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APPLYING PRINCIPAL COMPONENT AND CLUSTERING ANALYSES IN STUDYING N-USE PARAMETERS AMONG EGYPTIAN WHEAT GENOTYPES

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ABSTRACT: This study aimed to conclude the important components associated with the explained genotypic variation among Egyptian wheat genotypes and to estabilish particularity of each genotype with regard to N use parameters, under urea and nitrate supply treatments. The principal components analysis (PCA) indicated that the first four components accounted for more than 93% of the total variance over all studied traits under both treatments. While the first PC accounted for about 38% and 58% for urea and nitrate treatments, respectively. N uptake efficiency and N productivity were the major contributors to genetic divergence among urea and nitrate supply treatments, respectively, reflecting the varied genetic architecture of wheat genotypes under both treatments. The second principal component axis, PC2, separated the common parent "Sakha 8" and its F_1 hybrids "Sakha 8 x Sids 7" and "Sakha 8 x Giza 168" than the other parents and hybrids under urea supply treatments, while under nitrate treatment the PC2 separated the common parent Sakha 8 than its F_1 hybrids. Based on the extent of the relative dissimilarities between the studied genotypes, the five parents and the six F_1 hybrids were grouped into five and three clusters under urea and nitrate supply treatments, respectively.

Results of PC and clustering analyses appeared to be in complete accordance. Cluster analysis could efficiently describe the characteristics of groups of genotypes, however the components

analysis gave a special representation of each axis. Both the PC and cluster analysis gave a sensible and useful integration of the data.

Key words: Wheat, principal component analysis, clustering analysis, nitrogen use efficiency, genetic divergence.

INTRODUCTION

Environmental and economic considerations require the effective use of nitrogen (N) fertilizers in wheat production. Genotypic variations were reported in Nitrogen uptake and translocation between straw and grains (Moll *et al.*, 1982 and Van Sanford, Mackown, 1986 and May *et al.*, 1991). Moreover (Ehdaie *et al.*, 2001) reported that genotypic variation for N use efficiency among bread wheats appeared to be low and more emphasize should be given to N-uptake efficiency to improve this trait.

Principle components analysis (PCA) can elucidate patterns of associated variations among studied characters to initial factors explaining such variations using Eigen values. Also, PCA analysis can establish the particularty of each genotype with regard to studied character expressions (Hair *et al.*, 1987 and Brown, 1991). However, clustering patterns of genotypes provide visual idea about similarities and dissimlarties present in such genotypes, based on Euclidean distances (Johnson and Wichern, 1988).

The aim of this study is to conclude the important components associated with the explained genotypic variation among Egyptian wheat genotypes and to establish particularity of each wheat genotype with regard to N use parameters, under urea and nitrate supply treatments, using PCA analysis. Also, clustering technique was applied to detect genetic divergence among such genotypes.

MATERIALS AND METHODS

The used plant materials in this study were five Egyptian bread wheat (*Triticum aestivum*, L.) varieties. The pedigree and origin of these variations as follows in Table 1. These varieties were found to be different in their response to nitrogen reaction according to the results of Seham Mohamed (2002). This investigation was carried out at the Experimental Farm of the Faculty of Agriculture, Zagazig University during the two growing seasons of 2006 and 2007.

Table 1. Breeding history and pedigree of Egyptian wheat varieties

Entry	Name	Pedigree
1	Sakha 8	Indus66/.Norteno"s"PK3418-6s-1sw-0s
2	Giza 164	Veery"s"
3	Sids 7	Maya"s"/Mon"s"//CMH74A592/3/Sakha8*2 SD10002
4	Sids 1	HD2172/Pavon"s"//1158.57/Maya74"s"
5	Giza 168	Mtl/Buc//Seri CM93046-8M-0Y-0M-2Y-0B

In the season of 2006, the parental wheat grains were sown and crossing between them to obtain six F_1 crosses. Using Sakha 8 (P_1) and Giza 164 (P_2) as two common female parents and Sids 7 (P_3), Sids 1 (P_4) and Giza 168 (P_5) as male parents. In season of 2007, the parental and F_1 grains were grown in plastic bages. Each bag filled with 5kg of mixture of clay soil and sand (2 : 1). Nitrogen content in the used soil was 17.5 ppm. Three N different supply treatments were applied, no nitrogen supply as control, urea (46% N) and calcium nitrate (15% N). The bags were arranged in complete randomized block design experiment, with three replicates. Each replicate comprised 99 bags. Each genotype represented by nine bags per replicate i.e. three bags for each N treatment. In each bag, five grains were planted and after two weeks the seedlings were thinned to three seedlings. After thinning, stimulant dose of ammonium sulfate (20% N) was added to bags for all treatments with rate of 10 Kg N/Faddan ie, 50 mg N/bag. The available N in each control bag was 137.5 mg. For urea N supply, each bag received 637.7 mg N, while for calcium nitrate each bag received 621.2 mg N.

