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SSR allele frequency changes in response to recurrent selection for pearl millet grain yield and other agronomic traits

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Abstract

Allele frequency changes resulting from conventional recurrent selection were monitored at 34 SSR loci distributed across all 7 linkage groups using full-sib progenies (50 each) from released pearl millet open-pollinated variety CO (Cu) 9 and its four immediate progenitor populations (ICMP 87750, ICMP 91751, ICMP 93752 and UCC 23). Agronomic performance of these 250 full-sibs was assessed in two seasons of replicated field trials at ICRISAT, Patancheru, India, which demonstrated significant selection responses for flowering time and grain, stover and biomass yields, and their components. These selection responses were accompanied by significant, but non-linear, frequency changes for common alleles at many monitored SSR loci. Substantial genetic variability remains within CO (Cu) 9, suggesting further selection to improve its grain and stover yield performance should be effective.

Introduction

Dual-purpose, outcrossing food grain and fodder crop pearl millet (*Pennisetum glaucum*) provides the most reliable source of staple carbohydrates for millions of resource-poor subsistence farm families. However, pearl millet productivity is often low compared to staple cereals of more favorable environments as this crop is mostly grown on marginal lands in the hottest and driest of the world's agricultural environments. It is better adapted than other cereals to low fertility soils that receive minimal input applications. Recurrent selection approaches, similar to those used for maize (*Zea mays*) (which like pearl millet is highly cross-pollinated), have resulted in significant improvement of pearl millet grain and stover yields.

For long-term genetic improvement of pearl millet populations by continuous recurrent selection, enhancement of population mean performance must be accompanied by maintenance of genetic variability at trait-governing alleles. Large amounts of genetic variability can be created and maintained by repeated intermating during recurrent selection. It is presumed that recurrent selection increases the frequency of favorable alleles and decreases the frequency of those unfavorable for the trait(s) under selection (Hallauer 1985). Outcrossing and genetic drift during seed multiplication and generation advance also contributes to allele shifts. Rai et al. (1999) indicated that genetic shifts can occur in pearl millet when populations are grown in different environments, so that this crop can be fine-tuned for its adaptation to a specific growing environment.

Expression of quantitative traits such as grain and stover yields, and their components, is influenced by environment whereas DNA-based molecular markers are not. Therefore, molecular marker techniques offer advantages for monitoring shifts in allele frequencies in response to selection in pearl millet, as in other crops. Studies of shifts in allele frequencies at marker loci provide information on specific genomic regions that have responded to selection (Frascaroli and Landi 1998). Frequencies of marker alleles can also be used to study the effect of selection on genetic diversity of populations undergoing long-term recurrent selection. Recently, microsatellite markers or simple sequence repeat (SSR) markers have become the preferred marker technology for many plant breeding applications, because of their co-dominant inheritance, single-locus nature, and ease of automation. This study was conducted to quantify improvement in agronomic performance and shifts in SSR marker allele frequency that occurred over several generations of recurrent selection that led to the release of an improved pearl millet open-pollinated variety, and the effect of recurrent selection on diversity within the population, which is essential for its further genetic improvement.

Materials and methods

Commercially released pearl millet variety CO (Cu) 9 and four of its immediate progenitor populations (ICMP 87750, ICMP 91751, ICMV 93752 and UCC 23) were used as genetic materials for this study. ICMP 87750, ICMP 91751 and ICMV 93752 were bred at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India by phenotypic recurrent selection for higher yield and good agronomic performance including earliness and medium plant height. UCC 23 and CO (Cu) 9 were bred at Tamil Nadu Agricultural University (TNAU), Coimbatore, India by phenotypic recurrent selection within base population ICMV 93752 for higher grain and biomass yield. CO (Cu) 9 and its progenitor populations were synthesized as follows. At ICRISAT-Patancheru, ICMP 87750 (Initial cycle of Smut Resistant Composite II) was developed by random mating half-sibs generated by crossing genetically diverse Smut Resistant Composite and Inter-Varietal Composite plants. Full-sibs were then generated within ICMP 87750 and random mated to form ICMP 91751 (First cycle of Smut Resistant Composite II) after eliminating weaker full-sib progenies. ICMV 93752 was bred by random mating S_1 progenies from ICMP 91751 selection on the basis of higher grain yield and agronomic score at ICRISAT-Patancheru. At TNAU-Coimbatore, superior plant progenies from ICMV 93752 were selected on the basis of higher grain and biomass yield, and random mated to produce UCC 23. UCC 23 was then further refined for uniformity and high grain and biomass yield to synthesize CO (Cu) 9.

