African Journal of Biotechnology Vol. 9(29), pp. 4542-4549, 19 July, 2010
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB10.1740
ISSN 1684–5315 © 2010 Academic Journals

Full Length Research Paper

Study of genetic diversity in finger millet (*Eleusine coracana* L. Gaertn) using RAPD markers

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Accepted 3 June, 2010

Germplasm identification and characterization is an important link between conservation and utilization of plant genetic resources. The present study was conducted to characterize the genetic diversity using twelve germplasm of finger millet including two of the same variety (VL-149) but from different regions. Three replica of each germplasm was amplified using seventeen random primers. A total of 113 distinct fragments ranging from 117 bp to 2621 bp were amplified. Of these, 70 (61.9%) were found to be polymorphic. A fingerprint for GPU-28 was obtained. Another fingerprint for genotype VL-315 was generated where two primers (T10S6, T20S4) could distinguish it from other genotypes either by absence or presence of an allele, respectively. In addition to this, another interesting allele which was absent in genotypes of high altitudes (VL-324, VL-315, and VL-149) was discovered. The lowest and highest polymorphisms were obtained within individuals belonging to genotypes OUAT-2 and VL-324. Nei's analysis revealed the highest similarity between OUAT-2 and JWM-1 and the highest distance between BM-1 and VL-315. OUAT-2 and JWM-1, both white seeded germplasms, showed maximum closeness. The study helped in identifying the germplasm in a quick and reproducible manner and studying their relatedness.

Key words: *Eleusine coracana*, genetic diversity, RAPD markers, polymorphism.

INTRODUCTION

One of the aims of a breeder is to improve the resistance of a cultivated crop to diseases, drought, etc. To achieve this goal, usually a cross is performed between the susceptible cultivated forms with wild forms that possess the required tolerance/resistance. Thereafter, six or more back crossing steps are needed and the tolerance/ resistance is often difficult to detect. Hence, technologies that could make this procedure more efficient are of great interest. The use of molecular markers is one of such techniques.

Several types of molecular markers have been used to determine genetic divergence within and among plant species and are generally classified as hybridization based markers and polymerase chain reaction (PCR) based markers. PCR based markers use amplification of the regions of interest in the genome; subsequent gel electrophoresis is performed to size and/ or score the amplification products.

The characteristics of an ideal genetic marker is that, it detects qualitative and quantitative variation, shows no environmental or developmental influence, shows simple co-dominance inheritance, detects silent nucleotide changes, detects changes in coding and non-coding portions of genome, and detects evolutionary homologous changes. Such markers allow the possibility of un-ambiguously assigning a genotype to a taxon and then using these data either to estimate genetic variation present within and between populations or to compare taxonomies directly. The various molecular marker technologies which are extensively used in different studies

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Abbreviations: RAPD, Random amplified polymorphic DNA; **SCAR,** sequence characterized amplified region.

Table 1. Molecular markers used in biodiversity assessment.

Parameter	RFLP	RAPD	AFLP	PCR-RFLP	SSR	SSCP
Basis	Distribution of relative RE-site position	Distribution of random primers through genome	PCR of RE fragment subset using modified primers	RE digest of PCR products	PCR of simple sequence repeat regions	ssDNA takes up different structures in non-denaturing gels
Polymorphism	Nucleotide substitution; indels; insertions	Nucleotide substitution; indels; insertions	Nucleotide substitution; indels; insertions	Nucleotide substitution; indels; insertions	Repeat length changes	Nucleotide substitution; indels.
Abundance in genome	High	Very high	High	High	Medium	Medium
Level of polymorphism	Medium	Medium	Medium	Medium	High	High
Dominance	Co-dominant	Dominant	Co-dominant/ dominant	Co-dominant	Co-dominant	Co-dominant
Amount of material	Very little	2-10 µg DNA	10-25 ng DNA	1-2 µg DNA	50-100 ng DNA	50-100 ng DNA
Sequence information	No	No	No	Yes	Yes	No
Radioactive detection	Yes/No	No	Yes/No	No	Yes/No	Yes/No
Development costs	Medium	Low	Medium	Medium/High	High	Medium
Start-up costs	Medium/High	Low	Medium	High	High	Medium
Applications	Genetic diversity, polyploidy, hybridization, phylogeny, mating system	Fingerprinting, genetic diversity, polyploidy, hybridization, phylogeny	Fingerprinting, genetic diversity	Genetic diversity, polyploidy, hybridization, phylogeny	Genetic diversity, mating system	Genetic diversity, mating system
Automation	Limited	Yes	Yes	Limited	Yes	Limited
Reproducibility	High	Low	Medium	High	High	Medium

