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A comparative assessment of the utility of PCR-based marker systems in pearl millet

Mahendar Thudi · Senapathi Senthilvel · Andrew Bottley · C. Tom Hash · Arjula R. Reddy · Alex F. Feltus · Andrew H. Paterson · David A. Hoisington · Rajeev K. Varshney

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Abstract A set of 22 pearl millet inbred lines including the parents of eleven mapping populations, was screened with 627 markers including 100 pearl millet genomic SSRs (gSSRs), 60 pearl millet EST-SSRs (eSSRs), 410 intron sequence haplotypes (ISHs), and 57 exon sequence haplotypes (ESHs). In all, 267 (59%) of the markers were informative for at least one of the 11 mapping populations, which segregate for traits like drought and salinity tolerance; host plant resistance to downy mildew, rust and blast; fertility restoration and sterility and maintenance of cytoplasmic male sterility etc. An average of 116 polymorphic markers was identified per mapping population. The average PIC values and number of profiles (P) per polymorphic marker were: gSSRs (PIC = 0.62, P = 6.1), ISHs (PIC = 0.39, P = 2.6), eSSRs (PIC = 0.36, P = 3.1) and ESHs (PIC = 0.35, P = 3.1). A high correlation ($r > 0.97, P < 0.05$) was observed between the patterns of diversity exposed by the different marker systems. The polymorphic markers identified are suitable for the *de novo* construction, or the supplementation of pearl millet linkage maps. The genetic relationships identified among the panel of inbred lines may be useful in designing strategies to improve the use of available genetic variation in the context of pearl millet breeding.

Keywords Expressed sequence tags · Marker polymorphism · Pearl millet · Polymorphism information content (PIC)

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M. Thudi · S. Senthilvel · C. Tom Hash · D. A. Hoisington · R. K. Varshney
Center of Excellence in Genomics (CEG), International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Hyderabad 502324, AP, India

M. Thudi · A. R. Reddy
School of Life Sciences, University of Hyderabad, Hyderabad 500046, AP, India

A. Bottley
University of Nottingham, University Park, Nottingham NG7 2RD, UK

A. F. Feltus
College of Agriculture, Forestry and Life Sciences, Clemson University, Clemson, SC 29634, USA

A. H. Paterson
Center for Applied Genetic Technologies, University of Georgia, Athens, GA 30602, USA

R. K. Varshney (✉)
Genomics Towards Gene Discovery Sub Programme, Generation Challenge Programme, c/o CIMMYT, Int APDO Postal 6-641, 06600 Mexico, DF, Mexico
e-mail: r.k.varshney@cgiar.org

Introduction

Pearl millet (*Pennisetum glaucum* (L.) R. Br.) produces staple grain for about 90 million people living in the world's hottest, driest rain-fed crop/livestock production systems. It is widely grown as a multi-purpose cereal grain crop for feed, fodder, fuel and mulch over more than 26 million hectares predominantly in the semi-arid tropics of sub-Saharan Africa and the Indian subcontinent (FAO and ICRISAT 1996). Its grain protein content, concentration of essential amino acids and calorific content are all superior to those of maize (Davis et al. 2003). Despite being a hardy crop for dryland areas, the grain and stover production potential of pearl millet is limited by several biotic (fungal, parasitic and insect) and abiotic (high temperature, drought, and soil salinity, acidity, and infertility) stresses (Bidinger and Hash 2004).

