RAPD MARKER-BASED GENETIC DIVERSITY AMONG RELEASED FINGER MILLET (ELEUSINE CORACANA. GAERTN.) CULTIVARS WITH KNOWN PEDIGREE

The validity of concern regarding the narrow genetic base of released finger millet cultivars was explored.

Randomly Amplified Polymorphic DNA (RAPD) marker-based assay of 35 finger millet cultivars with known

pedigree indicated substantial inter-cultivar genetic distance ranging from 0.90 to 0.26. While the cultivars, MR

2 and HR 911 were highly similar, Indaf 9 and OEB 57 were highly divergent at RAPD loci. The cultivars could be grouped into nine clusters based on their genetic distance. The cultivars within a cluster had similar parentage

and those grouped into different clusters had diverse parental origin. The preliminary evidences from the study

did not completely support the hypothesis of narrow genetic base of finger millet cultivars developed and released

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INTRODUCTION

Finger millet (Eleusine coracana Gaertn.) is an allotetraploid cereal belongs to the family poaceae. It ranks third in importance among millets in the world after pearl millet and foxtail millet (Upadhyaya et al., 2007). It is grown as a rainfed crop under varied agro-climatic production environments in India. Wide adaptability, nutritional quality, dual-purpose (grain and dry fodder) nature of crop and excellent storability under ambient conditions of makes finger millet, one of the popular staple food crops and as an indispensable crop component in mixed crop-livestock system of farming prevalent in semi-arid tropics of India (Vietmeyer, 1996). Market forces and producer/consumer preferences for crop product uniformity have necessitated the finger millet breeders to induce variability and select varieties possessing most preferred traits that match the diverse production environments across India. As a result, most bred varieties are phenotypically similar for agronomic traits irrespective of the target location to which varieties were developed. This led us to hypothesise that finger millet varieties released in India have narrow genetic base which predispose them to biotic and abiotic stresses of large-scale proportions and jeopardize their sustainable productivity potential (Asins and Cabonell, 1989; Van Esbroeck et al., 1999). Duvick (1984) and Tanksley and McCouch (1997) have also expressed a great deal of concern

ABSTRACT

for commercial cultivation in India.

about narrow genetic base of crop cultivars. Devastation of commercial crop of single CMS-based maize hybrids in USA due to incidence of southern corn leaf blight (Tatum, 1971) and wheat crop of single variety due to severe winter in Soviet Union in 1972 (Fischbeck, 1981) are classical examples of production environments of crop cultivar genetic uniformity. However, reported evidence for narrowing of crop cultivar genetic diversity accompanying plant breeding in crops in general (Donini et al., 2000) and finger millet in particular is lacking.

Being environment neutral, crop stage non-specific and easily assayable, DNA markers are ideal tools for genetic diversity assessment in crop cultivars (Morrell et al., 1995; Virk et al., 1995; Powell et al., 1996; Nassiry et al., 2009). Among several DNA markers, Randomly Amplified Polymorphic DNA (RAPD) markers offer several advantages such as non-requirement of a priori DNA sequence information, low cost, whole genome screening, technical simplicity for genotyping, amenability for automation and possibility of simultaneous sampling of several loci by each primer per assay (Morrell et al., 1995; Powell et al., 1996; Subudhi and Huang, 1999). Panwar et al. (2010) and Prabhu and Ganesan (2013) have reported utility of RAPD and other DNA markers in assessing genetic diversity of finger millet germplasm accessions, advanced breeding lines and released varieties. These considerations have prompted us to undertake an investigation with an objective of examining the hypothesis of narrow genetic base of finger millet cultivars developed and released for commercial cultivation in India using RAPD-based marker assay.

MATERIALS AND METHODS

Plant material

The material for the study consisted of 35 finger millet cultivars, developed and released for cultivation all over India. The pedigree, maturity duration, grain yield potential and other salient characteristics of the released cultivars are furnished in Table 1. Most of these cultivars are developed in University of Agricultural Sciences (UAS), Gandhi Krishi Vigana Kendra (GKVK), Bangalore. The seeds of these genotypes were procured from all India co-ordinated small millets improvement project (AICSMIP), UAS, GKVK, Bangalore, Karnataka, India.