These fertilizers were divided two portions and were added at 30 and 60 day from sowing in form of soluble solutions. There fore, each plant received 45.8 mg in control, 212.5 mg in urea treatment and 207.1 mg in calcium nitrate treatment. At maturity, individual plants of each entry per replicate were harvested. Grains and straw were obtained per plant, air dried and weighted. N nitrogen content in grains and straw was determined according to method described in A.O.A.C. (1984). Data obtained from N content in both N supply treatments, urea and nitrate, were used in estimating the following nitrogen use parameters: N uptake efficiency (NUP), N utilization efficiency (NUT), N use efficiency (NUE), and N translocated (NTS) to grains, according to Moll *et al.* (1982) and outlined by Raun and Johonson (1999). However, data obtained from N content control treatment was used to extract the following N use parameters: N response index (NRS), N sufficiency index (NSF), N productivity index (NPR) and agronomic N use efficiency (ANUE), according to Singh and Arora (2001).

The original data of all studied characters were statistically analyzed after transforming the percentages to angular scale. Principal components analysis, PCA, according to (Hair *et al.*, 1987), was calculated from a matrix based on correlations between the contributed characters for all genotypes. The PCA analysis of the contributed characters, associated with each of urea and nitrate supply treatments, were expressed as Eigen values, (latent roots) and manifested in Eigen vectors in each PC axis. Moreover, The PCA was also plotted simultaneously in a "joint plot" diagram displaying the component score of each genotype based on all characters. Since each component score is a liner combination of the characters. The maximal amounts of variations could be shown on the first PC axis.

Likewise hierarchical clustering procedure using ward's minimum variance method was applied to determine the genetic similarities and dissimilarties. The procedure used a method performing a disjoint cluster analysis on the basis of Euclidean distance, represented as a dendrogram as outlined by Johnson and Wichern (1988). All these computations of multivariate analysis were performed using SPSS (1995) computer software package.

RESULTS AND DISCUSSION

PCA analysis seemed to elucidate patterns of variation among the studied characters. In an analysis with eight variables, eight axis were existed, however only those which exhibited high multivariate variation were considered. The first four PC axes accounted for more than 95% and 93% of the total variation under urea and nitrate treatments, associated with these first four PC axes, as well as their vectors of each character, respectively as shown in Tables 2 and 3. PCA analysis showed that the first PC axis accounted for about 58% and 38% of the multivariate variation among genotypes, showing the highest joint Eigen values, for nitrate and urea treatments respectively. But the second PC axis accounted for about 28% and 14%. While, the fourth PC axis contributed with about 9% under urea and 6% under nitrate treatment. Similar results were obtained by Rharrabti *et al.* (2003) and Biljana *et al.* (2005). Also, Hallu *et al.* (2006) evaluated 121 accessions form Ethiopia for 23 yield characters using multivariate analysis and reported that the first three and first two principal components were explained about 83% and 80% of the total variance among wheat germplasm with respect to regions and altitudenal classes, respectively. There results agreed with out results under either nitrate or urea supply treatment. However, Yifru *et al.* (2007) noticed that the first two PC axes explained altogether 27% of the total variation among 73 accessions of emmer wheat, reflecting low contribution toward the explained variance associated with both PC axes. Under urea supply treatment, PCA analysis showed that the N uptake efficiency was the primary source of variation with the largest coefficient in the first PC axis. Also, N Response index appeared to have the second largest coefficient, followed by N utilization efficiency. Likewise, the second PC axis showed that N productivity index was a primary source of variation with the largest coefficient. This trend was changed under nitrate supply treatment. Since, PC analysis showed that N utilization efficiency was the primary source of variation having the largest coefficient in the first PC axis followed by N productivity index then N uptake efficiency. However N translocation showed the largest coefficient in the second PC axis. In this regard, Rharrabti *et al.* (2003) performed PCA for quality characters of durum wheat genotypes and found that the first PC axis separated pigment content from kernel weight. But the observed variation on the second PC axis caused by sedimentation volume.