In total, 250 full-sib progenies (50 full-sibs each from the five populations) were generated by plant-to-plant crosses. The full-sibs were evaluated during the 2005 rainy season and the 2006 summer season at ICRISAT-Patancheru. Full-sibs were sown in alpha-lattice designs with 3 replications and 20 blocks per replication. Each block consisted of 13 full-sibs. Each full-sib was sown in a single-row plot. Interrow and intra-row distance was maintained at 60 cm and 20 cm, respectively. All management practices needed for good crop establishment and expression of phenotypic potential were followed during evaluation of the full-sibs. Observations were recorded on yield and phenological traits: flowering time (days), plant height (cm), panicle length (cm), panicle diameter (mm), effective tillers per plant, panicle yield (g m^{-2}), grain yield (g m^{-2}), panicle threshing percentage, stover dry matter yield (g m^{-2}), biomass yield (g m^{-2}), 1000-grain weight (g), panicle grain number and harvest index (%). Observations on plant height, panicle length and panicle diameter were recorded on five randomly selected plants while remaining traits were recorded on a whole plot basis and converted to per m^2 prior to analysis. Data analyses were performed using GenStat (version 8).

Genotyping of full-sib progenies was done by using genomic DNA isolated from two-week-old pot grown seedlings of each full-sib. DNA was extracted from pooled leaf tissues of at least 20 seedlings from each full-sib progeny using a modified CTAB/β-mercaptoethanol method (Mace et al. 2003). Polymerase chain reaction (PCR) conditions of circa 70 pearl millet SSR primer pairs available in public domain were optimized for the concentrations of primers, template DNA, Mg^{++}, dNTP, enzyme and annealing temperature. The PCR products were size-separated by capillary electrophoresis using an ABI Prism 3700 DNA analyzer (Applied Biosystems Inc.). For this purpose, forward primers were labeled with 6-FAM (blue), VIC (green), NED (yellow) or PET (red) fluorophores. SSR primer pairs were initially screened by using DNA pooled from diverse inbreds to identify pairs that reliably differentiate between allelic frequency ratios expected in full-sib progenies (ie, 0:0:0:1, 0:0:1:1, 0:0:3:1, 0:2:1:1 and 1:1:1:1). Generally, segregating populations such as backcross progenies, F_2 individuals or recombinant inbred lines are the product of crosses between two non-identical inbred lines, and in diploid species these segregate for only two alleles at any polymorphic locus. In contrast to this, a cross between two non-identical heterozygous plants of a diploid out-breeder produces progeny segregating for up to four alleles per locus and this number may vary between loci. If the two parents of a full-sib progeny were both heterozygous at a given locus and had four non-identical alleles (eg, a and b in parent 1 and c and d in parent 2), then the full-sib progeny would be comprised of ac, ad, bc and bd genotype individuals in equal frequencies and allelic frequencies of the four alleles in the full-sib progeny would all be 0.25 (1:1:1:1; the same as in the parents of this progeny). Similarly, if both parents were homozygous for this locus and had the same allele (d in this case), then their full-sib progeny would all have the same genotype with only the d allele for that locus and this would be recorded as 0:0:0:1.

