Plant Prod. Sci. 7 (3) : 301 — 308 (2004)

Sorghum Diversity Evaluated by Simple Sequence Repeat (SSR) Markers and Phenotypic Performance

Anas and Tomohiko Yoshida*

*(United Graduate School of Agricultural Science, Tokyo University of Agriculture and Technology; *Faculty of Agriculture, Utsunomiya University, Utsunomiya 321-8505, Japan)*

Abstract : Analysis of phenotypic performance in the field in combination with molecular analysis provides useful information to increase the efficiency in plant breeding programs. We investigated: (i) the relationship between phenotypic performance and genetic diversity determined using simple sequence repeat (SSR) markers, (ii) the possibility of grouping inbred lines based on phenotypic performances and (iii) the genotypic and phenotypic variance of yield, yield components and primary agronomic traits among inbred lines. Among 22 sorghum inbred lines, grain yield per plot and days to flowering were less representative as phenotypic markers. However, the following six phenotypic traits showed high heritability; 1000-grain weight, ear length, plant height, stalk diameter, dry weight and harvest index, and were useful as phenotypic markers. The data obtained using SSR markers significantly correlated with those of phenotypic performance in this study, and the grouping of inbred lines based on the combination of the performance of six phenotypes was similar to that based on SSR markers. On the basis of phenotypic performance, four inbred lines, D12, H11, C9xH13 and C9xH11, were selected as promising parents for plant breeding programs.

Key words : Correlation, Phenotype, Sorghum, SSR.

Measurement of phenotypic and genotypic variance in field trials is a common and traditional approach to examine the genetic differences among genotypes. A field experiment is simpler and cheaper than DNA-based technology. However, unavoidable environmental variation in the field trials may mask the actual genetic potency of a genotype.

Simple sequence repeat (SSR) markers are generally highly discriminative and are often used to distinguish varieties, or even individuals, and reveal parentage and identity (Karp et al., 1996). The use of SSR markers has been proved as a powerful technique for studying the diversity of sorghum (Dje et al., 2000; Ghebru et al., 2002; Smith, et al., 2000; Yang et al., 1996); and genetic redundancy among sorghum germplasm (Dean et al., 1999). However, there are few reports about the relationship between molecular data and the phenotypic performance in sorghum.

The phenotypic and genotypic variance among inbred lines based on the field performance will supply valuable information to the plant breeder. Combining the molecular data analysis and phenotypic data performance will be effective for determining useful inbred lines in future sorghum breeding programs. The characterization of parents that have specific superior characters should improve the efficiency in cultivar development.

The objectives of this study were: (i) to elucidate the relationship between phenotypic performance and genetic diversity determined with SSR markers; (ii) to study the possibility of grouping inbred lines based on phenotypic performances and (iii) to estimate the genotypic and phenotypic variation of yield, yield components and primary agronomic traits among inbred lines.

Materials and Methods

1. Field experiment

Twenty-two inbred lines (*Sorghum bicolor*) were grown in the field (Table 1). Most of the parental inbred lines were obtained from the Institute for the Semi-Arid Tropics (ICRISAT), India, University of Kansas, USA and Chugoku Agriculture Experimental Station, Japan. The experimental design was a randomized complete block design with two replications. The plot size was 1.5×2.0 cm^2 with 25 cm spacing between plants and 75 cm spacing between rows. The nominal plant density was 10 plants m^{-2}. Fertilizer was applied at the rate of 2 g m^{-2} of N, P_2O_5, and K_2O as basal dressing. Hand weeding and watering were practiced as necessary.

Seed yield (grain weight per plot) was recorded for every plot. The yield components consisted of the ear length (from the base of head to tip) and harvest index (ratio of grain weight to total above-ground weight). The primary agronomy traits involved plant height at maturity (from the ground to the tip of panicle of the main stalk), days to flowering (50% of plants in the plot was heading), dry weight, stalk

Received 6 October 2003. Accepted 20 January 2004. Corresponding author: T.Yoshida (yoshidat@cc.utsunomiya-u.ac.jp, fax +81-28-649-5401).

Table 1. List of inbred lines and their origin.

