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Karyomorphological and Morphometric Studies of Ploidy Levels in Some Wheat (*Triticum aestivum* L.) Genotypes

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Abstract. Karyomorphological and morphometric investigations of different ploidy levels of 14 genotypes of *Triticum aestivum* L. and one genotype of *Triticum durum* Desf. showed that, total chromosomal length (TCL) varied between genotypes. The highest value (56.21 μm) was recorded with mean chromosomal length of 8.03 ± 0.81 μm, while the lowest value of TCL (31.65 μm) was found with mean chromosomal length (MCL) of 4.52 ± 0.41 μm. Simple Pearson correlation coefficient (r) between TCL and MCL was the highest (r = 1.0 and P = 0.000). While the correlation coefficients between mean arm ratio (MAR) and parameters: total form (TF), intrachromosomal asymmetry index (A_1) and m (karyotype; metacentric region chromosome) as well as the coefficients between TF and m and between A_1 and m were the only significant (P<0.01) ones. Intrachromosomal asymmetry had a significant (P = 0.000) effect of total form percent than interchomosomal index. TCL and MCL were the most important karyological features influencing the principal component analysis and had 81.7 % variation, while in combination with MAR revealed 94% variation. Cluster dendrogram revealed close association and adjacent phylogenetic relatedness of tri- and hexaploid and also tetra- and hexaploid genotypes.

Keywords: cluster analysis, karyotype features, principal component analysis, wheat (*Triticum aestivum* L.), genotypes

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Introduction

The genus *Triticum*, both wild and cultivated, has been one of the most intensively studied groups of plants. Hexaploid wheat (2*n* = 6*x* = 42, *Triticum aestivum* L.) (~600 million tons is produced annually) is the most widely adapted of the major crops, thus offering potential for increased food production. It includes domesticated diploid and tetraploid wheats as well as rye (*Secale cereale*) and barley (*Hordeum vulgare*) (Huang *et al*., 2002).

Cytogenetical investigation is one of the best documented experimental proofs for the elucidation of the mode of speciation on different groups of plants (Zohary, 1984; Kumar and Rai, 2007).

The morphological and chromosomal studies are necessary for inducing genetic variations, transfer of useful characters and for study of genetic and cytogenetic variations using cluster analysis between genotypes (Siahsar *et al*., 2005). Studies of the morphology of chromosomes in hexaploid wheat have been made by many workers (Jahan and Vahidy, 1989; Kimber, 1971; Khan, 1963; Schulz-Schaeffer and Haun, 1961).

Karyotype analysis has played an important role in the identification and designation of chromosomes in many plant species. Among others, morphometric investigation of the karyological data can be studied through multivariate procedures including principal component and cluster analysis. Principal component analysis (PCA) is a multivariate statistical technique for exploration and simplifying complex data sets through transforming a number of possibly correlated variables into a smaller number of variables called principal components (Everitt and Dunn, 1992).

Cluster analysis can be used to identify variables which can be classified into main groups and subgroups based on similarity and dissimilarity. This technique is useful for parental selection in breeding programs (El-Deeb and Mohamed, 1999) and crop modeling (Leilah and Al-Khateeb, 2005; Siahsar *et al.*, 2005; Jaynes *et al.*, 2003; Morphy, *et al.*, 1992; Souza and Sorvells, 1991).

In this experiment, we performed karyological and morphometric evaluations in a population of diplo-tetra- and hexaploid wheat genotypes to estimate the best parameters interpreting the genetic diversity using karyological features.

