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RESEARCH ARTICLE

Phenotypic Diversity and Molecular Diversity of Finger Millet Composite Collection and Identification of Trait Specific Germplasm for Use in Crop Improvement

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ABSTRACT

A composite collection of finger millet consisting of 1000 accessions representing the diversity of the entire germplasm at ICRISAT gene bank was developed, including 622 accessions of ICRISAT core collection. Phenotyping of the composite collection for 15 quantitative traits and 20 SSR markers genotyping data resulted in the identification of promising trait-specific accessions. Principal component analysis with seven components indicated relative importance of the traits (days to 50 % flowering, plant height, peduncle length, ear head length, and panicle exertion) to total divergence. Clustering analysis grouped biological races into three clusters wherein cultivated races *vulgaris*, *plana*, *elongata*, and *compacta* were grouped in Cluster I and wild races *spontanea* in Cluster II and *africana* in Cluster III. Accessions were identified as useful for important traits such as early flowering (34), high grain yield (38), fodder yield (19); more fingers (29); basal tiller number (25) and ear head length (28).

Keywords:*Phenotypic diversity; Finger millet; Trait specific germplasm; Principal component analysis; Cluster analysis.*

INTRODUCTION

Finger millet (*Eleusine coracana L. Gaertn*) is an important crop in several countries of Asia and Africa used for food, fodder, and industrial purpose. Precise data of area and production under finger millet is not known because the production statistics of this crop has often been clubbed with other millets. At present 55 to 60 per cent of the finger millet crop is grown in Southern and Central Africa, and most of the remaining areproduced in India and Nepal. The Consultative Group on International Agricultural Research (CGIAR) has estimated that 10 per cent of the area under millets is with finger millet. The global area under finger millet, is 3.38 million hectares with aproduction of 3.76 million tonnes. Finger millet was domesticated about 5000 years ago in Eastern Africa (possibly Ethiopia) and introduced into India 3000 years ago. The closest wild relative of finger millet is *E. coracana* subsp. *africana,* which is a native of Africa.

Finger millet can perform better under adverse soil and weather conditions compared to other crops. The finger millet germplasm consisting of5949 accessions was conserved in International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) gene bank. A composite collection that constituted 1000 accessions was developed (Upadhyaya *et al.,* 2006b) under the Generation Challenge Program to unlock genetic diversity. The aim of the present investigation was to evaluate global composite collection for various morpho -agronomic traits at multi-locations and to identify trait-specific diverse accessions for use in breeding programme.

MATERIAL AND METHODS

The composite collection of 1000 accessions along with the four control cultivars, VR708, VL149, PR202 and RAU8 were evaluated in an augmented design in three environments (2005 -2006 post rainy (E1) at Coimbatore, 2006 rainy (E2) and 2007 rainy (E3) seasons at ICRISAT Centre, Patancheru, India. The Total 111 blocks were maintained with 9 test entries and one check variety. The length of each row is 6 m, the spacing between plants were maintained 30 X 15 cm. Data was recorded on 15 quantitative traits such as days to 50% flowering, plant height, basal tillers, culm branching, flag leaf blade length and width, flag leaf sheath length, peduncle length, panicle exsertion, inflorescence length and width, length and width of longest finger, and grain yield. The random model of residual maximum likelihood (REML) in GenStat 10 (Payne *et al.,* 2007) was used to analyze data of 15 quantitative traits for the individual location. Variance components owing to genotype (σ^2g) and its standard errors (SE) were

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estimated for individual and combined analysis. Best Linear Unbiased Predictors (BLUPs) for individual location and combined analysis were worked out for all quantitative traits. Correlations were calculated on BULP for all the traits. The Shannon- Weaver diversity index (H`) (Shannon and Weaver, 1949) was calculated for the entire set, different races for quantitative traits. Principal Component Analysis (PCA) on Genstat 10 (Payne *et al.*, 2007) was used to know the importance of different traits in explaining multivariate polymorphism. Cluster analysis was performed based on biological races using the scores of first four PCs following Ward (1963). These 1000 accessions of finger millet composite collection were genotyped with 20 polymorphic SSR markers. Based on the dissimilarity index, the most diverse pair of accessions were identified.

