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ANALYSIS OF GENETIC DIVERGENCE IN DURUM WHEAT (*TRITICUM DURUM* DESF.) AND SELECTION OF ELITE PARENTS FOR HYBRIDIZATION

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ABSTRACT: A field experiment was conducted at Geregera and Kone testing sites of Sirinka Agricultural Research Center, northeastern Ethiopia. The objective of the experiment was to estimate the genetic divergence among durum wheat germplasm of diverse origin and clustering them into homogenous groups for further hybridization program. Genetic divergence analysis was computed based on multivariate analysis using Mahalanobis's D^2 statistics. Based on D^2 values, 64 durum wheat genotypes were grouped into ten clusters. The highest inter-cluster distance was exhibited between cluster-II and cluster-III (D^2=57.15). Analysis within the indigenous durum wheat germplasm indicated that there was no correspondence between geographic and genetic distances. That is, germplasm collected from the same geographic area were placed into different cluster groups indicating their differences. Thus, to get more genetic variability, further collection mission should be targeted in major durum wheat growing regions of Ethiopia. On the other hand, indigenous and exotic germplasm were grouped into different clusters except in cluster-VI and cluster-X. Cluster-VI consisted of seven indigenous and one exotic germplasm. Cluster-X on the contrary, consisted of seven exotic and one indigenous germplasm, implying the presence of parallelism between genetic and geographic distances. Thus, there is an opportunity to improve grain yield through hybridization of genotypes from genetically divergent clusters and subsequent selection from the segregating generations. Crossing of parents involving cluster-IX (indigenous) with cluster-III (exotic) would complement each other and could result in high genetic variability and superior segregates having good combinations of characters from both parents.

Key words/phrases: Clustering, Genetic divergence, Genetic variability

INTRODUCTION

Both tetraploid (*Triticum durum* Desf.) and hexaploid (*Triticum aestivum* L.) wheat are among the most important cereal crops in Ethiopia ranking, third in total production (17%), next to maize and *tef* (CSA, 2002). Similarly, they are important cereal crops in North and South Wollo Administrative Zones, ranking third in area of production next to tef and

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sorghum (BFED, 2001). They cover a total arable land of 110, 434 ha with an average productivity of about 8.4 qt ha^{-1}, which is below the national average of 14.4 qt ha^{-1} owing to different production-limiting factors.

Most of the tetraploid wheat varieties currently grown in Ethiopia are landraces consisting of a large number of different genetic lines. Purseglove (1975) reported the presence of greatest diversity of durum wheat in Ethiopia. Due to the presence of genetic diversity, Vavilov (1951) and Zohary (1970) identified Ethiopia as the center of origin for tetraploid wheat. However, the criteria formulated by Harlan (1971) on the place of origin of cultivated plants (due to the absence of ancestral forms and wild relatives) ruled out Ethiopia as the center of origin of cultivated wheat (Pecetti *et al.,* 1992).

The major breeding objective in durum wheat is to develop new genotypes improved in features that contribute to greater yield potential, increased yield stability, and improved product quality (Poehlman and Sleper, 1995). To make the crossing program effective, parents should belong to different clusters. The more distant the parents within overall limits of fitness, the greater are the chances of obtaining higher amount of heterotic expression in F_1's and broad spectrum of variability in segregating populations (Norden, 1980; Rao *et al.,* 1981*;* Reddy, 1988). However, crossing of genotypes belonging to the same cluster would not be expected to yield desirable recombinants.