Inbred lines	Note
H11*, H13*, H2, TX403*	Parental inbred lines, Kansas, USA
C9*, C8*	Parental inbred lines, Chugoku Natl. Agric. Exp. Station, Japan.
SPA2*, SPAD*, D12*	Parental inbred lines, ICRISAT, India
C9xD12, C9xH11*, C9xH13*, C9xH2, C9xTX403, H11xD12, C8xD12, C8xH2, TX403xH11, TX403xH2, TX403xD12, H13xD12, H13xH2	Developed inbred lines. F_6 breeding lines, Crop Sci. Lab., Utsunomiya Univ., selected from the cross mentioned in the name.

*Inbred lines were used both in field experiment and SSR analysis marker. SPA2;SPA2 94039B. SPAD;SPAD 940006B.

Table 2. Characteristics of nine SSR loci analyzed.

SSR locus	RM[1)]	LG[2)]	A_L[3)]	FS[4)]	A_T[5)]	DI[6)]
Sb5-236	$(AG)_{20}$	G	3; 7-8; 16*; 15	165-229	6	0.810
Sb6-342	$(AC)_{25}$	A	3; 4; 9	265-304	5	0.737
Sb1-1	$(AG)_{16}$	H	3; 10-11; 12	227-295	6	0.763
Sb1-10	$(AG)_{27}$	D	4; 7-8; 20	225-492	8	0.905
Sb5-256	$(AG)_8$	C	1; 3; 1**; 15	154-188	3	0.607
Sb6-84	$(AG)_{14}$	F	5; 5; 16	164-218	5	0.736
Sb4-72	$(AG)_{16}$	B	1; 3-4; 12	181-209	5	0.655
Sb5-206	$(AC)_{13}/(AG)_{20}$	E	3; 7-8; 28	101-149	3	0.605
Sb6-34	$(AC)/(AG)_{15}$	I	1; 5; 10	180-202	2	0.602

[1)]RM=repeat motif in original publications (Brown et al., 1996); [2)]LG=linkage group (Dean et al., 1999); [3)]A_L=number of alleles in Brown et al., 1996; Dean et al., 1999; *Dje et al., 2000; **Smith et al., 2000 and Ghebru et al., 2002; [4)]FS=fragment size (bp); [5)]A_T=total number of observed alleles; [6)]DI=diversity index (Saghai-Maroof et al., 1994).

diameter (three cm above the ground) and 1000-grain weight.

The plant height, stalk diameter and days to flowering were measured before harvesting. The grain weight/plot, ear length, dry weight, 1000-grain weight and harvest index were recorded after harvesting. Stalks (including leaves) were dried at 80°C for four days and weighed to determine total plant dry matter. Heads were dried at 40°C for three days, weighed and threshed to determine grain yield, head yield and 1000-grain weight.

2. SSR analysis

Ten inbred lines evaluated by SSR analysis are listed in Table 1. Genomic sorghum DNA was isolated from leaves collected from 10- to 15-day old seedlings using Nucleon Phytopure (Amersham). DNA, for analysis by SSR, was extracted following supplied instructions (Nucleon Phytopure-Amersham).

In all, nine primer pairs were used for genotyping (Table 2). PCR amplification, electrophoresis and data analysis were conducted by protocols as described by Anas and Yoshida (2004). PCR was performed in a volume of 20 μL containing 20ng of templete, 1.23 μg to 4.55 μg of each primer, 1 U buffer, 200 μM of each dNTP, 15 mM $MgCl_2$ and 2.5 U *Taq* DNA Polymerase (Core Kit-Qiagen). Reactions were run in a Takara PCR Thermocycler MP TP3000 (Takara, Biomedicals) with an initial denaturation step for 4 min at 94°C; followed by 30 cycles of 94°C for 1 min, 60°C to 70°C

Table 3. ANOVA of performance of eight phenotypes in 21 inbred lines.