Principal components analysis was also used as an additional tool to establish the particularity of each wheat genotype with regard to N use component expressions. Therefore, each genotype could be plotted at the component score on each PC axis. The two dimensional distance between genotypes on the first two PC axes, PC_1, and PC_2, might reflect a summary of differences based on all characters. Thus, the first two PC axes were used for representing the eleven genotypes under urea and nitrate supply treatments as shown in

Figure 1. Under urea supply, the PC_2 axis separated the parental genotypes Sids 1 and Giza 168 from the other parents Sids 7, Giza 164 and Sakha 8. However, F_1 of the crosses "Sakha 8 x Sids 7" and "Sakha 8 x Giza 168" were the nearest to their parent Sakha 8. the same trend was observed for Giza 164 and it's F_1 cross "Giza 164 x Giza 168", but the two F_1 crosses "Giza 164 x Sids 7" and "Giza 164 x Sids 1" were separated from Giza 164 by PC_2 axis. On the other hand, in the case of nitrate treatment, the PC_2 axis separated Sids 7 form the other parental genotypes. Likewise, the same PC axis, PC_2, separated the F_1 crosses "Sakha 8 x Sids 1", Sakha 8 x Sids 7" and "Sakha 8 x Giza 168" from their common parent Sakha 8. However, F_1 the crosses "Giza 164 x Sids 7" and "Giza 164 x Giza 168" were grouped with their parent Giza 164 but the F_1 cross "Giza 164 x Sids 1" was separated by PC_2. In this regard, Rharrabti *et al.* (2003) reported that wheat genotypes grouped by the two PC axes in groups related to zone. PC_1 discriminated clearly between rainfed zone and superimposed zones. However, wheat genotypes were largely distributed along PC_2.

The clustering pattern of these wheat genotypes under urea and nitrate supply are graphically illustrated as dendograms (Figure 2). Such linkage dendrogram could provide visual idea about clustering and variability present in each wheat population. Cut of point at 7 dissimilarity point, Euclidean distance was fixed as minimum dissimilarity point in clustering pattern. Based on the extent of relative dissimilarity among genotypes, the five parents and their six F_1's hybrids were grouped into five clusters under N urea supply and three clusters under N nitrate supply treatments. The distribution of genotypes into clusters are given in Table 5. The clustering pattern indicated that there was inconsistent relationship between the parental genotypes and their F_1 hybrid performances. Cluster I, was the largest one in both urea and nitrate treatments, and included five members under urea supply and six members under nitrate supply. The parental genotypes, as common parents, were not completely grouped with all their F_1 progenies in one cluster.

For instance, cluster I under urea treatment included three parents and two F_1's. However, the same cluster under nitrate supply included four parents and two F_1's having also their common parent

Table 2. Principal components analysis of N use characters associated with wheat genotypes under urea supply showing Eigen value* and Eigen vectors of characters in the first four PC axes

	Parameters	PC axes			
		1	2	3	4
	Eigen values	3.090	2.304	1.436	0.778
	PC% of variation	38.630	28.798	7.952	9.727
	Cumulative proportion of var	38.630	67.428	85.380	95.108
		Eigen value			
1	N Uptake efficiency	0.922	0.291	0.001	0.115
2	N Utilization efficiency	-0.686	0.420	0.464	0.251
3	N Use efficiency	0.526	0.699	0.352	0.315
4	N Translocated to grains	0.557	0.169	0.535	-0.607
5	N Response index	0.846	-0.104	-0.283	0.373
6	N Sufficiency index	-0.680	0.618	-0.150	0.089
7	N Productivity	0.055	0.961	-0.089	-0.170
8	Agronomic N use efficiency	-0.028	-0.457	0.837	0.241

* latent root.