Molecular markers are already having an impact on pearl millet improvement (Hash and Witcombe 2001). Restriction fragment length polymorphism (RFLP) markers were developed some time ago (Liu et al. 1992), but have been superseded by PCR-based markers, including amplified fragment length polymorphism (AFLP) (vom Brocke et al. 2003) and microsatellites or simple sequence repeats (SSRs) (Kapila et al. 2008). SSR markers developed from the sequencing of both genomic DNA (gSSRs) and cDNA (eSSRs) libraries are available in pearl millet (Senthilvel et al. 2004; Senthilvel et al. 2008; Mariac et al. 2006; Yadav et al. 2007). With the development of high throughput DNA sequencing (Varshney et al. 2009), SSRs are now increasingly seen as less interesting (for various reasons) than single nucleotide polymorphisms (SNPs). There are several genotyping platforms for direct SNP detection, but these are still rather capital intensive; in the meanwhile SSCP gives the capability of indirect SNP detection, and has been used to define haplotype variation in both introns (Feltus et al. 2006; Bertin et al. 2005) and exons (Bottley et al. 2006). The variation in haplotypes present in introns and exons has been referred to as intron sequence haplotype (ISHs) and exon sequence haplotype (ESHs), respectively. Since expressed sequence is well conserved across the grasses, many cross species primer pairs, developed from exonic sequences have proven to be functional in pearl millet (Thudi et al. 2007). The present study was undertaken to examine the comparative utility of above mentioned marker systems (gSSRs, eSSRs, ISHs and ESHs) using a set of 22 genetically diverse pearl millet inbred genotypes. As the germplasm set includes parental genotypes of 11 mapping populations, the study also provides marker polymorphism information for populations which may be exploited to improve the saturation of existing linkage maps.

Materials and methods

Plant material and, DNA isolation

DNA was extracted following Mace et al. (2003) from a set of 22 inbred lines i.e. H 77/833-2, PRLT 2/89-33, ICMB 841-P3, 863B-P2, Tift $23D_2B_1$-P5, WSIL-P8, PT 732B-P2, P1449-2-P1, LGD 1-B-10, ICMP 85410-P7, 81B-P6, ICMP 451-P8, ICMP 451-P6, H 77/833-2-P5(NT), W 504-1-P1, P310-17-Bk, IP 18293-P152, Tift $238D_1$-P158, ICMB 89111-P6, ICMB 90111-P6, IPC 804 and 81B-P8. The pedigree information and the salient features of these lines are provided in Senthilvel et al. (2008). A total of 627 markers including 100 pearl millet genomic simple sequence repeats (gSSRs; Qi et al. 2004), 60 pearl millet expressed sequence tag (EST)-SSRs (eSSRs; Senthilvel et al. 2004), 100 pearl millet SSCP–SNPs (Bertin et al. 2005), 57 wheat SSCP–SNPs (Bottley et al. 2006), and 310 conserved intron spanning primers (CISPs; Feltus et al. 2006) were screened against these 22 inbred lines. As SNPs are well-defined as a single base difference in sequence and the polymorphisms observed in the current study are both due to sequence variation and indels, based on origin of polymorphism, we have chosen to refer to CISPs and SSCP–SNPs as "intron sequence haplotypes" (ISHs) and the wheat SSCP–SNPs as "exon sequence haplotypes" (ESHs) in the present study. Therefore data generated by CISP and SSCP–SNP primer pairs were analyzed together under ISHs class.

PCR amplification

For all the marker types, except for the wheat SSCP-SNPs (considered as ESHs in this article), PCRs were performed in a 5 µl volume containing 5 ng DNA, 1 pmol of each primer, 2 mM dNTP, 10 mM Mg^{2+}, 0.1U *Taq* DNA polymerase (Bioline, USA) and

0.5 µl 10 × PCR buffer (Bioline, USA). A touchdown amplification cycle was adopted for all reactions (except those based on exon sequence haplotype primers), consisting of a 3 min denaturation at 95°C, followed by five cycles of 94°C/20 s, 61°C/20 s (decreasing by 1°C per cycle) and 72°C/30 s, then 35 cycles of 94°C/20 s, 56°C/20 s and 72°C/30 s, and ending with a 20 min incubation at 72°C. For 60 of the fluorescent dye labelled genomic SSR primers, 30 (instead of 35) amplification cycles were imposed. For PCRs using the wheat SSCP–SNP primers, the reaction volume was 10 µl, made up of 5 ng DNA, 5 pmol of each primer, 2 mM dNTP, 10 mM Mg^{2+}, 0.1U *Taq* DNA polymerase (Bioline, USA) and 1 µl 10 × PCR buffer (Bioline, USA); and the amplification profile was 94°C/5 min, followed 34 cycles of 94°C/30 s, 59°C/60 s and 72°C/60 s, and ending with an incubation of 72°C for 5 min (Bottley et al. 2006).

