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# Hybrid performance and genetic distance as revealed by the $(GATA)_4$ microsatellite and RAPD markers in pearl millet

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**Abstract** Genetic diversity in five cytoplasmic male-sterile and seven restorer lines of pearl millet was determined by DNA fingerprinting using a $(GATA)_4$ microsatellite and randomly amplified polymorphic DNAs (RAPDs). A total of 160 polymorphic loci were generated and, based on the polymorphism data, similarity index values ranged from 0.81 to 0.50. Cluster analysis was performed and relationships among these lines revealed that they were not in agreement with the available pedigree data. The per se performance of parents and hybrids was analyzed for days-to-50% flowering, plant height, productive tillers, ear length, ear width, 1000-grain weight and grain yield per plot. Path co-efficient analysis revealed that productive tillers, ear width and days-to-50% flowering had a relatively large positive effect. The correlation values were mostly not significant with respect to genetic distance, except for days-to-50% flowering, ear length and ear width. Our results have indicated that genetic-distance measures based on the $(GATA)_4$ microsatellite and RAPDs may be useful for the grouping of parents, but not for predicting heterotic combinations, in pearl millet.

**Key words** *Pennisetum glaucum* · Heterosis · Genetic distance · Microsatellites · RAPDs

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## Introduction

Pearl millet is a cross-pollinated crop and its genetic improvement has been carried out by developing hybrids through conventional breeding procedures. The average yield of pearl millet in India fluctuates around 500 kg/ha, which is extremely low compared with other kharif crops as well as pearl millet production in other countries (Gill 1991). The primary reason for such a low yield is the cultivation of pearl millet on too-poor or too-dry soils where other cereals would fail to produce a crop. The improvement in yielding-ability envisages assembling the best combination of yield genes into a pearl millet variety or hybrid along with the improvement of performance under natural conditions. Pearl millet is highly heterozygous because of the cross-pollinating system of the crop. Recurrent selection is used to pool genes for a particular quantitative characteristic in an open-pollinated population, without a marked loss of genetic variability (Govil et al. 1982).

The exploitation of cytoplasmic male-sterility in pearl-millet hybrid development started in 1962 with the availability of Tift23A1 and indicated great scope for the genetic upgrading of this crop. Heterosis, which results from gene dispersion, directional dominance and non-allelic interactions, is computed as the deviation of the $F_1$ from the higher parent, which is sometimes referred to as heterobeltiosis. Heterosis over the mid-parent corresponds to dominance deviations on the scale of Mather (Mather and Jinks 1971). It can be exploited commercially; for example, in pearl millet single crosses have been used to exploit heterosis. Positive correlations between the genetic distance of the parents with heterosis in hybrids were reported by Paterniani and Lonquist (1963), Moll et al. (1965), Grant and Beversdorf (1985) and Lefort-Buson et al. (1987). In another study, Prasad and Singh (1986) found that heterosis was not linearly related to genetic divergence in ten maize varieties.

Molecular markers can be effectively used to precisely assess genetic diversity. For example, RFLPs have been employed in corn to study the relationship between genetic diversity and heterosis (Lee et al. 1989; Melchinger et al. 1990; Smith et al. 1990). In recent years, PCR-based markers (RAPDs) have proved to be useful in the analysis of genetic diversity and its relationship to hybrid performance and heterosis in rice (Xiao et al. 1996). Apart from RFLPs and RAPDs, DNA fingerprinting with mini- and micro-satellites can also be used to study the genetic diversity of parents to be used in breeding (Ramakrishna et al. 1994, 1995; Meng et al. 1996). To-date various contrasting results have been reported in relation to the prediction of heterosis and, in general, the correlation values have been shown to be dependent on the type of germplasm employed (Lee et al. 1989; Godshalk et al. 1990; Zhang et al. 1994, 1995, 1996; Saghai Maroof et al. 1997). Additionally, an increased correlation between marker heterozygosity and closely related germplasm is reported by Zhang et al. (1996).

In pearl millet, combining-ability studies have mostly been carried out for morphological and biochemical characters and yield parameters. It was observed that the characters tiller number, 500-grain weight, and plant height all had high heritability and high genetic advance, an effect of additive gene action. A highly significant and positive correlation for tiller number, followed by 500-grain weight with yield, indicated a major role for these characters in their contribution to yield (Kunjir et al. 1986). So far there has been no account of the use of molecular markers for variability and correlation studies in pearl millet. In our earlier work (Chowdari et al. 1998), we have shown the potential of a $(GATA)_4$ microsatellite and RAPDs in the evaluation of genetic diversity of pearl millet varieties and landraces. In the present study, we have evaluated the pearl millet CMS and restorer lines for their hybrid performance and heterosis and their relation to genetic distance for the prediction of heterotic combinations.