Table 3. PC analysis of N use characters associated with wheat genotypes under nitrate supply showing Eigen value* and Eigen vectors of characters in the first four PC axes

	Parameters	PC axes			
		1	2	3	4
	Eigen values	4.662	1.187	1.097	0.510
	PC% of variation	58.281	14.843	13.717	6.378
	Cumulative proportion of var	58.281	73.124	86.840	93.219
		Eigen value			
1	N Uptake efficiency	-0.921	0.375	0.016	0.105
2	N Utilization efficiency	0.994	0.103	-0.002	0.037
3	N Use efficiency	0.351	-0.236	0.592	0.490
4	N Translocated to grains	0.418	0.754	0.063	-0.501
5	N Response index	0.839	0.357	-0.109	-0.041
6	N Sufficiency index	0.898	-0.326	0.009	0.013
7	N Productivity	0.935	-0.293	0.033	-0.003
8	Agronomic N use efficiency	0.380	-0.303	0.854	-0.069

* latent root.

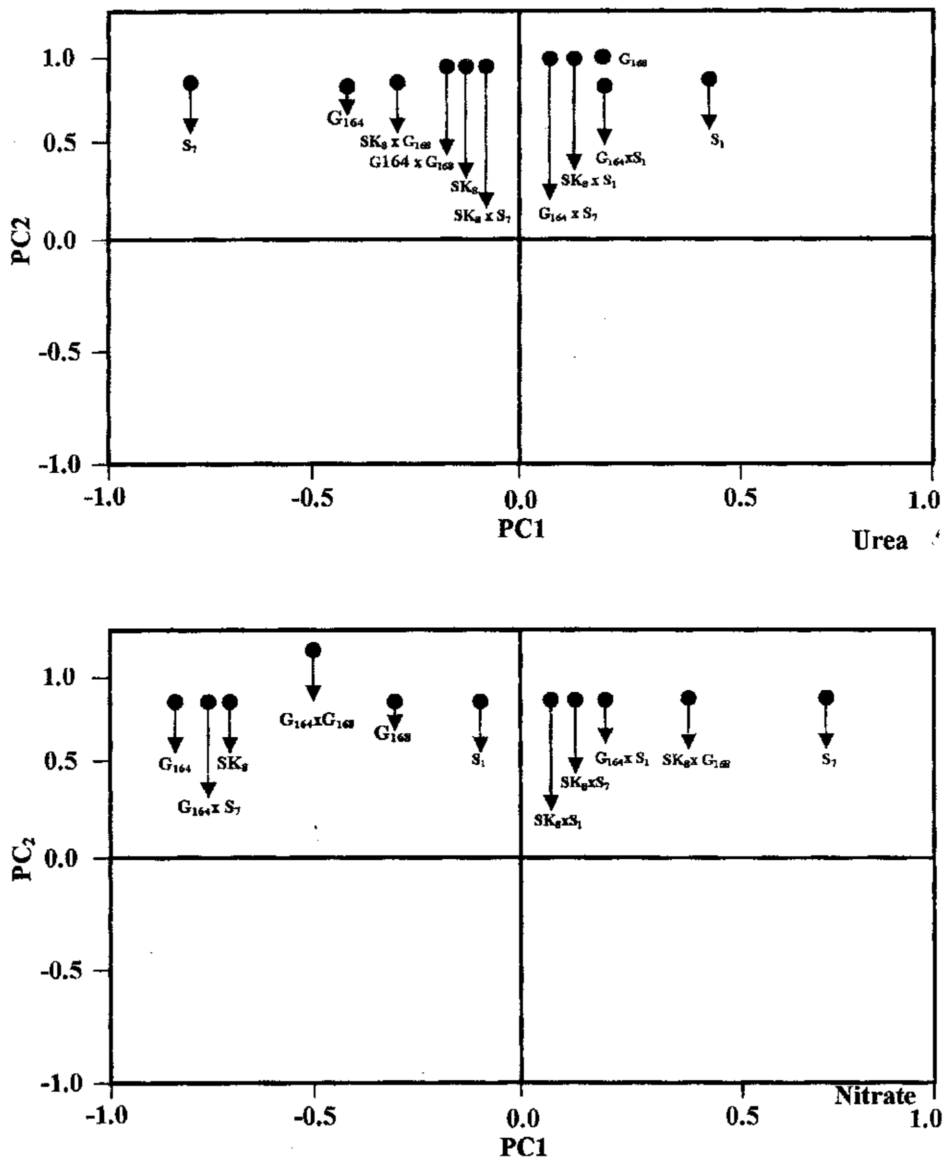
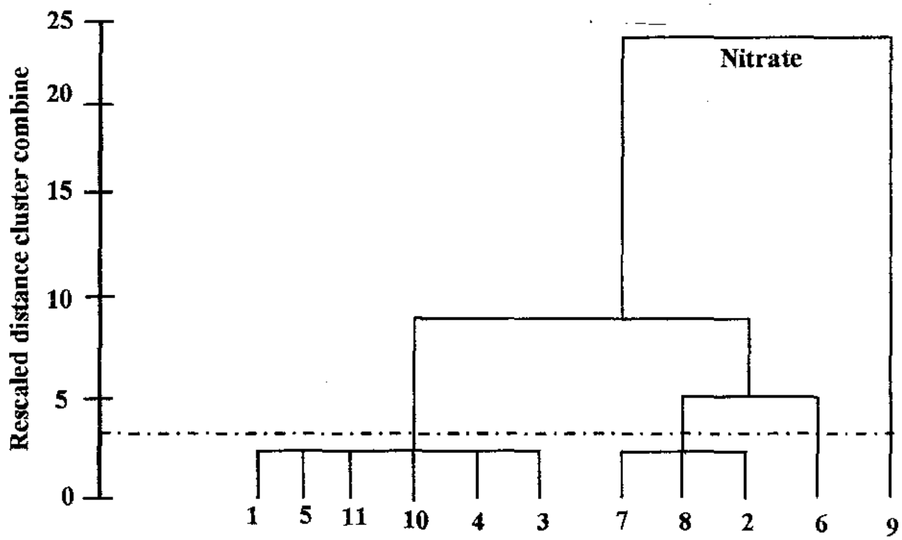