	Range	Means squared	σ^2_g	σ^2_{ph}	σ^2_e	H
Grain weight / plot (g)	62.99 – 226.20	235.82 ^{ns}	9.590 (2.07)	117.91 (1.27)	9.85	0.08
Harvest index (%)	20.00 – 60.24	8.56**	3.120 (4.27)	4.278 (5.00)	3.68	0.73
1000 grain weight (g)	7.09 - 31.19	2.27**	0.808 (4.91)	1.133 (5.81)	4.40	0.71
Length of ear (cm)	13.76 - 29.70	1.34**	0.614 (4.18)	0.672 (4.37)	1.80	0.91
Plant height (cm)	75.83 - 179.83	83.45**	39.558 (4.69)	41.727 (4.82)	1.55	0.95
Stalk diameter (cm)	1.07 - 1.93	0.004**	0.002 (2.90)	0.002 (3.31)	2.26	0.77
Dry weight (g)	29.27 - 51.51	3.61**	1.439 (2.98)	1.806 (3.34)	2.13	0.79
Days to flowering (d)	40.50 – 59.0	2.13 ^{ns}	0.426 (1.89)	1.066 (2.99)	3.27	0.40

SPA2 was excluded from the analysis of variance; Number in parentheses are coefficient of variance; σ^2_g = genotypic variance; σ^2_{ph} = phenotypic variance; σ^2_e = environment (error) variance; H (σ^2_g / σ^2_{ph}) = heritability; **= significant at the 0.01 level; ns= not significant.

(depend on primers, Table 2) for 2 min and 72°C for 1 min; followed by a final extension at 72°C for 10 min.

PCR products were electrophoresed on 8% non-denaturing polyacrylamide gels and amplified fragments were visualized using LAS-1000UV mini (Fuji Photo Film Co., LTD). Size of DNA fragments was determined using Science Lab. 2001 — Image Gauge Ver.4.0 software (Fuji Photo Film Co., LTD). Only allele size of loci that was recognized previously (Brown et al., 1996; Ghebru et al., 2002) as an amplification product of SSR was used for genotyping (Table 2). The size of each DNA fragment was determined relative to the size standard from the 1-kb size marker Gibco/Brl.

3. Statistical analysis

The yield components and primary agronomy traits were examined for six plants in the middle of each plot. The components of variances were obtainable from the analysis of variance (ANOVA) (Halloran et al., 1979). Phenotypic variance (σ^2_{ph}) and genotypic variance (σ^2_g) among population of inbred lines were estimated from expected mean square (EMS). All phenotypic observation variables were subjected as phenotypic markers in genetic population analysis. Associations among the populations were revealed by principal component analysis (PCA) based on Pearson's correlation coefficients (StatView for Windows Version 5.0.1, SAS Institute Inc.).

Genotypes of individual plants were represented by the allele size at all SSR loci. Genetic distance was estimated by the pairwise difference method and was calculated for each haplotype pair using the parameters F_{ST} in Arlequin ver. 2.000 software program (Schneider et al., 2000). The resulting dissimilarity matrix was employed to construct dendrograms by the neighbor-joining method of Saitou and Nei (1987). The genetic variation of each locus was measured in term of the number of alleles and diversity index (Saghai-Maroof et al., 1994) as follow: $DI=1-\Sigma p_i^2$, where p_i is the frequency of the i^{th} SSR allele.

The Spearman rank dissimilarity matrix (SDM) analysis was performed to estimate the similarity among inbred lines for both SSR marker data (base pair size) and phenotypic marker data (phenotypic performance). The Mantel test using Pearson's correlation coefficient was performed to study the correlation between two matrices and to evaluate the significance of the matrices.

Results

1. Field experiment

Six traits of the 21 sorghum lines, 1000-grain weight, harvest index, ear length, plant height, stalk diameter and dry weight, showed highly significant ($p<0.01$) differences in analysis of variance (ANOVA), but for grain weight per plot and days to flowering did not (Table 3). Generally, coefficient of phenotypic variance was slightly higher than coefficient of genotypic variance in the six traits. The highest coefficient of genotypic variance was observed in 1000-grain weight (4.91) and the lowest in days to flowering (1.89). Phenotypic variance of grain weight per plot and days to flowering greatly differed from their genotypic variance.

The six traits showed moderately high heritability. The highest heritability was observed in plant height

Table 4. Phenotypic performance and ranking position of ten inbred lines in the field experiment.