Materials and Methods

Plant materials. The experiment consisted of five F_8 double-haploid lines, obtained as somaclones *via* regeneration of

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plants from callus derived from immature inflorescences/ embryos of three hexaploid bread wheat genotypes and two d11887825 generations up to F_8 derived from a single R_0 doubled-haploid plant through selfed progeny for each line. These five lines were compared with nine bread wheat and one durum wheat (ID-10) genotypes. Features of the studied 15 genotypes are given in Table 1. Wheat genotypes were grown at Siwa Oasis, Tegzerty Experimental Farm of Desert Research Center during 2004 - 2005 winter seasons. Soil of the experimental site was characterized to be of sandy loam texture, saline (ECe 12.3 dS/m), calcareous ($CaCO_3$, 18.1 %) with 0.7 % organic matter. Deep artesian well irrigation water of EC about 4.1 dS/m was used for supplying nine irrigations throughout the growing seasons.

Methodology. Cytological preparations were carried out on root tips obtained from seeds germinated on sterile moist filter paper in petri dishes at 25 °C. Roots were pretreated with 0.05 % colchicine solution for 2-3 h, fixed in Carnoy for 24 h and stored in 70 % ethanol at 4 °C. Cytological preparations were made using the Feulgen squash method. The well-spread c-metaphase chromosomes were photographed from temporary preparations at magnification, 2000×. Slides of the original karyotypes are preserved in the Laboratory of Cytogenetics of Biological Sciences and Geology Department, Faculty of Education, Ain Shams University, Roxy, Cairo, Egypt. A karyogram for each genotype was constructed by arranging the chromosomes in homologous pairs by order of their length and arm ratio as measured from the photographic prints. The number of chromosome types was determined as described by Levan *et al.* (1965). Measurement of chromosomal length was taken on the same photographs of the karyogram. Variation in mean chromosomal length (MCL) and chromosome arm ratio (MAR) within the karyotype had been estimated by calculating the standard error (SE) of these parameters. Karyotype asymmetry deduced from the ratio between the short arms of the chromosomes and their total length was expressed as total form percent (TF %) as proposed by Huzwara (1962). Karyotype asymmetry expressed by the ratio between chromosome arms has also been estimated as the intrachromosomal asymmetry index (A_1) (Romero-Zarco, 1986). The value of A_1 was considered close to zero if all chromosomes were metacentric and approx. one if all chromosomes are telocentric. Karyotype asymmetry due to the ratio between sizes of different chromosomes was also estimated as the interchromosomal asymmetry index (A_2) using Pearson's dispersion coefficient, which is the ratio between the standard deviation and the mean chromosome length (Romero-Zarco 1986).

Table 1. Name, source, pedigree and selection history of the wheat genotypes used in the study

Genotype no.	Genotype	Source	Pedigree/selection history
1	Mexipak 65	ICARDA	II 8156-OPAK
2	Sahel - 1	Egypt	Ns. 732/Pima//Veery "S" #5 Sd735-4Sd-1Sd-1Sd-0Sd
3	Mar – 3	Egypt*	Cham 4/Sakha 8//2* Sakha 8 Su74-3Mr-32Mr-5Sw-13Sw-0Sw
4	ID - 10	ICARDA	ICD88-1233-ABL-8AP-0AP-3AP-0AP
5	Gem - 7	Egypt	CMH74A-630/Xs//Seri82/3/Agent/C Gm4611-2Gm-3Gm-16Gm-0Gm
6	Giza - 168	Egypt	MRL/BUC//SERI CM93046-8M-0Y-0M-2Y-0B-0GZ
7	Mar- 5	Egypt*	Giza 162//Bch'S/4/PI-ICW79 Su5-11Mr-38Mr-1Mr-0Mr
8	Cham - 4	Syria	CM 39816-1S-1AP-0AP
9	S_8 / 17	Egypt	R_8 tissue culture regenerated double haploid plant
10	LR / 1	Egypt	R_8 tissue culture regenerated double haploid plant
11	LR / 2	Egypt	R_8 tissue culture regenerated double haploid plant
12	Giza - 160 / 1	Egypt	R_8 tissue culture regenerated double haploid plant
13	Giza - 160	Egypt	L.2188/1131 – Chenab 70/ Giza 155
14	Lerma Rojo - 64	Spain	Long – term check
15	L R / 3	Egypt	R_8 tissue culture regenerated double haploid plant

*Newly bred line released through Desert Research Center, Wheat Breeding Program; ICARDA = International Center of Agricultural Research in the Dry Areas.