## Materials and methods

### Plant material and field evaluation

Seven cytoplasmic male-sterile lines (81A, 863A, Tift23A, 5141A, 841A, 393A and 267A) and five restorers (D23, ICMP451, PPMI301, PPMI493 and J254) of pearl millet were used in the present study. The crossing work was conducted in an off-season nursery at Coimbatore, India, during the summer of 1995. Bagging and pollination were carried out in the morning from 8 am to 12 noon. Thirty five $F_1$ hybrids were developed and the experiments were laid out in a completely randomized block design during the kharif of 1995 at IARI, New Delhi, India. The crosses and parents were restricted to separate, but consecutive, blocks within each replication and randomization was done separately for crosses and parents. Agronomic practices were carried out in accordance with the recommendations for this area and season. A spacing of 75 cm between rows and 10 cm between plants was employed. The traits evaluated were days-to-50% flowering, plant height, productive tillers, ear length, ear width, 1000-grain weight and grain yield per plot.

### DNA markers and data analysis

Twelve lines of pearl millet were surveyed for DNA polymorphism with 20 primers (OPA1–OPA20) from Operon Technologies and with a $(GATA)_4$ microsatellite probe. The DNA extraction procedure followed was as described by Sharp et al. (1988). DNA was digested with the restriction enzymes *Dra*I, *Hin*fI and *Hae*III (6–8 units/μg), separated on 1% or 1.4% agarose gels in 1 × TPE buffer (0.09 M Tris phosphate, 0.002 M EDTA pH 8.0), and gels were dried on a vacuum gel dryer. The $(GATA)_4$ hybridization procedure was as described by Ramakrishna et al. (1995). Hybridizations were performed at Tm-5°C and the hybridized gels were first washed with 5 × SSPE, 0.1%SDS twice for 15 min at RT and then at the hybridization temperature for 2 min, and were exposed to X-ray films at − 70°C with intensifying screens. PCR reactions were performed in 25-μl volumes consisting of 100 μM each of dATP, dTTP, dCTP and dGTP, 5 pmol of primer, 20 ng of genomic DNA, 1 × reaction buffer and 0.5 U of *Taq* DNA Polymerase. Samples were amplified in a Perkin Elmer Cetus 48-well DNA thermal cycler programmed with a 3-min step at 94°C for initial denaturation. This was followed by 45 cycles of 1 min at 94°C, 1 min at 36°C and 2 min at 72°C. The final cycle was followed by a 5-min final extension step at 72°C. Amplified products were separated on 1.4% agarose gels in 1 × TAE buffer (0.04 M Tris-acetate, 0.001 M EDTA, pH 8.0).

The 12 lines in the experiment were assessed for all seven characters by giving a final score for each line across the seven characters. To assess the crosses, the mean performance of the check, Pusa 23, was used as a standard. For each character, the overall mean was taken as a standard and those lines expressing values greater than the mean + SE were assigned a high status (H) while the others were given a low status (L). Similarly, for days to-50% flowering and plant height the lines expressing values below the mean + SE were considered desirable (D) and the others as undesirable (UD). For the status of H or D, a score of +1 was given, while L or UD received a zero score. For each line, a total score was obtained by adding the score across the characters. For combining-ability analysis, only crosses were considered and these were assessed based on the line × tester analysis. Heterosis for each cross was computed as a deviation from the mid-parent value.

In the molecular analysis, band-profiles for each parent were designated as 1 for presence or 0 for absence of a given band. Genetic similarities (GS) were calculated for all pairwise combinations of parents using the methods described by Nei and Li (1979). Rank correlations were calculated between $F_1$ performance, heterosis over the midparent and better parent, and the genetic distance.

## Results

### Per se performance of parents and hybrids

The seven female lines and the five testers in the experiment were assessed for all seven characters by giving a final score for each line across the seven characters, as mentioned in Materials and methods. Line 863A was ranked first with a score of five. The lines 81A and 393A stood second with a score of four each and the third place was occupied by 5141A with a score of three. 841A, with a score of 2, was placed fourth whereas Tift23A and 267A were placed in the fifth position with a score of one. The tester PPMI301 ranked second with a score of four; however, it was undesirable both for

days-to-50% flowering and plant height. The three testers D23, ICMP451 and PPMI493, each with a score of three, ranked third. The tester J254 was in fourth position with a score of two (Table 1).

Based on the total score, the 35 crosses between all CMS and tester lines were divided into five groups, as described in Materials and methods. Group I had the maximum score (4) and group V the minimum score (0). Only two crosses, 841A × PPMI301 and 393A × J254 occupied group I and were desirable both for days-to-50% flowering and for plant height. 81A × D23 cross had given a score of three, out of a maximum possible five, for yield components. In group II, six crosses were grouped whereas group III included 16 crosses. Nine crosses were placed in group IV (Table 2).