Among the 70 SSR primer pairs, 34 SSR loci distributed across all 7 pearl millet linkage groups were selected based on preliminary screening and used for genotyping the 250 full-sib progenies. Marker allele frequency was calculated as the ratio of the sum of copies of particular SSR marker allele to the total number of full-sibs studied and divided by four [since each full-sib progeny was the product of four parental chromosome complements (two from each parent)], which is a modification of the formula described by de Vicente et al. (2004). Rare alleles were identified as those of frequency less than 5% among genotyped full-sib sets. Nei's unbiased estimation of genetic diversity (expected heterozygosity) was calculated for each SSR locus. Allele frequency-based genetic distance and cluster analysis was performed with PowerMarker (v 3.25) using the Neighbor-Joining method. Dendrograms of both phenotypic data and marker allele data clusters were constructed with DARwin (v 5.0).

Results and discussion

Agronomic traits. Increasing grain yield and yield stability has been the major breeding objective for genetically improving pearl millet. Increasing grain yield through recurrent selection should be feasible due to pearl millet's tremendous genetic variability (Rattunde et al. 1989). In the present study, 250 full-sib progenies generated from CO (Cu) 9 and its four progenitor populations and 10 check entries [CO (Cu) 9 bulk] were evaluated for grain and stover yield and related traits in alpha-lattice designs. Mean performance of the five populations over two seasons are presented in Table 1. For flowering time, there was not much variation between the base population (ICMP 87750) and improved population [CO (Cu) 9] despite two days earlier flowering in the mid-generation population (ICMV 93752). Earlier flowering time was recorded in the rainy season (54 days) than in the summer season (59 days). Despite the shorter photoperiod expected to induce earlier flowering, suboptimal temperatures during critical early stages of plant development perhaps delayed summer season flowering in this experiment. Plant height influences both stover dry matter and biomass yield. Population mean plant height revealed that advanced generation CO (Cu) 9 recorded higher plant height (7.8% in the rainy season and 4.6% in the summer season) than the mid-generation ICMV 93752. The trend of changes in flowering time and plant height from the initial to mid-generations indicates that populations initially responded

Table 1. Performance of pearl millet populations over two seasons, 2005 rainy season and 2006 summer season, ICRISAT, Patancheru, India.

Characters	Season[1]	ICMP 87750	ICMP 91751	ICMV 93752	UCC 23	CO (Cu) 9	SE±
Flowering time (days)	E1	54	52	52	56	55	0.69
	E2	59	58	57	60	59	0.47
	Mean	57	55	55	58	57	0.73
Plant height (cm)	E1	215	205	207	225	223	3.6
	E2	179	174	173	185	181	2.7
	Mean	197	190	191	204	201	4.0
Panicle length (cm)	E1	26.3	26.9	26.9	29.0	27.9	0.58
	E2	26.9	27.4	27.3	29.5	28.4	0.56
	Mean	26.7	27.2	27.2	29.1	28.1	0.77
Panicle diameter (mm)	E1	25.8	25.0	25.3	26.2	26.8	0.40
	E2	25.4	24.3	23.8	25.5	25.9	0.44
	Mean	25.6	24.8	24.7	25.8	26.2	0.55
Grain yield (g m^{-2})	E1	207	208	215	225	231	7.3
	E2	159	166	175	162	161	6.4
	Mean	188	190	194	193	195	7.7
Stover yield (g m^{-2})	E1	310	300	291	346	344	13.2
	E2	269	262	245	278	276	9.2
	Mean	291	285	274	308	306	13.6
Biomass yield (g m^{-2})	E1	594	586	583	651	657	20.1
	E2	504	507	501	513	516	14.3
	Mean	555	554	550	578	581	19.5
1000-grain weight (g)	E1	9.58	9.55	9.47	9.29	9.62	0.24
	E2	8.55	8.23	8.39	8.29	8.30	0.18
	Mean	9.05	8.91	8.94	8.83	8.97	0.26
Panicle grain number	E1	2004	1831	1891	2253	2194	97.74
	E2	2036	2026	1978	2233	2195	81.41
	Mean	2033	1955	1966	2218	2176	110.96
Harvest index (%)	E1	34.8	35.4	36.7	34.5	34.8	0.59
	E2	31.5	32.7	34.6	31.2	31.5	0.80
	Mean	33.4	34.0	35.1	33.2	33.4	0.80