DNA extraction

The seeds of all 35 finger millet cultivars are gown in green house located in experimental plots of Department of Genetics and Plant Breeding (GPB), College of Agriculture (CoA), UAS, GKVK, Bangalore, India. The genomic DNA was isolated from leaves of 15 days-old seedlings by CTAB mini-preparation method (Doyle and Doyle, 1987). The extracted DNA was quantified on 0.8 % agarose using standard \ddot{e} uncut DNA (50ng/µL) and the DNA was diluted to 10ng/µL using T₁₀E_{0.1} (Tris- EDTA) buffer. The diluted DNA was used for RAPD profiling in Kirkhouse Trust, UK funded Plant Molecular Biology Laboratory (PMBL) located in the Department of GPB, CoA, UAS, GKVK, Bangalore, India.

RAPD assay

Fifty random decamer primers with 50-70% GC content (Table 2) were used for RAPD profiling. The polymerase chain reactions (PCR) were performed in a $20 \,\mu$ L mixture containing 40 ng of genomic DNA, $10 \mu M/\mu$ L RAPD decamer primer (Operon technology, Almeda, Calif, USA), 1.25 mM of each of dATP, dCTP, dGTP and dTTP (New England Biolabs, England), 1 U Taq polymerase (Enzene biosciences), 1X Taq assay buffer (Enzene biosciences) and 0.5 mM MgCl_a.



Figure 1: RAPD banding profile of 35 finger millet genotypes for the primer OPA 3

Amplifications were performed in a thermal cycler (PTC, M.J research). The standardized PCR cycle include: initial denaturation temperature 94°C for 3 min, followed by 35 cycles of denaturation at 94°C for 30sec; primer annealing 36°C for 1 min; primer extension: 72°C for 1 min; and a final primer extension at 72°C for 10 min. The amplicons were assessed by loading on to 1.5% (w/v) agarose along with bromophenol blue as loading dye. Amplicons were resolved on an electrophoresis unit at 100 V for 3 hours using 1X TBE (Tris-Boric acid-EDTA) buffer and stained with ethidium bromide (50mg/100mL agarose).

Band scoring and data analysis

The RAPD assay and size separation of amplicons through agarose gel electrophoresis resulted in banding pattern at different levels (loci). The amplicons generated in RAPD assav are inherited as dominant markers. Therefore, RAPD loci were scored for two alleles, the 'band-presence allele' and 'bandabsence allele'. The score '1' was assigned for the presence of band and '0' for absence of the band at each loci. The variation in band intensity was not considered to avoid ambiguity in scoring. Assuming that the marker alleles from different loci do not co-migrate to the same position on a gel and each locus could be unambiguously scored. Considering the difficulty in distinguishing heterozygous individuals from homozygous individuals for the band-presence allele (van Haeringen et al., 2002), two approaches were followed to estimate population genetic parameters based on the RAPD profile data. The first one referred to as 'band-based' approach corresponds to direct counting of band 'presence' or 'absence'. The second one referred to as 'allele frequency-based' approach involves estimating allelic frequency at each locus. Estimates of these allele frequencies were used to compute population genetic parameters (Mariette et al., 2002).

'Band-based' population genetic parameters

The polymorphism of all 50 RAPD primers was calculated using the formula, Polymorphism (%) = (Total number of loci - Number of monomorphic loci)/Total number of loci \times 100 (Blair *et al.*, 1999). Resolving power (Rp) defined as the ability of the primers to differentiate the cultivars was estimated to



Figure 2: Dendrogram showing clustering pattern among 35 finger millet cultivars based on RAPD marker profile

