of dry fodder. Breeding for highly nutritive and productive varieties of forage crops will reduce the existing gap between demand and supply of fodder. Napier grass *(Pennisetum purpureum* K. Schum) is a perennial and autotetraploid native grass of Africa. In India 19 species of *Pennisetum* have been reported (Bor, 1960). Many triploid hybrids between pearl millet and Napier grass have been developed and are in cultivation. A large germplasm of Napier grass has been assembled at the Department of Forage Crops, Tamil Nadu Agricultural University and it was felt that the genetic diversity of this species has to be studied in detail so as to identify the best genotypes for further exploitation in crossing programmes.

Table 1. Composition of D^2 clusters in Napier grass

Cluster Number	Total Number of genotypes	Genotype Number	Origin
I	14	FD: 441, 449, 45 1, 453,455, 456, 458, 459, 470, 472, 473, 4749, 476 and 483	Kenya, Australia, Puerto Rico
II.	35	FD: 430, 433, 434, 435, 436, 437, 438, 439, 440, 442, 444, 445, 446, 447, 448, 449, 450, 451, 452, 460, 461, 462, 463, 464, 465, 466, 467, 468, 471, 477, 478, 479, 480, 481, 482, and 485	Puerto Rico, Kenya, Australia, USA
III	1	FD: 443	Puerto Rico
IV	1	FD: 457	Kenya
V	1	FD: 432	Puerto Rico
VI	1	FD: 431	Puerto Rico

Table 2. Inter and intra (diagonal) cluster average of D^2 and D values (within parenthesis) among the clusters in Napier grass

Clusters	I	II	III	IV	V	VI
I	7.897 (2.81)	11.634 (3.41)	8.949 (2.99)	9.691 (3.11)	14.853 (3.85)	10.660 (3.26)
II		7.448 (2.72)	9.007 (3.00)	8.948 (2.99)	8.942 (2.99)	15.121 (3.88)
III			0.000 (0.00)	5.546 (2.35)	11.397 (3.37)	14.111 (3.75)
IV				0.000 (0.00)	10.276 (3.20)	13.962 (3.73)
V					0.000 (0.00)	17.238 (4.15)
VI						0.000 (0.00)

Table 3. Cluster means for nine characters in Napier grass genotypes.

Characters / Clusters	Plant height (cm)	Leaf Length (cm)	Leaf Width (cm)	Stem Thickness (cm)	No. of tillers per plant	Panicle Length (cm)	Green fodder yield (g)	Crude Protein (%)	Crude Fibre
I	293.69	46.16	1.65	1.40	1.48	6.53	78.11	9.56	25.26
II	**313.15**	57.29	1.64	1.53	23.57	24.39	82.07	9.85	**27.71**
III	246.80	57.33	1.97	0.78	9.67	19.00	78.70	9.93	23.23
IV	309.43	53.33	2.33	1.43	9.33	18.23	103.73	**13.03**	26.20
V	269.33	68.00	2.37	2.17	**32.00**	**30.67**	**104.23**	8.83	25.07
VI	265.67	**94.00**	**3.27**	**3.00**	17.00	Q.00	60.33	10.30	24.37
General Mean	282.88	62.68	2.14	1.72	15.51	16.47	84.53	10.25	25.31

Materials and Methods

All the 53 genotypes of Napier grass germplasm assembled from different countries of the world were evaluated during 1999 - 2000. The genotypes were planted using rooted slips on the sides of ridges 3m long adopting a spacing of 50 cm x 50 cm in a randomized block design with two replications. Each genotype was planted in two ridges with six plants in each row. Uniform cultural operations were undertaken. Observations on nine traits were recorded on five plants selected at random per genotype per replication.

The data were subjected to Mahalanobis D^2 analysis and the group constellations were determined using Tocher's method (Rao, 1952). The intra and inter cluster distances were also calculated.

The isozyme analysis for esterase and polyphenol oxidase was carried out with five day old leaf samples adopting standard procedures of gel electrophoresis as given by Vallejos (1993).