AFLP-Amplified fragment length polymorphism; RFLP-Restriction fragment length polymorphism; PCR- Polymerase chain reaction; SSR-Simple sequence repeats; PCR-RFLP- Polymerase chain reaction - restriction fragment length polymorphism.

are shown in Table 1 (Harris, 2003).

Among the use of molecular marker, the most simple, fast and easy to perform assay is the use of RAPD markers to determine the existing genetic diversity and variation among and within the population. The first use of RAPD markers was reported by Williams et al. (1990) and Welsh and McClelland (1990).

Finger millet (*Eleusine coracana* L. Gaertn), an allotetraploid cereal, is widely cultivated in the arid and semiarid regions of the world. Being rich in protein, iron and calcium, finger millet serves as an important staple food for rural populations in developing tropical countries where calcium deficiency and anemia are widespread (Babu et al., 2006). Scientific intervention with regards to improvement and development of this important nutri-cereal has been negligible. However, it has been grown as a subsistence crop for decades and local farmers have employed methods of selection to preserve/ improve germplasm. It is imperative that these germplasm are documented and identified with the help of molecular markers for protection of intellectual property and for future use in breeding programmes. An attempt was made to identify the germplasm readily available through the use of RAPD markers. Commercially available and self designed markers have been used in combination with eleven germplasms to look for specific fingerprints.

Table 2. Scorable DNA bands of finger millet germplasm generated by different random primers.

S/N	Primer	Total number of amplification products	Number of polymorphic products	Percent polymorphism	Size range (bp)
1	T10S4	5	4	80 %	195-1633
2	T10S5	9	9	100 %	125-2586
3	T10S6	15	1	6.6%	221-1381
4	T20S4	4	1	25%	238-1916
5	T20S5	2	1	50%	286-474
6	C3	7	2	28.4%	552-1322
7	C4	10	5	50%	117-674
8	C5	6	3	50%	201-983
9	C6	7	4	56.8%	231-941
10	RPI#1	14	11	78.5%	132-1547
11	RPI#2	10	10	100%	269-1138
12	RPI#3	5	5	100%	237-2621
13	RPI#4	7	6	85.2%	268-2024
14	RPI#5	6	5	83.3%	160-1316
15	RPI#9	4	2	50%	216-696
16	RPI#10	2	1	50%	257-794

1 - 9 = Self designed random primers.
10 -16 = Random primers obtained from Bangalore GeNei, India.

MATERIALS AND METHODS

Collection of germplasm and isolation of DNA

Seeds of eleven genotypes were obtained from Department of Plant Breeding and Genetics, Birsa Agricultural University (B.A.U.), Ranchi, India and Vivekananda Parvatiya Krishi Anusandhan Station (V.P.K.A.S.), Almora, India. The seeds were sown at the College of Biotechnology, B.A.U. Three replicates of each genotype were used for DNA extraction. These were A4O4, OUAT-2, BM-2, GPU-28, JWM-1, R+, BM-1, VR-708, VL-149 (Almora), VL-149 (Ranchi), VL-315 and VL-324. The extraction of genomic DNA was done according to the protocol given by Dellaporta et al. (1983).
Plant DNA was extracted from 5 g fresh, young, healthy leaf tissues of finger millet. The DNA was resolved on a 0.7% agarose gel. Its concentration was determined by visual assessment of the DNA band intensity compared with that of a known concentration of λ DNA/ Hind III-digested DNA. Aliquots of these samples were used for PCR amplification.