Inbred lines	100-grain weight (g)	r	Dry weight (g)	r	Ear Length (cm)	r	Plant height (cm)	r	Stalk diameter (cm)	r	Harvest index (%)	r
D12	31.19	1	30.55	10	24.82	1	137.08	4	1.20	9	48.34	2
H11	16.93	6	39.81	8	18.93	5	124.75	6	1.19	10	46.27	3
C8	17.23	5	44.11	4	15.41	8	131.67	5	1.31	6	34.34	5
C9	21.16	2	46.87	2	19.43	3	144.67	3	1.25	7	30.33	6
C9xH11	18.19	4	51.51	1	20.03	2	162.33	2	1.24	8	40.86	4
H13	15.07	7	42.38	6	13.76	10	119.08	7	1.41	4	28.62	7
C9xH13	18.30	3	41.63	7	17.11	6	172.17	1	1.43	3	52.00	1
TX403	7.09	10	38.05	9	19.35	4	75.83	10	1.93	1	22.84	8
SPAD	12.12	8	44.57	3	15.76	7	101.58	8	1.37	5	28.27	9
SPA2	10.55	9	43.01	5	14.00	9	100	9	1.58	2	20.00	10

r=ranking position

(0.94) and the lowest in grain weight per plot (0.08) (Table 3). The days to flowering also showed a low heritability in comparison with other traits. The days to flowering ranged from 40 days to 62 days. The SPA2 inbred line flowered very late and produced only a few seeds.

Table 4 shows the phenotypic performance and ranking position of inbred lines. The ranking position of inbred lines for each trait was subjected to principal component analysis (PCA). D12 line showed the highest 1000-grain weight and TX403 line the lowest 1000-grain weight (Table 4). D12 line also showed the highest harvest index (48.34), whereas, H13, TX403, SPAD and SPA2 line showed a low harvest index. These inbred lines also showed a low plant height.

In the principal component analysis using Pearson's correlation coefficient for six phenotypes in 22 lines, the first and second principal components (PC 1 and PC 2) explained 48% and 24% of the total variation, respectively (Fig. 1). These principal components could explain 71% of the total variation. Sixteen lines were pooled in one group and six lines were clearly separated from the group (D12, H2, TX403, SPA2, SPAD and H13) (Fig. 1).

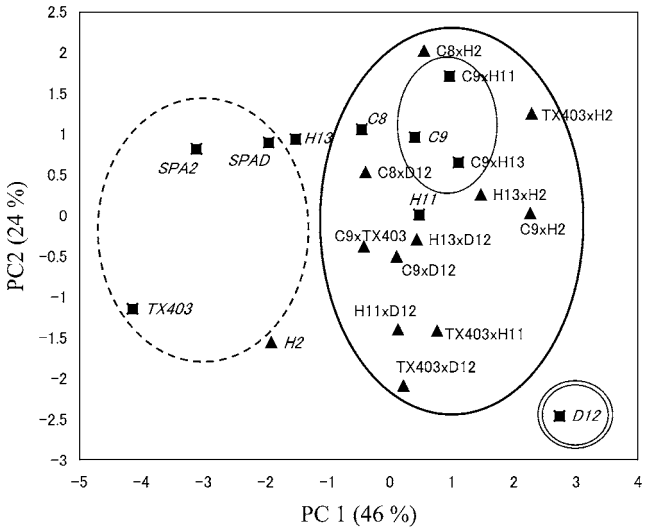

Fig. 1. Principal component analysis of 22 inbred lines using data for six phenotypic data. PC1 and PC2 are the first and second principal components, respectively. ■ inbred lines were used in both field experiment and SSR analysis marker; ▲ inbred lines were used only in field experiments; Italic letters are parental inbred lines; Plain letters are developed inbred lines.

2. Simple sequence repeat marker

In total, 43 putative alleles (different fragment size) were observed in the ten inbred lines. All nine loci were polymorphic. Table 2 shows the number of alleles and diversity indices for each SSR marker. In this study, the numbers of alleles per locus ranged from two (Sb6-34) to 8 (Sb1-10), and the average number of alleles per locus was 4.8. Diversity indices of each locus ranged from 0.602 (Sb6-34) to 0.905 (Sb1-10) with a mean value of 0.713.

