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Principal Components Analysis of Some F_1 Sunflower Hybrids at Germination and Early Seedling Growth Stage

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ABSTRACT: Estimating genetic diversity inside breeding materials using morphological, physiological and biochemical data and selection of superior hybrids are essential in sunflower breeding programs. Principal components analysis is one of the multivariate statistical methods that can be utilized for genetic diversity estimation and grouping of genotypes through biplot diagrams. In order to study the principal components analysis of some F_1 sunflower hybrids at germination and early seedling growth stage, a randomized complete block design has been conducted with three replications. Plant materials consisted of 18 single cross sunflower hybrids set of six male restorer lines crossed with 18 female (CMS) lines using the North Carolina Design I scheme. The first calculated two components accounted for 80.93 % of the variability in the original data. Seed vigor index (SVI) has a highest weight in the first component while second component was more associated with the shoot/root ratio (SHRR). Based on the created biplot diagram, three distinct groups could be differentiated.

Key words: genetic diversity, hybrid, principal components analysis, sunflower

Çimlenme ve Erken Fide Gelişim Aşamasında Bazı F_1 Ayçiçeği Melezlerinin Temel Bileşenler Analizi

ÖZET : Ayçiçeği ıslah programlarında morfolojik, fizyolojik ve biyokimyasal veriler üzerinden üstün hibritlerin seçilmesi ve ıslah materyallerinin genetik çeşitliliğinin belirlenmesi oldukça önemlidir. Biplot diagramlar yoluyla genotiplerin gruplandırılmasında ve genetik çeşitliliğinin belirlenmesinde kullanılan, temel bileşenler analizi çok değişkenli istatistik metotlarından birisidir. Çimlenme ve erken fide gelişim aşamasında olan bazı F_1 ayçiçeği hibritlerinde, temel bileşenler analizini uygulamak için tamamen tesadüfi blok denemesi 3 tekerrürlü olarak kurulmuştur. Bitki materyalleri, 6 erkek restorer hattın 18 erkek kısır dişi hatla bir North Carolina Design I planında melezlenmesi ile oluşturulmuş 18 tek melez ayçiçeği hibrit setinden oluşmaktadır. İki bileşen, orjinal verilerin değişkenliğinin % 80.93'ünü oluşturmaktadır. Birinci bileşende tohum canlılık indeksi (SVI) en yüksek ağırlığa sahip iken ikinci bileşen daha çok sürgün/kök oranı (SHRR) ile ilişkilidir. Biplot diagramına göre bileşenler ile 3 farklı grup oluşturulmuştur.

Anahtar kelimeler: Genetik çeşitlilik, hibrit, temel bileşenler analizi, ayçiçeği

INTRODUCTION

Today, sunflower is one of the most important crops in the world grown for edible oil, after soybean (*Glycine max* L.), rapeseed (*Brassica rapa* L.) and peanut (*Arachis hypogaea* L.) (Putt, 1997) but its growing area has been very variable and decreased from 105,000 ha in 1994 to 30,000 ha in Iran (Anonymous, 2010).

Sunflower breeding programs in Iran focus on producing new hybrid cultivars to replace with open pollinated ones. To reach this purpose, development and evaluation of parental lines and corresponding hybrids is important (Ghaffari et al., 2011). Genetic diversity estimation of obtained materials and selection of superior hybrids are essential in such programs. Based on morphological, physiological and biochemical data, many methods were used in estimating genetic diversity of sunflower genotypes (Dong et al., 2007). Principal Component Analysis (PCA) can be utilized to derive a two or three-dimensional scatter plot of individuals, such that the geometrical distances among individuals in the plot reflect the genetic distances among them with minimal distortion. Aggregations of individuals in such a plot will reveal sets of genetically similar individuals (Mohammadi, 2003).

This will allow visualization of the differences among the individuals and identify possible groups. The reduction is achieved by linear transformation of the original variables into a new set of uncorrelated variables known as principal components (Mohammadi, 2003). Because PCs are orthogonal and independent of each other, each PC reveals different properties of the original data and may be interpreted independently. In this way, the total variation in the original data set may be broken down into components that are cumulative (Mohammadi, 2003). Tersac et al., (1993) used PCA based on specific combining ability (SCA) to show the structure of sunflower populations by country of origin. De la Vega and Chapman (2001) also used PCA for revealing two-dimensional structures among genotypes and their environments

based on their interactions. They reported the effectiveness of PCA for revealing genotype × environment interactions. Ghafari (2004) used this method for selection of superior three-way cross hybrids in sunflower**.** Logical orientation of genotypes under the impression of agronomic traits could be used as an effective tool for rapid selection of high yielding and early maturing hybrids. Due to considerable conformity with conventional method and by presenting a bright view of genotype's potential, PCA method contributed for selection of 10 superior hybrids and could be used in cultivar development programs. Tabrizi (2009) and Ghaffari et al., (2011) used this method for genetic diversity estimation of single cross hybrids based on agronomic traits. In Arshad et al., (2010) study, the principal components analysis could help for identification of the best sunflower hybrids. The study of Maruthi Sankar et al., (2004) using principal components analysis indicated that plant traits stomatal conductance, photosynthesis, root length, stem nitrogen, leaf nitrogen, flower head diameter and flower head weight are dominant and consistent traits for sunflower growth in different seasons.

