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Multivariate analysis of genetic diversity in wheat (*Triticum aestivum* L.) recombinant inbred lines using agronomic traits

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ABSTRACT

Determination of genetic diversity is useful for plant breeding and hence production of more efficient plant species under different conditions. A set of 94 bread wheat recombinant inbred lines derived from cross between Roshan and Superhed#2 varieties was evaluated using a randomized complete block design (RCBD) with two replications. Days to heading, flag leaf area, peduncle length, spike length, plant height, number of spikelet per spike, number of spikes, number of grain per spike, 1000 grain weight, grain yield, shoot biomass, percent of grain protein, straw yield and harvest index were measured. Analysis of variance revealed significant differences among the lines for all the studied traits. The level of genetic variation was higher for peduncle length, flag leaf area, number of spikes, grain yield, straw yield and shoot biomass. Cluster analysis based on all the traits using Ward's algorithm and squared Euclidean distances assigned the lines into three groups. In these grouping, group two lines showed highest mean of grain yield. In factor analysis, five first factors explained 80.26% of total variation. First factor determining 23.94% of the variation was named as grain yield factor. Cluster analysis based on the five factors grouped the lines into three groups. The first group lines were superior with respect to grain yield.

Key words: Genetic diversity, Cluser analysis, Factor analysis, Recombinant inbred lines, Agronomic traits, Grain yield.

INTRODUCTION

Bread wheat (*Triticum aestivum* L.) is the most important crop cultivated in the world and planting in the extensive area of environmental conditions in the world and also it produces about 20 percent food resources of the world people [1]. Genetic diversity of plants determines their potential for improved efficiency and hence their use for breeding, which eventually may result in enhanced food production [2]. Many modern cultivars in wheat and in other crops as well, are often genetically similar, with a rather narrow genetic base. Therefore, in breeding we need to also utilize sources of new diversity. New variation can be created by hybridization between different parental cultivars [3].

One of the important approaches to wheat breeding is hybridization and subsequent selection. Plants' choice is the first step in plant breeding program through hybridization. In order to benefit transgressive segregation, genetic distance between parents is necessary [4]. The higher genetic distance between parents, the higher hetrosis in progeny can be observed [5,6]. Estimation of genetic distance is one of appropriate tools for parental selection in wheat hybridization programs [7]. Appropriate selection of the parents is essential to be used in crossing nurseries to enhance the genetic recombination for potential yield increase [8].

Some appropriate methods, cluster analysis, PCA and factor analysis, for genetic diversity identification, parental section, tracing pathway to evolution of crops, center of origin and diversity, and study interaction between the environments are currently available [9,10,11,12]. Factor analysis is a method for investigation whether a number of variables of interest are linearly related to a smaller number of unobservable factors. It is also used in general to reduce a larger set of variables to a smaller set of variables that explain the important dimensions of variability. A method widely used for determining a first set of loadings is the principal component method. This method seeks values of the loadings that bring the estimate of the total communality as close as possible to the total of the observed variances [13].

Khodadadi et al. [7] determined the genetic diversity of 36 winter wheat cultivars from Iran and by using cluster analysis, seven clusters were determined. Mollasadeghi et al. [1] reported that clusters analysis of data was placed 8 genotypes of bread wheat on two groups. Zaeifizadeh et al. [14] clustered 30 wheat cultivars into three groups based on performance of genotypes. Ahmadzadeh et al. [15] classified 37 durum wheat genotypes from Iran and Azerbaijan republic in normal irrigation and drought stress conditions using cluster analysis and in both conditions, genotypes divided into three groups. Narouee Rad [16] determined the genetic diversity of wheat landraces in the west of Iran by using cluster analysis, six clusters were determined for different areas. Fang et al. [17] clustered 120 genotypes of durum wheat into five groups based on maturity date, plant height, spike length, number of seeds per spike, 1000- seed weight and spike seed yield. Gupta et al. [18] reported that the generations of 40 advanced lines of wheat with 11 controls were evaluated. Factor analysis, 15 traits associated with yield and grain quality to address five main characteristics of spike, grain characteristics and quality protein and reduced tillering. Heydari et al. [19] performed factor analysis based on maximum likelihood in bread wheat doublehaploid population that indicated five factors explaining 80.4% and 73.9% of total variation in 2003 and 2004, respectively. In other research five first factors explained 82.58% of variation. First factor explaining 26.79% of variation was identified as an effective factor in plant weight and performance [20].