1. E1 = 2005 rainy season; E2 = 2006 summer season.

to phenotypic recurrent selection at ICRISAT-Patancheru for medium plant height and earliness with higher grain yield, whereas subsequent selection cycles at TNAU-Coimbatore were effective for later flowering and moderately increased plant height while further increasing grain yield (at least under rainy season conditions). Panicle length and panicle diameter are important components of panicle yield. Population means revealed that advanced generation cycles had greater panicle length than previous cycle populations. About 6% improvement for panicle length was observed in CO (Cu) 9 and 10% improvement was in UCC 23 over the initial cycle ICMP 87750, indicating that panicle length was one of the targets for improvement in the recurrent selection program. Unlike panicle length, improvement of panicle diameter over the generations followed a trend similar to those of flowering time and plant height.

Population mean data showed that the advanced generation population CO (Cu) 9 recorded a mean grain yield of 231 g m^{-2} in the rainy season trial, which was a 11.6% improvement over the initial generation ICMP 87750 (207 g m^{-2}). However, during the summer season ICMV 93752 recorded higher grain yield than CO (Cu) 9. This was probably a result of higher temperatures and greater evaporative demand during grain filling resulting in moderate drought stress in the summer season trial, despite weekly irrigation, and this stress had more adverse effects on grain filling of later-flowering full-sib progenies of UCC 23 and CO (Cu) 9. Support for this supposition is provided by the data on 1000-grain weight, which indicate that grain filling – especially of the later-flowering progenies – was compromised in the summer season trial. Mean stover and biomass yields showed that initial and advanced cycles recorded more stover and biomass yields than the mid-generation ICMV 93752. This type of trend was also observed in flowering time and plant height and indicates that the direction of selection for these traits changed when the selection environment was shifted from ICRISAT-Patancheru to Tamil Nadu. Harvest index was higher in ICMV 93752 (36.7% and 34.6% in the rainy season and the summer season, respectively) than in advanced generation cycle UCC 23 and CO (Cu) 9 in contrast to stover yield. Overall, grain and stover yield and related traits have responded to directional selection both at ICRISAT-Patancheru and in Tamil Nadu.

In plant breeding programs, yield improvement can be achieved by improving yield component traits that exhibit significant correlation with yield. Phenotypic correlations, which are associations between two characters that we observe and measure, are presented in Table 2. Flowering time was positively correlated with grain yield (0.152, *P* <0.05) and stover yield (0.552, *P* <0.01). In earlier studies, Unnikrishnan et al. (2004) reported positive correlation between these traits. When plant height was taken as an independent variable, grain and stover yield exhibited highly significant positive correlations with this trait (0.292, *P* <0.01 and 0.640, *P* <0.01) for grain yield and stover yield, respectively) as reported earlier by Thangasamy and Gomathinayagam (2003). Panicle length and panicle diameter had significant positive correlations with both grain and stover yield. In this study, grain yield had highly significant positive correlations with stover yield (0.551, *P* <0.01) and biomass yield (0.806, *P* <0.01). These observations were in agreement with Yadav et al. (2001). Stover dry matter yield had significant negative correlation with harvest index (–0.507, *P* <0.01). From these correlation studies, it can be expected that grain yield of CO (Cu) 9 can be enhanced further by improvement of yield component traits like panicle length, panicle diameter, panicle grain number and harvest index. Because of highly significant and positive correlations between grain yield and stover yield in the materials studied, simultaneous improvement of grain

Table 2. Correlation coefficients of pearl millet grain yield and component traits (data are mean of 2005 rainy season and 2006 summer season, ICRISAT, Patancheru, India)[1].

Characters	Flowering time	Plant height	Panicle length	Panicle diameter	Grain yield	Stover yield	Biomass yield	1000-grain weight	Panicle grain number
Plant height	0.677**								
Panicle length	0.315**	0.556**							
Panicle diameter	0.340**	0.358**	0.215**						
Grain yield	0.152*	0.292**	0.177**	0.145*					
Stover yield	0.552**	0.640**	0.259**	0.207**	0.551**				
Biomass yield	0.425**	0.529**	0.259**	0.230**	0.806**	0.897**			
1000-grain weight	−0.207**	0.005	−0.078	0.246**	−0.006	0.075	0.043		
Panicle grain number	0.557**	0.521**	0.437**	0.320**	0.445**	0.348**	0.410**	−0.493**	
Harvest index	−0.448**	−0.392**	−0.127*	−0.116	0.398**	−0.507**	−0.169**	−0.097	0.084

* = Significant at 0.05 level of probability; ** = Significant at 0.01 level of probability.

and stover yield should be possible in further cycles of recurrent selection, provided that sufficient genetic variability exists.