| Sl.no | Genotype | Pedigree | Duration (days) | Grain yield (t ha ⁻¹) | Other salient characteristics | |
|-------|----------------------------|--|--------------------|--------------------------------------|--|--|
| 1 | GPU-26 | INDAF 5 \times IE 1012 | 95-100 | 3.0-4.0 | Neck and finger blast resistant, high grain yield | |
| 2 | GPU-28 | INDAF 5 \times IE 1012 | 110-115 | 3.5-4.5 | Neck and finger blast resistant, high grain yield | |
| 3 | GPU-45 | GPU 26 \times L 5- BAN | 95-100 | 2.7-2.9 | Neck and finger blast resistant, high grain yield | |
| 4 | GPU-48 | GPU 26 × L 5 | 100-110 | 3.0-3.4 | Pigmented, neck and finger blast resistant | |
| 5 | GPU-66 | PR 202 × GPU 28 | 110-115 | 3.8-4.0 | Neck and finger blast resistant | |
| 6 | INDAF-8 | Hullubele $	imes$ IE 929 | 120-125 | 2.5-3.0 | Blast tolerant | |
| 7 | INDAF-9 | K1(Selection) \times IE 980 R | 95-105 | 2.0-2.5 | High yielding | |
| 8 | INDAF-11 | - | 118-120 | 2.5-2.8 | White | |
| 9 | INDAF-15 | IE 67 × IE 927 | 125-135 | 4.0-4.5 | Neck and finger blast resistant | |
| 10 | L-5 | Malvi × INDAF 9 | 120-125 | 4.0-2.5 | Long duration and high yielding | |
| 11 | MR-1 | Hamsa $	imes$ IE 927 | 120-125 | 4.5-5.0 | Moderately tolerant to neck and finger | |
| | | | | | blast, high grain yield | |
| 12 | MR-2 | PR 202 × IE 927 | 120-125 | 3.8-4.0 | Highly tolerant to diseases | |
| 13 | HR-911 | UAS 1 × IE 927 | 115-120 | 4.0-5.0 | High yielding | |
| 14 | PR-202 | Pure-line selection from Peddapuram local | 118-120 | 3.0-3.2 | Medium duration suitable for late planting | |
| 15 | VL-149 | VL 201 × IE 882 | 110-115 | 3.2-3.5 | Resistant to neck and finger blast | |
| 16 | PES-400 | Pure-line selection from Panthnagar local | 100-105 | 2.8-3.0 | Early duration variety | |
| 17 | VR-708 (champayathi) | Selection from VMEC-32, | 93 -100 | 2.5-3.0 | Drought and blast resistant | |
| 18 | $O[\Delta T ^2 (Shubba)]$ | Selection from CO 9 | 110 115 | 3035 | White sheath blast resistant | |
| 10 | PES_{110} | Germplasm selection | 110-115 | 3.0-4.5 | Released from Littar Pradech | |
| 20 | MR 6 | $\Delta_{W} \times R_0 H_2$ | 120 125 | J.0-4.J | Highly resistant to diseases | |
| 20 | | C_{21} Very \times IE 927 | 105 110 | 4.5-5.0 | Cultivated in all seasons except rabi | |
| 21 | HR_374 | $E_{-4840} \times 1E_{-927}$ | 95-100 | 4.5-5.0 1 0-1 2 | Dwarf variety Karnataka | |
| 23 | | $FC = 4971 \times GPU 26$ | 118-120 | 3 0-3 5 | White seeded han | |
| 23 | GPU-67 | Selection from GE 5331 | 115-118 | 3 5-4 5 | Semi dwarf variety neck and finger | |
| 24 | | Selection nom de 5551 | 115 110 | 5.5 4.5 | blast resistant | |
| 25 | GPU-71 | GE-4971 × VL 147 | 115-118 | 3.0-3.5 | Neck and finger blast resistant | |
| 26 | GPU-72 | PR 202 × GPU 48-10 | 115-120 | 3.5-3.8 | Neck and finger blast resistant | |
| 27 | GPU-76 | PR 202 × GPU 48-7 | 115-120 | 3.5-3.8 | Neck and finger blast resistant | |
| 28 | VL-332 | VI 127 × IE 121 | 100-105 | 2.2-2.4 | Almora, neck and finger blast resistant | |
| 29 | VL-333 | IE 881 × VL 298 | 105-110 | 2.5-2.7 | Almora neck and finger blast resistant | |
| 30 | RAU-8 | BR 407 × Ranchi Local | 100-105 | 2.5-3.0 | Bihar, lodging resistant, neck and finger blast resistant | |
| 31 | OEB-211 | Mutant of PR 202 | 115-118 | 3.0-3.2 | Bhuvaneshwar | |
| 32 | VR-888 | GPU 26 \times selection 16 | 115-120 | 3.0-3.2 | Andhra Pradesh | |
| 33 | OEB-57 | CO 12 × CO 13 | 118-120 | 2.8-3.0 | Bhuvaneshwar | |
| 34 | OEB-265 | Mutant of PR 202 | 119-120 | 3.0-3.2 | Bhuvaneshwar | |
| 35 | GPU 65 | GE 4971 × VL 149 | 112-115 | 3.0-3.2 | Neck and finger blast resistant | |

Table 1: Pedigree, maturity duration, grain yield potential and other salient characteristics of 35 finger millet released varieties used in the study

assess the informativeness of bands. $Rp = \acute{O}$ lb (Prevost and Wilkinson, 1999), where lb (band informativeness) = $1-[2 \times (0.5-p)]$, where, p is the proportion of the 35 cultivars containing the band.