Heterosis over the mid parent and the better parent was represented for all 35 crosses in Table 3. For days-to-50% flowering, eight and two crosses recorded significant heterosis over the midparent and the better parent, respectively, in the desired direction (for earliness). The cross 393A × PPMI493 showed the maximum heterosis for earliness as − 10.77% over midparent and − 9.38% over the better parent. For plant height, none of the crosses showed significant heterosis over the mid parent and the better parent in a negative direction (dwarfness). For productive tillers, the 81A × D23 cross showed significant heterosis in the desired direction with a maximum value of 125% over the mid as well as the better parent. For ear length and ear width, the crosses Tift23A × D23 and 393A × J254, respectively, showed significant heterosis over both the mid parent and the better parent compared with other crosses. For 1000-grain weight and grain yield, significant heterosis was found, but none of the crosses performed significantly better than Pusa 23 (the check) for 1000-grain weight.

**Table 1** Score performance and overall status of parents based on per se performance (L × T analysis)

| Parents | Score | | | Rank |
|---|---|---|---|---|
| | Desirability | Yield components | Total | |
| Lines | | | | |
| 81A | 1 | 3 | 4 | II |
| 863A | 1 | 4 | 5 | I |
| Tift23A | 1 | 0 | 1 | V |
| 5141A | 0 | 3 | 3 | III |
| 841A | 1 | 1 | 2 | IV |
| 393A | 1 | 3 | 4 | II |
| 267A | 0 | 1 | 1 | V |
| Testers | | | | |
| D23 | 1 | 2 | 3 | III |
| ICMP451 | 0 | 3 | 3 | III |
| PPMI301 | 0 | 4 | 4 | II |
| PPMI493 | 0 | 3 | 3 | III |
| J254 | 2 | 0 | 2 | IV |

**Table 2** Score performance and overall status of hybrids based on per se performance in comparison with Pusa 23

| Hybrid | Score | | | Rank |
|---|---|---|---|---|
| | Desirability | Yield components | Total | |
| 81A × D23 | 0 | 3 | 3 | II |
| 81A × ICMP451 | 0 | 1 | 1 | IV |
| 81A × PPMI301 | 0 | 2 | 2 | III |
| 81A × PPMI493 | 0 | 1 | 1 | IV |
| 81A × J254 | 0 | 1 | 1 | IV |
| 863A × D23 | 1 | 1 | 2 | III |
| 863A × ICMP451 | 1 | 1 | 2 | III |
| 863A × PPMI301 | 0 | 2 | 2 | III |
| 863A × PPMI493 | 0 | 2 | 2 | III |
| 863A × J254 | 1 | 1 | 2 | III |
| Tift23A × D23 | 1 | 1 | 2 | III |
| Tift23A × ICMP451 | 1 | 0 | 1 | IV |
| Tift23A × PPMI301 | 1 | 2 | 3 | II |
| Tift23A × PPMI493 | 1 | 2 | 3 | II |
| Tift23A × J254 | 2 | 0 | 2 | III |
| 5141A × D23 | 0 | 2 | 2 | III |
| 5141A × ICMP451 | 0 | 1 | 1 | IV |
| 5141A × PPMI301 | 1 | 0 | 1 | IV |
| 5141A × PPMI493 | 0 | 2 | 2 | III |
| 5141A × J254 | 0 | 0 | 0 | V |
| 841A × D23 | Check | Check | Check | Check |
| 841A × ICMP451 | 0 | 1 | 1 | IV |
| 841A × PPMI301 | 2 | 2 | 4 | I |
| 841A × PPMI493 | 1 | 1 | 2 | III |
| 841A × J254 | 2 | 0 | 2 | III |
| 393A × D23 | 1 | 1 | 2 | III |
| 393A × ICMP451 | 2 | 1 | 3 | II |
| 393A × PPMI301 | 0 | 2 | 2 | III |
| 393A × PPMI493 | 1 | 2 | 3 | II |
| 393A × J254 | 2 | 2 | 4 | I |
| 267A × D23 | 0 | 1 | 1 | IV |
| 267A × ICMP451 | 1 | 1 | 2 | III |
| 267A × PPMI301 | 2 | 1 | 3 | II |
| 267A × PPMI493 | 0 | 2 | 2 | III |
| 267A × J254 | 1 | 0 | 1 | IV |