Shifts in marker allele frequencies. Thirty-four SSR loci showing polymorphism among 250 full-sib progenies were used to estimate marker allele frequencies. The SSR marker map locations were assumed to be as in the integrated genetic map (Qi et al. 2004). Estimated frequencies of SSR marker alleles exhibiting directional shifts are presented by linkage groups in Table 3.

In total, 159 alleles were detected from all 34 SSR loci. Among 159 alleles, 49 (30.8%) were rare alleles having frequencies less than 5% among the 250 full-sib progenies studied. The number of alleles ranged from 2 (*Xpsmp*2059, *Xpsmp*2211 and *Xpsmp*2202) to 8 (*Xpsmp*2069) with an average of 4.68 alleles per locus (Table 4). Three to four alleles were detected at most of the loci. The relative information value of each marker can be evaluated on the basis of its polymorphic information content (PIC) (Table 4). The average PIC value observed was 0.54 and ranged from 0.13 (*Xpsmp*2018) to 0.80 (*Xpsmp*2231). Lowest allele frequency of 0.00 was observed for *Xpsmp*2088_144 (in ICMV 93752). *Xpsmp*2018_161 had the highest allele frequency of 0.98 in ICMP 87750. Allele detected in early and late selection cycles and undetected in the mid-selection cycle might be the result of genetic drift due to the modest sample sizes (50 full-sib progenies derived from 100 parental plants per cycle) used in this study, or a change in direction of selection in the early and late selection cycles.

Among 110 common alleles detected across 34 SSR loci covering all 7 linkage groups, 17 alleles (15%) of 13 SSR loci had linear changes in allele frequencies that either increased or decreased to the mid-generation cycles and reversed their direction in advanced generations (Table 3). However, 46 alleles (42%) of 26 SSR loci showed non-linear shifts over recurrent selection cycles. Of these 63 alleles (exhibiting both linear and non-linear shifts), 32 alleles (51%) decreased their frequencies from ICMP 87750 (initial cycle) to ICMV 93752 (middle cycle) and then increased to CO (Cu) 9 (advanced generation cycle), while remaining 31 alleles (49%) exhibited shifts in the opposite directions.

The overall mean expected genetic diversity recorded was 0.551 in ICMP 87750 (Table 4). The mean genetic diversity (expected heterozygosity) reduced slightly to 0.537 in ICMP 91751, and 0.523 in CO (Cu) 9. However, ‘t’ tests revealed that these mean genetic diversity changes between populations were not significant. This non-significant reduction of mean genetic diversity was in agreement with the reports of Revilla et al. (1997), following three cycles of S_1 recurrent selection for yield in maize, and Holthaus and Lamkey (1995) following 11 cycles of reciprocal recurrent selection in maize. Our study suggests that due to the maintenance of mean genetic diversity over recurrent selection generations, further response to recurrent selection can be achieved for the improvement of yield and its component traits in CO (Cu) 9. As described in materials and methods section, the first three populations (ICMP 87750, ICMP 91751 and ICMV 93752) were bred at ICRISAT-Patancheru, with selection primarily based on higher grain yield, uniform maturity and medium plant height in June sowings (with relatively long day lengths), whereas the last two selection cycles producing UCC 23 and CO (Cu) 9 were conducted at TNAU-Coimbatore, with selection for higher grain and biomass yields in October sowings (with relatively shorter day lengths). Hence, directional shifts of the observed marker alleles were expected in response to phenotypic recurrent selection at ICRISAT-Patancheru (for higher grain yield, earlier maturity and medium plant height) and at TNAU-Coimbatore (for higher grain and biomass yields). Results of cluster analyses performed using the Neighbor-Joining method are presented in Figure 1. Cluster analysis revealed that populations bred at ICRISAT-Patancheru and TNAU-Coimbatore grouped separately when both phenotype and marker allele data sets were used. Populations bred in a particular