Based on frequency of band presence and absence, the average phenotypic genetic diversity (APD) often referred to as Nei's expected heterozygosis (Nei, 1978) was estimated (Mariette *et al.*, 2002). For each RAPD locus, APD was computed as APD = $1-(P_i^2-Q_i^2)$, where P_i and Qi are the frequencies of band presence and absence, respectively. The estimates of Hp were averaged over loci sampled by each primer. Shannon-Weaver Diversity index (SDI) (Shannon, 1948) for each locus was computed as SDI = $\acute{O} P_i \log P_i$. The estimate of 'SDI' was averaged over loci sampled by each primer.

'Allele frequency-based' population genetic parameters

Frequencies of band-presence allele (p,) and band absence

allele (q_i) were computed first. Frequencies of q_i at each RAPD loci sampled by a primer were computed as the square root of the ratio of number of individuals with band absences to the total number of individuals. The Frequencies of p_i were computed as (1-q_i) (Mariette *et al.*, 2002). The estimates of p_i and q_i were used to compute average gene diversity (AGD) for each locus as AGD = 1- (p_i² - q_i²) (Mariette *et al.*, 2002). The estimates of AGD were averaged over the loci sampled by each primer. Assessment of over 50 genotypes is usual requirement for sampling RAPD loci from the genome. Unbiased genetic diversity (UGD) was estimated to correct for less than 50 genotypes (as only 35 genotypes were used in the present investigation).

Inter-cultivar genetic distance and grouping of cultivars

The binary data was used to estimate inter-cultivar genetic distance based on simple matching coefficient (SMC) using 'NTSYS' software (Rohlf, 1999). The cultivars were grouped

into different clusters using Un-weighted Pair Group Method using Arithmetic Average (UPGMA) algorithm based on distance matrix.

RESULTS AND DISCUSSION

RAPD marker assay-based polymorphism among finger millet varieties

Amplification of genomic DNA segments complementary to 50 RAPD primers resulted in 445 scorable loci with an average of 8.9 loci per primer. Among these 445 amplicons, 154 loci were polymorphic. The number of loci per primer varied from 15 to solitary. The Primer 57 could amplify eight loci, seven of these being polymorphic. Contrastingly, primer 4 could amplify 10 loci, of which only one of them was polymorphic (10% polymorphism). On an average, the 50 primers sampled 34.5%

Table 2: Estimates of Population genetic parameters based on RAPD marker profile of 35 finger millet released cultivars with known pedigree

