*The Andhra Agric. J* 63 (1): 58-62, 2016

# Morphological and Molecular Diversity in Relation to Hybrid Performance in Fodder Pearl Millet

**G Naga Raju and B L Bhardwaj**
National Institute of Plant Genome Research, J.N.U. Campus, NewDelhi-110067, India.

**ABSTRACT**

The investigation with five male-sterile lines, eight pollinator lines, 40 $F_1$ crosses, which were generated through Line x Tester matings was conducted to assess the association of genetic diversity of parental lines with mean performance of hybrids, mid parent heterosis, better parent heterosis and sca effects. All the parental lines were screened to detect polymorphism in the form of RAPD markers. Genetic diversity among the parental lines was determined by RAPD markers and morphological characters. The genetic distances so obtained were correlated with $F_1$ mean performance and heterosis. Positive correlation was obtained between molecular marker diversity and $F_1$ mean performance, heterosis over better parent but the value of correlation coefficient was found to be non-significant. In contrary, negative correlation was obtained between taxonomic distance and $F_1$- mean performance; better parent heterosis. This study clearly indicated that genetic-distance measures based on RAPDs may be useful for the grouping of parents, but not for predicting heterotic combinations in pearl millet.

**Key words :** Fodder pearl millet, Genetic diversity, Hybrids, RAPD.

Pearl millet, *Pennisetum glaucum* (L.) R. Brown[ =*Pennisetum typhoides* (Bum.) Stapf et Hubb., *Pennisetum americanum* (L.)Schumann ex Leeke] *(2n* = 14) is a valuable grain and forage crop. It is widely cultivated in different parts of the worldin an area 27 million hectares, primarily in Asia and Africa. In terms of annual production, pearl millet is the sixth most important cereal crop in the world, following wheat, rice, maize, barley and sorghum. Among the millets, it is second only to sorghum. Pearl millet is the only cereal that reliably provides both grain and fodder on poor, sandy soils under hot and dry conditions. It is remarkable that it produces nourishment from the poorest soils in the driest regions in the hottest climates. In the drier regions of Africa and Asia, the crop is a staple food grain. In more favored areas, however, pearl millet grain is fed to bullocks, milch animals, and poultry.

Heterosis is significant in pearl millet for both grain and forage production. Use of hybrids is increasing each year in all of the pearl millet growing areas except Africa and Pakistan. Most cultivars grown outside the major pearl millet growing areas of Africa, India, and Pakistan are hybrids and are used for forage. However, there is an increased emphasis on production of grain hybrids in the United States. Researchers estimate that 40% of the cultivars in India are hybrids, but the areas planted to hybrids range from about 95% in Gujarat to about 10% in Rajasthan(Andrews, 1987; Dave, 1987).

Development of $F_1$ hybrid involves crossing of newly developed inbred lines to generate several cross combinations ($F_1$s) and subsequent evaluation of these $F_1$ plants in order to choose the best combination.But no sound genetic theory is available which can help us to predict the performance of two parents and the development of hybrids thus remaining to be a number game where large number of crosses are produced and tested to see if anyone is worth cultivation.

In recent times, recognition of lines with superior cross performance is still the most expensive and time consuming process in hybrid development programme. In general, parental lines with superior cross performance are identified by genetic divergence analysis and performance of crosses between diverse lines is assessed under extensive field trials. With the advent of molecular markers of different types, the molecular diversity between parental lines has been viewed as a potential tool for predicting hybrid performance.

The advent of Random Amplified Polymorphic DNA (RAPD) technique by Welsh and McCleIf and (1990) and Williams *et al* (1990) had made it possible to detect polymorphism at many loci. The present study was carried to estimated genetic diversity of parental lines using morphological and molecular characters and association of genetic diversity with hybrid performance in order to predict the performance of crosses.

## MATERIAL AND METHODS

The experimental material consisted of five male sterile lines viz, Pb 220A, Pb 311A, Pb 408A, Pb 502 and Pb 601 belonging to different cytoplasmic male sterile sources and 8 different restorer lines viz, PIB 250, PIB 253, PIB 258, PIB 262, PIB 280, PIB 314, PIB 366 and PIB 481. All the thirteen parental lines were assayed for DNA polymorphism in the form of RAPDs, and the whole analysis involved the following steps.Total DNA was isolated from the leaves by CTAB (Cetyl Trimethyl Ammonium Bromide) method. DNA concentration and its quality were measured by fluorescence after staining with Ethidium bromide. Amplification reactions of 25ml contained 10 mM Tris-HCL (pH 8.0), 50 mM KCl, 2.0mm $MgCl_2$, 0.1mM of each doxynucloside triphosphate, 0.4 mM of one oligonucleotide decamer primer (operon technologies, California, U.S.A.), Ca, 25mg of genomic DNA, and one unit of Taq DNA polymerase A total number of 13 different operon primers were used in separate amplification reactions.