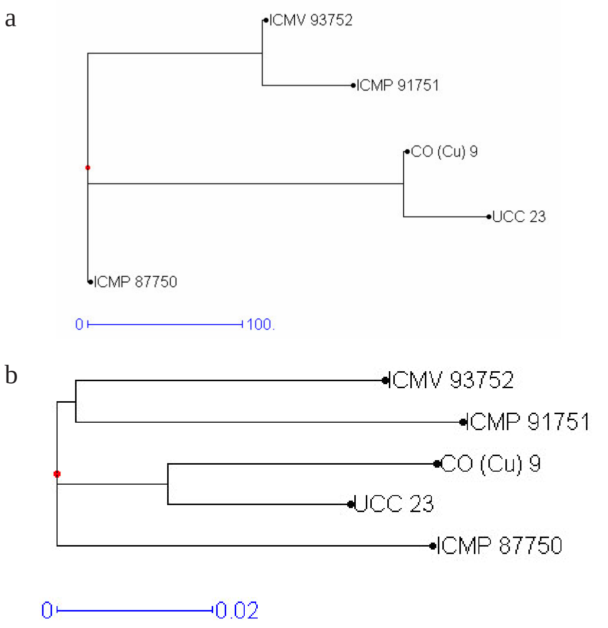

Figure 1. Dendrograms constructed by cluster analysis (Neighbor-Joining method) using (a) mean phenotypic values; and (b) SSR-based genetic similarities among the five cycles of the recurrent selection program.

Table 3. Changes in frequencies of non-rare marker alleles in response to four cycles of phenotypic recurrent selection.