Fragment analysis

Unlabelled SSR amplicons were separated by 6% w/v polyacrylamide gel electrophoresis (PAGE) and visualized by silver staining (Tegelstrom 1992). Labelled amplicons were separated on an ABI 3100 Genetic Analyzer. The amplicons of both ISHs and ESHs were denatured and separated using mutation detection enhancement (MDE) gel electrophoresis on 310 × 380 × 0.4 mm gels containing 24% v/v MDE monomer (Cambrex Bio-science Rockland, Rockland, ME, USA) for 16 h at 200 V at room temperature, before being silver stained as described by (Tegelstrom 1992).

Data analysis

The unlabelled markers (40 gSSR, all 410 ISH and all 57 ESHs) were scored as continuous variables, using "a" to indicate the first band, "b" the second band and so on. For the fluorescent-dye labelled gSSR markers (60), allelic data were collected in the form of numbers of base pairs, and these were binned using in house software called "AlleloBin" which automates the process of assigning allele size into an appropriate allele "bin". The allelic data was further converted to continuous variables as mentioned above. Profile data were used to calculate the polymorphism information content (PIC) as suggested by Botstein et al. (1980).

The combined continuous variable dataset from all the marker systems was used to calculate Manhattans distance (Sneath and Sokal 1973) and resulting Manhattan similarity distance matrix was used to construct a neighbor joining (NJ) dendrogram employing DARWin 5 (Perrier et al. 2003). Support for clusters was estimated by bootstrap analysis using DARwin 5 (Perrier et al. 2003). The relationship between Manhattan similarity distance matrices was given by the product-moment correlation coefficient (Mantel 1967) with a *t*-testing performed to assign statistical significance to each correlation, based on 1000 random permutations.

Results and discussion

Recent advances in high-throughput genotyping have enabled the cost-effective and routine use of molecular markers to characterize germplasm (Varshney et al. 2006). Several marker genotyping platforms are currently available for SSR and SNP markers. The present study employed PAGE for unlabelled gSSR markers, capillary electrophoresis for labeled gSSR and eSSR markers and MDE gel electrophoresis for ISHs and ESHs (CISP and SSCP-SNP markers). Estimates of genetic diversity can be biased both by the choice of marker system and statistical methodology. Here, we have assessed the comparative utility of various PCR-based marker systems for pearl millet germplasm analysis.

Marker polymorphism

Of the 627 primer pairs screened, 455 (73%) yielded a scorable amplicon. The highest success rate was achieved with gSSRs (82%) and the poorest rate was for the ESHs (44%). The most informative marker type were the eSSR markers (73% of which showed at least one polymorphism across the 22 accessions) while ESHs were least informative (25%; Table 1). However, the eSSR primer pairs were not associated with a high number of profiles, so their mean PIC value was low. As eSSR markers are developed from coding sequence, which is relatively well conserved, polymorphism is typically limited (Varshney et al. 2005). The ESHs primers also target coding sequence, and so are similarly expected to be rather non-informative. In all, 45% of the gSSR and 40% of

Table 1 Summary on marker polymorphism and marker informativeness attributes

Marker attributes	Marker systems			
	gSSRs[a]	eSSRs[b]	ISHs[c]	ESHs[d]
Number of markers screened	100	60	410	57
Number of markers amplified	82	45	303	25
Polymorphic with one or the other populations	45	44	164	14
Percent marker polymorphism	45.0	73.3	40	24.6
Profile number range	2–14	2–8	2–4	2–4
Average number of profiles	6.1	3.1	2.6	3.1
Total number of profiles	273	136	436	44
PIC value range	0.08–0.91	0.08–0.84	0.09–0.50	0.17–0.49
Average PIC value	0.62	0.36	0.39	0.35
Genetic similarity range	0.23–0.82	0.46–0.92	0.58–0.84	0.33–0.97
Average genetic similarity	0.31	0.64	0.72	0.62

[a] gSSRs, genomic SSRs

[b] eSSRs, EST-SSRs

[c] ISHs, intron sequence haplotypes

[d] ESHs, exon sequence haplotypes

the ISHs were informative, and this high proportion reflects the multi-allelic nature of the former, and the targeting of variable intronic DNA by the latter.