## DNA polymorphism and cluster analysis

We have earlier employed $(GATA)_4$ and RAPDs as DNA fingerprinting markers for the analysis of the pearl millet genome (Chowdari et al. 1998). In the present study, 12 genotypes were analyzed with the $(GATA)_4$ microsatellite using *Dra*I, *Hin*fI and *Hae*III restriction enzymes and 20 Operon primers (OPA series). The 12 genotypes analyzed using the microsatellite probe exhibited unique DNA fingerprint profiles and generated 37, 28 and 36 polymorphic loci with *Dra*I, *Hin*fI and *Hae*III, respectively. Out of 20 primers tested, 14 (OPA2, 3, 5, 8, 10 to 17, 19 and 20) were used for analysis and generated 59 polymorphic loci. OPA 20 was shown to be highly polymorphic and generated 14 polymorphic bands. Using $(GATA)_4$ and 14 Operon primers, a total of 160 polymorphic loci were generated. Based on the polymorphism data, similarity index values were calculated which ranged from 0.81 to 0.50

and cluster analysis was performed (Table 4). The dendrogram generated four clusters as shown in Fig. 1. Three of the clusters (D23 and Tift23 A; ICMP451, PPMI301, 863A and 267A; PPMI493, J254 and 393A) joined at a similarity value of 68%. The fourth cluster (81A, 5141A and 841A) joined with the remaining three at a 61% similarity value.

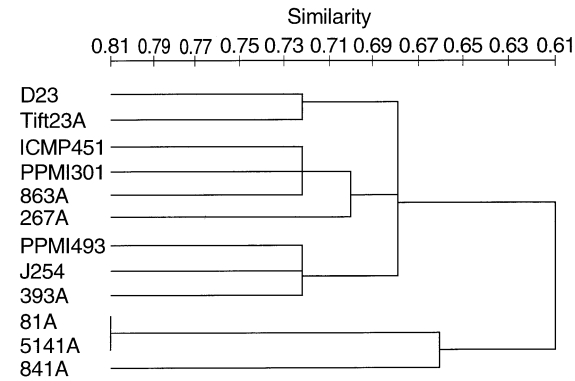


**Fig. 1** Genetic relationships among lines and testers of pearl millet used in the present study

## Correlation of genetic distance with hybrid performance and heterosis

Table 5 depicts the correlations of genetic distance with hybrid performance and heterosis over the mid parent and the better parent in the pearl millet line and tester analysis. The correlation values were mostly not significant with respect to genetic distance, except for days-to-50% flowering. For ear length and ear width, heterosis over the mid parent and the better parent were significantly correlated, but 1000-grain weight was not correlated with hybrid performance and heterosis.