The concept of "genetic distance" has been of vital utility in many contexts and more in differentiating well-defined populations. Several measurements of distance have been proposed over the past several decades to suit various objectives. Of these Mahalanobis's generalized distance D^2, (Mahalanobis, 1936; Rao, 1952) occupies a unique place in plant breeding. Mahalanobis's generalized distance utilizing multiple measurements which are subjected to multivariate statistical analysis and can provide such a measure of generalized distance between populations (Mahalanobis, 1936). A number of workers observed that Mahalanobis's D^2 statistics was a powerful biometrical technique in discerning divergence among lines based on multiple characters. Selection of parents based on the extent of genetic divergence has been successfully utilized in different crop species. For instance, the technique has been widely used in upland cotton (Miller and Marani, 1963; Singh and Gupta, 1968), spring wheat (Bhatt, 1970; Singhal and Upadhyay, 1977; Jatasra and Paroda, 1978), pearl millet (Singh and Gupta, 1979), chickpea (Jain *et al.,* 1981), dry edible bean and faba bean

(Ghaderi *et al.*, 1984) and winter wheat (Shoran and Tandom, 1995).

Durum wheat is mostly grown on vertisols by small-scale farmers under rain fed conditions. It tolerates water logging much more than bread wheat and is thus usually called *Yekoticha Sinde* (*in Amharic)*, which means wheat of heavy black clay soils. Therefore, the largest proportions of vertisol were covered by durum wheat in both North and South Wollo. It is, therefore, imperative to improve the genetic background of durum wheat so as to improve productivity and production of durum wheat through crossing of divergent genotypes followed by selecting filial generations for desirable traits. But, information on genetic divergence in durum wheat is limited. Therefore, this experiment was aimed to identify genetically divergent durum wheat parents having desirable traits for further hybridization.

MATERIALS AND METHODS

Description of the study areas

The experiment was conducted at Geregera and Kone testing sites of Sirinka Agricultural Research Centre, northeastern Ethiopia. The trial was evaluated for two cropping seasons at Geregera (2003 and 2004) and for one cropping season at Kone (2004). Geregera is located at 11^0 45' North latitude and 038^0 45' East longitude and at an altitude of 2846 m a.s.l. The soil type for Geregera is generally characterized as clay (70.6%); containing 1.0% organic carbon, 0.8% organic matter, 0.2% N_2 and 9.2% P_2O_5 with pH of 6. On the other hand, Kone is located at 11^0 36' North latitude and 038^0 55' East longitude and at an altitude of 2890 m a.s.l. The rainfall for both locations is erratic in distribution and less predictable with uni-modal pattern. Delayed onset (during sowing time), early cessation (during grain filling period), and sometimes torrential rainfalls followed by long dry spells are the major and common problems of the area.

Experimental materials

The experimental material consisted of 64 durum wheat genotypes of which 20 were exotic (received from CIMMYT) and the remaining 44 genotypes were randomly taken from the indigenous germplasm collections (Table 1). The indigenous durum wheat germplasm were collected from the central and western highlands of the country where durum wheat is widely cultivated.

Table 1 List of 64 durum wheat accessions, their origin and collection area.