The objective of this study was the use of principal components analysis and biplot diagram to find a relationship among traits and grouping the genotypes based on the biplot diagram at germination and early seedling growth stage of F_1 sunflower hybrids.

MATERIAL AND METHODS

In order to study the principal components analysis of some F_1 sunflower hybrids at germination and early seedling growth stage, a randomized complete block design has been conducted with three replications at Plant Genetic Laboratory of Department of Field Crops, Faculty of Agriculture, Ataturk University. Plant materials consisted of 18 single cross sunflower hybrids set of six male restorer lines crossed with 18 female (CMS) lines using the North Carolina Design I scheme, which have been developed by Agricultural and Natural Resources Station of Khoy, Iran (Table 1 and 2).

Table 1 and 2. F_1 sunflower single cross hybrids

First Set		
A line	**R line**	**Hybrid**
CMS_{28}	R_{43}	A
CMS_{128}		B
CMS_{346}		C
CMS_{330}	R_{27}	D
CMS_{78}		E
CMS_{328}		F
CMS_{336}	R_{34}	G
CMS_{52}		H
CMS_{148}		I

Second Set		
A line	**R line**	**Hybrid**
CMS_{344}	R_{56}	J
CMS_{260}		K
CMS_{32}		L
CMS_{222}	R_{25}	M
CMS_{96}		N
CMS_{356}		O
CMS_{356}	R_{32}	P
CMS_{196}		Q
CMS_{376}		R

Seeds were sterilized 1 min in alcohol 70 %, followed by 15 min in sodium hypochloride 25 % and then washed twice with sterilized water.

To prevent fungal infection, Benomyl 4 % fungicide was also added to each petri dish with 3 ml sH_2O. Numbers of seeds per petri dishes were ten. All petri dishes were placed in germinator (growth-chamber) with 25±1 with 12 hr light + 12 hr darkness for seven days (ISTA, 1996).

Germination tests and seed counting were started after 24 hours. After three days of germination, 3 ml water added each petri dish to prevent drought of genotypes.

After 7th day, observations were started including germination percentage (GP), germination rate (GR), germination duration (GD), root length (RL), shoot length (SHL), root dry weight (RDW), shoot dry weight (SHDW), root fresh weight (RFW), shoot fresh weight (SHFW), root/shoot ratio (RSHR), shoot/root ratio (SHRR) and seed vigor index (SVI).

After measuring root+shoot fresh weights, all samples were placed in a foil and kept in Owen for 48 hours at 70°C. The data were analyzed using STATGRAPHICS Centurion XV statistical software (Statgraphics, 2007).

RESULTS AND DISCUSSION

The purpose of the principal components analysis is to obtain a small number of linear combinations, which account for most of the variability in used data. In this study, two components have been extracted, since two components had eigen values greater than or equal to 1. They account for 80.93 % of the variability in the original data (Table 3).

Maruthi Sankar et al., (1999) have assessed the variability of eight plant traits for growth of sunflower and reduced the dimensionality to two principal components, which extracted about 80% of variance in the original data.

Table 3. Principal Components Analysis of F_1 sunflower single cross hybrids

Component Number	Eigen value	Percent of Variance	Cumulative Percentage
1	7.79796	64.983	64.983
2	**1.91313**	**15.943**	**80.926**
3	0.852437	7.104	88.029
4	0.609778	5.081	93.111
5	0.278329	2.319	95.430
6	0.199531	1.663	97.093
7	0.169944	1.416	98.509
8	0.086283	0.719	99.228
9	0.0747685	0.623	99.851
10	0.00931116	0.078	99.929
11	0.00675377	0.056	99.985
12	0.00177299	0.015	100.000

The portion of each two components was approximately 65 and 16 percent of total variance, respectively. Greatness of these variances influences good separation of genotypes. If there would be correlations among traits or similarities among genotypes, these components can provide suitable grouping and separate same genotypes in distinct groups (Tabrizi, 2009).