Table 2. Karyological features of the studied genotypes of *Triticum aestivum* L

Genotype	*x*	2*n*	TCL (μm)	MCL ± SE (μm)	MAR ± SE (*r*-value)	TF %	A_1	A_2	SAT	Chromosome type		
										M	m	sm
1	7	21	37.59	5.37 ± 0.30	1.30 ± 0.07	43.76	0.22	0.15	-	-	7	-
2	7	28	56.21	8.03 ± 0.81	1.35 ± 0.13	43.50	0.23	0.27	-	-	6	1
3	7	28	51.40	7.34 ± 0.41	1.42 ± 0.08	41.67	0.29	0.15	+	-	6	1
4	7	14	45.82	6.55 ± 0.79	1.63 ± 0.11	38.02	0.37	0.32	+	-	5	2
5	7	42	42.41	6.06 ± 0.51	1.45 ± 0.06	41.19	0.30	0.22	+	-	7	-
6	7	28	33.48	4.78 ± 0.35	1.30 ± 0.07	43.79	0.22	0.19	-	1	6	-
7	7	42	32.38	4.63 ± 0.45	1.28 ± 0.04	43.45	0.22	0.26	-	-	7	-
8	7	42	31.65	4.52 ± 0.41	1.23 ± 0.06	44.49	0.18	0.24	-	-	7	-
9	7	42	40.44	5.78 ± 0.42	1.32 ± 0.06	43.18	0.23	0.19	-	-	7	-
10	7	28	35.02	5.00 ± 0.38	1.33 ± 0.04	42.89	0.24	0.20	-	-	7	-
11	7	28	47.95	6.85 ± 0.48	1.24 ± 0.05	44.75	0.18	0.18	+	-	7	-
12	7	42	38.53	5.50 ± 0.48	1.48 ± 0.08	40.85	0.31	0.23	+	-	6	1
13	7	21	38.67	5.52 ± 0.40	1.51 ± 0.13	40.29	0.31	0.19	+	-	4	3
14	7	42	40.51	5.79 ± 0.43	1.22 ± 0.04	45.40	0.17	0.20	+	-	7	-
15	7	42	37.71	5.39 ± 0.52	1.38 ± 0.08	41.69	0.26	0.26	+	-	7	-

NS = Non-significant at 0.05 of statistical level; ** = significant at 0.05 of statistical level; *** = significant at 0.001 of statistical level. TLC = mean chromosome length; MCL = mean chromosome length; MAR = mean arm ratio; SE = standard error; M = metacentric chromosome; m = metacentric region chromosome; sm = submetacentric chromosome; TF% = total form percent; SAT = satellite; A_1 = intrachromosomal asymmetry index; A_2 = interchromosomal asymmetry index.

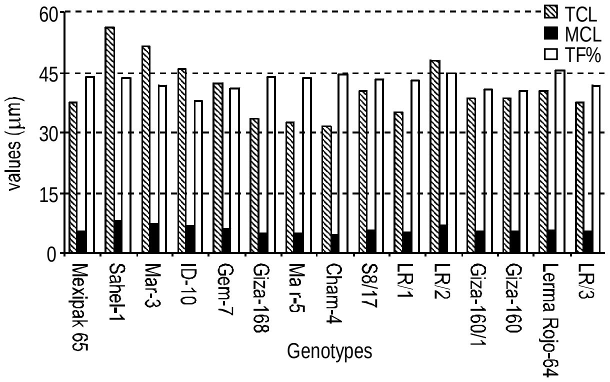

Fig. 1. Comparison of 15 genotypes on the basis of total chromosome length (TCL), mean chromosome length (MCL) and total form percentage (TF %).