DNA amplification

Total seventeen 10-mer, 15-mer and 20-mer random primers were used. These were either commercially available or ordered from Bangalore GeNei, India or Hysel India Pvt Ltd, India. PCR reaction mixture of 20 µl was prepared by adding 2 µl 10 X buffer, 1.6 µl dNTPs (200 µM of each dNTP), 1 µl (200 pmole) random primer, 0.33 µl (1 Unit) of Taq polymerase (Bangalore GeNei), 1 µl (50 ng) of genomic DNA and 14.07 µl sterile water. A control tube without any template DNA was also prepared. Amplification was performed in a master cycler epgradient S thermal cycler (Eppendorf) according to the following programme: initial denaturation at 94°C for 5 min, followed by 40 cycles at 94°C for 30 s, annealing temperature was selected between 40 - 60°C for 30 s and elongation at 72°C for 45 s. A final extension was given at 72°C for 5 min.

DNA electrophoresis

The amplicons obtained after PCR were run on 1.5% agarose gels (Sigma A9539) in 1X TAE buffer and electrophoresed at a constant voltage of 100V for 3 h. Ethidium bromide was added to the gel for staining and visualized under a UV transilluminator and photographed using Alpha digi doc system (Alpha Innotech). The sizes of amplified fragments were estimated against Φ x 174/ HaeIII digest ladder (Bangalore GeNei).

Gel scoring and data analysis

Each unambiguous band was assumed to represent a dominant allele at a unique genetic locus and was recorded as 1 (present) or 0 (absent) for each sample. The amplified products were analyzed using the software Popgene (Yeh et al., 1999).

RESULTS

Amplification of genomic DNA from each of the eleven germplasms, using seventeen random primers, revealed a variety of RAPD patterns. A total of 113 scorable DNA fragments were observed, of which 70 (61.9%) were polymorphic among the finger millet genotypes (Table 2).

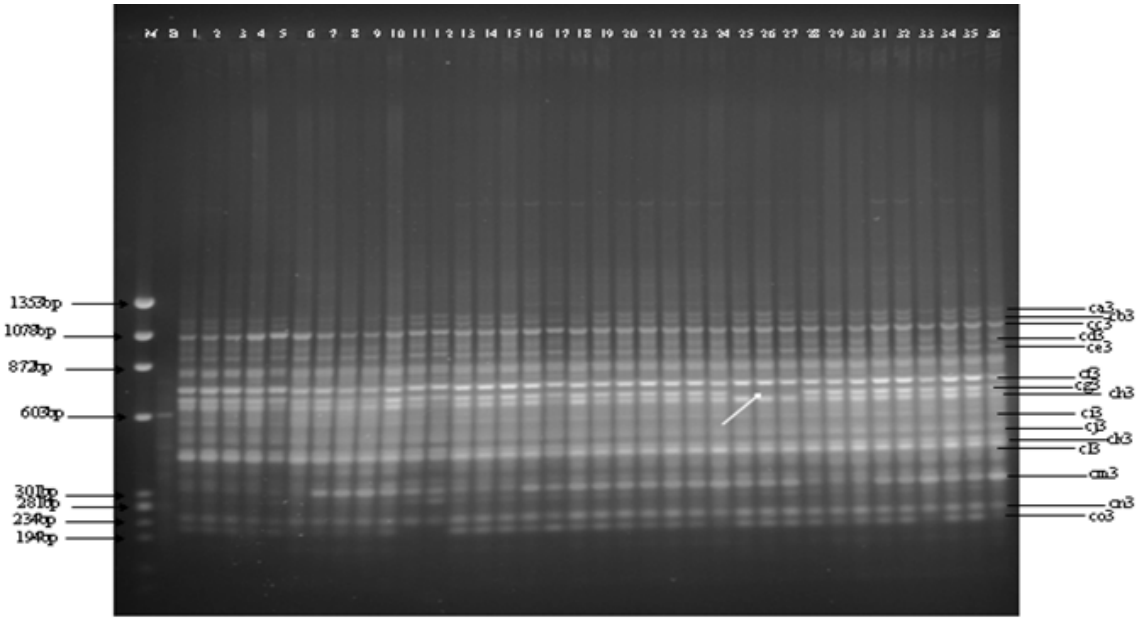

Figure 1. Amplicons obtained using primer T10S6 from finger millet germplasm: VR-708 (1-3), BM-1(4-6), A404 (7-9), VL-324 (10-12), R+(13-15), GPU-28 (16-18), OUAT-2 (19-21), JWM-1 (22-24), VL-315 (25-27), BM-2 (28-30), and VL-149 (31-36)

One primer (T20S6) did not show any amplification. The average number of bands per primer was found to be 7 and average numbers of polymorphic bands was found to be 4.3. Among these 17 primers, four primers (T10S6, T20S4, C3 and RPI#9) were able to generate specific marker for the genotypes VL-315, BM-2, VL-324, VL-149 and GPU-28.