Table 4 shows each component weight. It is considerable that seed vigor index (SVI) has a highest weight in first component and from the aspect of this trait; genotypes can be grouping using this component. In decreasing importance, root dry weight (RDW), germination percentage (GP), root length (RL), shoot fresh weight (SHFW) and root fresh weight (RFW) are other traits that have high weights, also and can be explained by the first component. Second component was more associated with shoot/root ratio (SHRR) and shoot length (SHL). These relations can be easily seen in Fig. 1, where SVI, RDW, GP, RL, SHFW and RFW are at the right side of the biplot diagram (first component) and GD and SHRR are at the left side of the biplot diagram (second component).

Genotypes scattered around these vectors in the biplot diagram cause to comprising distinct groups of genotypes. Therefore, selection for one of these traits should be accompanied by the associated traits, and this would provide the opportunity to exert multi-traits

selection in sunflower breeding programs (Ghaffari et al., 2011). For example, B, C, E and Q hybrids scattered around the germination duration (GD) vector. This is explained by the attention to the table 5 and the mean values of these hybrids in GD trait. Another consideration of the biplot diagram is the angles of vectors. Kroonenberg (1995) concluded that the angle of vectors shows correlations of vectors and therefore, among traits. In Fig. 1, there were some vectors of traits, which had a small angle with each other that means they had positive correlations. On the other hand, GP and GD vectors had a completely opposite direction that means they had negative correlation (Table 5). Therefore, the smaller angle among vectors, indicates the greater positive correlation among related traits and vice versa.

Table 4. Component Weights

	Component 1	Component 2
GP	**0.316874**	-0.135055
GR	0.294845	-0.0958438
GD	-0.319817	-0.0649776
RL	**0.316617**	0.0099945
SHL	0.13702	**0.636659**
RDW	**0.329312**	-0.0622228
SHDW	0.265267	0.152731
RFW	**0.315539**	-0.105055
SHFW	**0.316283**	0.0935852
SVI	**0.345801**	-0.0280496
RSHR	0.305378	-0.129749
SHRR	-0.010311	**0.705905**

In Fig. 1, three distinct groups were formed by components. Hybrid M, because of the lowest value on all measured traits, was in the negative section of two components and far from vector loadings and their origin. In contrast, P and R hybrids, because of their high value in most traits were located close to the correlated vectors of first component. The third group, including B, C, E and Q hybrids, because of their low values (close to GD vector) were well distinguished from other hybrids. Rest of the hybrids with moderate means values were located between high and low groups (Table 6).

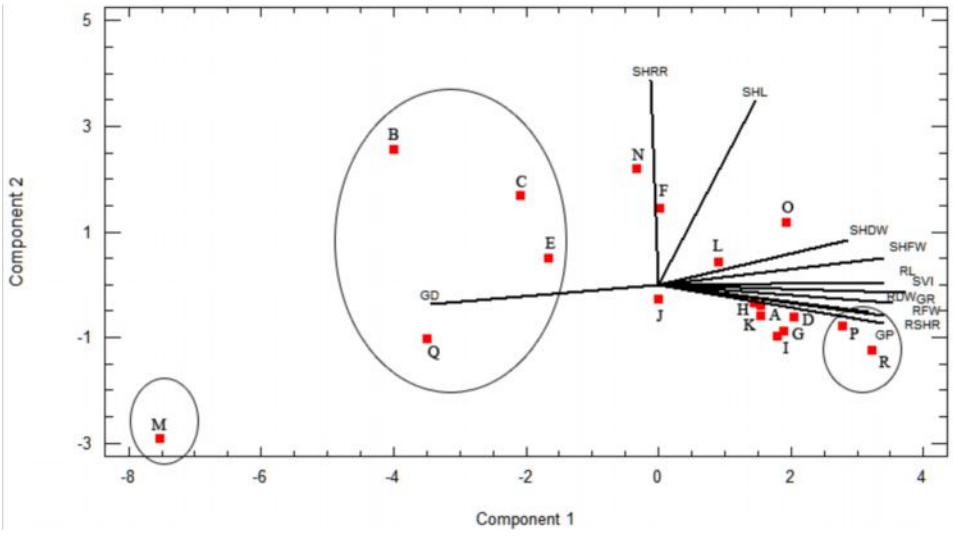

Figure 1. Biplot diagram of F_1 sunflower hybrids based on first 2 principal components