			Allele frequencies				
SSR Locus	LG[1]	Allele size[2] (bp)	ICMP 87750	ICMP 91751	ICMV 93752	UCC 23	CO (Cu) 9
*Xpsmp*2030	1	112 (n)	0.20	0.27	0.19	0.23	0.23
		114	0.58	0.52	0.69	0.55	0.59
		126 (n)	0.07	0.12	0.03	0.07	0.05
*Xpsmp*2090	1	179	0.42	0.38	0.43	0.32	0.21
		183 (nl)	0.11	0.03	0.03	0.06	0.08
*Xpsmp*2246	1	257 (n)	0.34	0.16	0.21	0.33	0.33
		261	0.49	0.69	0.63	0.51	0.52
*Ximep*3017	1	187 (n)	0.32	0.29	0.24	0.34	0.26
		193	0.32	0.33	0.46	0.33	0.39
		196 (n)	0.36	0.38	0.30	0.33	0.35
*Ximep*3032	1	189	0.30	0.35	0.41	0.35	0.38
		192 (n)	0.36	0.37	0.32	0.33	0.38
*Xpsmp*2059	2	112	0.87	0.93	0.90	0.87	0.80
		114 (n)	0.13	0.07	0.10	0.13	0.20
*Xpsmp*2072	2	137 (l)	0.25	0.35	0.46	0.43	0.36
		139 (nl)	0.28	0.12	0.07	0.10	0.12
		141 (n)	0.14	0.08	0.13	0.10	0.15
*Xpsmp*2077	2	136 (n)	0.75	0.77	0.72	0.77	0.78
		140 (l)	0.04	0.05	0.11	0.09	0.06
		146 (nl)	0.13	0.04	0.02	0.05	0.06
		148	0.05	0.07	0.06	0.06	0.03
*Xpsmp*2088	2	144 (nl)	0.12	0.02	0.00	0.06	0.18
*Xpsmp*2201	2	364 (nl)	0.89	0.89	0.78	0.88	0.90
*Xpsmp*2211	2	257 (nl)	0.26	0.20	0.06	0.23	0.31
		258 (l)	0.74	0.80	0.94	0.77	0.69
*Xpsmp*2225	2	220 (nl)	0.48	0.47	0.46	0.49	0.58
		222	0.10	0.09	0.14	0.12	0.06
*Xpsmp*2237	2	252 (n)	0.14	0.01	0.07	0.10	0.11
*Xpsmp*2227	3	194 (n)	0.17	0.09	0.15	0.22	0.22
*Xpsmp*2008	4	184 (n)	0.29	0.29	0.06	0.17	0.07
		186 (nl)	0.41	0.21	0.18	0.20	0.26
		188	0.05	0.14	0.29	0.23	0.24
		190	0.25	0.36	0.47	0.39	0.43
*Xpsmp*2076	4	148	0.29	0.57	0.52	0.27	0.44
		160 (n)	0.67	0.28	0.40	0.64	0.48
*Xpsmp*2085	4	169	0.45	0.54	0.54	0.44	0.52
*Xpsmp*2086	4	116	0.12	0.05	0.19	0.06	0.07
		118	0.33	0.50	0.38	0.55	0.24
		120 (n)	0.33	0.39	0.30	0.33	0.56
*Xpsmp*2208	5	246 (l)	0.82	0.88	0.94	0.84	0.84
		252 (n)	0.08	0.05	0.03	0.12	0.08
		313 (nl)	0.09	0.07	0.01	0.04	0.05
*Xpsmp*2220	5	125	0.34	0.17	0.39	0.32	0.29
*Xpsmp*2233	5	256 (n)	0.66	0.40	0.59	0.67	0.77
		260	0.10	0.10	0.19	0.07	0.09
		262	0.04	0.12	0.10	0.05	0.04
*Xpsmp*2277	5	244	0.31	0.35	0.34	0.35	0.25
*Ximep*3027	5	200	0.53	0.32	0.70	0.36	0.27
		202 (n)	0.40	0.29	0.31	0.63	0.71
*Xpsmp*2018	6	161 (nl)	0.98	0.90	0.89	0.94	0.95
*Xpsmp*2248	6	162	0.34	0.47	0.36	0.17	0.18
*Xpsmp*2275	6	273 (n)	0.60	0.55	0.59	0.67	0.68
		276	0.03	0.06	0.08	0.04	0.05
*Ximep*3058	6	191 (nl)	0.22	0.22	0.15	0.16	0.16
		193 (n)	0.78	0.69	0.65	0.58	0.71
		199	0.01	0.08	0.18	0.10	0.13
*Xpsmp*2027	7	229	0.10	0.06	0.11	0.03	0.04
*Xpsmp*2087	7	118 (n)	0.44	0.45	0.39	0.42	0.53
		120	0.08	0.05	0.11	0.06	0.03
		122 (l)	0.42	0.44	0.46	0.45	0.40
*Xpsmp*2224	7	153	0.16	0.16	0.20	0.22	0.16
		155	0.61	0.54	0.78	0.62	0.68
		157 (nl)	0.12	0.10	0.01	0.14	0.14

1. LG = Linkage group.
2. l = Allele frequency either linearly increased or decreased to the mid-generation and then reversed its direction.
   n = Allele frequency decreased (non-linear) from ICMP 87750 to ICMV 93752 and then increased to CO (Cu) 9.

environment grouped together. It reflects effects of selection environment and directional recurrent selection on different population cycles leading to released CO (Cu) 9. This result was in accordance with reports of Rai et al. (1999) that genetic shifts occur when pearl millet populations are grown in different selection environments, permitting this crop to be fine-tuned for its adaptation to the growing environment.

Pearl millet composites are highly heterozygous and heterogenous due to their out-breeding nature. This attribute creates large variability for quantitative traits and makes them amenable for recurrent selection as has been practiced in case of maize. From this study, we concluded that CO (Cu) 9 progenitor populations have responded to recurrent selection for improvement of grain and stover yield, and related traits that led to the improved composite CO (Cu) 9. Further, directional selection and selection environment had significant effects on shifts in marker allele frequencies. Nevertheless, genetic diversity was maintained within the populations. It is presumed that alleles having positive effect on traits under selection should increase their frequencies while those with negative effect on such traits should decrease their frequencies. Also, the frequency of alleles with large effects should increase or decrease faster than the frequency of alleles with relatively small effects (Delaney and Bliss 1991). Since substantial genetic variability is still maintained in the improved

Table 4. Estimation of genetic diversity for each SSR locus[1].