| SI. No | Primer name | Sequence | TNL | NPL | % P | RP | AGD | UGD | PGD | SDI |
|--------|-------------|---------------|------|------|--------|------|------|------|------|------|
| 1 | OPC- 5 | GAT GAC CGC C | 13 | 11 | 84.61 | 4.43 | 0.46 | 0.47 | 0.37 | 0.18 |
| 2 | OPB -8 | GTC CAC ACG G | 10 | 03 | 30.00 | 2.57 | 0.48 | 0.48 | 0.31 | 0.22 |
| 3 | OPA-11 | CAA TCG CCG T | 06 | 03 | 50.00 | 4.40 | 0.40 | 0.40 | 0.30 | 0.14 |
| 4 | OPA-12 | TCG GCG ATA G | 06 | 02 | 33.33 | 1.08 | 0.42 | 0.43 | 0.24 | 0.24 |
| 5 | OPA-2 | TGC CGA GCT G | 05 | 02 | 40.00 | 3.82 | 0.36 | 0.37 | 0.32 | 0.08 |
| 6 | OPA-3 | TGT CAG CCA C | 13 | 04 | 30.76 | 3.48 | 0.53 | 0.53 | 0.34 | 0.23 |
| 7 | OPD-12 | CAC CGT GCA C | 04 | 01 | 25.00 | 1.08 | 0.69 | 0.70 | 0.44 | 0.30 |
| 8 | OPB-10 | CTG CTG TTA C | 08 | 05 | 62.5 | 5.31 | 0.48 | 0.49 | 0.33 | 0.20 |
| 9 | OPE-3 | CCA GAT GCAC | 08 | 02 | 25.00 | 3.20 | 0.64 | 0.65 | 0.49 | 0.21 |
| 10 | OPB-5 | TGC GCC CTT C | 08 | 05 | 62.50 | 5.08 | 0.45 | 0.45 | 0.30 | 0.19 |
| 11 | OPE-3 | CCA GAT GCAC | 01 | 01 | 100.00 | 0.86 | 0.61 | 0.62 | 0.37 | 0.30 |
| 12 | OPC-6 | GAA CGG ACT C | 13 | 04 | 30.76 | 4.80 | 0.61 | 0.62 | 0.42 | 0.25 |
| 13 | OPC-1 | TTC GAG CCA G | 11 | 04 | 36 36 | 4 28 | 0.51 | 0.52 | 0.34 | 0.22 |
| 14 | OPA-1 | | 11 | 02 | 18 18 | 2.97 | 0.63 | 0.64 | 0.46 | 0.22 |
| 15 | OPF-8 | GGG ATA TCG G | 03 | 01 | 33 33 | 0.51 | 0.43 | 0.44 | 0.24 | 0.25 |
| 16 | OPB-4 | | 07 | 01 | 14 28 | 0.17 | 0.45 | 0.17 | 0.08 | 0.13 |
| 17 | | | 05 | 02 | 40.00 | 3 20 | 0.10 | 0.17 | 0.00 | 0.15 |
| 18 | Drimer 4 | | 10 | 01 | 10.00 | 1.02 | 0.67 | 0.68 | 0.00 | 0.22 |
| 19 | Primer 5 | | 09 | 01 | 11 11 | 1.02 | 0.31 | 0.00 | 0.42 | 0.30 |
| 20 | Primer 1/ | | 07 | 07 | 28.57 | 1.37 | 0.31 | 0.31 | 0.10 | 0.20 |
| 20 | Primor 90 | | 10 | 02 | 20.57 | 1.51 | 0.47 | 0.47 | 0.20 | 0.24 |
| 21 | Primer 81 | | 10 | 05 | 50.00 | 4.00 | 0.30 | 0.39 | 0.43 | 0.20 |
| 22 | Primer 80 | | 12 | 00 | 19 19 | 0.02 | 0.40 | 0.40 | 0.50 | 0.16 |
| 23 | Primer 09 | | 11 | 02 | 10.10 | 2.97 | 0.69 | 0.70 | 0.50 | 0.25 |
| 24 | Primer 90 | | 10 | 04 | 30.30 | 5.42 | 0.65 | 0.64 | 0.45 | 0.24 |
| 25 | Primer 04 | | 07 | 04 | 40.00 | 4.74 | 0.57 | 0.56 | 0.39 | 0.23 |
| 26 | Primer 92 | | 07 | 02 | 20.57 | 2.60 | 0.59 | 0.60 | 0.42 | 0.23 |
| 27 | Primer 4/ | | 0/ | 02 | 20.57 | 2.34 | 0.63 | 0.64 | 0.42 | 0.26 |
| 20 | | | 14 | 02 | 14.20 | 1.94 | 0.63 | 0.64 | 0.40 | 0.29 |
| 29 | Primer o | | 07 | 03 | /1.42 | 2.21 | 0.59 | 0.39 | 0.40 | 0.24 |
| 30 | Primer 12 | | 09 | 03 | 33.33 | 2.34 | 0.57 | 0.58 | 0.34 | 0.28 |
| 31 | Primer 10 | | 11 | 02 | 18.18 | 3.14 | 0.66 | 0.67 | 0.49 | 0.22 |
| 32 | Primer 46 | | 10 | 04 | 44.44 | 3.37 | 0.52 | 0.53 | 0.33 | 0.24 |
| 33 | Primer 6 | | 10 | 02 | 20.00 | 2.28 | 0.66 | 0.67 | 0.43 | 0.28 |
| 34 | Primer 26 | | 08 | 02 | 25.00 | 3.// | 0.42 | 0.42 | 0.36 | 0.10 |
| 35 | Primer 33 | | 08 | 02 | 25.00 | 2.17 | 0.67 | 0.68 | 0.43 | 0.29 |
| 36 | Primer 35 | | 08 | 05 | 62.50 | 5.3/ | 0.50 | 0.51 | 0.35 | 0.20 |
| 3/ | Primer 37 | | 05 | 04 | 80.00 | 2.80 | 0.58 | 0.59 | 0.37 | 0.27 |
| 38 | Primer 69 | | 12 | 07 | 58.33 | 7.60 | 0.65 | 0.66 | 0.42 | 0.28 |
| 39 | Primer 5/ | | 08 | 07 | 87.50 | 7.71 | 0.43 | 0.43 | 0.31 | 0.17 |
| 40 | Primer /I | | 11 | 03 | 27.27 | 2.97 | 0.62 | 0.63 | 0.39 | 0.28 |
| 41 | Primer 53 | GAG ICA CGA G | 12 | 04 | 33.33 | 4.91 | 0.62 | 0.63 | 0.43 | 0.25 |
| 42 | Primer 64 | | 12 | 06 | 50.00 | 4.80 | 0.40 | 0.41 | 0.28 | 0.17 |
| 43 | Primer 73 | CAG GCG GCG I | 15 | 04 | 26.66 | 5.65 | 0.59 | 0.59 | 0.43 | 0.21 |
| 44 | Primer /4 | | 15 | 01 | 06.66 | 0.9/ | 0.66 | 0.6/ | 0.41 | 0.30 |
| 45 | Primer 7 | | 08 | 03 | 37.50 | 3.14 | 0.55 | 0.56 | 0.3/ | 0.23 |
| 46 | Primer 49 | AGC AGC GTG G | 80 | 02 | 25.00 | 3.48 | 0.56 | 0.57 | 0.45 | 0.16 |
| 47 | Primer 2 | GGT GGG GAC T | 06 | 01 | 16.66 | 1.31 | 0.71 | 0.72 | 0.49 | 0.28 |
| 48 | OPL 12 | GGG CGG TACT | 07 | 02 | 28.57 | 2.63 | 0.69 | 0.70 | 0.48 | 0.27 |
| 49 | Primer 88 | GCT GGA CAT C | 08 | 03 | 37.50 | 4.17 | 0.67 | 0.68 | 0.47 | 0.25 |
| 50 | Primer 54 | TCC ATG CCG T | 08 | 01 | 12.50 | 1.26 | 0.71 | 0.72 | 0.48 | 0.29 |
| Total | | | 445 | 155 | - | - | | - | - | - |
| Averag | e | | 8.90 | 3.10 | 36.72 | | 0.55 | 0.56 | 0.38 | 0.22 |