The main objective of this study was to capture the potential genetic diversity among wheat recombinant inbred lines by using cluster analysis and cluster analysis based on factor analysis and to identify effective factors on genetic improvement.

MATERIALS AND METHODS

In this research 96 bread wheat recombinant inbred lines derived from Roshan and superhed#2 cross with their parents were cultivated in the research field of faculty of Agriculture, Tabriz University, Tabriz, Iran in 2007. Roshan is a resistant cultivar to salinity and drought stresses with high height and Superhead is a dwarf cultivar and susceptible to salinity and drought stresses with high grain yield. These lines provided from ABRI[1] that had been numbered from 1-96. Genotypes number 22 and 77 was removed from experiment because didn't reach to the heading stage and finally 94 recombinant inbred lines were evaluated with their parents. A randomized complete block design with two replications was used. Each plot consisted of three rows 1m long. The interrow and interplant spacings were 20 and 5cm, respectively. Nitrogen fertilizer was applied at a rate of 40kg ha^{-1} at pre-emergence, tillering and stem elongation stages. Plots were irrigated as needed to keep soil moisture optimal for plant growth. Observation were recorded on 12 traits, namely days to heading (days to 50% heading), plant height, flag leaf area, peduncle length, spike length, number of spikelet per spike, number of spikes per m^2, number of grain per spike, 1000 grain weight, grain yield per m^2, shoot biomass per m^2 and percent of grain protein. Harvest index was determined from the ratio of grain yield to shoot biomass. Straw yield was determined from the difference between shoot biomass and grain yield. Ten plants per plot were collected and the mean data points were used for statistical analysis.

The performed statistical analysis including Shapiro-Wilk normality test, analysis of variance, cluster analysis based on all measured traits using Ward's algorithm and standardized means [11], factor analysis using principle components analysis and Varimax rotation of provisional factors and cluster analysis based on the five extracted factors. Discriminant analysis was performed to identification of the cutting point but because of the similarity in the signification results, dendrograms was incised from maximum distance among groups. Due to the abnormality of input data for days to heading, the inverse transform procedure was utilized for it. For statistical analysis, MSTATC and SPSS were used. The variance components, genotypic and phenotypic coefficient of variation were determined as suggested by Burton and De Vane [21] and Johnson et al. [22].

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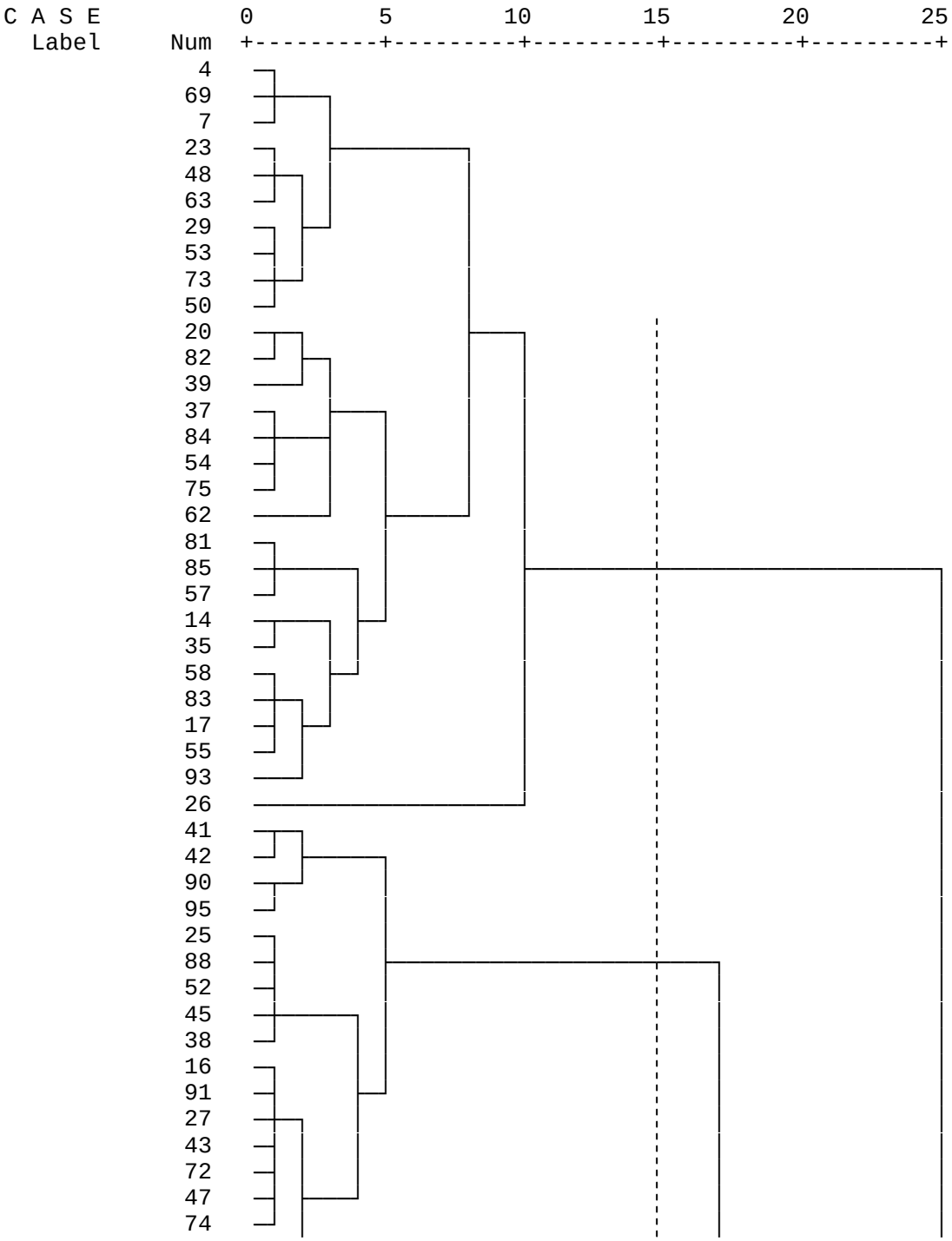
RESULTS AND DISCUSSION