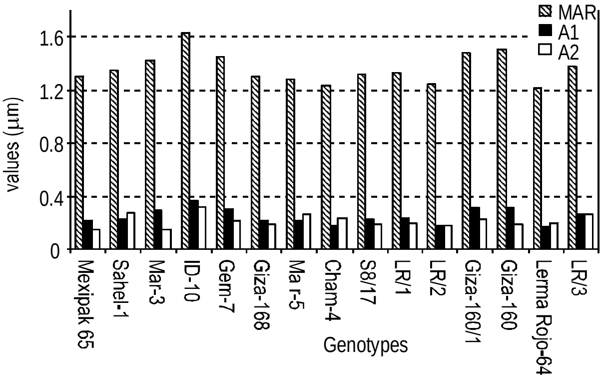

Fig. 2. Comparison of 15 genotypes on the basis of total chromosome arm ratio (MAR), intrachromosomal asymmetry index (A_1) and interchromosomal asymmetry index (A_2).

For the numerical characterization of the karyotypes, the following parameters were calculated (Seijo and Fernandez, 2003): (1): total chromosomal length of the haploid complement (TCL); (2) mean chromosome length (MCL); (3) intrachromosomal asymmetry index (A_1) = 1 - [$\sum(b/B)/n$]; where b and B are the mean length of short and long arms of each pair of homologues, respectively, n is the number of homologues, (4) interchromosomal asymmetry index (A_2) = s/x, were s is the standard deviation and x the mean chromosome length. Karyotype asymmetry was determined using A_1 and A_2 indices (Romero-Zarco, 1986) and the categories of Stebbins (1971) and (5) total form percentage (TF %) which measures the symmetry of the chromosomes over the whole karyotype (El-Bakatoushi and Richards, 2005).

Statistical analysis. In order to determine the association of the karyotype features, simple Pearson coefficient of correlation (r) and also the linear regression analysis between MAR and A_1 and A_2 were applied. For grouping the lines showing similar karyotype characteristics, clustering method ward as well as ordination based on principal components analysis (PCA) were performed (Sheidai *et al*., 2006). Statistical analysis was performed using Minitab V.15 statistical software (Minitab Inc, 2008).

Results and Discussion

A summary of the karyological features of the studied genotypes of *Triticum aestivum* L. is given in Table 2 and Fig. 1 and 2. Among the 15 genotypes studied different ploidy levels appeared including a diploid (genotype 4), two triploid (genotypes 1 and 13), five tetraploid (genotypes 2, 3, 6, 10 and 11) and seven hexaploid (genotypes 5, 7, 8, 9, 12, 14 and 15). It is well known that the mode of chromosome pairing in triploid ($2n = 3x = 21$) and pentaploid ($2n = 5x = 35$) hybrids helped Kihara (1944) to uncover the ancestral species of allopolyploid wheats.

The genotypes had different total chromosomal lengths (TCL) (Table 2; Fig. 1 and 2) ranging from 56.21 (in genotype 2) to 31.65 µm (in genotype 8). The karyotypes of the examined genotypes are considerably symmetrical with regard to chromosomal length. The most similar chromosomes are scored in genotype (1) (SE of MCL = 0.30 µm). The degree of karyotype asymmetry as indicated by TF (%) value ranges between 38.02 % in genotype 4 and 45.40 % in genotype 14. In general, A_1 and A_2 values show high degree of karyotype symmetry in the majority of the genotype studied (Table 2). Eight of the studied genotypes (3, 4, 5, 11, 12, 13, 14 and 15) are characterized by the presence of SAT in their chromosome arms (Table 2).

Hexaploid wheat ($2n = 6x = 42$) is an allohexaploid and contains three genomes. Karp and Maddock (1984) studied chromosomes of 192 regenerated plants derived from immature embryo callus of hexaploid wheat cultivars. A total of 71 % of the regenerants carried the expected $2n = 42$ chromosomes and 29 % of the plants were aneuploid ($2n$ = 38 to 45). It is thought that somaclonal variation possibly occurs during the process of plant tissue culture which is considered to provide a source of new germplasm. Polyploidy is one of the most frequent incidents among the somaclonal variations (Sangthong *et al*., 2004).