Results and Discussion

In the present study, as many as nine characters related to the developmental as well as economic importance besides quality characters were considered and Wilks criterion was used for simultaneous test of significance of the

Table 4. The markers obtained for the two isozyme systems in the different genotypes

S.No.	Genotype	Esterase	Polyphenol oxidase
1	FD. 430	4,5,6,7,8,9,10.	1,6,7,8,10,13
2.	FD. 431	4,5,6,7,8,9.	1,2,3,4,6,7,8,9,11,13.
3.	FD. 432	1,2,3,4,5,6,8,9	1;2,6,7.
4.	FD. 433	1,3,6,8,9,10.	1,2,3,4,5,6.
5.	FD. 434	3,4,6,8,9,10,11,12.	1,2,3,4,5,6,7,8,10.
6.	FD. 435	10,111.	1,5,6,7,8,10,11.
7.	FD. 436	5,7,8,10,11,12.	3,4,6,7,8,9,10,12,13.
8.	FD. 437	2,3,5,7,8,9.	1,2,3,4,6,7,8,9,10,12.
9.	FD. 438	10,11,12.	7,8.
10.	FD. 439	11,12.	3,7,8,10.
11.	FD. 440	2,3,4,6,8,9,10,11.	1,5,6,7,8.
12.	FD. 441	2,3,4,6,8,9,10,11.	1,5,6,7,8.
13	FD. 442	10,11.12	1,5,7,8
14.	FD. 443	1,2,3,4,5,6,7,8,9, 1 0.	2,3,5,7
15.	FD. 444	10,11,12.	1,7,8.
16.	FD. 445	12.	3,7.
17.	FD. 446	3,4,5,6,7,8,9,10.	7,9,10.
18.	FD. 447	Il.	5,7,8,9,10,11.
19.	FD. 448	2,4,5,7,10.	1,6,7,10,11.
20.	FD. 449	1,2,3,4,5,6,7,8,9, 1 0.	3,7,10,11.
21.	FD. 450	2,3,6,8,9,10,11,12.	1,2,6,7,89,10,11,12.
22.	FD. 451	3,4,5,6,7,8,9,11,12.	1,8.
23.	FD. 452	10,11,12.	1,7,9,10,11,12.
24.	FD. 453	2,4,6,7,8,9,10,11,12.	1,8.
25.	FD. 454	7,9,10,11,12.	1,2,3,7,8,9,10,11,12.
26.	FD. 455	1,2,3,4,5,7,8,9,10.	1,2,7,8,9,10,12.
27.	FD. 456	11.	1,2,7,12.
28	FD 457	11	1,2,3,4,6,7,11,12
29.	FD 458	2,3,4,6,9,10,11	1,2,5,6,7,10
30.	FD 459	4,5,6,7,8,9,11,12	3,4,6,6,8,9,11,12
31.	FD 460	3,4,10,11912	1,3,4,6,7,8, 11,12
32.	FD. 461	4,10,11,12	2,3,4,5,6,7,8
33.	FD. 462	7,8,9,10,11	5,6,7
34.	FD. 463	6,7	3,4,5.6,7
35.	FD. 464	10,14	4,5,6,7
36.	FD. 465	5,10,11	2,3,495
37.	FD. 466	6,7,9,10,11	1,2,4,5,6,7
38.	FD. 467	6	2,4
39.	FD. 468	3,4,5,6,7,8,9,10,11,12,13	4,5
40	FD. 470	5,8,9.io	4,5,6,7
41.	FD. 471	2,3,4,5,7,9	1,2,3,4,6
42.	FD. 472	8,10,11	1,2,3,5,7,8,9,10,11
43.	FD. 473	3,4,5,7,8,9,10,1	1, 3,5,7,9,1 . I 1
44.	FD. 474	2,3,4,5,6,7,8,9,10,11,12,13	1,4,5,6,7,9,1
45.	FD. 476	7,10,11	1,495
46.	FD. 477	1,2,3,4,5,7,8	1,5,7,8,9
47.	FD. 478	2,3,4,596,10,	1,5,7
48	FD. 479	4,6,9	1
49.	FD. 483	1,2,3,5	1
50.	FD. 481	294,596579	2,8
51.	FD. 482	5,7	1,8
52.	FD. 483	4,5,7,12	1,9, 1 0
53.	FD. 485	4,5,10,11	1

differences in the mean value of the characters. The pooled value was found to be significant indicating the wide spectrum of variability among the genotypes tested. Sokal and Daly (1961) have stressed the importance of the proper choice of the characters for studies on genetic divergence, as such a choice of characters will reflect the usefulness of D^2 analysis. The square of the distance between the genotypes, calculated as the sum of squares of the difference between mean values of all the nine transformed variables, was used for final grouping of the genotypes. By the application of clustering technique, the 53 genotypes were grouped into six clusters (Table 1). The first cluster included 14 genotypes from different geographical regions *viz.*, Kenya, Australia and Puerto Rico. The second cluster, the biggest included 35 genotypes and the third, fourth and sixth clusters included only one entry each.