Highly significant genotypic differences were observed among the 96 genotypes for the various characters measured (Table1). The highest coefficient of variation (CV) was shown by straw yield, followed by grain yield and flag leaf area. The lowest values were shown by days to heading. The phenotypic coefficient of variation (PCV) and genetic coefficient of variation (GCV), estimates of components of variance are shown in table 1. The PCV was higher than the GCV for all of the characters. The highest values were shown by peduncle length, flag leaf area and numbers of spikes, followed by grain yield, straw yield and shoot biomass. The least value was shown by percent of grain protein, followed by number of spikelet per spike and days to heading.

Cluster analysis

Dendrogram was achieved from cluster analysis of 96 genotypes on the basis of 14 agronomic traits (Fig. 1). According to this grouping under- study wheat genotypes divided to three groups.



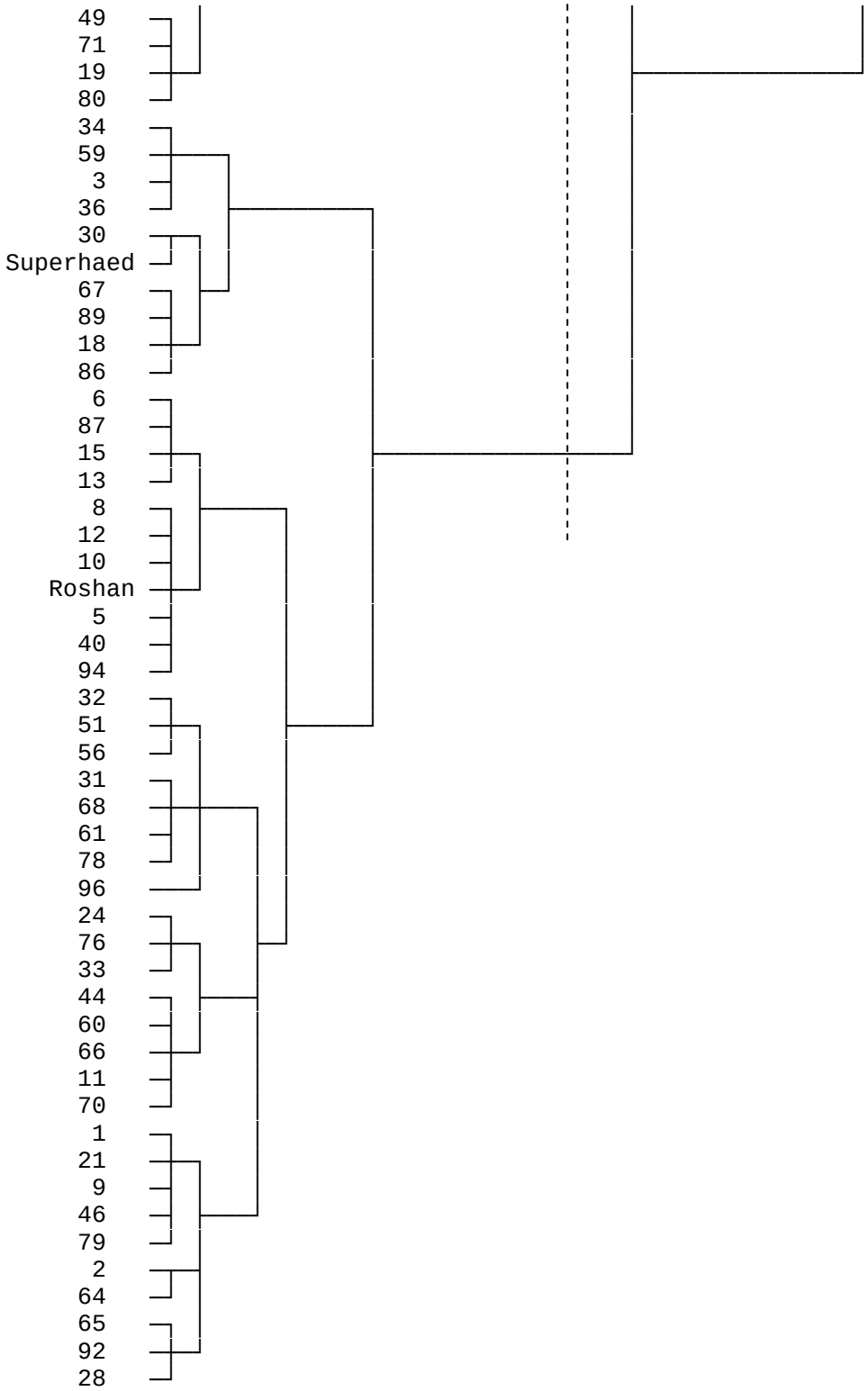


Fig. 1. Dendrogram of 96 genotypes for 14 studied variables using hierarchical cluster analysis (Ward's method and squared Euclidean distance)

The average of traits for each cluster and the percent of their deviation from ground mean are shown in table2. In first cluster 29 lines were classified including 30.85% of total genotypes.

The average values of genotypes in this cluster for days to heading, plant height, flag leaf area, spike length and 1000 grain weight were higher than the total mean of all genotypes and for other traits were less than the total mean. Genotypes in this cluster had greatest values for number of spike, straw yield and harvest index. Second group comprises 20 lines including 19.15% of total genotypes. Genotypes in this group were in the highest rate with respect to plant height, peduncle length, spike length, number of spikes, 1000 grain weight, grain yield, shoot

biomass, number of spikelet per spike and straw yield and they were in the lowest rate with respect to percent of grain protein. Members of this group are suitable for breeding programs aimed at improving the yield. Crossing among existing genotypes in first and second groups provided more possibility to having more genetic variance and optimal genotypes with respect to yield performance. In the third cluster 47 genotypes were classified including 50% of total lines. Values of number of spikes, number of grain per spike, grain yield, number of spikelet per spike, percent of grain protein and harvest index in this cluster were greater than the total mean. Genotypes in this cluster were in the highest rate with respect to number of grain per spike, percent of grain protein and harvest index. These lines can be used for increase in production quality and harvest index. The harvest index as a quantitative trait indicating plant efficiency to distribute dry matter for grain and it is one of the main purposes at the breeding programs of cereals, which introduced genotypes with high harvest index [23]. Crossing among existing lines in first and third groups provided more possibility to having more genetic diversity and optimal genotypes with respect to harvest index.