Id no.	Accession name	Origin	Collection area	Id no.	Accession name	Origin	Collection area
1	B-I 100	Indigenous	Bichena	33	A-II-139	Indigenous	Ambo
2	B-I 113	Indigenous	Bichena	34	A-II-158	Indigenous	Ambo
3	B-I 157	Indigenous	Bichena	35	A-III 59	Indigenous	Ambo
4	B-I 163	Indigenous	Bichena	36	A-III 130	Indigenous	Ambo
5	B-I 118	Indigenous	Bichena	37	A-III 159	Indigenous	Ambo
6	B-II 102	Indigenous	Bichena	38	A-III 163	Indigenous	Ambo
7	B-II 159	Indigenous	Bichena	39	A-III 186	Indigenous	Ambo
8	B-II 187	Indigenous	Bichena	40	A-IV 9	Indigenous	Ambo
9	B-II 188	Indigenous	Bichena	41	A-IV 12	Indigenous	Ambo
10	B-II 191	Indigenous	Bichena	42	A-IV 29	Indigenous	Ambo
11	K-I-73	Indigenous	Kotu	43	A-IV 52	Indigenous	Ambo
12	K-I-90	Indigenous	Kotu	44	A-IV 71	Indigenous	Ambo
13	K-I-95	Indigenous	Kotu	45	CD I-49	Indigenous	CD
14	K-I-108	Indigenous	Kotu	46	CD I 76	Indigenous	CD
15	K-I-128	Indigenous	Kotu	47	CD I 104	Indigenous	CD
16	K-II-9	Indigenous	Kotu	48	CD I 131	Indigenous	CD
17	CIGM91-347-1B-O'	Exotic	CIMMYT	49	Laste (standard check)	Released variety	CIMMYT
18	CDSS93Y33	Exotic	CIMMYT	50	ICD91	Exotic	CIMMYT
19	K-II-126	Indigenous	Kotu	51	CDSS92B128	Exotic	CIMMYT
20	CD91Y7	Exotic	CIMMYT	52	1/CD92	Exotic	CIMMYT
21	CD91989	Exotic	CIMMYT	53	CD97383	Exotic	CIMMYT
22	B-III 38	Indigenous	Bichena	54	CIGM91-349-6B-O'	Exotic	CIMMYT
23	B-III 122	Indigenous	Bichena	55	CDSS93Y104	Exotic	CIMMYT
24	B-III 114	Indigenous	Bichena	56	CD98206	Exotic	CIMMYT
25	B-III 132	Indigenous	Bichena	57	CD94523-1Y	Exotic	CIMMYT
26	A-I 123	Indigenous	Ambo	58	CDSS93Y107	Exotic	CIMMYT
27	A-I 130	Indigenous	Ambo	59	CIGM91-347-6B-O'	Exotic	CIMMYT
28	A-I 138	Indigenous	Ambo	60	CDWS9TM447	Exotic	CIMMYT
29	A-I 171	Indigenous	Ambo	61	1CDSS92B1136	Exotic	CIMMYT
30	A-I 184	Indigenous	Ambo	62	CDSS92B193	Exotic	CIMMYT
31	A-II 124	Indigenous	Ambo	63	CD89239	Exotic	CIMMYT
32	A-II-127	Indigenous	Ambo	64	98OSN PATHO	Exotic	CIMMYT

CD= Chefe Donsa

Experimental design and cultural practices

The trial was laid-out in 8 x 8 triple lattice design. Each genotype was planted in four rows of 2.5 meter length with a row spacing of 20 cm. Seed rate was adjusted based on the kernel size where seed rates of 125 and 150 kg ha^{-1} were used for small and large kernelled genotypes, respectively as per the national recommendation (Tanner *et al.*, 1991). Urea and DAP fertilizers were applied at the rate of 50 and 100 kg ha^{-1}, respectively. The whole of the DAP was applied at sowing while Urea was applied in split, where the first half was applied at sowing and the second half top-dressed at full tillering stage. The trial was hand weeded at 20 and 45 days after emergence (DAE).

Data on plant height, number of spikeletes $spike^{-1}$, number of kernels $spike^{-1}$ and kernel yield $plant^{-1}$ were recorded from five randomly taken plants from

the central two rows, which were tagged ahead of heading. While data for days to heading, days to maturity, biomass yield, thousand kernels weight, grain yield and harvest index were recorded from plots basis (from the central two rows).

Statistical procedures

To compare the total variability present within the evaluated genotypes, the data were subjected to Analysis of Variance (ANOVA) using MSTAT-C computer program (Michigan State University, 1988) following triple lattice design as per Cochran and Cox (1957). To estimate the relative efficiency of lattice design to RCBD, the data were analyzed with both the designs and it was found that the CV for the two designs was non-significant indicating RCBD is as efficient as lattice design. Lattice design is flexible for its analysis (Cochran and Cox, 1957) and RCBD design is convenient in computing combined analysis. Therefore, combined analysis of variance for the three environments (Geregera 2003, Geregera 2004 and Kone 2004) was worked-out using RCBD after testing the homogeneity of error variances for each environment.