Only the most intense and reproducible DNA bands were considered for analysis. These were scored as 1 (for presence) and O (for absence).

### Computation of genetic distance

Genetic distance among the parental lines was estimated using both morphological and molecular marker data. Average taxonomic distance between all pair of parents was estimated from the morphological characters as well as molecular data using the computer programme Numerical Taxonomy System.

Similarly, using the molecular marker data, genetic distance between all pairs of parental lines was measured based on Dice coefficient (Nei and Li, 1979) using the same computer programme NTSYS. With distance measures, cluster analysis was performed using Unweighted Pair-Group Method Arithmetic Average (UPGMA) programme. Karl Pearson coefficient of correlation (r) was measured between genetic distances and $F_1$ performance. The significance of correlation coefficient (r) was tested with the help of 't'test.

## RERESULTS AND DISCUSSI

### Genetic diversity of parental lines

Two measures of genetic divergence were employed to estimate the genetic distance between pairs of parental lines.

### Nei and Li distance based on RAPD marker data

All the parental lines were surveyed for DNA polymorphism in the form of RAPD markers. Fifteen 10-mer primers were pre-screened for their ability to detect polymorphism in four randomly chosen parental lines. Thirteen primers were selected based on the amplification products. All the thirteen parental lines were then surveyed with the selected thirteen primers. The total number of fragments amplified are shown in the Table-1. The primer OPO-16 generated a maximum of 13 amplification products and all were polymorphic. A total of 129 fragments were amplified, out of which 122 were found to be polymorphic. On an average 9.38 polymorphic fragments were obtained with each of the thirteen primers. Amplification shown by one of the primers (OPE-16) was shown in the Fig-1.

Molecular marker information genetic distance matrix was prepared using the computer programme NYSYS. Maximum Nei and Li distance recorded was 1.31 between the parental lines Pb408A and PIB250 and the minimum distance was 0.47 between the parental lines Pb311A and Pib262.

### Taxonomic distance based on morphological data

The data for all the 9 morphological characters under study was compiled and analysed for all the parental lines. A standardized matrix was prepared using standardization coefficient, then the genetic distance was worked out in the form of average taxonomic distance using the computer programme NTSYS. Based on the taxonomic distances genetic distance matrix was constructed. The maximum taxonomic distance for any pair of

Table 1. Molecular marker data for the parental lines.

| Sr.No. | Random Primer | Sequence | Total fragments amplified |
|---|---|---|---|
| 1 | OPZ-15 | 5[1]CAGGGCTTTC3[1] | 9 |
| 2 | OPP-10 | 5[1]TCCCGCCTAC3[1] | 12 |
| 3 | OPD-9 | 5[1] CTCTGGAGAC3[1] | 9 |
| 4 | OPE-16 | 5[1]AAGACCCCTC3[1] | 10 |
| 5 | OPJ-10 | 5[1]CAAGGCCTTC3[1] | 7 |
| 6 | OPK-13 | 5[1]GGTTGTACCC3[1] | 12 |
| 7 | OPG-2 | 5[1]GGCACTGAGG3[1] | 11 |
| 8 | OPE-17 | 5[1]CTACTGCCGT3[1] | 7 |
| 9 | OPD-12 | 5[1]CACCGTATCC3[1] | 11 |
| 10 | OPO-16 | 5[1]TCGGCGGTTC3[1] | 13 |
| 11 | OPC-16 | 5[1]CACACTCCAG3[1] | 11 |
| 12 | OPG-13 | 5[1]CTCTCCGCCA3[1] | 8 |
| 13 | OPP-18 | 5[1]GGCTTGGCCT3[1] | 9 |
| Total | 13 | | 129 |