TNL: Total number of loci; NPL: number of polymorphic loci; %P: percentage of polymorphic loci; RP. Resolving power; AGD: Average genetic diversity; UGD: Unbiased genetic diversity; PGD: Phenotypic genetic diversity; SDI: Shannon Weaver diversity Index.

polymorphic loci with an average of 4.5 polymorphic loci per primer. Frakruddin et *al.* (2001) and Kalyana Babu et *al.* (2007) have reported 479 informative amplified fragments from 50 primers which are comparable to that reported in the present study but with a higher number (9.6) of polymorphic loci per primer among released finger millet cultivars. Sankar Reddy et *al.* (2013) in rice, Holeyachi and Savithramma (2013) in mungbean and Bahurupe et *al.* (2013) in chilli have also reported substantial degree of DNA marker-based polymorphism. A wide range in the estimates of resolving power from 7.71 to 0.17 (Table 2) indicated differential ability of RAPD primers to discriminate 35 finger millet cultivars.

Genetic diversity among the cultivars

It has been demonstrated that a minimum of 50 genotypes need to be used for DNA marker-based genetic diversity assessment (Bonin, 2007). In the present study, marginal differences between standard average genetic diversity (AGD) estimate and unbiased genetic diversity (UGD) suggested that 35 finger millet cultivars considered for the study is adequate for genetic diversity assessment. AGD as a measure of variability is more appropriate for inbreeding species such as finger millet and is loosely referred as average heterozygosis (Weir, 1996). The diversity was as low as 0.08 at loci captured by primer OPB-4 and as high as 0.71 at loci sampled by primers 2 and primer 54. At loci sampled by several RAPD primers, AGD e" 0.6 suggesting substantial polymorphism among the finger millet cultivars investigated in the present study. The estimates of AGD are allele frequency dependent. Estimation of allelic frequencies requires precise determination of heterozygosis and Hardy-Weinberg equilibrium assumption. With dominant markers, both the requirements are seldom met. Shanon's diversity index (SDI), being relatively insensitive to bias caused by inability to detect heterozygous individuals (Dawson et al., 1995), is more appropriate measure of population diversity with dominant marker data (Bussel and Bussel, 1999). SDI is being largely used as a measure of diversity in plant genetic resources (Upadhayaya et al., 2007). In the present study, estimates of SDI complemented those of AGD.