Profiles of nine SSR markers were collectively able to discriminate all inbred lines (Fig. 2). All of these SSR markers were frequently used for genetic diversity study in sorghum and useful method for studying diversity in sorghum ((Dje et al., 2000; Ghebru et al., 2002; Smith et al., 2000; Yang et al., 1996).

3. Relationship between phenotypic marker and SSR marker

Ten inbred lines were analyzed by the field

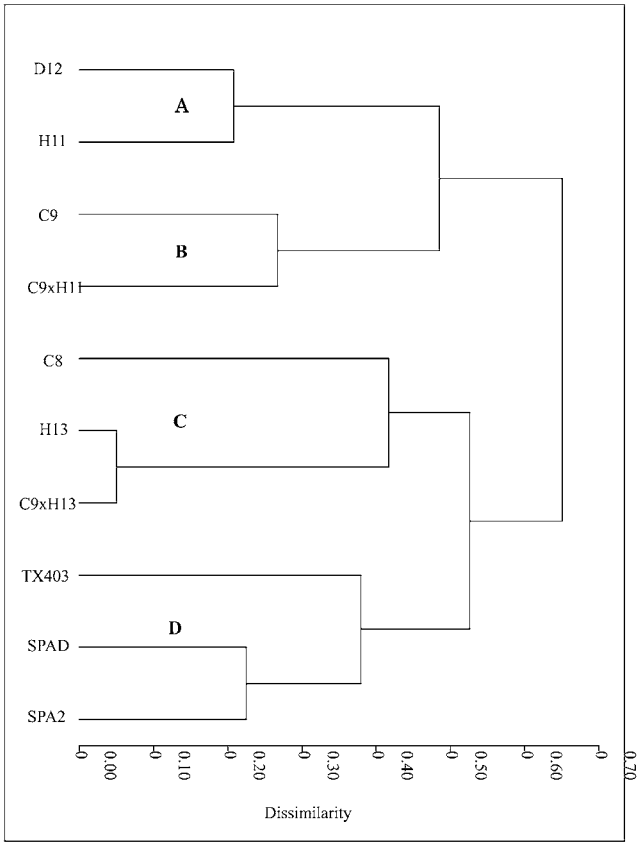

Fig. 2. Dendrogram showing genetic relationship among ten sorghum-inbred lines assessed by SSR markers. The bold capital letters are cluster group.

Table 5. Correlations of dissimilarity distance matrix between SSR markers and all possible combinations of phenotypic markers data based on Pearson correlation.

Correlation between SSR data and single phenotypic data.					
	DW	LE	PH	SD	HI
SSR	**ns**	**Ns**	**ns**	**ns**	**ns**
Correlation between SSR data and a combination of two phenotypic data.					
	DW,LE	DW,PH	DW,SD	DW,HI	
SSR	**ns**	**Ns**	**ns**	**ns**	
	LE,PH	LE,SD	LE,HI		
SSR	0.325*	0.574**	**ns**		
	PH,SD	PH,HI			
SSR	0.320*	0.306*			
	SD,HI				
SSR	0.446*				
Correlation between SSR data and a combination of three phenotypic data.					
	DW,LE,PH	DW,PH,SD	DW,SD,HI		
SSR	**ns**	0.297*	0.396*		
	LE,PH,SD	LE,SD,HI			
SSR	0.606**	0.589**			
	PH,SD,HI				
SSR	0.460**				
Correlation between SSR data and a combination of four phenotypic data.					
	DW,LE,PH,SD	DW,PH,SD,HI	LE,PH,SD,HI		
SSR	0.549**	0.409*	0.630**		
Correlation between SSR data and a combination of five and six phenotypic data.					
	DW,LE,PH,SD,HI	GW,DW,LE,PH,SD,HI			
SSR	0.568**	0.444**			

Correlations between matrixes were analyzed by the Mantel test; GW was included only in a combination of six phenotypic data. DW=dry weight; LE=ear length; PH=plant height; SD=stalk diameter; HI=harvest index; GW=1000-grain weight; *, ** =significant at 0.05 and 0.01 level, respectively; ns=not significant.

experiment and with SSR markers. The dissimilarity matrix of molecular data was plotted against the dissimilarity matrix of single phenotypic to a combination of six phenotypic data in a mantel test and was analyzed using Pearson's correlation (Table 5). Generally, the Spearman rank dissimilarity obtained using SSR markers was significantly correlated with that obtained with phenotypic markers (r=0.444**). Table 6 shows the Spearman dissimilarity distance of SSR markers for ten inbred lines.