Multivariate Analysis of Variance (MANOVA) was computed after aggregating all the traits. Pooled differences among genotypes were tested using Wilk's criterion of aggregate variation, following V-statistics as described in Singh and Chaudhary (1985). The calculated V-value was tested against the tabulated χ^2 value for $p*(g-1)$ degree of freedom at 5% probability level, where;

p = number of characters studied and

g-1= degree of freedom for genotypes

Then, genetic divergence analysis was computed based on multivariate analysis using Mahalanobis's D^2 statistics (Mahalanobis, 1936) using Statistical Package for Agricultural Research (SPAR-1) software.

Estimation of squared distances

Squared distances (D^2) for each pair of genotype combinations were computed using the following formula: $D^2_{ij} = (X_i-X_j)' S^{-1} (X_i-X_j)$, where

D^2_{ij} = the square distance between any two genotypes i and j,

X_i and X_j = the vectors for the values for i^{th} and j^{th} genotypes and

S^{-1} = the inverse of pooled variance covariance matrix

Clustering of genotypes

Clustering of genotypes was done based on the squared distance (D^2) values using Tocher's method as described by Singh and Chaudhary (1985). Average intra- and inter- cluster D^2 values were estimated using the formula $\frac{\sum D_i^2}{n}$, where $\sum D_i^2$ is the sum of distances between all possible combinations (n) of durum wheat genotypes included in a cluster. Significance of the squared distances for each cluster was tested against the tabulated χ^2 values at P degree of freedom at 5% probability level, where P represents the number of characters used for clustering genotypes.

RESULTS AND DISCUSSION

Combined analysis of variance over the three environments indicated highly significant (P<0.001) mean sum of squares due to genotypes for all the characters considered, revealing the existence of substantial amount of variations among the genotypes. Likewise, the performance of durum wheat genotypes for different quantitative traits were significantly varied (P<0.001) across different environments (Table 2), implying the contribution of environmental variations for the phenotypic expression of traits

Table 2 Combined Analysis of Variance over three environments (Geregera 2003, Geregera 2004 and Kone 2004) of 64 durum wheat genotypes for ten quantitative traits.

Source of Variations		Mean square									
	DF	DH	DM	PH	NSS	NK	KYP	BY	TKW	GY	HI
Replication (R)	2										
Genotypes (G)	63	**	**	**	**	**	**	**	**	**	**
Environments (E)	2	**	**	**	**	**	**	**	**	**	**
GxE	126	**	**	**	*	ns	ns	ns	*	ns	**
Residuals	382										
CV %		5.3	5.0	8.4	6.2	16.3	17.8	17.2	12.5	18.1	8.6

Multivariate Analysis of Variance (MANOVA) showed significant differences among genotypes when all the characters were pooled, justifying the need to estimate squared distance (D^2) values for the genotype combinations using these characters.

D^2 values corresponding to 2016 possible comparison among 64 genotypes, taking two genotypes at a time, were computed separately. Based on these estimates of genetic divergence, the 64 durum wheat genotypes were grouped into ten distinct clusters (Table 3). Cluster-I and cluster-VII consisted of maximum number of nine genotypes each, followed by cluster-

VI, cluster-VIII and cluster-X with eight genotypes each. On the other hand, cluster-III and cluster-IX comprised the lowest number of genotypes, each having three genotypes.

Table 3 Summary of ten cluster groups of 64 durum wheat germplasm.