Cytological investigations revealed that the number of chromosomes varied highly in anther-derived calli and in their regenerants (Nishibayashi *et al*., 1989); high variability was also reported in chromosomal number during callus induction and plant regeneration from mature barley embryos (Lupotto, 1984). In an experiment, González *et al.* (1996) determined chromosomal number in calli of barley plants cultures regenerated from two kinds of explants, immature embryos and seedling leaves. They pointed out diploid cells were predominant in all cases; although in leaf-derived cultures, tetraploid cells ($2n = 4x = 28$) showed a tendency to increase as duration of culture increased and after more than six months in culture, diploid cells decreased down to almost 70 %. Aneuploid cells were generally infrequent in all cases. The source of explant had been more important than the genotype (cultivar) and the type of callus (morphogenic *vs.* non-morphogenic) in the chromosomal stability of cultures as time increased. From short term cultures, only 1.85% of the regenerated plants were tetraploid; the remaining were diploids.

Brasileiro *et al*. (1999) reported that in anther derived tomato plant cultures, the regenerated plants presented tetraploid cells and rare diploid cells. These tetraploid plants could be used as source of further trisomic lines, for the purpose of genetic localization studies and analysis of protein compound.

The pair-wise simple Pearson correlation coefficients between all karyotype features is given in Table 3. Significant correlation may be observed between total chromosomal length (TCL) and mean chromosomal length (r =1.0 and P<0.001). This relationship is well expected because total and mean chromo-

Table 3. Simple pair-wise Pearson correlation coefficients between karyotype features

	TCL	MCL	MAR	TF(%)	A_1	A_2
MCL	1.00000***					
MAR	0.27831^{NS}	0.27802^{NS}				
TF %	-0.17402^{NS}	-0.17391^{NS}	-0.98475***			
A_1	0.22976^{NS}	0.22946^{NS}	0.98891***	-0.98691***		
A_2	0.04543^{NS}	0.04688^{NS}	0.38636^{NS}	-0.42095^{NS}	0.34446^{NS}	
m	-0.23761^{NS}	-0.23646^{NS}	-0.72690**	0.66574**	-0.66292**	0.16694^{NS}

NS = Non-significant at 0.05 of statistical level; ** = significant at 0.05 of statistical level; *** = significant at 0.001 of statistical level. TLC = mean chromosome length; MCL = mean chromosome length; MAR = mean arm ratio; SE = standard error; M = metacentric chromosome; m = metacentric region chromosome; sm = submetacentric chromosome; TF% = total form percent; SAT = satellite; A_1 = intrachromosomal asymmetry index; A_2 = interchromosomal asymmetry index.

somal lengths are associated positively. So the karyological features function in the same direction, each of which could be applied to the karyological studies. The next significant correlation coefficients include a negative value for mean arm ratio (MAR) with TF % (r ~ -0.98 and P<0.001), a positive value for MAR with A_1 (r ~ -0.99 and P<0.001) and a negative coefficient for MAR with m (r ~ -0.73 and P<0.01). It is presumably logical that intrachromosomal asymmetric index (A_1) is well related to mean arm ratio. The same holds for MAR and m (chromosome type). There are also negative significant correlation coefficients between total form percentage (TF %) and A_1 (r ~ -0.99 and P<0.001) and A_1 and m (r ~ -0.66 and P<0.01). The other pair-wise correlation coefficients between other karyotype features were not significant (P>0.05). Interestingly, the interchromosomal asymmetry (A_2) in this study showed no relation to individual chromosomal characteristics.