**Table 3** Heterosis over the mid parent and the better parent (L × T mating design)

| Hybrid | Days-to-50% flowering | | Plant height | | Productive tillers | | Ear length | |
|---|---|---|---|---|---|---|---|---|
| | MP | BP | MP | BP | MP | BP | MP | BP |
| 1. 81A × D23 | 11.48** | 20.57** | 55.84** | 22.95** | 125.00** | 125.00** | 40.02** | 24.88** |
| 2. 81A × ICMP451 | 6.71** | 12.08** | 54.76** | 33.33** | 84.62** | 71.43** | 10.11* | 7.27 |
| 3. 81A × PPMI301 | 5.99** | 9.80** | 34.44** | 10.50 | 25.00** | 25.00 | 16.44** | 13.89* |
| 4. 81A × PPMI493 | 3.34* | 3.66* | 41.04** | 19.23** | 36.00** | 30.77 | 21.02** | 12.63* |
| 5. 81A × J254 | 13.71** | 25.93** | 57.65** | 45.78** | 85.10** | 66.67** | 26.44** | 14.02* |
| 6. 863A × D23 | 4.86** | 7.09** | 25.11** | 15.57** | − 13.51 | − 36.00* | − 0.32 | − 11.93* |
| 7. 863A × ICMP451 | 3.38* | 4.08* | 20.86** | 24.51** | − 23.00 | 7.14 | 3.45 | 1.85 |
| 8. 863A × PPMI301 | 7.33** | 9.52** | 19.40** | 16.16** | 2.70 | − 24.00 | − 0.40 | − 3.59 |
| 9. 863A × PPMI493 | 4.49** | 10.88** | 26.15** | 26.09** | − 21.00 | − 40.00** | − 10.36* | − 15.74** |
| 10. 863A × J254 | 9.93** | 14.81 | 30.90** | 17.99** | − 25.00 | 0.00 | 7.09 | − 4.35 |
| 11. Tift23A × D23 | 1.38 | 4.62* | 11.70* | − 1.80 | 38.46 | 28.57 | 46.80** | 42.12** |
| 12. Tift23A × ICMP451 | 4.70** | 4.70** | 21.58** | 18.46** | 21.43 | 28.43 | 19.93** | 7.57 |
| 13. Tift23A × PPMI301 | − 0.66 | 0.67 | 8.91 | 0.46 | 12.31 | 4.29 | 25.66** | 17.84* |
| 14. Tift23A × PPMI493 | − 3.82** | 1.34 | 31.63** | 24.64** | 107.41** | 100.00** | 35.68** | 16.75** |
| 15. Tift23A × J254 | 5.63** | 11.11** | 27.64** | 21.08** | 31.03 | 26.67 | 30.19** | 27.56** |
| 16. 5141A × D23 | 7.99** | 19.86** | 30.60** | 21.23** | 76.30** | 58.67* | 45.86** | 36.20** |
| 17. 5141A × ICMP451 | 8.41** | 16.78** | 46.14** | 41.24** | − 10.34 | − 13.33 | 30.67** | 21.33** |
| 18. 5141A × PPMI301 | − 7.69** | − 1.96 | 23.83** | 21.00** | 11.11 | 0.00 | 11.42* | 8.35 |
| 19. 5141A × PPMI493 | − 2.67* | − 0.61 | 26.62** | 26.32** | 28.57 | 20.00 | 20.73** | 7.35 |
| 20. 5141A × J254 | 7.49** | 22.22** | 28.00** | 14.83** | 36.00 | 36.00 | 38.82** | 31.14** |
| 21. 841A × D23 | − 7.53** | − 4.26* | 15.79** | 1.56 | 15.38 | 7.14 | 29.01** | 12.57* |
| 22. 841A × ICMP451 | 10.00** | 10.74** | 32.98** | 29.23** | 0.00 | 0.00 | 21.47** | 21.33* |
| 23. 841A × PPMI301 | 1.32 | 1.99 | 13.65* | 4.57 | 15.38 | 7.14 | 15.64** | 10.39 |
| 24. 841A × PPMI493 | − 5.70** | − 1.32 | 35.55* | 28.02** | 11.11 | 7.14 | 18.62** | 13.04** |
| 25. 841A × J254 | 6.99** | 13.33** | 28.57** | 22.28** | 44.83* | 40.00 | 23.33** | 8.78 |
| 26. 393A × D23 | 7.64** | 14.89** | 9.36 | − 9.02 | − 5.88 | − 27.27 | 23.06** | 12.71* |
| 27. 393A × ICMP451 | − 1.62 | 2.01 | 27.73** | 16.93** | 0.00 | − 80.18 | 14.25** | 8.18 |
| 28. 393A × PPMI301 | 4.79** | 7.19** | 35.08** | 17.50** | − 11.76 | − 31.82 | − 1.41 | − 2.14 |
| 29. 393A × PPMI493 | − 10.77** | − 9.38* | 37.67** | 22.71** | 2.86 | − 18.18 | 11.34* | 0.85 |
| 30. 393A × J254 | − 1.02 | 8.15** | 43.29** | 41.57** | 18.92 | 0.00 | 30.63** | 21.02** |
| 31. 267A × D23 | 11.40** | 21.28** | 30.01** | 10.25* | 41.18* | 9.09 | 34.90** | 30.67** |
| 32. 267A × ICMP451 | − 2.86* | 2.68 | 37.06** | 28.21** | 20.00 | − 1.82 | 13.53* | 1.79 |
| 33. 267A × PPMI301 | − 7.21** | − 3.27 | 21.40** | 7.76 | 23.53 | − 4.55 | 11.01* | 4.04 |
| 34. 267A × PPMI493 | 2.72* | 3.03 | 41.72** | 28.99** | 8.57 | − 13.64 | 8.62 | − 6.57 |
| 35. 267A × J254 | 4.32 | 16.30 | 39.73** | 38.16** | 8.11 | − 9.09 | 26.80** | 24.33** |