Consequences of differential marker informativeness

The differential informativeness of particular marker systems has implications for their suitability for germplasm analysis and maintenance. Marker informativeness can be assessed by a number of different criteria, including the number of profiles present, allele frequency and PIC, which are presented in Table 1 and ESM 1.

The total number of profiles ranged from 44 (ESHs) to 436 (ISHs). The ranges of profiles detected by each marker system were 2–14, 2–8, 2–4, and 2–4, for the informative gSSRs, eSSRs, ISHs, and ESHs, respectively (Table 1). The average number of profiles per polymorphic marker ranged from 2.6 (ISHs) to 6.1 (gSSRs). The range in profile number (2–14) and the average number of profiles (6.1) detected by the gSSRs were higher than those reported both by Budak et al. (2003) (2–9 profiles across 15 inbred lines) and Chandra-Shekara et al. (2007) (2.62 profiles/locus; 21 genotypes). In contrast, Mariac et al. (2006) reported 2–18 profiles (6.8 profiles per locus) for 15 informative eSSRs screened against a sample of 32 plants from a collection of 46 wild accessions and 421 of highly heterozygous, cross-pollinated African landraces. The reason for these differences, if statistically significant, presumably relates to the quantity and/or type of germplasm sampled and the nature of the markers used.

Polymorphism information content (PIC)

The gSSRs were associated with the highest average PIC value (0.62), followed by the ISHs (0.39), eSSRs (0.36) and ESHs (0.35) (Table 1). The PIC value calculated for the gSSRs and ISHs were in line with what others have shown (Feltus et al. 2006; Mariac et al. 2006; Chandra-Shekara et al. 2007).

Marker polymorphism among the parents of the mapping populations

About 59% (267 out of 455) of the markers were informative in at least one of the populations (Table 1). On a per population basis, the number of polymorphic markers ranged from 103 to 122 (mean 116; Table 2). The (ICMB 841-P3 × 863B-P2)-based population had the highest number of informative markers, while that based on (Tift $23D_2B_1$-P5 × WSIL-P8) had the least. The international pearl millet reference mapping population based on (81B-P6 × ICMP 451-P8) has

Table 2 Marker polymorphism in parental combinations of eleven pearl millet mapping populations

S. no.	Name of mapping population	gSSRs[a]	eSSRs[b]	ISHs[c]	ESHs[d]	Total polymorphic markers	% polymorphism
1	ICMB 841-P3 × 863B-P2	27	18	72	5	122	26.81
2	W 504-1-P1 × P310-17-Bk	39	11	67	2	119	26.15
3	IPC 804 × 81B-P8	40	17	57	4	118	25.93
4	LGD 1-B-10 × ICMP 85410-P7	39	14	62	3	118	25.93
5	81B-P6 × ICMP 451-P8	35	15	63	5	118	25.93
6	ICMP 451-P6 × H 77/833-2-P5(NT)	33	15	67	3	118	25.93
7	PT 732B-P2 × P1449-2-P1	30	18	65	3	116	25.49
8	IP 18293-P152 × Tift $238D_1$-P158	37	19	58	2	116	25.49
9	ICMB 89111-P6 × ICMB 90111-P6	36	23	55	2	116	25.49
10	H 77/833-2 × PRLT 2/89-33	30	20	59	3	112	24.62
11	Tift $23D_2B_1$-P5 × WSIL-P8	31	16	54	2	103	22.64

[a] gSSRs, genomic SSRs

[b] eSSRs, EST-SSRs

[c] ISHs, intron sequence haplotypes

[d] ESHs, exon sequence haplotypes

been used to generate a 198 point genetic map (Qi et al. 2004), and some of the 118 polymorphic markers identified in the present study can be readily incorporated to enhance the density of this genetic map. Sets of independent maps are particularly useful in trait mapping, as it is uncommon to for all traits of interest to segregate within a single mapping population. The integration of independently constructed maps is only feasible where a common subset of markers is present (Varshney et al. 2007b). The present study provides 60 common polymorphic markers across two parental genotype combinations (H 77/833-2 × PRLT 2/89-33 and ICMB 841-P3 × 863B-P2), 29 among three parental combinations (H 77/833-2 × PRLT 2/89-33, ICMB 841-P3 × 863B-P2 and LGD 1-B-10 × ICMP 85410-P7) and 26 among four parental combinations (H 77/833-2 × PRLT 2/89-33, ICMB 841-P3 × 863B-P2, LGD 1-B-10 × ICMP 85410-P7 and 81B-P6 × ICMP 451-P8). The gSSRs provided the highest number of such common markers (ESM 2).