Table 2 shows the difference in the number of bands that were obtained by the RAPD primers that were used. A dendrogram was constructed on the basis of the RAPD profiles. Close relationship was detected between the germplasms OUAT-2 and JWM-1 as well as between A404 and BM-2. All four gemplasms fall under the same sub-sub group. The phenotypic and genotypic trait similarities between OUAT-2 and JWM-1 exist since both are white seeded germplasms of finger millet. The data generated through Nei's genetic analysis revealed that the highest similarity was present between populations OUAT-2 and JWM-1 and highest distance existed between BM-1 and VL-315.

DISCUSSION

RAPD markers proved to be very informative and useful in monitoring the genetic diversity present in a sample of eleven germplasms. The use of RAPD has led to the identification of genetic markers in many finger millet germplasm (Das et al., 2006; Fakrudin et al., 2004; Hilu et al., 1995; Salimath et al., 1995). From these studies, it is apparent that RAPD markers can be used with a great degree of confidence in finger millet in a reproducible manner. The data of the present study indicates that the band pattern obtained with the use of 16 primers can identify the different germplasm of finger millet. The experiments were primarily based on 16 different 10, 15, and 20-mer oligonucleotide primers that were scored in eleven germplasm of finger millet. The maximum number of bands generated by a single primer was 15 (T10S6), and three different primers, C3, C6 and RPI#4, generated 7 bands each. The primers T20S5 and RPI#10 only amplified one polymorphic and one monomorphic band in each samples. Some primers such as T10S5, RPI#2 and RPI#3 could detect polymorphism across all samples. This result shows the ability of RAPD to discriminate among genotypes and their application for cultivar identification.

One of the immediate use of this study has been to identify primers which are likely to be efficient in revealing diversity in other genotypes of finger millet, for instance out of a set of 16 primers used 4 could be useful in detecting polymorphism among 5 germplasms of finger millet. Short-listing such primers will be useful for further analysis of germplasm (Virk et al., 1995).

A specific marker for the germplasm VL-315 was generated. The allele cg3 was found to be absent in all VL-315 genotypes (Figure 1). However, it was present in all individuals of the remaining genotypes. Thus this primer can be used to distinguish VL-315 from the