RESULTS AND DISCUSSION

Variances

The variances of the cultivated and wild races were homogenous for most of the trait except days to 50% flowering and ear head width in E1, days to 50% flowering, ear head length and length of longest finger in E2, flag leaf blade length, ear head length, the width of longest finger, and the number of fingers in E3, days to 50% flowering, width of longest finger, ear head length, ear head width in combined (data not given).

Table 1. Correlation coefficients more than 0.500 or less -0.500 among different traits in finger millet composite collection

Traits	Plant height	Panicle excertion	Length of longest finger	Width of longest finger	Finger number
Days to 50% flowering	0.981 (E1) 0.542 (E3)				
Basal tiller number	0.770 (E1)				
Flag leaf blade length	0.844 (pooled)				
Flag leaf blade width	0.801 (E1) 0.650 (pooled)				
Peduncle length		0.656 (E2) 0.662 (E3)	-	-	-
Ear head length		-	0.817(E2); 0.716(E3), 0.502 (pooled)	-	-
Grain yield		-	0.774 (E2); 0.583 (E3)	0.720 (E3)	0.507 (E1) 0.575 (E3)

Any correlation coefficient for global finger millet composite (998 degrees of freedom) with an absolute value greater than 0.05 will be significant at $P = 0.05$ and greater than 0.09 will be significant at $P = 0.01$.

Correlation

The correlation coefficient helps to understand the degree of association among the different traits using phenotypic values obtained in different environments. Phenotypic correlation coefficients were calculated for the global composite collection to understand the nature of associations between different quantitative traits in all the three environments separately and overall in the three environments. In total, 367 correlations were estimated in the E1, E2, E3 and combined analysis. At probability 0.05 or less, 14 out of 66 correlations were significant in E1, 68 out of 105 in E2, 68 out of 105 in E3, and 31 out of 91 in the combined analysis of data (data not given).

The proportion of variance in one trait can be attributed to its linear relationship with a second trait and is indicated by the square of the correlation coefficient (coefficient of determination) (Snedecor and Cochran, 1980). In the present study, we have considered only those correlations, which are greater than 0.500 and smaller than -0.500 as meaningful as at least 25% of the variation of one trait is predicted by another.The pairs of correlations wereobserved such as plant height with days to 50% flowering (0.981 in E1, 0.542 in E3), with basal tiller number (0.770 in E1), with flag leaf blade length (0.844 in pooled), with flag leaf blade width (0.801 in E1, 0.650 in pooled), peduncle length with panicle excertion (0.656 in E2, 0.662 in E3), ear head length with length of longest finger (0.817 in E2, 0.716 in E3, 0.502 in pooled), grain yield with length longest finger (0.774 in E2, 0.583 in E3), with width of longest finger (0.720 in E3), with finger number (0.507 in E1, 0.575 in E3) (Table 1). This information would help in selecting the useful traits and thus optimize the data recording by taking observations on a few related traits in the preliminary trials involving a larger number of germplasm accessions.

Diversity

The Shannon- Weaver diversity index (H`) was calculated to compare phenotypic diversity for all characters in the entire set, accessions among the races in each environment separately and also

overall environments. Out of five morphological traits studied, The traits such as, number of the basal tiller (0.648), flag leaf sheath length (0.623), ear head width (0.607), number of fingers (0.611) in E1, days to 50% flowering (0.619), culm branching (0.615), panicle length (0.627), panicle excertion (0.626), length of longest finger (0.603) in E2, plant height (0.623), flag leaf blade length (0.624), ear head length (0.603), width of longest finger (0.616) and grain yield (0.634) in E3 showed high H` compared to other two environments. The combined analysis revealed low H` for flag leaf blade width (0.412) and high H` for panicle excertion (0.618) (Table 2).