Genetic similarity and cluster analysis

The Mantel test was applied to compare the diversity patterns exposed by the various marker systems (Table 3). The diversity patterns detected by these marker systems were all highly inter-correlated, indicating that these genotypic data can legitimately be pooled for further analysis. In other species, EST-derived markers have been shown to be highly correlated eg., barley (Kota et al. 2001; Varshney et al. 2008) and rye (Varshney et al. 2007a; Khlestkina et al. 2004). The average genetic similarity detected between pairs of inbred lines was consistently around 0.7 for the gene-associated markers (ISHs 0.72, eSSRs 0.64, ESHs 0.62), but only 0.31 for the gSSRs (Table 1).

Table 3 Cophenetic correlation coefficients among marker systems

Marker systems	Marker systems			
	gSSRs[a]	eSSRs[b]	ISHs[c]	ESHs[d]
gSSRs	1			
eSSRs	0.92	1		
ISHs	0.89	0.98	1	
ESHs	0.90	0.97	0.97	1

[a] gSSRs, genomic SSRs

[b] eSSRs, EST-SSRs

[c] ISHs, intron sequence haplotypes

[d] ESHs, exon sequence haplotypes

A Neighbour Joining based phylogeny was constructed based on the combined genotypic data set (Fig 1). Although the phylogenies derived from the individual marker data sets varied slightly from one