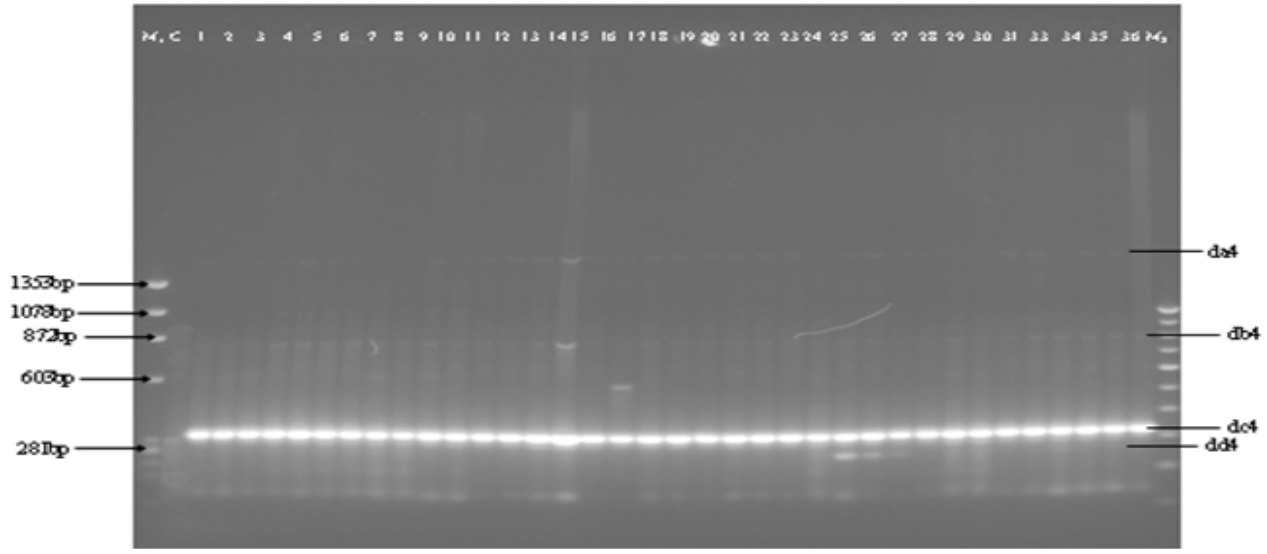

Figure 2. Amplicons obtained using primer T20S4 from finger millet germplasm: VR-708 (1-3), BM-1(4-6), A404 (7-9), VL-324 (10-12), R+(13-15), GPU-28 (16-18), OUAT-2 (19-21), JWM-1 (22-24), VL-315 (25-27), BM-2 (28-30), and VL-149 (31-36).

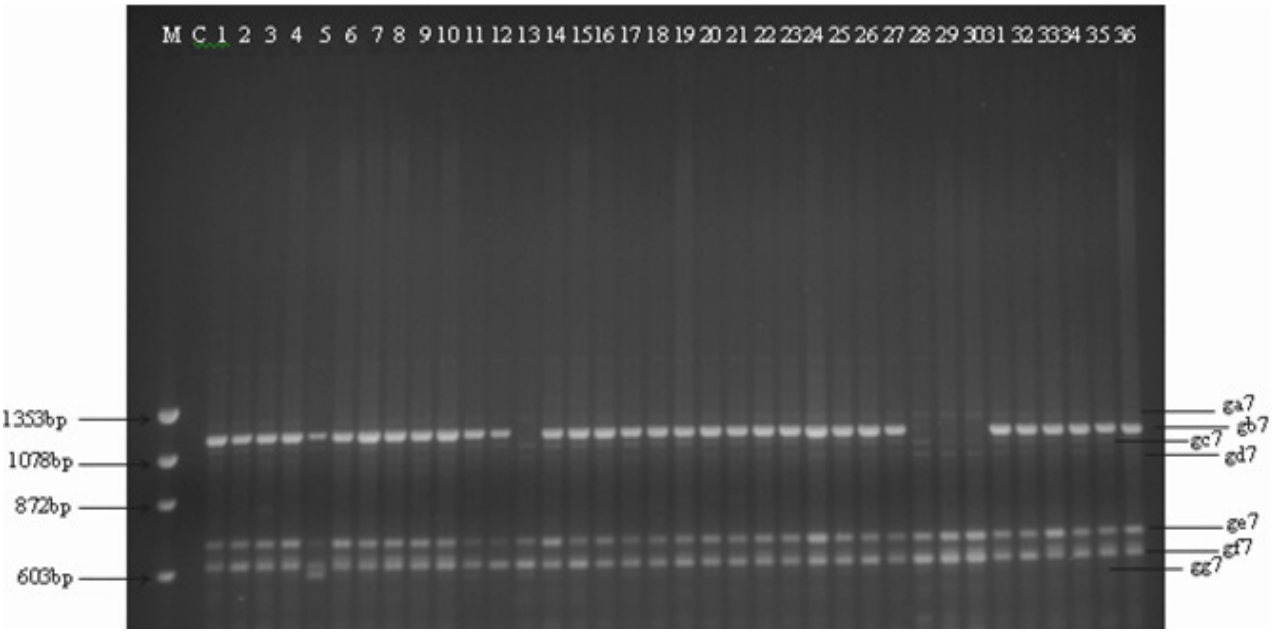

Figure 3. Amplicons obtained using primer C3 from finger millet germplasm: VR-708 (1-3), BM-1(4-6), A404 (7-9), VL-324 (10-12), R+(13-15), GPU-28 (16-18), OUAT-2 (19-21), JWM-1 (22-24), VL-315 (25-27), BM-2 (28-30), and VL-149 (31-36).

remaining genotypes.