Cluster group	Total number of germplasm	Entries by identification	Origin
I	9	50, 53, 54, 57, 58, 59, 61, 63, 64	All of them are exotic
II	5	26, 27, 41, 44, 47	All of them are indigenous
III	3	49, 60, 62	All of them are exotic
IV	4	2, 3 ,5, 24	All of them are indigenous
V	7	6, 8, 10, 11, 19, 25, 38	All of them are indigenous
VI	8	1, 4, 13, 16, 18, 22, 42, 46	All of them are indigenous except one exotic genotype (CDSS93Y33)
VII	9	12,14,23,28,29,30,34,36,37	All of them are indigenous
VIII	8	31, 32, 33, 35, 39, 43, 45, 48	All of them are indigenous
IX	3	7, 9, 40	All of them are indigenous
X	8	15, 17, 20, 21, 51, 52, 55, 56	All of them are exotic except one indigenous genotype (K-I-128)

Analysis within the indigenous germplasm indicated that there was no correspondence between geographic and genetic distances. That is, germplasm collected from the same geographic area were placed into different clusters indicating their differences and those collected from different geographic regions were placed into the same cluster indicating their closeness. Such occurrences could be due to the same genetic background (base population) of the indigenous germplasm. Therefore, it could be concluded that germplasms collected from different regions might have the same genetic background. Thus, to get more genetic variability, further collection missions should be targeted in all durum wheat growing regions of Ethiopia. The present finding is in agreement with the earlier workers (Garg and Gautam 1988; Walia and Garg 1996; Singh *et al*., 2003) who reported lack of parallelism between genetic and geographic diversity.

However, indigenous and exotic germplasm were grouped into different clusters except in cluster-VI and cluster-X. Cluster-VI consisted of seven indigenous and one exotic germplasm while cluster-X consisted of seven exotic and one indigenous genotype, implying the presence of parallelism between genetic and geographic distances. The standard check (*Laste*), being exotic in origin, was grouped in cluster-III with two exotic germplasm. This clearly showed that wider geographic distances for indigenous and exotic germplasm created wider genetic variability because of adaptation to different environmental conditions. The present finding is in agreement with Adary (1978), who reported the relation of genetic divergence to geographical distance among countries of origin and to

environmental differences among sites of collection.

Mean values of cluster-III (Table 4) encompassed desirable combinations of characters having maximum number of kernels plant^{-1} (47.83), maximum grain yield (4.3 ton ha^{-1}), maximum harvest index (43.5%) and high thousand-kernel weight (39.2 g). Therefore, it could serve as valuable component for future crossing program. Similarly, cluster-IX comprised tall plant height (93.3 cm) and early maturing (127 days) genotypes. Therefore, genotypes from these two clusters could be used as parents for future breeding program to develop superior varieties.

Table 4 Mean values of the eleven quantitative characters of ten clusters of durum wheat germplasms.

Characters	Clusters										% Contribution to divergence
	I	II	III	IV	V	VI	VII	VIII	IX	X	
DH	72.00	81.00	76.00	77.00	71.00	76.00	74.00	70.00	71.00	71.00	10.1
DM	128.00	135.00	133.00	136.00	125.00	130.00	135.00	137.00	127.00	130.00	6.2
PH	70.00	78.40	74.60	86.40	93.20	85.50	99.20	90.40	93.30	79.80	15.1
NSS	14.70	15.33	15.39	15.80	15.04	15.09	16.18	17.18	14.84	14.51	10.4
NK	35.60	25.50	47.83	28.80	27.07	26.65	30.53	28.28	25.60	33.50	11.0
KYP	1.37	0.87	1.90	1.17	1.03	0.97	1.29	1.08	1.13	1.54	10.5
BY	8.80	7.20	10.00	7.80	9.30	7.90	10.20	9.00	8.20	9.10	9.5
TKW	37.80	36.30	39.20	40.10	38.10	34.90	42.50	38.60	40.30	45.50	9.3
GY	3.60	2.60	4.30	3.00	3.50	2.90	3.70	3.10	3.00	3.70	11.1
HI	41.20	36.30	43.50	38.60	37.40	36.40	36.50	34.50	37.40	40.30	6.8

DH=Days to heading, DM=Days to maturity, PH= Plant height, NSS=Number of spikelets spike^{-1}, NK= Number of kernels spike^{-1}, KYP=Kernel yield plant^{-1} (g), BY= Biological yield (ton ha^{-1}), TKW= Thousand kernel weight (g), GY=Grain yield (ton ha^{-1}) and HI=harvest index (%)

Plant height accounted for the highest contribution to total genetic divergence (15.1%) followed by grain yield (11.1%) and number of kernels spike^{-1} (11.0%), while days to maturity had the least contribution (6.2%) (Table 4). This finding is partly in agreement with Das and Brothakur (1973) who reported the highest contribution of days to heading, thousand kernels weight and plant height to genetic divergence in rice.