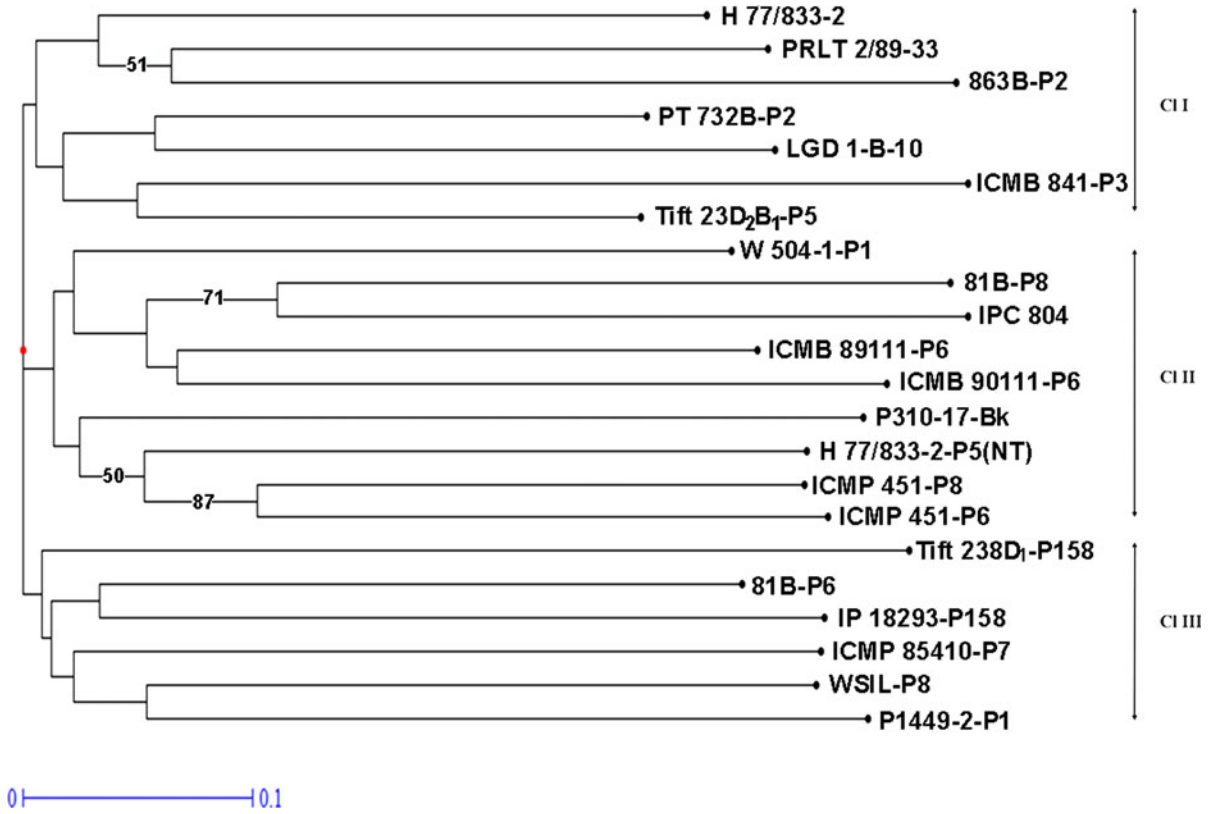

Fig. 1 Neighbour Joining dendrogram of 22 pearl millet inbred lines constructed using a Manhattan's similarity distance matrix obtained from profile data generated using all marker systems (eSSRs, gSSRs, ISHs and ESHs), showing three distinct clusters

another, this was thought to reflect sampling error resulting from small (marker) sample size, particularly for the exon sequence haplotypes. The 22 inbred lines were grouped into three clusters, I, II and III with seven, nine and six inbred lines, respectively. Moderate to high bootstrap values (51–87) were observed in case of inbred lines that grouped in Cluster I and Cluster II. Of seven inbred grouped in Cluster I, five were derived from landraces, with PRLT 2/89-33, 863B-P2, and LGD 1-B-10 all derived from the Iniadi landrace from Togo and Ghana, PT 732B-P2 reportedly derived from a spontaneous dwarf mutant that appeared in a landrace from Andhra Pradesh, and H 77/833-2 reportedly derived from a Rajasthani landrace population (Fig 1). However, the implied genetic relationship between H 77/833-2 and PT 732B-P2, and these two inbreds of Indian landrace origin with each other and with the three *Iniadi*-derived inbreds were completely unexpected and remain unexplained. The other two inbreds grouped in Cluster I, genetically tall ICMB 841-P3 and d_2 dwarf Tift $23D_2B_1$-P5, are expected to cluster together as they share genetically tall Tift $23B_1$ as a common ancestor. However, the two subselections of 81B, namely 81B-P6 and 81B-P8, were expected to cluster with these as 81B is a product of an induced mutation breeding program based on Tift $23D_2B_1$. The association of PT 732B-P2 with Tift $23D_2B_1$-P5 is likely due to outcrossing of the original landrace accession from which PT 732B was derived with Tift $23D_2B_1$, as the dwarfing genes of the two lines are allelic. Among nine members of Cluster II, IPC 804 is a tall, long-bristled triple-restorer of the A_1, A_4 and A_{egp} cytoplasmic male-sterility (CMS) systems, with profuse pollen producing capacity, while ICMP 451-P6 and ICMP 451-P8 are subselections of phenotypically similar elite A_1 and A_4 restorer LCSN 72-1-2-1-1; 81B-P8 and ICMB 89111-P6 are d_2 dwarf maintainer lines of the A_1, A_4 and A_{egp} CMS systems, and ICMB 90111-P6 is a tall weak restorer of A_1 and maintainer of A_{egp}. Two inbreds in this cluster are downy mildew resistance

donor lines (ICMB 90111-P6 and P310-17-Bk), and two are highly susceptible (H 77/833-2-P5(NT) and W 504-1-P1). Cluster III included three downy mildew resistance donor lines (IP 18293-P152, P1449-2-P1 and WSIL-P8) and two downy mildew susceptible lines (81B-P6 and Tift $238D_1$). It also includes d_1 dwarf restorer line (Tift $238D_1$), three d_2 dwarf lines (maintainer 81B-P6, restorer ICMP 85410-P7, IP 18293-P152, and WSIL-P8), and genetically tall line P1449-2-P1.