The genotype VL-315 can also be distinguished from other genotypes with the use of T20S4 primer. The allele dd4 shows amplification in only VL-315 (Figure 2). Since multiple bands are seen with both T10S6 and T20S4 but only one band is absent (cg3) or present (dd4) in all the three individuals of genotype VL-315, use of either primer can help to differentiate VL-315 from others.

Similarly, BM-2 can be distinguished by absence of allele gb7 (Figure 3). However, though all individuals of genotype BM-2 show absence of allele gb7 one individual of R^+ genotype also follows the same pattern.

Table 3. Unique markers used to discriminate finger millet germplasm at the inter-specific level.

Discriminated germplasm	Unique marker	Criteria
VL-315	T10S6 (cg3)*	Absence
VL-315	T20S4 (dd4) *	Presence
BM-2	C3 (gb7) *	Absence
VL-315,VL-324,VL-149	RPI#9 (pa16) *	Absence
GPU-28	RPI#9 (pd16) *	Absence

*The figures in parenthesis refer to the band name given in different gel photographs.

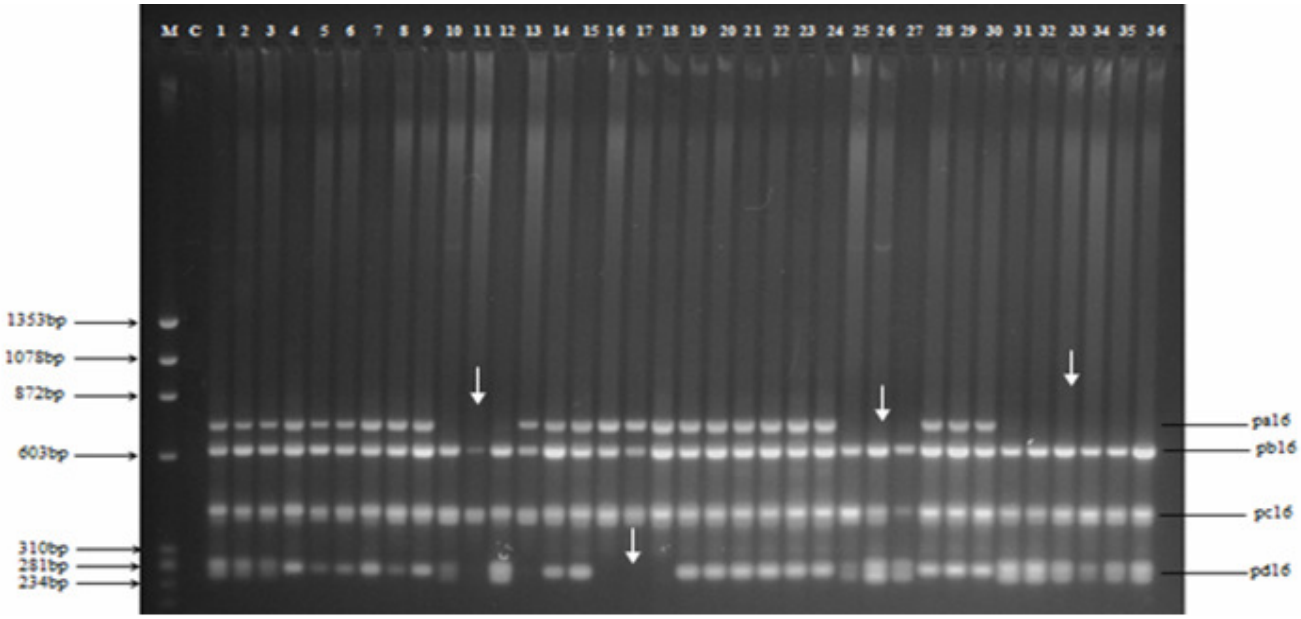

Figure 4. Amplicons obtained using primer RPI#9 from finger millet germplasm: VR-708 (1-3), BM-1 (4-6), A404 (7-9), VL-324 (10-12), R+ (13-15), GPU-28 (16-18), OUAT-2 (19-21), JWM-1 (22-24), VL-315 (25-27), BM-2 (28-30), and VL-149 (31-36).