SSR locus	LG	No. of alleles	PIC values	Nei's genetic diversity				
				ICMP 87750	ICMP 91751	ICMV 93752	UCC 23	CO (Cu) 9
*Xpsmp*2030	1	6	0.60	0.613	0.649	0.488	0.633	0.596
*Xpsmp*2069	1	8	0.59	0.514	0.480	0.552	0.675	0.638
*Xpsmp*2090	1	7	0.65	0.680	0.590	0.662	0.649	0.612
*Xpsmp*2246	1	3	0.58	0.623	0.486	0.539	0.613	0.605
*Ximep*3017	1	3	0.66	0.672	0.669	0.648	0.673	0.664
*Ximep*3032	1	3	0.66	0.671	0.668	0.664	0.673	0.660
*Xpsmp*2059	2	2	0.22	0.229	0.125	0.180	0.228	0.329
*Xpsmp*2072	2	6	0.77	0.797	0.764	0.725	0.740	0.782
*Xpsmp*2077	2	7	0.42	0.421	0.399	0.472	0.404	0.393
*Xpsmp*2088	2	7	0.66	0.717	0.710	0.566	0.543	0.630
*Xpsmp*2201	2	3	0.25	0.210	0.199	0.378	0.224	0.172
*Xpsmp*2211	2	2	0.33	0.389	0.323	0.114	0.358	0.433
*Xpsmp*2225	2	4	0.59	0.599	0.609	0.624	0.601	0.536
*Xpsmp*2231	2	7	0.80	0.793	0.651	0.803	0.793	0.793
*Xpsmp*2237	2	4	0.31	0.538	0.040	0.285	0.318	0.316
*Xpsmp*2227	3	4	0.40	0.349	0.326	0.428	0.416	0.458
*Xpsmp*2008	4	4	0.72	0.693	0.730	0.666	0.729	0.691
*Xpsmp*2076	4	6	0.58	0.473	0.587	0.579	0.523	0.582
*Xpsmp*2085	4	5	0.66	0.693	0.619	0.630	0.696	0.659
*Xpsmp*2086	4	6	0.68	0.758	0.600	0.721	0.586	0.618
*Xpsmp*2202	5	2	0.49	0.457	0.505	0.499	0.504	0.502
*Xpsmp*2208	5	4	0.25	0.322	0.221	0.109	0.290	0.296
*Xpsmp*2220	5	7	0.72	0.775	0.761	0.650	0.730	0.625
*Xpsmp*2233	5	5	0.57	0.529	0.714	0.596	0.509	0.399
*Xpsmp*2277	5	5	0.71	0.673	0.712	0.684	0.731	0.741
*Ximep*3027	5	5	0.59	0.559	0.769	0.428	0.484	0.423
*Xpsmp*2018	6	3	0.13	0.044	0.193	0.198	0.115	0.106
*Xpsmp*2248	6	4	0.52	0.542	0.597	0.508	0.444	0.414
*Xpsmp*2275	6	4	0.53	0.539	0.596	0.573	0.479	0.487
*Ximep*3002	6	4	0.53	0.524	0.575	0.514	0.548	0.519
*Ximep*3058	6	4	0.49	0.357	0.466	0.530	0.611	0.458
*Xpsmp*2027	7	6	0.70	0.780	0.657	0.727	0.683	0.578
*Xpsmp*2087	7	4	0.61	0.626	0.604	0.633	0.615	0.567
*Xpsmp*2224	7	5	0.54	0.585	0.652	0.354	0.556	0.505
Mean genetic diversity				0.551	0.537	0.521	0.541	0.523

[1] LG = Linkage group; PIC = Polymorphic information content.

population, further recurrent selection can be expected to lead to genetic gain for grain and stover yield and related traits.

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