The clustering of both downy mildew resistant with susceptible lines, of tall and dwarf lines, and CMS sterility maintainers with fertility restorers indicates that while members of a cluster may be genetically more similar to one another than to those in another cluster, the genetic differences underlying economically important traits have not contributed to the marker-based phylogeny. The genetic basis for this discordance reflects the fact that these traits are likely controlled by few genes, and thus their influence is swamped by that of the rest of the genome. In conclusion, we have assessed here the relative informativeness of gSSRs, eSSRs, ISHs and ESHs. Each marker type provided polymorphic markers in the germplasm analyzed. These are suitable for the de novo construction, or the supplementation of pearl millet linkage maps. The most informative marker type was the gSSRs. We have identified sets of >100 informative markers for each of 11 mapping populations. The genetic relationships identified among the panel of inbred lines may be useful in designing strategies to improve the use of available genetic variation in the context of pearl millet breeding.

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References

Bertin I, Zhu JH, Gale MD (2005) SSCP-SNP in pearl millet—a new marker system for comparative genetics. Theor Appl Genet 110:1467–1472

Bidinger FR, Hash CT (2004) Pearl millet. In: Nguyen HT, Blum A (eds) Physiology and biotechnology integration for plant breeding. Marcel Dekker, New York, pp 225–270

Botstein D, White RL, Skolnick M, Davis RW (1980) Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am J Hum Genet 32: 314–331

Bottley A, Xia GM, Koebner RMD (2006) Homoeologous gene silencing in hexaploid wheat. Plant J 47:897–906

Budak H, Pedraza F, Cregan PB, Baenzinger PS, Dweikat I (2003) Development and utilization of SSRs to estimate the degree of genetic relationships in a collection of pearl millet germplasm. Crop Sci 43:2284–2290

Chandra-Shekara AC, Prasanna BM, Bhat SR, Singh BB (2007) Genetic diversity analysis of elite pearl millet inbred lines using RAPD and SSR markers. J Plant Biochem Biotechnol 16:23–28

Davis AJ, Dale NM, Ferreira FJ (2003) Pearl millet as an alternative feed ingredient in broiler diets. J Appl Poult Res 12:137–144

FAO and ICRISAT (1996) The world sorghum and millet economies: facts, trends and outlook. Food and Agriculture Organization of the United Nations (FAO): Rome, Italy, and International Crops Research Institute for the Semi-Arid Tropics (ICRISAT). Patancheru, India, p 72

Feltus FA, Singh HP, Lohithaswa HC, Schulze SR, Silva TD, Paterson AH (2006) A comparative genomics strategy for targeted discovery of single-nucleotide polymorphisms and conserved-noncoding sequences in orphan crops. Plant Physiol 140:1183–1191

Hash CT, Witcombe JR (2001) Pearl millet molecular marker research. Intl Sorghum Millets Newsl 42:8–15

Kapila RK, Yadav RS, Plaha P, Rai KN, Yadav OP, Hash CT, Howarth CJ (2008) Analysis of genetic diversity in pearl millet inbreds using microsatellite markers. Plant Breed 127:33–37

Khlestkina EK, Than MHM, Pestsova EG, Röder MS, Malyshev SV, Khorzum V, Börner A (2004) Mapping of 99 new microsatellite-derived loci in rye (*Secale cereale* L.) including 39 expressed sequence tags. Theor Appl Genet 109:725–732

Kota R, Varshney RK, Thiel T, Dehmer KJ, Graner A (2001) Generation and comparison of EST derived SSR and SNP markers in barley (*Hordeum vulgare* L.). Hereditas 135:141–151

Liu CJ, Witcombe JR, Pittaway TS, Nash M, Hash CT, Gale MD (1992) Restriction fragment length polymorphism in pearl millet, *Pennisetum glaucum*. In: Mounolou JC, Sarr A (eds) Complexes d'espèces, flux de gènes et resources génétiques des plantes. Colloque international, Publications du Bureau des Ressources Génétiques, 8–10 janvier, Paris, pp 233–241

Mace ES, Buhariwalla HK, Crouch JH (2003) A high throughput DNA extraction protocol for tropical molecular breeding programs. Plant Mol Biol Rep 21:459a–459h

Mantel N (1967) The detection of disease clustering and a generalized regression approach. Can Res 27:209–220

Mariac C, Luong V, Kapran I, Mamadou A, Sagnard F, Deu M, Chantereau J, Gerard B, Ndjeunga J, Bezancon G, Pham J-L, Vigouroux Y (2006) Diversity of wild and cultivated pearl millet accessions [*Pennisetum glaucum* (L.) R. Br.]