Fig. 1. Plot of first and second principal components, PC1 and PC2 from PC analysis between genotypes under urea and nitrate supply treatments



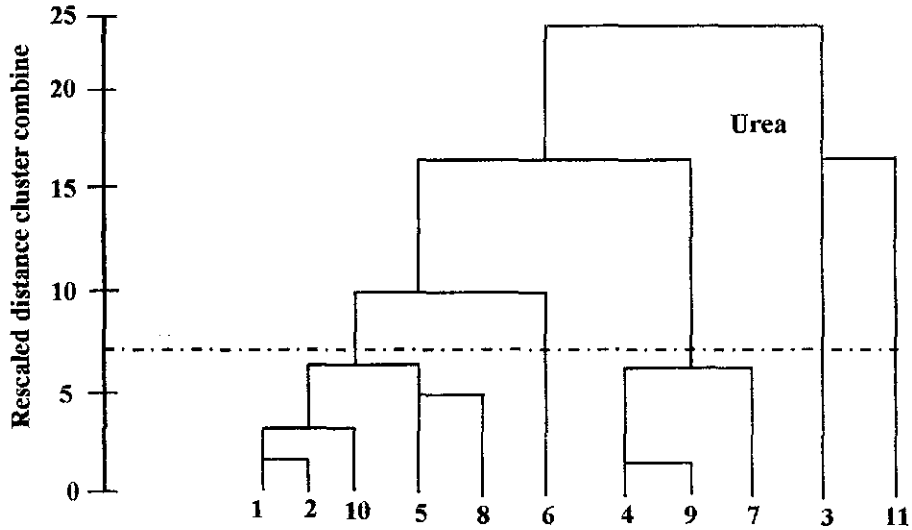


Fig. 2. Dendrogram representation of studied wheat genotypes showing clustering pattern

Giza 164. Cluster II included only three genotypes, Giza 168 and two F_1's (Giza 164 x Sids 1" and "Sakha 8 x Sids 1") under urea, but it included four members, Sids 7 and three F_1's "Sakha 8 x Sids 7", "Giza 164 x Sids 1" and "Sakha 8 x Giza 168" under nitrate treatment. Cluster III consisted only of one F_1 hybrid "Sakha 8 x Sids 7" under urea supply treatment and "Sakha 8 x Sids 1" under nitrate supply treatment. Each of clusters IV and V, under urea treatment, comprised a single genotype, Sids 1 and "Giza 164 x Sids 7", respectively. It is interest to note that the parent "Sids 1" showed wide divergent distance from all the other genotypes, forming unique cluster by it self. Such results might indicate that considerable divergence could be created by hybridization, Since F_1's were widely dispersed from their parents. Rania-Heakal (2004) studied clustering pattern of Egyptian wheat genotypes based on allelic differences of glutenin loci and stated that all studied genotypes distributed into two clusters in addition to a single genotype (Giza 168) forming a unique cluster. All Sakha and Sids varieties were grouped together in cluster I, and the Giza 164, 165 and 167 varieties grouped in cluster II. However Gemmiza varieties distributed in the two clusters I and II. Salem *et al.* (2008) studied genetic diversity of the seven wheat (*Triticum aestivum L.*) varieties and reported that the Egyptian wheat genotypes were grouped into two main clusters, the first one included Sakha 69, Sakha 93 and Gemiza 3. The second main cluster was divided into two sub clusters, sub-cluster I included Gemmiza 7 and Mariam, while sub-cluster II included Sids 4 and Baviacora.