Table 1. analysis of variance, components of variance, coefficient of variation (CV), genetic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV)

Trait	Mean of squars			Estimates of components of variance		CV (%)	GCV (%)	PCV (%)
	Replication	Genotype	Error	σ_g^2	σ_{ph}^2			
DF	1	95	95					
Day to heading	7×10^{-8} ns	4×10^{-6}**	2×10^{-7}	2.11×10^{-6}	2.21×10^{-6}	2.5	8.07	8.26
Plant height (cm)	140.13ns	176.82**	37.71	69.56	88.91	7.66	10.40	11.73
Flag leaf area (cm^2)	65.46**	24.35**	9.00	7.67	12.17	20.28	18.72	23.58
Peduncle length (cm)	0.09ns	35.26**	6.50	14.38	17.63	17.61	26.20	29
Spike length (cm)	9.75**	2.72**	0.7	1.01	1.36	8.95	10.71	12.43
No. spikes/m^2	59591.19**	5957.63**	2232.40	1862.61	2978.81	199.95	18.22	23.05
No. grain per spike	268.18**	49.98**	19.71	15.13	24.99	14.21	12.44	16
1000 grain weight (gr)	30.33ns	50.80**	18.17	16.31	25.40	10.24	9.70	12.10
Grain yield (gr/m^2)	31043.09*	12998.84**	5434.93	3751.95	6499.42	21.09	17.42	22.93
Shoot biomass (gr/m^2)	738.95ns	54004.29**	26024.46	13989.91	27002.14	19.49	14.29	19.85
No. spikelet per spike	4.23ns	2.40**	1.42	0.49	1.20	8.79	5.17	8.09
Grain protein (%)	88.02**	1.07**	.058	0.24	0.53	.07	3.93	5.78
Straw yield (gr/m^2)	41360.99ns	20628.43**	10723.49	4952.47	10314.21	21.75	14.78	21.32
Harvest index	0.04**	5×10^{-3}**	2×10^{-3}	15×10^{-4}	25×10^{-2}	10.61	9.13	11.79

**, ** and ns significant at $p \leq 0.05$, $p \leq 0.01$ and non-significant, respectively*

Table2. The average of traits for each cluster (above number) and the percent of difference between each cluster with the total mean (below number)

cluster	Days to heading	Plant height (cm)	Flag lea area (cm^2)	Peduncle length (cm)	Spike length (cm)	No. spikes/m^2	No. grain per spike	1000 grain weight (gr)	Grain yield (gr/m^2)	Shoot biomass (gr/m^2)	No. spikelet per spike	Grain protein (%)	Straw yield (gr/m^2)	Harvest index
1	60.16	80.46	176.61	14.03	9.61	187.07	28.96	4.66	270.98	7/3.60	13.46	12.46	442.62	0.38
	8.92	0.36	18.98	-3.09	2.38	-21.01	-7.34	2.45	-22.92	-13.79	-2.86	-1.11	-7.06	-10.14
2	53.60	89.34	15.37	18.32	9.73	279.83	31.43	42.92	423.87	1018.2	13.91	12.36	594.32	0.42
	-2.95	11.44	3.88	26.56	3.74	18.16	0.58	3.09	20.57	23.00	2.37	-1.87	24.80	-2.12
3	52.88	76.09	12.82	13.12	9.10	249.22	32.59	40.46	370.49	817.19	13.62	12.78	446.70	0.46
	-4.25	-5.09	-13.36	-9.40	-3.06	5.24	4.28	-2.83	5.39	-1.28	0.60	1.46	-6.20	7.31
Total mean	52.23	80.17	14.80	14.48	9.38	236.82	31.25	41.69	351.55	827.77	13.59	12.60	476.22	0.42

Factor analysis

Factor analysis was performed based on principle component analysis and provisional factors were rotated by Varimax method. The first five factors explained 80.26% of total variation (Table 3). First factor determining 23.94% of the variation has an important role to justify alteration of number of spikes, grain yield, shoot biomass and straw yield (Table 3). So, this factor was named as grain yield factor. If the selection had complemented on the basis of first factor, this selection will has the most effectiveness in the grain yield. These results are compatible with Golabadi and Arzani [24], Yildrim et al. [25], Mollasadeghi et al. [1], Sorkhi Lelahlou et al. [26], Tousi Mojarad and Bihamta [27], Damania and Jacson [28]. Second factor had justified 18.37% of total variance. The factorial coefficients of days to heading and flag leaf area were high and negative while grain yield and harvest index had high and positive factorial coefficients. In the third factor that had explained 14.80% of alteration, number of grain per spike and number of spikelet per spike had the greatest effect and therefore this factor called as effective factor

to number of grains. Forth factor had justified 14.42% of total variance and mostly affected by plant height, peduncle length, spike length and 1000 grain weight. The most effective trait in the fifth factor was percent of protein. So, it can be called as production quality factor.