Thus, more individuals of these two genotypes would have to be studied for amplification of this allele before it can be used to distinguish BM-2 with a high degree of confidence. Table 3 summarizes the unique markers obtained.

The highest polymorphism was obtained with the primer RPI#9 (Figure 4). The pa16 allele was absent in all genotypes obtained from region of high altitude, that is, Almora, India, which brings up the intriguing possibility of linkage with an area of specific phenotype. Presence of the allele in genotypes which grow at lower altitudes indicates it is linked to some phenotypic trait associated with the climatic/edaphic conditions prevalent. Large number of genotypes would need to be screened for this allele before linkage can be clearly established. This allele is being developed as a SCAR marker and the sequence obtained will be compared with those in DNA banks to look for homology.

The allele pd16 was found to be absent from all individuals of the genotype GPU-28. However, it was also absent in one individual each of genotype VL-324 and R^+. Further investigation of these genotypes would be necessary before this could be used as a fingerprint for GPU-28. Thus primer RPI #9, which helped in amplifying pa16 and pd16 alleles mentioned above, proved to be highly informative.

No single primer could discriminate all 11 germplasms. However, the band pattern of a combination of different primers offers a powerful method to discriminate them. There are many successful examples for using RAPD markers to discriminate germplasms at both intra and inter-specific levels. The DNA fingerprinting analysis also provides a good method for the discrimination of germplasms at the interspecific level (Jia et al., 2000;

Table 4. Nei's analysis of genetic distance and genetic similarity of finger millet germplasm.

pop ID	1	2	3	4	5	6	7	8	9	10	11	12
1	****	0.9237	0.9594	0.9215	0.9728	0.9547	0.9724	0.9757	0.8946	0.9626	0.9440	0.9560
2	0.0794	****	0.9250	0.9415	0.9023	0.9075	0.8934	0.8956	0.8802	0.9186	0.8993	0.8934
3	0.0415	0.0779	****	0.9092	0.9514	0.9303	0.9452	0.9692	0.9241	0.9793	0.9542	0.9556
4	0.0818	0.0603	0.0952	****	0.9270	0.9395	0.9133	0.9000	0.9235	0.9071	0.9378	0.9157
5	0.0275	0.1028	0.0499	0.0759	****	0.9702	0.9850	0.9781	0.9126	0.9716	0.9552	0.9585
6	0.0464	0.0971	0.0722	0.0624	0.0302	****	0.9744	0.9649	0.9252	0.9463	0.9384	0.9317
7	0.0280	0.1127	0.0563	0.0907	0.0151	0.0259	****	0.9915	0.9011	0.9631	0.9533	0.9542
8	0.0246	0.1103	0.0313	0.1054	0.0222	0.0357	0.0085	****	0.9014	0.9746	0.9521	0.9615
9	0.1114	0.1277	0.0789	0.0796	0.0914	0.0777	0.1041	0.1038	****	0.9138	0.9453	0.9255
10	0.0382	0.0849	0.0209	0.0975	0.0288	0.0552	0.0376	0.0257	0.0901	****	0.9617	0.9652
11	0.0576	0.1062	0.0468	0.0642	0.0458	0.0636	0.0479	0.0491	0.0563	0.0391	****	0.9893
12	0.0450	0.1127	0.0454	0.0881	0.0424	0.0708	0.0469	0.0393	0.0774	0.0354	0.0108	****

Germplasms: 1- VR-708; 2- BM-1; 3-A404; 4- VL-324; 5- R+; 6- GPU-28; 7- OUAT-2; 8- JWM-1; 9- VL-315; 10- BM-2; 11-VL-149 (ALMORA); 12- VL-149 (RANCHI).