The results of stepwise regression revealed that among six karyotype features (TCL, MCL, MAR, A_1, A_2 and m), the first four features had the most effect on total form (TF %) and A_2 had not any special effect. The multiple linear regression analysis showed a significant causative relationship between the four predicting features and TF percentage. The simple regression analysis individually showed significant relationships between MAR, A_1 and m and TF %. Other features individually did not have significant effect on total form percentage. So the contributions of these three features are more notable and effective in determining total form of chromosomes and can be definitely applied in the karyological studies.

Though the MAR, A_1 and m indicated that they determine the total form of a chromosomal set, but according to morphometric investigations (principal component analysis and cluster categorization) they cannot be applied in interpretation of all aspects of the karyotype studies. The reason is that, though these three parameters visually depict the karyotype, but there remains the problem of grouping and classifying different genotypes in the diversity studies. Applying principal component analysis (PCA) of some of the features, this problem can be solved. For evaluating and grouping 15 wheat genotypes of various ploidy levels, principal component analysis of this experiment has been performed, standardizing the data of karyotype features and using correlation coefficient matrix for PCA as shown in Table 4.

PCA results indicated that the first principal component had variance (eigenvalue) of 3.915 and accounted for 55.9 % of the total variance. This data (Table 4), graphically shown in Fig. 3, demonstrated that an increase in the number of components was associated with a decrease in the eigenvalue. This trend reached its maximum at three factors. Accordingly, it is reasonable to assume that the principal components analysis had grouped the estimated wheat variables into three main components which all together accounted for 94.0 % of the total variation of karyotype features. The coefficients listed under PC_1 show how to calculate the principal component scores. Results showed that PC_1 correlated moderately well with MAR, TF %, A_1 and m. Meanwhile, the PC_2 correlated moderately with TCL and MCL. The third component i. e. PC_3 had a moderate correlation with ‘m’ and and the highest correlation with ‘A_2’ karyotype features. Variables which significantly correlated with the first three eigenvectors were the variables with the greatest variability. The factor loadings (Fig. 2) refer to the coefficients in each principal component or the correlation between the component and the variables. A high correlation between PC_1 and a variable indicates that the

Table 4. (a) Principal component analysis: (b) Eigenanalysis of the correlation matrix

(a) Principal component analysis

Variable	PC_1	PC_2	PC_3	PC_4	PC_5	PC_6	PC_7
TCL	-0.226	-0.664	-0.079	-0.024	-0.025	0.023	0.707
MCL	-0.226	-0.664	-0.081	-0.025	-0.033	0.019	-0.707
MAR	-0.492	0.128	0.074	-0.177	0.272	-0.794	0.000
TF %	0.476	-0.207	-0.030	0.270	0.802	-0.117	-0.004
A_1	-0.478	0.158	0.102	-0.336	0.527	0.587	-0.004
A_2	-0.220	0.174	-0.919	0.270	0.056	0.038	0.001
m	0.390	-0.062	-0.356	-0.842	0.009	-0.094	0.001

(b) Eigen analysis of the correlation matrix

Eigenvalue	3.9150	1.8032	0.8602	0.4090	0.0083	0.0043
Proportion	0.559	0.258	0.123	0.058	0.001	0.001
Cumulative	0.559	0.817	0.940	0.998	0.999	1.000