Inter-variety genetic distance

Most published studies do not explain the choice of coefficient of diversity in relation to the type of marker and or ploidy level and pollination control system of the crop being investigated (Kosman and Leonard, 2005). While investigating the diversity of related genotypes in predominately self pollinated crops such as finger millet used in the study, Simple Matching Coefficient (SMC) is the most appropriate diversity measure as it takes care of 'absence' as well as 'presence' bands in both the compared genotypes as causes of similarity (Laurentin, 2009). SMC among 35 varieties ranged from 0.90 (between the cultivars MR 2 and HR 911) to 0.26 (between Indaf 9 and OEB 57). Differences in inter-cultivar genetic distance among the cultivars could be attributed to wide differences in heterozygosis loci at RAPD loci.

Organization of genetic diversity

Based on UPGMA algorithm, 35 finger millet varieties could be grouped into nine clusters (Fig. 2). While some of the clusters consisted of cultivars ranging from 2 to 5, a few others were solitary. As the bred cultivars were derived from crosses between locally adapted germplasm lines and high yielding lines with desirable agronomic background and selections were based on farmers/consumers/end-user preferences and weather and edaphic factors, it was not surprising to find grouping of the some of the cultivars into different clusters. The cultivars such as GPU 26, GPU 28, GPU 45 and GPU 48 sharing similar pedigree and bred with similar selection pressure and released to a narrow range of production environments representing southern dry zone of Karnataka state have predictably grouped into a single cluster. The cultivars bred for different agro-climatic conditions and with distinct pedigree have remained solitary. For example, Indaf 9 bred for southern dry zone of Karnataka state, VR 888 bred for dry zone of Andhra Pradesh, VL 332 bred for temperate climatic of Uttaranchal and OEB 57 bred for tropical humid climate of Orissa state have segregated into different clusters. The derivatives of these crosses among the cultivars are expected to have wide spectrum of variability with high frequency of productive recombinant inbred lines. The strategy of crossing genotypes with marked differences in DNA marker loci has resulted in appearance of higher frequency (>50%) of new and useful guantitative trait loci alleles in rice and tomato (Tanksley and McCouch, 1997).

All the four varieties in the I cluster have one common parent, i.e. GPU 26 and GPU 28 with SMC of 0.86 have both the parents in common (Indaf $5 \times IE 1012$) justifying their inclusion in single cluster. Other varieties included in I cluster, GPU 45 and GPU 48 with SMC of 0.83 are the selections among the segregating generations derived from GPU 26 \times L 5 cross. These two sets of varieties have single common parent GPU 26. Similarly, the varieties (L5, MR 1, MR 2, HR 911, VL 149) included in the cluster 4 have one parent in common. Inclusion of varieties with similar pedigree into a single cluster and those with dissimilar pedigree into different clusters based on RAPD assay is a clear evidence for the utility of RAPD markers to distinguish the finger millet cultivars at DNA level (Kalyan Babu et al., 2007). Though RAPD marker-based assay sample nucleotide sequence polymorphism randomly (mostly from non-coding regions) distributed throughout the genome (Subudhi and Huang, 1999), they are powerful tools for assessing the extent of genetic similarity/dissimilarity among crop varieties and complement the conventional phenotypic assavs.

To conclude, higher genetic diversity atleast among a few of finger millet cultivars considered in the present study is in agreement with that reported by Ofori *et al.* (2008) in *Brassica rapa* cultivars. Our results (preliminary) do not completely support concerns about narrow genetic base of finger millet varieties bred and released for commercial cultivation in India. Our views auger well with those of Fu (2006) who reported only a marginal genome-wide reduction in crop cultivar genetic diversity accompanying crop improvement. Based on extensive review on several crop plants Witcombe (1999) argues favorable role of plant breeding in widening crop cultivar genetic diversity.

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