in Niger assessed by microsatellite markers. Theor Appl Genet 114:49–58

Perrier X, Flori A, Bonnot F (2003) Data analysis methods In: Hamon P, Seguin M, Perrier X, Glaszmann JC (eds) Genetic diversity of cultivated tropical plants. CIRAD/science, Montpellier, France, pp 43–76

Qi X, Pittaway TS, Lindup S, Liu H, Waterman E, Padi FK, Hash CT, Zhu J, Gale MD, Devos KM (2004) An integrated genetic map and a new set of simple sequence repeat markers for pearl millet, *Pennisetum glaucum*. Theor Appl Genet 109:1485–1493

Senthilvel S, Mahalakshmi V, Sathish Kumar P, Reddy AR, Markandeya G, Reddy MK, Misra R, Hash CT (2004) New SSR markers for pearl millet from data mining of expressed sequence tags. In: Proceedings of 4th international crop sci congress, 26th Sept to 1st Oct 2004, Brisbane, Australia, p 232 (http://www.cropscience.org.au/icsc2004/poster/3/1/1223_hashct.htm)

Senthilvel S, Jayashree B, Mahalakshmi V, Sathish Kumar P, Nakka S, Nepolean T, Hash CT (2008) Development and mapping of simple sequence repeat markers for pearl millet from data mining of expressed sequence tags. BMC Plant Biol 8:119

Sneath PHA, Sokal RR (1973) Numerical taxonomy: the principles and practice of numerical classification. Freeman, SanFrancisco, p 573

Tegelstrom H (1992) Detection of mitochondrial DNA fragments. In: Hoelzel AR (ed) Molecular genetic analysis of populations: a practical approach. IRL Press, Oxford, pp 89–114

Thudi M, Hash CT, Senthilvel S, Bottley A, Koebner RMD, Paterson AH, Reddy AR, Hoisington DA, Varshney RK (2007) Polymorphism assessment of SSR, SNP and CISP marker systems in pearl millet [*Pennisetum glaucum* (L.) R. Br.]. In: Plant and animal genomes XV conference, 13–17th Jan 2007, San Diego, California (http://www.intlpag.org/15/abstracts/PAG15_P05d_373.html)

Varshney RK, Graner A, Sorrells ME (2005) Genic microsatellite markers in plants: features and applications. Trends Biotech 23:48–55

Varshney RK, Hoisington DA, Tyagi AK (2006) Advances in cereal genomics and applications in crop breeding. Trends Biotech 24:490–499

Varshney RK, Beier U, Khlestkina E, Korzun V, Graner A, Börner A (2007a) Single nucleotide polymorphisms in rye (*Secale cereale* L.): discovery, frequency and applications for genome mapping and diversity studies. Theor Appl Genet 114:1105–1116

Varshney RK, Marcel TC, Ramsay L, Russell J, Röder MS, Stein N, Waugh R, Langridge P, Niks RE, Graner A (2007b) A high density barley microsatellite consensus map with 775 SSR loci. Theor Appl Genet 114:1091–1103

Varshney RK, Thiel T, Sretenovic-Rajicic T, Baum M, Valkoun J, Guo P, Grando S, Ceccarelli S, Graner A (2008) Identification and validation of a core set of informative genic SSR and SNP markers for assaying functional diversity in barley. Mol Breed 22:1–13

Varshney RK, Nayak SN, May GD, Jackson SA (2009) Next-generation sequencing technologies and their implications for crop genetics and breeding. Trends Biotech 27:522–530

vom Brocke K, Christinck A, Weltzien RE, Presterl T, Geiger HH (2003) Farmers' seed system management practices determine pearl millet genetic diversity patterns in semi-arid regions of India. Crop Sci 43:1680–1689

Yadav OP, Mitchell SE, Zamora A, Fulton TM, Kresovich S (2007) Development of new simple sequence repeat markers for pearl millet. SAT eJournal 3(1). http://www.icrisat.org/Journal/mpii/v3i1/Sorgu_Millet%20_other_Cereals/ISMN%2022_Development%20of%20new…%20(OPYadav%20et%20al.).pdf