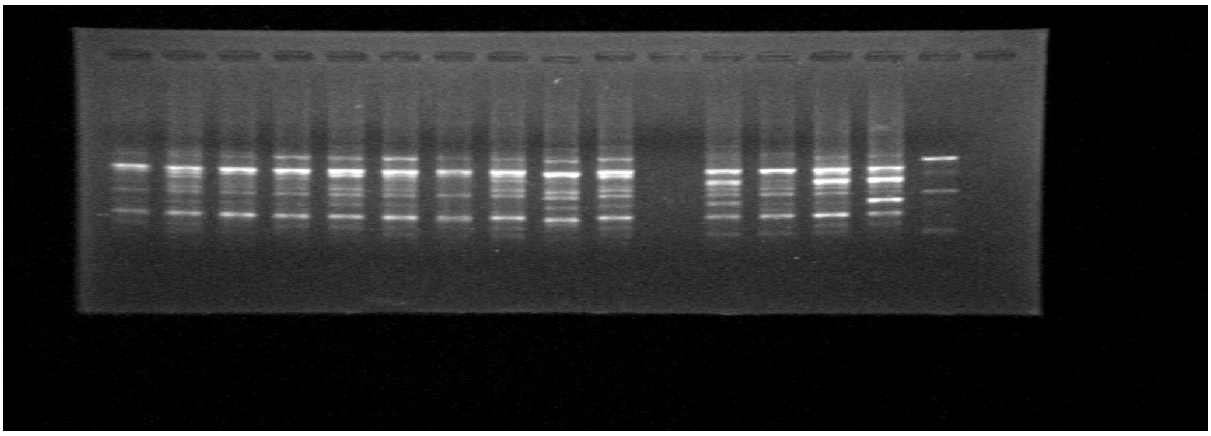

Fig-1 RAPD profile on parents with one of the decamer primer

parental lines was 0.27 for the parents Pb220A and PIB481. The lowest taxonomic distance was recorded between the parental lines Pb502A and PIB262 (0.04).

**Correlation of genetic distances with hybrid performance and heterosis**

Genetic distances based on taxonomic and Nei and Li correlated with hybrid performance, heterosis over mid parent, heterosis over better parent and sca of the cross (Table-2).

Most of the correlation coefficients were found to be non-significant when Nei and Li distance was correlated with mean performance, heterosis over mid and better parent and sca for green fodder yield without any group division. The distances were positively correlated with mean performance of hybrid, heterosis over better parent and sca effects but the correlation coefficient values were non-significant and not useful in to predicting the performance of hybrid based on genetic distances.

Even with group division also, all the correlation coefficient values were found to be non-significant, when the crosses among the clusters were compared. Where as significant negative correlation was obtained between genetic distance and $F_1$ mean performance, when the crosses within the clusters are compared

Table 2. Correlation of various parameters of hybrid performance for green fodder yield with genetic distance of parental lines.

| Cross | Nei & Li distance | Taxo-nomic distance | Mean performance of hybrid | Heterosis over mid parent | Heterosis over better parent | SCA |
|---|---|---|---|---|---|---|
| Pb 220A x PIB 250 | 0.94 | 0.16 | 924.6 | 247.50 | 36.3 | 207.70 |
| Pb 220A x PIB 253 | 0.79 | 0.19 | 623.3 | -46.17 | -265.0 | -93.70 |
| Pb 220A x PIB 258 | 0.75 | 0.10 | 633.0 | -144.50 | -255.3 | -12.60 |
| Pb 220A x PIB 262 | 0.68 | 0.20 | 548.3 | -132.17 | -340.0 | -185.30 |
| Pb 220A x PIB 280 | 0.81 | 0.22 | 703.3 | 46.83 | -185.0 | 267.50 |
| Pb 220A x PIB 314 | 0.89 | 0.23 | 556.3 | -79.33 | -332.0 | -3.70 |
| Pb 220A x PIB 366 | 0.65 | 0.18 | 420.6 | -275.50 | -467.6 | -198.70 |
| Pb 220A x PIB 481 | 0.54 | 0.27 | 580.0 | -28.67 | -308.3 | 18.90 |
| Pb 311A x PIB 250 | 0.67 | 0.09 | 881.0 | 425.50 | 415.0 | -19.70 |
| Pb 311A x PIB 253 | 0.52 | 0.10 | 931.6 | 483.80 | 1481.0 | 30.70 |
| Pb 311A x PIB 258 | 0.53 | 0.12 | 626.6 | 70.83 | -40.0 | -202.80 |
| Pb 311A x PIB 262 | 0.47 | 0.09 | 103.2 | 573.10 | 559.3 | 114.40 |
| Pb 311A x PIB 280 | 1.1 | 0.10 | 534.0 | 99.17 | 89.0 | -85.60 |
| Pb 311A x PIB 314 | 1.17 | 0.13 | 731.3 | 317.33 | 286.3 | -12.50 |
| Pb 311A x PIB 366 | 0.77 | 0.10 | 867.0 | 392.50 | 363.0 | 63.70 |
| Pb 311A x PIB 481 | 0.59 | 0.15 | 856.6 | 469.60 | 44.6 | 111.70 |
| Pb 408A x PIB 250 | 1.31 | 0.12 | 675.0 | 86.50 | -36.0 | -105.40 |
| Pb 408A x PIB 253 | 1.21 | 0.14 | 755.0 | 174.10 | 44.0 | -25.60 |
| Pb 408A x PIB 258 | 1.15 | 0.06 | 101.7 | 328.10 | 306.0 | 307.80 |
| Pb 408A x PIB 262 | 1.06 | 0.15 | 805.0 | 213.50 | 94.33 | 8.00 |
| Pb 408A x PIB 280 | 0.59 | 0.17 | 400.6 | -167.10 | -310.3 | 98.60 |
| Pb 408A x PIB 314 | 0.58 | 0.18 | 584.0 | 37.00 | -127.0 | -39.50 |
| Pb 408A x PIB 366 | 0.71 | 0.13 | 631.0 | 23.50 | -80.0 | -51.90 |
| Pb 408A x PIB 481 | 0.77 | 0.22 | 630.0 | 110.00 | -81.0 | 5.40 |
| Pb 502A x PIB 250 | 1.01 | 0.11 | 668.6 | 234.50 | 202.6 | -115.70 |
| Pb 502A x PIB 253 | 0.9 | 0.07 | 953.6 | 527.17 | 503.0 | 169.00 |
| Pb 502A x PIB 258 | 0.78 | 0.14 | 602.0 | 67.50 | -64.6 | -111.10 |
| Pb 502A x PIB 262 | 0.76 | 0.04 | 982.0 | 544.50 | 509.3 | 180.70 |
| Pb 502A x PIB 280 | 0.76 | 0.06 | 432.6 | 19.17 | 8.0 | -70.60 |
| Pb 502A x PIB 314 | 0.8 | 0.12 | 691.0 | 298.3 | 288.6 | 63.40 |
| Pb 502A x PIB 366 | 0.58 | 0.11 | 635.3 | 182.1 | 131.3 | -51.50 |
| Pb 502A x PIB 481 | 0.59 | 0.11 | 564.3 | 198.6 | 162.0 | -64.20 |
| Pb 601A x PIB 250 | 1.22 | 0.19 | 656.0 | 287.6 | 190.0 | 33.20 |
| Pb 601A x PIB 253 | 1.14 | 0.13 | 654.6 | 182.0 | 92.0 | -80.30 |
| Pb 601A x PIB 258 | 1.06 | 0.23 | 570.3 | 101.6 | -96.3 | 18.80 |
| Pb 601A x PIB 262 | 0.99 | 0.12 | 521.6 | 150.0 | 49.0 | -117.90 |
| Pb 601A x PIB 280 | 0.62 | 0.11 | 329.0 | -18.6 | -95.6 | -12.60 |
| Pb 601A x PIB 314 | 0.67 | 0.17 | 458.3 | 131.5 | 75.3 | -7.50 |
| Pb 601A x PIB 366 | 0.67 | 0.18 | 763.6 | 376.3 | 259.6 | 238.40 |
| Pb 601A x PIB 481 | 0.72 | 0.12 | 395.0 | 95.1 | 66.0 | -71.90 |