**Table 3** (Continued)

| Hybrid | Ear width | | 1000-grain weight | | Grain yield per plot | |
|---|---|---|---|---|---|---|
| | MP | BP | MP | BP | MP | BP |
| 1. 81A × D23 | 26.26** | 5.72 | 13.89 | 0.35 | 157.25** | 95.14** |
| 2. 81A × ICMP451 | 6.10 | − 11.26** | 5.49 | − 2.35 | 113.12** | 66.46* |
| 3. 81A × PPMI301 | 25.60** | 4.11 | 26.07* | 18.00 | 135.82** | 56.20** |
| 4. 81A × PPMI493 | 7.79 | − 7.78 | 12.56 | 10.09 | 85.60** | 34.62 |
| 5. 81A × J254 | 9.23 | − 2.46 | 20.33 | 0.46 | 182.69** | 150.21** |
| 6. 863A × D23 | 13.47** | 3.88 | 12.52 | 5.17 | 82.09** | 71.43** |
| 7. 863A × ICMP451 | − 3.48 | − 11.51** | 14.87 | 2.13 | 53.09* | 50.00 |
| 8. 863A × PPMI301 | 17.43** | 8.76 | 25.73** | 10.64 | 82.89** | 42.27** |
| 9. 863A × PPMI493 | 8.61 | − 2.88 | 6.64 | − 9.73 | 125.93** | 96.88** |
| 10. 863A × J254 | 16.87** | 0.13 | 25.05 | − 9.73 | 112.50** | 87.06** |
| 11. Tift23A × D23 | 21.47** | 1.81 | 51.60** | 7.34 | 115.66** | 57.43* |
| 12. Tift23A × ICMP451 | 0.45 | − 15.92** | 40.27** | 2.73 | 145.55** | 84.16** |
| 13. Tift23A × PPMI301 | 24.60** | 3.28 | 75.07** | 29.20** | 53.58* | − 1.62 |
| 14. Tift23A × PPMI493 | 7.14 | − 8.25 | 112.20** | 61.40** | 104.85** | 42.07* |
| 15. Tift23A × J254 | 9.72 | − 1.93 | 98.49** | 80.14** | 147.47** | 108.51** |
| 16. 5141A × D23 | 20.04** | − 0.05 | 12.92 | − 17.48 | 156.74** | 134.00** |
| 17. 5141A × ICMP451 | 6.55 | − 12.01* | 36.08** | 3.13 | 30.49 | 23.60 |
| 18. 5141A × PPMI301 | 14.8** | − 6.02 | 24.61 | − 4.80 | 62.56** | 23.38 |
| 19. 5141A × PPMI493 | 15.23* | − 2.70 | 35.00** | 6.58 | 104.55** | 73.08** |
| 20. 5141A × J254 | 4.70 | − 7.72 | 46.76** | 39.73* | 121.80** | 101.39** |
| 21. 841A × D23 | 7.92 | − 7.68 | 50.94** | 25.87** | 76.99** | 71.43** |
| 22. 841A × ICMP451 | 7.91 | − 7.81 | 22.60* | 7.03 | 78.15** | 76.52* |
| 23. 841A × PPMI301 | 18.13** | 0.00 | 63.24* | 44.00** | 65.16** | 31.20* |
| 24. 841A × PPMI493 | 15.61** | 1.11 | 27.92* | 17.54 | 26.08 | 12.74 |
| 25. 841A × J254 | 12.86* | 3.16 | 47.18** | 29.84* | 99.29** | 71.04** |
| 26. 393A × D23 | 13.21** | 9.04 | 25.40** | 10.49 | 75.52** | 68.00** |
| 27. 393A × ICMP451 | 12.88** | 8.56 | 55.27** | 43.75** | 73.83** | 73.20** |
| 28. 393A × PPMI301 | 25.62** | 19.53** | 40.17** | 31.20** | 71.00** | 34.71* |
| 29. 393A × PPMI493 | 11.16* | 9.84 | 54.71** | 51.32** | 87.77** | 66.11** |
| 30. 393A × J254 | 29.45** | 24.72** | 64.29** | 37.16** | 189.37** | 150.94** |
| 31. 267A × D23 | 27.91** | 6.63 | 47.45** | 5.94 | 118.33** | 87.14** |
| 32. 267A × ICMP451 | 19.93** | − 0.15 | 44.88** | 7.81 | 125.52** | 100.31* |
| 33. 267A × PPMI301 | 25.27** | 3.38 | 29.07* | − 3.20 | 14.39 | − 17.09 |
| 34. 267A × PPMI493 | 23.39** | 5.08 | 51.27** | 17.11 | 124.32** | 79.57** |
| 35. 267A × J254 | 16.29** | 3.33 | 57.93** | 46.58* | 100.10** | 94.40** |

* Significant at the 5% level
** Significant at the 1% level

**Table 4** Genetic similarity values among the seven CMS and the five restorer lines used in the present study

| Line | 81A | 863A | Tift23A | 5141A | 841A | 393A | 267A | D23 | ICMP451 | PPMI301 | PPMI493 | J254 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 81A | 1.00 | | | | | | | | | | | |
| 863A | 0.57 | 1.00 | | | | | | | | | | |
| Tift23A | 0.70 | 0.54 | 1.00 | | | | | | | | | |
| 5141A | 0.81 | 0.57 | 0.79 | 1.00 | | | | | | | | |
| 841A | 0.66 | 0.68 | 0.54 | 0.61 | 1.00 | | | | | | | |
| 393A | 0.70 | 0.64 | 0.59 | 0.70 | 0.50 | 1.00 | | | | | | |
| 267A | 0.59 | 0.57 | 0.57 | 0.59 | 0.57 | 0.61 | 1.00 | | | | | |
| D23 | 0.61 | 0.54 | 0.73 | 0.57 | 0.54 | 0.64 | 0.66 | 1.00 | | | | |
| ICMP451 | 0.61 | 0.73 | 0.64 | 0.61 | 0.68 | 0.68 | 0.70 | 0.68 | 1.00 | | | |
| PPMI301 | 0.57 | 0.59 | 0.64 | 0.66 | 0.68 | 0.68 | 0.52 | 0.64 | 0.73 | 1.00 | | |
| PPMI493 | 0.66 | 0.68 | 0.73 | 0.66 | 0.59 | 0.73 | 0.57 | 0.68 | 0.59 | 0.59 | 1.00 | |
| J254 | 0.70 | 0.59 | 0.73 | 0.70 | 0.59 | 0.68 | 0.61 | 0.68 | 0.64 | 0.68 | 0.73 | 1.00 |