Table 2. Shannon-Weaver diversity index for quantitative traits of global finger millet composite collection evaluated in three environments and pooled

Traits	2005-2006 Coimbatore (E1)	2006 Rainy Patancheru (E2)	2007 Rainy Patancheru (E3)	Pooled
Days to 50 per cent flowering (days)	0.609	0.619	0.571	0.599
Plant height (cm)	0.616	0.599	0.623	0.612
Basal tiller numbers (number)	0.648	0.587	0.581	0.605
Culm branching (number)	0.531	0.615	0.531	0.573
Flag leaf blade length (mm)	0.607	0.623	0.624	0.618
Flag leaf blade width (mm)	0.447	0.343	0.446	0.412
Flag leaf sheath length (mm)	0.623	0.576	0.559	0.586
Peduncle length (mm)	0.557	0.627	0.623	0.602
Panicle exertion (mm)	0.531	0.626	0.611	0.618
Ear head length(mm)	0.602	0.596	0.603	0.6
Ear head width (mm)	0.607	0.569	0.569	0.581
Length of longest finger (mm)	0.531	0.603	0.592	0.597
Width of longest finger (mm)	0.531	0.487	0.616	0.551
Finger number per inflorescence (number)	0.611	0.408	0.607	0.542
Grain yield (t ha^{-1})	0.617	0.596	0.634	0.615

The mean and range of H` for all the traits in the present study is comparable with the H` of the entire and core collection of finger millet (Upadhyaya *et al.,* 2006a), indicating that the composite collection represents the entire diversity.

Principal component analysis

The principal component analysis is used to provide a reduced dimension model that would indicate measured differences among groups. In all the three environments and also in the pooled analysis, a large proportion of the total variation was explained by the first seven PCs were 59.63 (E1), 62.50 (E2), 68.29 (E3) and 88.90 % (pooled) variation was accounted. In the present study, from the first seven PCs, over three environments and combined analysis, the traits such as days to 50 % flowering, plant height, peduncle length, ear head length and panicle exertion contributed repeatedly to total divergence at least in two environments (data not given). It revealed that these traits were contributing more towards divergence.

Clustering

Clustering was performed using a score of the first four PCs (68.29% variation) on the pooled data based on biological race and geographical origin. Four cultivated races (*elongata*, *plana*, *vulgaris* and *compacta*) were delineated into Cluster I, whereas accessions of wild races were grouped separately in two clusters *viz.,* Cluster II (wild *spontanea*) and Cluster III (wild *africana*) (Figure 1). The linkage distance between the wild races *spontanea* and *africana* was more than 50 %, so it was grouped into two different clusters. The variation of these wild races was reported at mitochondrial DNA level (Muza *et al.,* 1995). Cytogenetically, *E. coracana and E. africana* are reported to be an allotetraploid with genomic notation of AABB with distinct genetic background (Chennaveeraiah and Hiremath, 1974). This could be the reason for difference in clustering pattern of these major sub species. This clustering observed in this study is in agreement with earlier reports based on molecular markers (RFLP, Salimath et al*.,* 1995; RAPD, Fakrudin *et al.,* 2004; SSR, Dida *et al.,* 2008)

Identification of diverse and traits specific diverse accessions

By evaluating the global finger millet composite collection over three seasons, we could identify few accessions performed repeatedly better than the best control cultivar for the particular trait(s) in all environments. The number of accessions identified specific for traits were 34 for early flowering (<50 days), 38 for high grain yield (>2 tha^{-1}), 29 for more fingers (>10), 28 for ear head length (>150mm). Phenotypic diversity was calculated between each

Table 3. Promising diverse accessions in the finger millet composite collection based on phenotypic and genotypic diversity