Table 3. Factor analysis of under study traits via principal components analysis in 96 wheat genotypes

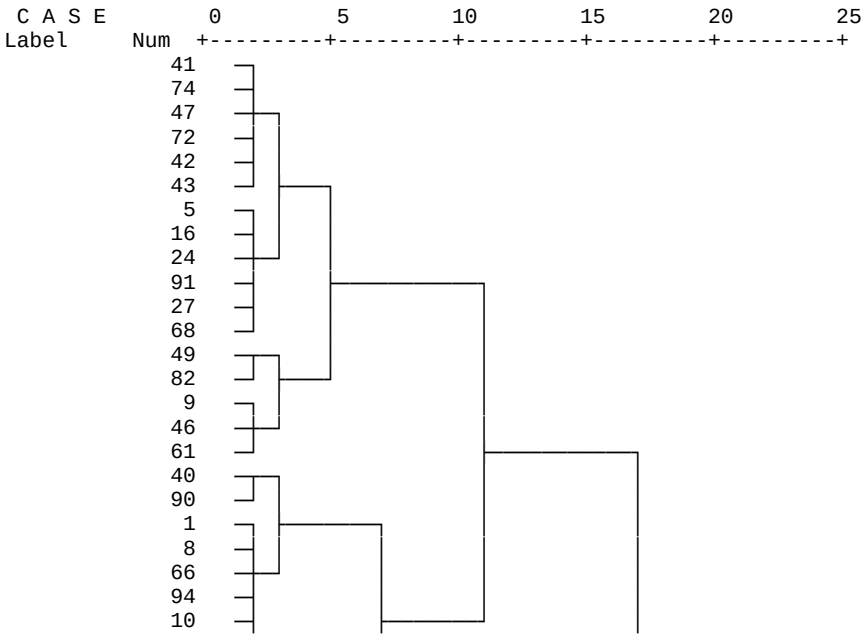
Trait	Rotated factor loadings					communality
	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	
Days to heading	-0.31	-0.80	0.06	-0.06	-0.13	0.78
Plant height (cm)	0.49	-0.24	-0.13	0.68	-0.17	0.82
Flagleaf area (cm^2)	-0.12	-0.66	0.41	0.38	0.08	0.79
Peduncle length (cm)	0.39	-0.02	-0.25	0.56	-0.28	0.62
Spike length (cm)	0.01	-0.11	0.30	0.56	0.49	0.68
No. spikes/m^2	0.076	0.49	-0.03	-0.16	-0.02	0.85
No. grain per spike	0.08	0.12	0.87	-0.17	0.08	0.82
1000 grain weight (gr)	0.01	0.06	-0.16	0.76	-0.007	0.61
Grain yield (gr/m^2)	0.67	0.56	0.33	0.12	-0.10	0.92
Shoot biomass (gr/m^2)	0.91	0.18	0.21	0.19	-0.04	0.95
No. spikelet per spike	0.16	0.02	0.80	-0.096	-0.06	0.69
Grain protein (%)	-0.05	0.018	-0.06	-0.12	0.88	0.80
Straw yield (gr/m^2)	0.94	-0.15	0.07	0.20	0.01	0.95
Harvest index (%)	-0.17	0.86	0.35	0.02	-0.09	0.91
Eigen values	3.35	2.57	2.08	2.01	0.21	
Proportional variance	23.94	18.37	14.89	14.42	8.64	
Cummulative variance	23.99	42.31	57.20	71.62	80.26	

Cluster analysis based on extracted factors

Cluster analysis based on the five factors grouped the lies into the three groups(Fig. 2). Average of factors for each cluster is shown in table 4. In the first cluster, 40 lines were classified including 42.55% of total genotypes. Lines in this cluster were in the highest rate with respect to first and second factors. Genotypes of this cluster can be used for increase in grain yield in breeding programs. Second group comprises 33 lines including 35.11% of total genotypes. Lines In this cluster had greatest values for fourth and fifth factor. Therefore, these genotypes were superior with respect to plant height, 1000 grain weight and percent of grain protein. In the third group, 21 genotypes were classified including 22.34% of total lines. Genotypes in this cluster had highest mean with respect to third factor. Members of this group can use for increase in number of grain per spike in breeding programs.

Table 4. The average of traits for achieved groups from cluster analysis based on factor analysis in 96 wheat genotypes.