Data in Tables 4 and 6 illustrated mean performance of genotypes and cluster means of the eight studied N use characters, respectively. Under urea treatment, cluster IV, which included only Sids 1, characterized by its high N utilization, N response index and Agronomic N use efficiency than the other clusters. Also, cluster III, which comprises F_1 Sakha 8 x Sids 7, characterized by its highest values of N utilization, N response index, N productivity and Agronomic N use efficiency. While under nitrate treatment, cluster I characterized by it's high N uptake and N use efficiency, however, cluster III, which comprises F_1 Sakha 8 x Sids 1, characterized by it's high values of most N use parameters. Characterization of cluster I, under both N supplies, might reflect the

Table 4. Mean performance of agronomic N use efficiency of wheat genotypes under different N fertilizers

Genotypes	N Response index		N sufficiency index		N productivity index		Agronomic NUE		N uptake eff.		N utilization eff.		N use eff.		N translocated	
	Urea	Nitrate	Urea	Nitrate	Urea	Nitrate	Urea	Nitrate	Urea	Nitrate	Urea	Nitrate	Urea	Nitrate	Urea	Nitrate
Sakha 8	5.18	4.97	0.19	0.20	7.69	8.49	25.43	24.74	59.32	47.40	59.42	65.60	34.95	31.00	91.88	89.51
Giza 164	4.69	4.42	0.21	0.21	7.80	8.93	23.83	25.27	54.91	47.91	56.73	64.94	31.10	31.11	92.51	90.09
Sids 7	5.46	5.57	0.18	0.18	7.14	11.08	25.17	26.44	53.02	36.58	59.55	73.96	31.43	27.05	90.05	89.43
Sids 1	5.99	5.67	0.17	0.18	6.80	7.04	25.90	24.91	48.31	50.50	61.12	63.36	29.50	31.95	90.99	91.79
Giza 168	4.91	4.90	0.20	0.21	8.09	9.12	24.40	24.94	50.51	46.16	60.62	68.63	30.57	31.48	92.34	88.90
Sakha 8 x Sids 7	5.02	5.00	0.20	0.20	7.87	11.17	25.04	25.58	52.64	40.27	59.29	68.99	31.17	27.78	94.63	87.23
Sakha 8 x Sids 1	4.72	4.78	0.21	0.21	8.63	13.82	25.42	24.27	51.23	32.18	61.28	97.46	31.35	31.37	92.06	94.08
Sakha 8 x G 168	4.71	4.90	0.14	0.21	8.23	10.54	24.37	26.36	55.15	42.43	58.59	71.97	32.31	31.82	92.86	92.00
Giza 164 x Sids 7	4.70	4.65	0.22	0.22	7.77	8.54	22.75	23.39	49.74	46.39	58.46	54.20	29.05	29.77	90.27	91.46
Giza 164 x Sids 1	4.64	4.96	0.22	0.21	8.97	10.43	23.75	26.56	52.10	42.78	62.61	73.27	32.62	31.26	91.44	91.05
Giza 164 x Giza 168	4.69	4.95	0.22	0.20	8.11	9.18	23.49	25.75	53.19	45.91	59.30	66.96	31.53	30.72	91.99	93.02

Table 5. Distribution of studied wheat genotypes into clusters

Cluster No.	No. of genotypes in cluster	Members of each cluster
		1. Under urea treatment
I	5	Sakha 8, Sids 7, Giza 164, Sakha 8 x Giza 168, Giza 164 x Giza 168
II	3	Giza 168, Giza 164 x Sids 1, Sakha 8 x Sids 1
III	1	Sakha 8 x Sids 7
IV	1	Sids 1
V	1	Giza 164 x Sids 7
		2. Under nitrate treatment
I	6	Sakha 8, Sids 1, Giza 168, Giza 164, Giza 164 x Giza 168, Giza 164 x Sids 7
II	4	Sids 7, Sakha 8 x Sids 7, Giza 164 x Sids 1, Sakha 8 x Giza 168
III	1	Sakha 8 x Sids 1