Diversity nature	Pairs of accessions showing the diversity			
	Phenotypic diversity based on morpho-agronomic traits		Genotypic diversity based on SSR markers data	
Minimum diversity in composite collection	VR708	IE3455	IE143	IE1011
	VR708	IE4789	IE2921	IE3826
	VR708	IE61	IE143	IE6241
	VR708	IE3802	IE2365	IE4956
	VR708	IE3654	IE2332	IE2921
Maximum diversity in composite collection	IE5442	IE4221	IE2332	IE2765
	IE6541	IE2084	IE4108	IE3238
	IE2441	IE2364	IE2288	IE431
	IE4570	IE3291	IE2748	IE431
	IE4890	IE3130	IE4972	IE431
Trait specific accessions showing maximum diversity				
Early Flowering	IE4755	IE2275	IE49	IE2275
	IE4759	IE3543	IE4442	IE2323
	IE2393	IE3537	IE3537	IE2329
	IE4442	IE2957	IE583	IE4759
	IE4734	IE2293	IE2275	IE2083
	IE4866	IE4257	IE2578	IE3802
High finger number	IE4677	IE4563	IE96	IE2340
	IE5689	IE4476	IE3194	IE6236
	IE2957	IE6013	IE2773	IE96
	IE5877	IE4476	IE5198	IE2587
	IE6548	IE2108	IE6252	IE4677
More finger length	IE3046	IE3722	IE5689	IE5877
	IE3489	IE2789	IE3790	IE4586
	IE5321	IE3046	IE6059	IE4476
	IE2608	IE2223	IE2591	IE5956
	IE2773	IE5472	IE2678	IE3802
High grain yield/ha	IE2684	IE2299	IE6236	IE3194
	IE4600	IE2590	IE2340	IE2992
	IE4121	IE2827	IE5198	IE3194
	IE3663	IE4600	IE5198	IE2712

pair of accessions for 15 quantitative traits. The diversity index was calculated by averaging all the difference in the phenotypic value for each trait divided by their respective range (Johns *et al.,* 1997).

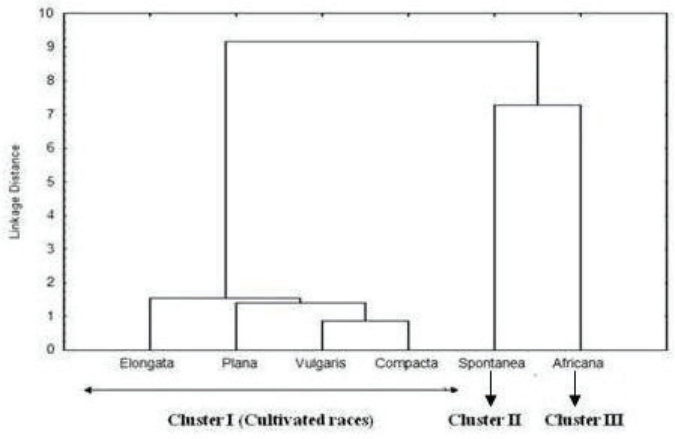

Figure 1. Ward's clustering of finger millet global composite collection concerning races based on scores of first four PCs

In the entire composite collection, the mean phenotypic diversity was 0.8423. The maximum phenotypic diversity (0.990) was observed between the accessions IE5442 and IE4221 and least diversity (0.156) was observed between the pair of accessions such as VR708 with IE3455. In the trait specific accessions, most phenotypic diversity was observed between the accessions IE4755 and IE2275 (early flowering); IE4866 and IE4257 (more finger number); IE6548 and IE2108 (high finger length); IE2773 and IE5472 (grain yield) (Table 3).

Based on the dissimilarity matrix of 20 SSR markers data on 959 accessions (41 accessions excluded from the analysis due to poor quality), the most genotypic diversity in the composite collection was identified between IE2332 and IE2765 and least diversity between IE5442 and IE4224. Among the trait-specific accessions, most genotypic diversity was observed between the accessions IE49 and IE2275 (early flowering); IE2578 and IE3802 (more finger number); IE6252 and IE4677 (high finger length); IE2678 and IE3802 (high grain yield) (Table 3). There was no correspondence between the highly diverse pair of identified accessions using phenotypic and genotypic diversity in any of the traits. This could be due to that the diversity detected by these limited number of SSR markers does not reflect the diversity associated with these important traits.

CONCLUSION

By evaluating the global finger millet composite collection in a different environment, the trait-specific accessions were identified, which would serve as new sources of variation in finger millet crop improvement. The most diverse pairs of accessions were identified in the composite collection based on the diversity index for phenotypic diversity and SSR based dissimilarity matrix for genotypic diversity. It would be interesting and to involve the most diverse lines in the hybridization program to see the extent of segregation for different traits. The inclusion of these diverse germplasm lines from such collections in the hybridization programs would increase the dominance effect and epistatic variation in the inheritance of quantitative traits.

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