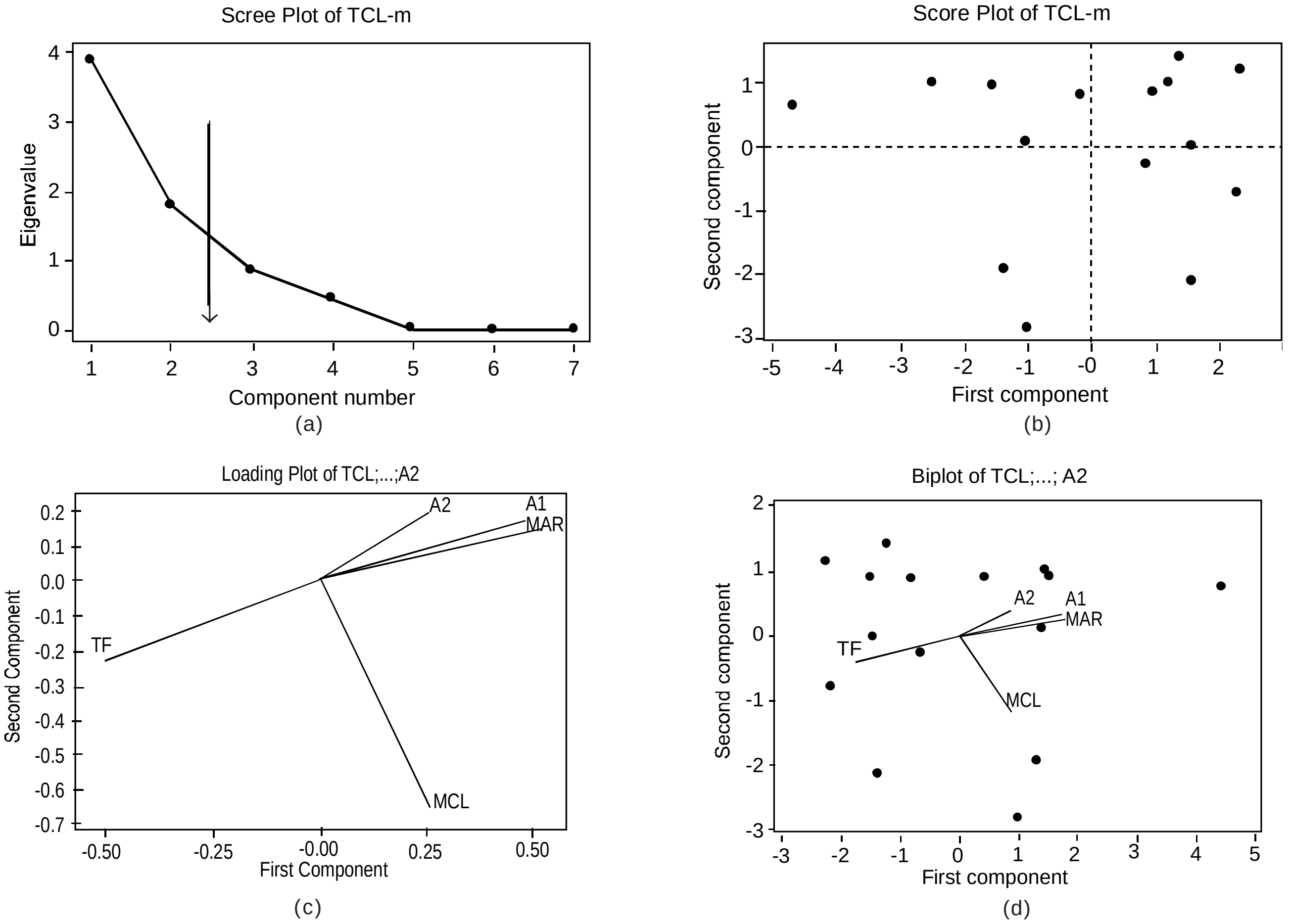

Fig. 3. (a) Scree plot showing eigenvalues in response to number of components for the estimated variables of wheat. (b) Score plot of the PCA. (c) Loading plot of the PCA. (d) Biplot of the PCA.

variable is associated with the direction of the maximum amount of variation in the data set (Leilah and Al-Khateeb, 2005).

$$PC_1 = -0.226\,(TCL) - 0.226\,(MCL) - 0.492\ (MAR) + 0.476\,(TF\,\%) - 0.478\,(A_1) - 0.220\,(A_2) + 0.390\,(m)$$

It should be noted that the interpretation of the principal components is subjective; however, obvious patterns emerge quite often. For instance, one could think of the first principal component as representing total chromosomal length, mean chromosomal length, mean arm ratio and total form percentage in the karyotype studies for grouping different genotypes, because the coefficients of the first three terms have the same sign and are not close to zero. This case could be followed for the second principal component which has variance 1.8032 and accounts for 25.8 % of the data variability, which has been calculated from the original data using the coefficients listed under PC_2. This component could be thought of as an important alternative to the first component for grouping. Together, the first two and the first three principal components represent 81.7 % and 94 %, respectively, of the total variability. Thus, most of the data structure can be captured in two or three underlying dimensions. The remaining principal components account for a very small proportion of the variability and are probably unimportant. The eigenvalue (scree) plot provides this information visually (Fig. 3 (b-d)) (Minitab Inc., 2008).