The clustering pattern showed that the genotypes originating from different centres were not necessarily grouped in the different clusters. Geographical diversity though important, may not necessarily be the only factor in determining genetic divergence as observed earlier by Murty and Tiwari (1967) and Suthamathi and Dorairaj (1994). The clustering pattern revealed that the tendency of genotypes from diverse geographic regions to group together in one cluster might be due to similarity of the nature of selection pressure operating under the respective domestic conditions as reported by Arunachalam and Jawahar Ram (1967), or might be due to identical genetic architecture of the genotypes. Further, certain genotypes fell separately into single genotype clusters *viz.*, cluster III, IV, V and VI. These genotypes originated either from Puerto Rico, Australia or Kenya. It was also observed that the genotypes originating from Puerto Rico were found scattered in different clusters. The existence of wide genetic diversity among the genotypes chosen from the same geographic region was thus obvious.

The intra and inter cluster D^2 and D values among the six clusters are furnished in Table 2. The intra cluster generalized distance was the highest in cluster I and zero in cluster III, IV, V and VI since they included only one entry each. Inter cluster distance was the highest between cluster V and cluster VI followed by that between cluster II and cluster VI. Considering the inter cluster distances it was observed that cluster I was highly divergent from cluster V and VI. Cluster II was highly divergent from clusters VI and III. Cluster III was highly divergent form cluster VI and V. Cluster IV was highly divergent from clusters VI and V.

The cluster mean values worked out for the different characters are presented in Table. 3. Cluster V exhibited the maximum mean values for green fodder yield, number of tillers per plant and panicle length. Cluster VI exhibited maximum mean value for leaf length, leaf width and stem thickness. Cluster IV exhibited maximum mean values for crude protein. Cluster II exhibited the maximum mean values for plant height and crude fibre.

The occurrence of multiple isozymes has been used as a tool to estimate genetic diversity and trueness to type (Smith and Smith, 1992). The biochemical characterization of the 53 genotypes for esterase and polyphenol oxidase isozyme markers revealed the following (Table.4). A total of 13 alleles were observed for esterase among the 53 genotypes. Among them the Est. 13 was present only in the genotypes FD 468 and FD 474. The genotypes FD 447, FD 456 and FD 457 showed monomorphic band for Est. 11 and genotypes FD 467 showed monomorphic band for Est. 6. For polyphenol oxidase also 13 alleles were observed. The allele Ppo. 13 was observed in FD. 430, FD 431 and FD.436. The genotypes FD 479, FD 480 and FD 485 showed monomorphic bands for Ppo. 1.

The clustering pattern based on isozyme markers was done by SHAN clustering programme using UPGMA method and the dendrogram was drawn. At 0.51 similarity coefficient two major clusters, A and B were formed, cluster A had two sub clusters A1 and A2 and no sub cluster was observed in B.

The utility of isozyme as genetic markers are generally attributed to their polymorphism, co-dominance, simple inheritance, rapid and simple assay and ubiquity in plant tissues and organs (Simpson and Withers, 1986). The isozyme markers have been extensively used for classifying the taxonomic and phylogenetic relationships in plants (Mowrey and Wemer, 1990, Lu and Pickergill, 1995). By comparison of genetic relationships among different genotypes of Napier grass using the D^2 and isozyme approaches, it was observed that the latter may be a more reliable method for studying the genetic diversity.

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Impact of green manuring on the availability of sulphur and zinc in the rice soils

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Abstract : A greenhouse experiment was conducted at Tamil Nadu Agricultural University, Coimbatore on two soils, deficient in S and Zn *viz.* Typic Ustochrept and Typic Haplustalf using rice as test crop to study the effect of green manure on soil Zn and S availability. Two sources of Zn ($ZnSO_4$ and EDTA-Zn @ 5 kg Zn ha^{-1}) and S (gypsum @ 50 kg S ha^{-1}) along with green manure *viz. Sesbania aculeata* @ 10 t ha^{-1} were applied. The GM application in sandy loam soil manifested higher availability of DTPA-Zn, more particularly with EDTA-Zn than in clay loam soil. Incorporation of GM with EDTA-Zn + NPK enhanced the availability of Zn (2.91, 3.60 and 2.80 mg kg^{-1}) at active tillering (AT), panicle initiation (PI) and harvest stages, respectively followed by GM aplication along with NPK + $ZnSO_4$ + gypsum. The highest sulphur availability was obtained with NPK + GM + $ZnSO_4$ + gypsum at AT (21.38 mg kg^{-1}) and PI (20.13 mg kg^{-1}) and with the treatment, NPK + GM + gypsum at harvest (26.38 mg kg^{-1}) stages. *(Key words : Sesbania green manuring, Zinc and Sulphur fertilizers. Zn and S availability, Rice soils).*

The deficiency of secondary and micronutrients have become common particularly in rice soils and causes reduction in crop yield. Occurence of zinc and sulphur deficiency is known to be associated with many factors like their status in soil, their interaction with other plant nutrients, both synergistically and antagonistically in rice soil system and adverse conditions of soil and