Estimates of intra- and inter-cluster squared distances (D^2)

Intra- and inter-cluster D^2 values among the ten clusters are presented in Table 5. The magnitude of intra-cluster distances indicates the extent of genetic variability between the durum wheat genotypes of the same cluster. The intra-cluster distance (D^2) varied from 1.66 to 5.06 where the maximum intra-cluster distance was obtained in cluster-VII while the lowest intra-cluster distance was recorded in cluster-IX. The relatively low value of intra-cluster distance suggests the presence of narrow genetic variations within a cluster.

Table 5 Average intra-cluster (bolded main diagonal) and inter-cluster (off diagonal) D^2 values among 10 clusters of durum wheat germplasm.

Cluster	I	II	III	IV	V	VI	VII	VIII	IX	X
I	**3.31**									
II	23.62**	**2.69**								
III	14.29	57.15**	**4.88**							
IV	18.75*	7.08	37.58**	**3.65**						
V	12.53	19.45*	35.52**	12.39	**2.72**					
VI	15.60	4.20	45.43**	5.48	6.86	**2.10**				
VII	18.75*	25.70**	26.83**	11.83	9.00	16.48	**5.06**			
VIII	25.40**	11.02	42.90**	5.62	14.06	8.94	7.67	**3.96**		
IX	20.52*	16.97	45.83**	7.56	4.71	6.50	14.59	14.98	**1.66**	
X	5.81	29.81**	15.76	17.14	12.46	20.70*	11.76	24.70**	16.48	**4.71**

The highest average inter-cluster distance was exhibited between cluster-II and -III (D^2=57.15) followed by cluster-III and -IX (D^2=45.83) and cluster-III and VI (D^2=45.43). The genotypes belonging to these clusters were found genetically most divergent. Minimum inter-cluster D^2 value was observed between cluster-II and-VI (D^2=4.20) indicating that genotypes of these clusters were genetically close. Thus, crossing of genotypes from these two clusters wouldn't produce higher amount of variability in the segregating (F_2) populations. Parents for hybridization could be selected on the basis of their large inter-cluster distance for isolating useful recombinants in the segregating generations. Increasing parental distance implies a greater number of contrasting alleles at the desired loci, and then to the extent that these loci recombine in the F_2 and F_3 generations following a cross of distantly related parents, the greater will be the opportunities for effective selection for yield (Ghaderi *et al.*, 1984). Hence, it would be logical to incorporate divergent genotypes having useful traits in the breeding program. From this study, germplasm from cluster-IX (indigenous) and cluster-III (exotic) were genetically divergent. Moreover, these clusters comprised desirable combinations of traits. Thus, the genotypes of these two clusters hold great promise as parents to obtain considerable variability in the segregating populations.

CONCLUSION

From this study, we can conclude the absence of correspondence between geographic and genetic distances among indigenous durum wheat germplasm. Germplsam collected from the same geographic area were clustered into different groups showing their genetic differences and those collected from different geographic regions were placed into the same cluster. This might be associated with the same genetic background. On the other hand, indigenous and exotic germplasm were grouped into different

clusters, implying the presence of parallelism between genetic and geographic distances. Thus, there is an opportunity to improve grain yield through hybridization of genotypes from genetically divergent clusters and subsequent selection from the segregating generations. Crossing of parents involving cluster-IX (indigenous) with cluster-III (exotic) would complement each other and could result in high genetic variability and superior segregates having good combinations of characters from both parents.

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