The next important part of the study is classifying 15 genotypes using multivariate schedule cluster analysis wherein the Ward method was utilized and cluster tree was drawn (Fig. 4) using Minitab software (Minitab Inc., 2008). The cluster analysis of karyological data and ordination of taxa on the first three PCA axes are given in Fig. 3 (A-D). Grouping by ordination of taxa based on the first three PCA axes supports the clustering results. Briefly, cluster methods start with the calculation of the distance of each variable in relation to other variables. Groups are then formed by the process of agglomeration division. In this process, all variables start individually. Close groups then gradually merge until

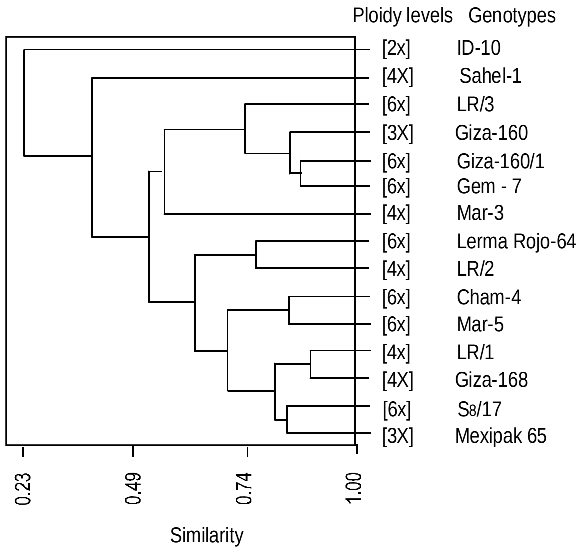

Fig. 4. Cluster categorization of 15 wheat (*T. aestivum* L.) genotypes of different ploidy levels obtained *via* Ward method. Similarity levels of the estimated 7 wheat characters (variables) using Ward cluster analysis, showing cluster 1 (ID-10, diploid genotype), cluster 2 (including Sahel-1, tetraploid genotype) and cluster 3 (including other genotypes without diploid, including triploids.

finally all variables form a single group (Leilah and Al-Khateeb, 2005).

As is apparent (Fig.4), 15 genotypes were classified in three major categories, so that ID-10, a diploid, and Sahel-1, a tetraploid genotype, were located in 2 discrete categories and LR/3, Giza-160, Giza-160/1 and Gem-7 in the next category. The tetraploid Mar-3 genotype was located in another separate cluster. The other category identified Lerma Rojo-64 and LR/2. And finally the last cluster belonged to Cham-4, Mar-5, LR/1, Giza-168, S8/17 and Mexipak 65. The most important finding, derived from the callus culture, is that these genotypes showed various ploidy levels ranging from diploid to hexaploid. It was found that the diploid and other ploidy levels especially triploid had never been placed adjacent to one another in a common category, i.e. the triploid and hexaploid genotypes were always in the same categories which may be due to their remotely different chromosomal set.

Moreover, tetraploid-tetraploid, hexaploid-hexaploid and even tetraploid-hexaploid genotypes were put in the same categories. Interestingly, the results showed that the closeness of triploid and hexaploid genotypes may prove a close evolutionary relationship between these two ploidy levels. Thus the hexaploid wheat plant could be obtained simpley through doubling the chromosomal set (diploidization) of a triploid plant, regenerated from endosperm-derivation.

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