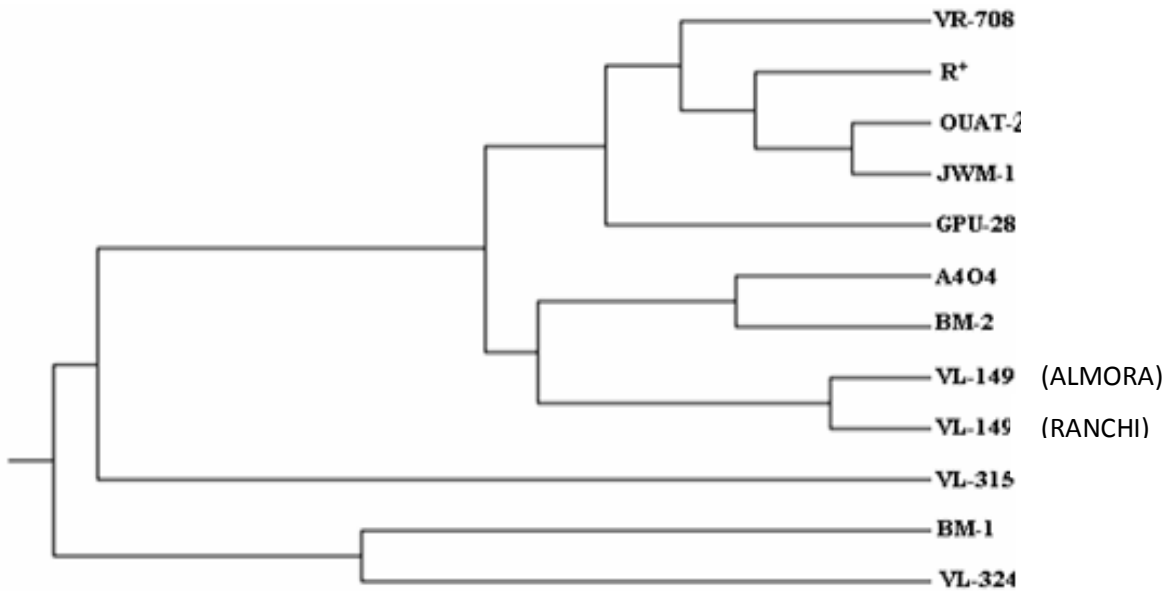

Figure 5. Dendrogram of finger millet germplasm based on Nei's analysis of genetic distance and genetic similarity.

Conner and Wood, 2001). Chen and Yamaguchi, (2005) performed multiplex PCR (using four primers) and succeeded in discriminating 24 tea germplasms in one step. Similarly a combination of primers T10S6, T20S4, C3 and RPI#9 can be used to discriminate between the genotypes VL-315, VL-324, BM-2, VL-149 and GPU-28 in a single step.

Nei's analysis (1978) of genetic distance and identity was used to study the genetic distance between the populations. The highest genetic similarity exists between the white germplasms of finger millet, that is, OUAT-2 and JWM-1 (Table 4). Inspite of their different sources of collection, these two germplasms fall under one sub-group. The lowest similarity was observed between BM-1 and VL-315. In plant breeding programmes, the most diverse genotypes are used in the hybridization programme. According to the dendrogram (Figure 5) based on Nei's analysis (1978), the whole population was divided into two groups. The smaller subgroup comprises two germplasms VL-324 and BM-1. The other subgroup contains the rest of the germplasms. This group consists

of the populations VR-708, R^+, OUAT-2, JWM-1, GPU-28, BM-2, VL-149 and VL-315. Close relatedness was observed between JWM-1, OUAT-2, R^+ and VR-708. OUAT-2 and JWM-1 are white germplasms of finger millet and maximum closeness was observed between them. The germplasm VL-149 taken from two different places falls under the same group.

We conclude that RAPD analysis can be extended to additional finger millet germplasm, especially those with unknown pedigree information. It would be possible to obtain information of their genetic relationship using this technique which could then help design breeding strategies. Since finger millet consumption is limited to indigenous populations, the local farmers have developed the germplasms over decades but these are not documented. Fingerprinting would help in identification and thus protection of intellectual property of these farmers.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the financial assistance in the form of fellowship provided by Birsa Agricultural University to Kanchan Kumari. This work was carried out at the College of Biotechnology, Birsa Agricultural University, Ranchi, Jharkhand, India.

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