None of the dissimilarity distance matrices based on single phenotypic data showed a significant correlation with the dissimilarity distance matrix of SSR markers (Table 5). Five matrices obtained for the combination of two phenotypic data significantly correlated with the SSR marker data. Only one matrix obtained for the combination of three phenotypic data (dry weight, ear length, plant height) was not significantly correlated with the molecular data. All combinations of four, five and six phenotypic data gave results highly correlated with SSR marker data. Table 6 shows the dissimilarity distance of SSR markers between 10 inbred lines.

Discussion

1. Variation of phenotypic marker

Six traits showed high heritability values in this study. The relative importance of a genotype as a determinant of phenotypic value is given by the ratio of genotypic to phenotypic variance, V_g/V_p (Falconer, 1970). Genetic factors greatly affected the 1000-grain weight, dry weight, ear length, plant height, stalk diameter and harvest index. On the other hand, environmental factors influenced performance of grain yield per plot. Grain weight per plot showed a very low heritabilty and, therefore, it is not a good phenotypic marker.

Including the grain yield per plot, traits in principal component analysis and Spearman rank dissimilarity analysis significantly reduced the correlations of their dissimilarity matrices (data not shown). Unfavorable environmental conditions may mask the factual genetic potency of a quantitative character such as yield trait. Therefore, grain yield per plot was not included as a phenotypic marker in the correlation analysis, hereafter.

We also found that the environmental factors

Table 6. Spearman rank dissimilarity distance of SSR markers between ten inbred lines.

Inbred lines	D12	H11	H13	TX403	C8	C9	SPAD	C9xH13	C9xH11	SPA2
D12	0									
H11	0.208	0								
H13	0.750	0.833	0							
TX403	0.608	0.733	0.500	0						
C8	0.492	0.650	0.358	0.458	0					
C9	0.675	0.558	0.475	0.775	0.592	0				
SPAD	0.475	0.600	0.625	0.350	0.750	0.583	0			
C9xH13	0.833	0.775	0.050	0.608	0.475	0.342	0.592	0		
C9xH11	0.458	0.250	0.758	0.658	0.517	0.267	0.742	0.658	0	
SPA2	0.817	0.792	0.333	0.408	0.617	0.408	0.225	0.250	0.750	0

affected the days to flowering more strongly than genetic factors. Can and Yoshida (1999) reported that days to heading of sorghum genotypes were greatly influenced by environmental factors and selection of this trait must be conducted for each season.

There was no significant difference in days to flower among inbred lines. All sorghum-inbred lines used in this study, except SPA2 and SPAD, were routinely planted in the same area. Craufurd et al. (1999) reported that adaptation of sorghum to the diverse environments was largely determined by photoperiod sensitivity and the minimum time to flower. Consequently, a very small genetic variation will be observed in days to flowering (Table 3), and therefore, days to flowering might not be a useful phenotypic marker in this study.

High phenotypic variation was observed among parental inbred lines. They were widely scattered along PC1 and PC2 (Fig.1). All parental inbred lines were clearly separated from each other. Three countries were recognized as origins of these parental inbred lines (Table 1). This may be why a high phenotypic variation among parental inbred lines was observed in this study.

2. Grouping of inbred lines based on principal component analysis

Principal component analysis clearly separated the developed inbred lines from the parental inbred lines. On the basis of their performance data, developed inbred lines may be included in one group (bold circle in Fig. 1) because the members of this group had a relatively similar phenotypic performance (Table 4). All inbred lines in this group were previously selected for short plant statue, early maturity and high yield. In addition, they were grown at the same place for many years.

Two inbred lines from ICRISAT (SPA2 and SPAD) showed a poor performance in the field experiment. SPA2, SPAD and TX403 showed a similar in 1000-grain weight, plant height and harvest index (Table 4). According to the principal component analysis (Fig. 1) TX403, SPA2 and SPAD were clearly separated from the above-mentioned group and positioned relatively close to each other (broken circle in Fig. 1).