Table 6. Cluster means of the contributed characters in each cluster

Cluster No.	The studied characters							
	NUP	NUT	NUE	NTS	NRS	NSF	NPR	ANUE
	1. Under urea treatment							
I	55.119	58.718	32.267	91.855	4.945	0.204	7.793	24.458
II	51.281	64.506	31.513	91.051	4.756	0.213	8.561	24.141
III	52.641	59.290	31.170	94.632	5.023	0.200	7.874	25.040
IV	48.307	61.124	29.499	90.986	5.987	0.168	6.793	25.902
V	49.743	58.457	29.053	90.268	4.643	0.216	7.767	22.746
	2. Under nitrate treatment							
I	47.379	65.613	31.004	90.745	4.993	0.202	8.549	24.832
II	40.515	72.797	29.484	89.917	5.109	0.198	10.803	26.234
III	32.183	97.461	31.365	94.079	4.779	0.210	13.822	25.421

N Uptake efficiency, N Utilization efficiency, N Use efficiency, N Translocated to grains, N Response index, N Sufficiency index, N Productivity, Agronomic N use efficiency.

importance of N uptake in N use efficiency components.

Generally, the results of (PCA) analysis and clustering analyses appeared to be in complete accordance. Cluster analysis could efficiently describe the characteristics of groups of genotypes, however the components analysis could provide no clear grouping but give a special representation of each mode. Both the cluster and principal components analyses gave a sensible and useful integration of the data. Routray *et al.* (2007) reported considerable interpretations were available through the complementary use of both methods.

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تطبيقات تحليل المكونات الأساسية وتحليل المجاميع فى دراسة مقاييس استخدام النيتروجين ما بين أصناف القمح المصرية

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تهدف هذه الدراسة إلى استنتاج أهم المكونات المرتبطة بوجود الاختلافات الوراثية ما بين أصناف القمح المصرية لمعرفة مدى تأثير كل صنف قمح على مقاييس استخدام النيتروجين (N use) وذلك تحت تأثير كلاً من معاملتى اليوريا والنترات. لقد أوضح تحليل المكونات الأساسية **(PCA) Principal components anslysis** أن المكونات الأربعة الأولى تمثل حوالى أكثر من ٩٣% من التباين الكلى بالنسبة لجميع الصفات المدروسة لكلا المعاملتين وبينما يمثل المكون الأول حوالى ٣٨% و ٥٨% من التباين بين التراكيب الوراثية لكلاً من معاملتى اليوريا والنترات على التوالى حيث كانت صفة كفاءة امتصاص النيتروجين **(NUP) N uptake efficiency** وصفة دليل إنتاجية المحصول **N (NPR) productivity index** هما الأكثر مساهمة فى التباعد الوراثى تحت تأثير كلاً من معاملتى اليوريا والنترات على التوالى مما يعكس اختلاف التراكيب الوراثية بكلا الصفتين. كما أوضح أن المحور الثانى للمكونات الأساسية **(PC_2)** فصل الأب المشترك سخا ٨ وهُجنه (سخا ٨ × سدس ٧) و(سخا ٨ × جيزة ١٦٨) عن الآباء والهجن الأخرى وذلك تحت تأثير المعاملة باليوريا بينما تحت تأثير النترات وجد أن **(PC_2)** فصل الأب المشترك سخا ٨ عن كل هُجنه. وعلى أساس عدم التشابه النسبى بين الأصناف المدروسة تم تقسيم التراكيب الوراثية (٥ أباء + ٦ هجن) إلى خمس، ثلاث مجاميع تحت تأثير معاملة اليوريا والنيترات على التوالى. أظهرت نتائج كلاً من تحليل المكونات الأساسية **(PCA)** وكذلك تحليل المجاميع **(cluster analysis)** أنها فى توافق تام حيث أن تحليل المجاميع بين خصائص كل مجموعة من التراكيب الوراثية بينما تحليل المكونات الأساسية **(PCA)** أعطى تمثيل خاص لكل محور وكلا التحليلان أعطوا تكاملاً مفيداً للبيانات المدروسة.