In case of correlation of taxonomic distance with mean performance of hybrid negative correlation coefficient was obtained. The correlation was negative with heterosis over mid parent and heterosis over better parent when group division was not done. But a positive correlation was obtained between taxonomic distance and sca effects when crosses within the cluster were considered.

These results are in agreement with the results obtained by Chen Shu *et al* (1996) in maize and Chowdari *et al* (1998) in Pearl Millet. The poor association between molecular genetic distance and hybrid performance could be because of The employed RAPD microsatellites markers detected polymorphism in the genomic regions, which are functionally not important (non-coding regions). So for accurate prediction of hybrid performance, very specific markers, which are very closely associated with the quantitative trait loci controlling the yield level of a genotype need to be generated.

## LITERATURE CITED

**Andrews D J 1987** Breeding pearl millet grain hybrids. *In* "Hybrid Seed Production of SelectedCereal Oil and Vegetable Crops" (W. A. Feistzer and A. F. Kelly, eds.), FA0 PlantProduction and Protection, Paper 82, Rome. pp. 83-109.

**Chen Shu, Hwu Kae Kang, Chen Cheng, Chen S, Hwu K K and Chen C 1996** Prediction of heterosis with genetic diversity among maize (*Zea mays L.*) inbred lines revealed by random amplified polymorphic DNA. *Journal of Agricultural Research China,* 45: 147-55.

Chowdari K V, Venkatachalam S R, Davierwala A P, Gupta V S, Ranjekar P K and Govila O P 1998 Hybrid performance and genetic distance as revealed by the $(GATA)_4$ microsatellite and RAPD markers in pearl millet. *Theor Appl Genet,* 97: 162-69.

**Dave H R 1987** Pearl millet hybrids. *Proc. Intl. Pearl Millet Workshop,* pp. 121-126.

**Nei M and Li W H 1979** Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proc Natl Academy Science USA* 76: 5269-73.

**Welsh J and McClefand 1990** Finger printing genomes using PCR with arbitrary primers. *Nucleic Acids Research* 18: 7213.

**Williams G K J, Kubelik A R, Livak K J, Rafalsk J A and Tingey S V 1990** DNA polymorphisms amplified by arbitrary primers are useful as genetic markers *Nucleic Acids Research,* 18: 6531-6535.

(Received on 30.01.2012 and revised on 13.10.2013)