D12 inbred line clearly differed from other inbred lines in 1000-grain weight, ear length and harvest index. D12 was clearly separated from SPA2, SPAD and TX403 inbred lines in PCA analysis (double circle in Fig. 1). Two inbred lines (C9xH11 and C9xH13) were generated from the same parent and have a genetic background closely related to C9 inbred lines. Actually C9, C9xH11 and C9xH13 inbred lines showed a similar phenotypic performance (Table 4), and in the principal component analysis they were close to each other (thin circle in Fig. 1).

3. Diversity of molecular markers

The number of putative alleles and average number of allele per locus in our study were slightly lower than those previously reported (Smith et al., 2000; Dean et al., 1999; Ghebru et al., 2002). However, in all cases the observed number of alleles was much higher than that reported in the original publication (Brown et al., 1996). The low number of putative alleles and average number of allele per locus could be attributable to the following: (i) closely related genotypes, (ii) limited number of DNA markers and (iii) DNA markers are not distributed widely across the plant genome.

However, we found higher gene diversity indices among SSR markers than the gene diversity reported by Smith et al. (2000) and Dean et al. (1999), which showed a large variation in the genetic background of our materials. The difference between the longest and

shortest alleles ranged from 22 to 267 bp. Ghebru et al. (2002), using Eritrian sorghum and world sorghum accessions, reported a variation in allele size similar to that in the present study. The locus Sb1-10 gave the highest gene diversity and showed high polymorphism for many alleles (Table 2). On the other hand, locus Sb6-34 gave the lowest gene diversity index and had few alleles. A significant correlation ($r=0.95$; $P<0.01$) was observed between the gene diversity index and average number of alleles per marker.

4. Correlations between phenotypic performance and molecular data

We investigated the correlation between the result of Spearman rank dissimilarity analysis with SSR markers and that of a combination for four, five and six phenotypic data because theoretically the phenotypic performance is the result of interaction between genotypic (particular assemblage of genes in each individual) and environmental factors (all non-genetic circumstances that influenced the phenotypic value) (Falconer, 1970).

This statement is supported by the fact that the correlation value increased in parallel with the increase in the number of phenotypes analyzed in combination (Table 5). The Spearman rank dissimilarity for less than four phenotypic data showed a low correlation with that for the molecular marker data. Single phenotypic data did not show any significant correlation with the molecular data (Table 5). This suggests that the use of more phenotypic markers will provide a good description of the genotype.

All combinations of phenotypic markers including ear length and stalk diameter always gave a high correlation with our nine SSR markers. Even the combination of those two phenotypic data showed a highly significant correlation with SSR markers (Table 5). This suggests that combination of ear length and stalk diameter is portrayed by nine SSR markers.

5. Grouping of inbred lines based on SSR markers

The clustering among the sorghum inbred lines assessed using SSR markers (Fig. 2) showed that all accessions were distinctly placed in this dendrogram, and classified at least into four groups (A–D).

Two inbred lines from ICRISAT (SPA2 and SPAD) and one from USA (TX403) were placed in the same cluster (Group D). These lines showed a poor performance in the field experiment (Table 4).

Two developed inbred lines (C9xH11 and C9xH13) were generated from the same parent, thus they should have a genetic background closely related to C9, H11 and H13 inbred lines. SSR markers accurately portrayed these pedigree histories. C9 and C9XH11 were neighboring in the same branch, while C9XH13 was also placed in the same branch as H13. Generally all these inbred lines were clustered in neighboring branches (Group B and C).

Conclusion

The result of analysis using a combination of more than four phenotypic data fairly correlated with SSR marker data. The SSR marker highly correlated with the combination of phenotypic markers that included ear length and stalk diameter. Generally, grouping of inbred lines based on their phenotypic performance data (combination of the six phenotypic data) was similar to that based on SSR markers. However, SSR markers gave more accurate and detailed grouping of inbred lines. On the basis of the phenotypic performance, D12, H11, C9xH13 and C9xH11 were promising parents that could be used in future plant breeding programs. Judging from the dissimilarity distances (Table 6), D12 x (C9xH13), H11 x (C9xH13) and SPAD x (C9xH11) seem to be distant genetically, giving a wide array of progenies, and might produce promising lines.

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