## Discussion

The analysis of variance indicated that the parents were diverse for all seven characters studied. Hallauer and Miranda (1988) and Smith (1986) have reported a poor relationship between per se performance of lines and hybrid combinations. However, in our study, the per se performance of both CMS lines and restorer lines was significantly correlated with hybrid performance for

**Table 5** Correlations of genetic distance (GD) with hybrid performance, heterosis over the mid-parent (MPH) and better-parent (BPH) in the pearl millet line and tester sets

| Item | Days-to-50% flowering | Plant height | Productive tillers | Ear length | Ear width | 1000-grain weight | Grain yield per plot |
|---|---|---|---|---|---|---|---|
| GD with hybrid performance | 0.223 | − 0.082 | 0.197 | − 0.027 | 0.025 | − 0.136 | 0.019 |
| GD with heterosis over mid parent | 0.209* | 0.125 | 0.062 | 0.326** | 0.434*** | 0.056 | 0.089 |
| GD with heterosis over better parent | 0.275* | − 0.090 | − 0.057 | 0.254* | 0.362** | − 0.046 | 0.194 |

* Significant at more than the 5% level
** Significant at the 5% level
*** Significant at the 1% level

three characters, viz. days-to-50% flowering, ear width and 1000-grain weight. The per se performance of hybrids indicated that many crosses were superior to the check, Pusa 23 (data not shown). The grain yield of Pearl millet is influenced by many component characters such as 1000-grain weight, ear length, ear width, productive tillers, plant height and days-to-flowering. The degree of influence of one variable on the other can be expressed in quantitative terms. Path co-efficient analysis, a method employed by Dewey and Lu (1959) in plants, was very efficient in this respect. The result of path analysis revealed that productive tillers, ear width and days-to-50% flowering were very important component characters since they had a relatively large positive direct effect on yield (data not shown).

In the present study, based on molecular analysis, all lines and testers grouped into four clusters and the relationship of inter- and intra-cluster genotypes with hybrid performance and heterosis for all the traits gave varied results. Even the field data for crosses produced similar information. Our results indicated that the days-to-flowering trait showed a significant correlation with hybrid performance and heterosis, whereas a significant association was found with ear length and ear width for heterosis.

The heterosis phenomenon has been reported by many authors for various traits, positively or negatively, in pearl millet. Positive heterosis for grain yield, plant height, tiller number, ear length, ear girth, harvest index and for forage traits was reported by Soundarpandian et al. (1964), Phul et al. (1973), Subramaniam and Rathiram (1980), Bajaj and Phul (1982), Burton (1982), and Rao et al. (1983). Negative heterosis for days-to-earing was found by Ahluwalia and Patnaik (1963). Our results showed very low correlation levels and were not useful for predicting $F_1$ performance; however, the information could be used for grouping and differentiating genotypes.

The exploitation of heterosis by breeding hybrid varieties offers considerable scope for the improvement of the pearl millet crop. Specific marker-heterozygosity studies showed highly significant correlations in rice (Xiao et al. 1996). However, few markers have so far been reported to be associated with QTLs for yield in pearl millet.

In summary, the microsatellite $(GATA)_4$, which is highly polymorphic, along with RAPDs, can be used for the grouping of parents and for identifying specific markers linked to QTLs. Predictions based on specific markers may help in developing promising hybrids in the pearl milllet crop.

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## References

Ahluwalia MM, Patnaik MC (1963) A study of heterosis in pearl millet. Indian J Genet Pl Breed 23 : 34–38

Bajaj RK, Phul PS (1982) Inheritance of harvest index and span of maturity in pearl millet. Indian J Agric Sci 52 : 285–288

Burton GW (1982) Improving the heterotic capacity of pearl millet lines. Crop Sci 22 : 655–657

Chowdari KV, Davierwala AP, Gupta VS, Ranjekar PK, Govila OP (1998) Genotype identification and the assessment of genetic relationships in pearl millet (*Pennisetum glaucum* L.) using the $(GATA)_4$ microsatellite and RAPDs. Theor Appl Genet (in press)

Dewey DR, Lu KH (1959) A correlation and path co-efficient analysis of components of crested wheat-grass seed production. Agron J 51 : 515–518

Gill KS (1991) Pearl millet and its improvement. IARI Publications, PUSA, New Delhi, India

Godshalk EB, Lee M, Lamkey KR (1990) Relationship of restriction fragment length polymorphisms to single-cross hybrid performance of maize. Theor Appl Genet 80 : 273–289

Govil JN, Pokhriyal SC, Murty BR (1982) Full-sib and reciprocal recurrent selection in relation to pearl millet improvement. Theor Appl Genet 62 : 25–30