Cluster	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5
1	0.376	0.148	-0.379	-0.0035	0.632
2	-0.0832	0.0029	-0.288	0.3023	0.960
3	-0.622	-0.302	1.211	-0.468	-0.243



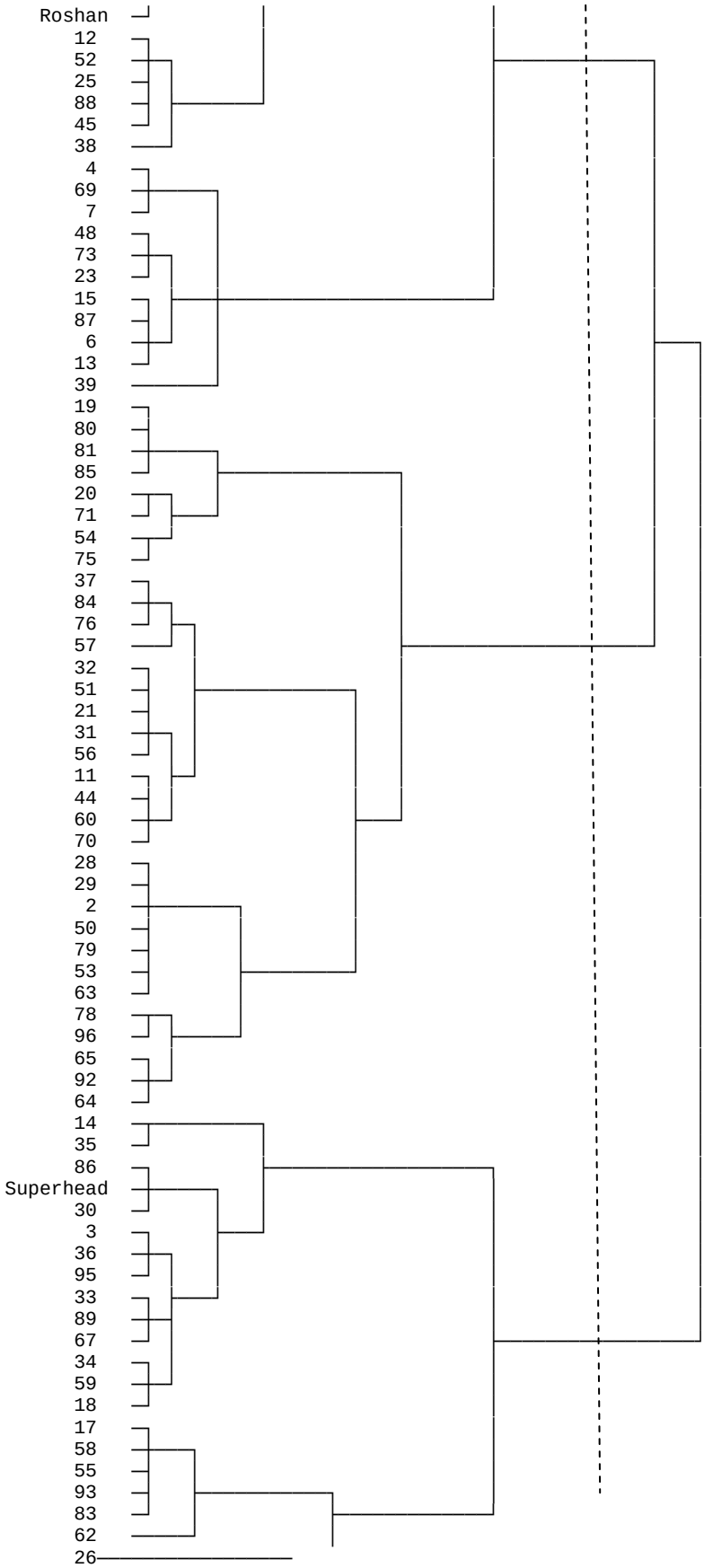


Fig.2. Dendrogram of 96 genotypes for 5 extracted factors using hierarchical cluster analysis (Ward's method and squared Euclidean distance)

CONCLUSION

This study has shown the existence of considerable genetic variation among the genotypes considered with may help for further selection and breeding. Parents may be selected from those clusters which had significant genetic distance for crossing in order to obtain genetic recombination and transgressive segregation in the subsequent generations. However further study across location and years needs to be done in order to corroborate the results obtained in the present investigation.

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