Grant I, Beversdorf WD (1985) Heterosis and combining ability estimates in spring-planted oil seed rape (*Brassica napus* L.). Can J Genet Cytol 27 : 472–478

Hallauer AR, Miranda JB (1988) Quantitative genetics in maize breeding. Iowa State University Press, Ames, Iowa

Kunjir AN, Nakhole RR, Harer PN, Navale PA, Harinarayana G (1986) Combining ability analysis in gray × white grained pearl millet. J Maharashtra Agric Univ 13 : 147–149

Lee M, Godshalk EB, Lamkey KR, Woodman WW (1989) Association of restriction fragment length polymorphisms among maize inbreds with the agronomic performance of their crosses. Crop Sci 29 : 1067–1071

Lefort-Buson M, Guillot-Lemoine B, Dattee Y (1987) Heterosis and genetic distance in rape seed (*Brassica napus* L.): crosses between European and Asiatic selfed lines. Genome 29 : 413–418

Mather KJ, Jinks JL (1971) Biometrical genetics, Chapman and Hall, London

Melchinger AE, Lee M, Lamkey KR, Hallauer AR, Woodman WW (1990) Genetic diversity for restriction fragment length polymorphisms and heterosis for two diallel sets of maize inbreds. Theor Appl Genet 80 : 488–496

Meng A, Gong G, Chen D, Zhang H, Qi S, Tang H, Gao Z (1996) DNA fingerprint variability within and among parental lines and its correlation with the performance of $F_1$ laying hens. Theor Appl Genet 92 : 769–776

Moll RH, Lonnquist JH, Velez Fortuno J, Johnson EC (1965) The relationship of heterosis and genetic divergence in maize. Genetics 52 : 139–144

Nei M, Li WH (1979) Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc Natl Acad Sci USA 76 : 5269–5273

Paterniani E, Lonnquist JH (1963) Heterosis in interacial crosses of corn (*Zea mays* L.). Crop Sci 3 : 504–507

Phul PS, Nanda GS, Gupta SP (1973) Combining ability in pearl millet. Indian J Genet Pl Breed 33 : 334–339

Prasad SK, Singh TP (1986) Heterosis in relation to genetic divergence in maize (*Zea mays* L.). Euphytica 35 : 919–924

Ramakrishna W, Lagu MD, Gupta VS, Ranjekar PK (1994) DNA fingerprinting in rice using oligonucleotide probes specific for simple repetitive DNA sequences. Theor Appl Genet 88 : 402–406

Ramakrishna W, Chowdari KV, Lagu MD, Gupta VS, Ranjekar PK (1995) DNA fingerprinting to detect genetic variation in rice using hypervariable DNA sequences. Theor Appl Genet 90 : 1000–1006

Rao MK, Kumar MA, Koduru PRK (1983) Role of early seed development in two heterotic hybrids and their parental lines in pearl millet. J Plant Breed 9 : 25–35

Saghai Maroof MA, Yang GP, Zhang Q, Gravios KA (1997) Correlation between molecular-marker distance and hybrid performance in U.S. southern long grain rice. Crop Sci 37 : 145–150

Sharp PJ, Kries M, Shewry PR, Gale MD (1988) Location of $\beta$-amylase sequences in wheat and its relatives. Theor Appl Genet 75 : 286–290

Smith OS (1986) Covariance between line per se and heterosis performance. Crop Sci 26 : 540–543

Smith OS, Smith JSC, Bowen SL, Tenborg RA, Wall SJ (1990) Similarities among a group of elite maize inbreds as measured by pedigree, $F_1$ grain yield, grain yield heterosis, and RFLPs. Theor Appl Genet 80 : 833–840

Soundarapandian G, Madhava Menon P, Ponnaiya BW (1964) Heterosis in pearl millet, effect of nitrogen fertilization on hybrids. Madras Agric J 51 : 356

Subramaniam R, Rathiram M (1980) Heterosis for grain yield and its components in pearl millet. Madras Agric J 67 : 561–566

Xiao J, Li J, Yuan L, Mc Couch SR, Tanksley SD (1996) Genetic diversity and its relationship to hybrid performance and heterosis in rice as revealed by PCR-based markers. Theor Appl Genet 92 : 637–643

Zhang QF, Gao YJ, Yang SH, Ragab RA, Saghai Maroof MA, Li ZB (1994) A diallel analysis of heterosis in elite hybrid rice based on RFLPs and microsatellites. Theor Appl Genet 89 : 185–192

Zhang QF, Gao YJ, Yang SH, Saghai Maroof MA, Li JX (1995) Molecular divergence and hybrid performance in rice. Mol Breed 1 : 133–142

Zhang Q, Zhou ZQ, Yang GP, Xu CG, Liu KD, Saghai Maroof MA (1996) Molecular-marker heterozygosity and hybrid performance in indica and japonica rice. Theor Appl Genet 93 : 1218–1224