Table 5. Correlation among traits of F_1 sunflower single cross hybrids

Traits	Germination percentage (%)	Germination rate (day)	Germination duration (day)	Root length (cm)	Shoot length (cm)	Root dry weight (gr)	Shoot dry weight (gr)	Root fresh weight (gr)	Shoot fresh weight (gr)	Seed vigor index	Root/Shoot ratio	Shoot/Root ratio
GP	1											
GR	0.511**	1										
GD	-0.575**	-0.981**	1									
RL	0.606**	0.584**	-0.630**	1								
SHL	0.273*	0.223	-0.328**	0.465**	1							
RDW	0.690**	0.566**	-0.620**	0.826**	0.356**	1						
SHDW	0.299*	0.130	-0.174	0.134	0.076	0.284*	1					
RFW	0.682**	0.547**	-0.620**	0.796**	0.474**	0.880**	0.269*	1				
SHFW	0.586**	0.492**	-0.562**	0.519**	0.444**	0.721**	0.287*	0.761**	1			
SVI	0.764**	0.575**	-0.640**	0.956**	0.551**	0.843**	0.178	0.831**	0.600**	1		
RSHR	0.564**	0.582**	-0.601**	0.882**	0.119	0.743**	0.181	0.685**	0.412**	0.786**	1	
SHRR	-0.526**	-0.511**	0.524**	-0.656**	0.019	-0.562**	-0.134	-0.509**	-0.276*	-0.618**	-0.693**	1

* and **: significant at 5 % and 1 %, respectively.

Table 6. Trait Means of F_1 sunflower single cross hybrids at germination and early seedlings growth stage

Hybrids	Germination percentage (%)	Germination rate (day)	Germinatign duration (day)	Root length (cm)	Shoot length (cm)	Root dry weight (gr)	Shoot dry weight (gr)	Root fresh weight (gr)	Shoot fresh weight (gr)	Seed vigor index	Root/Shoot ratio	Shoot/Root ratio
A	93.333 ab	0.647 abc	1.607 de	1.351 abcde	0.973 abc	0.022 abcde	0.411 abcd	0.153 abcde	1.218 bcdef	217.333 abc	1.461 abcd	0.736 bc
B	26.666 e	0.304 de	3.305 b	1.100 cdef	1.466 a	0.005 fgh	0.108 ef	0.031 de	0.385 g	68.666 ef	1.052 cde	1.571 a
C	66.666 cd	0.375 de	2.708 bc	0.880 def	1.268 ab	0.015 cdefg	0.160 def	0.086 bcde	0.869 ef	135.222 cde	0.723 e	1.410 ab
D	90.000 abc	0.733 a	1.374 e	1.714 ab	1.057 abc	0.024 abcd	0.346 bcde	0.141 abcd	0.966 def	247.666 a	1.655 abc	0.624 cd
E	53.333 d	0.496 bcd	2.027 de	1.035 cdef	0.975 abc	0.011 efgh	0.269 cdef	0.072 cde	1.030 def	107.444 de	1.082 cde	0.969 abc
F	66.333 cd	0.571 abc	1.866 de	1.450 abcd	1.483 a	0.016 cdefg	0.320 cde	0.089 bcde	1.095 cdef	192.500 abc	0.984 cde	1.020 abc
G	93.333 ab	0.682 ab	1.507 de	1.381 abcde	0.870 bc	0.026 abc	0.410 abcd	0.163 abcd	1.251 bcdef	211.111 abc	1.596 abc	0.628 cd
H	93.333 ab	0.669 ab	1.496 de	1.482 abc	0.871 bc	0.015 cdefg	0.478 abc	0.105 abcde	1.205 bcdef	219.000 abc	1.701 abc	0.796 cd
I	86.666 abc	0.356 de	2.810 bc	1.776 ab	0.931 abc	0.028 ab	0.377 abcde	0.215 ab	1.395 abcd	234.857 a	1.973 a	0.538 cd
J	83.333 abc	0.629 abc	1.657 de	1.297 bcde	1.016 abc	0.013 defg	0.162 def	0.112 abcde	0.961 def	194.074 abc	1.312 abcde	0.778 bc
K	83.333 abc	0.756 a	1.328 e	1.251 bcdef	0.907 abc	0.017 bcdef	0.364 abcde	0.237 a	1.271 abcdef	183.000 abcd	1.371 abcde	0.743 bc
L	93.333 ab	0.458 cd	2.200 cd	1.269 bcdef	1.116 abc	0.020 abcde	0.424 abcd	0.123 abcde	1.578 abc	223.666 ab	1.159 bcde	0.885 abc
M	26.666 e	0.229 e	4.400 a	0.200 g	0.000 d	0.000 h	0.000 f	0.000 e	0.000 g	5.333 f	0.000 f	0.000 d
N	73.333 abcd	0.467 cd	2.201 cd	0.845 ef	1.202 ab	0.016 bcdefg	0.646 a	0.118 abcde	1.357 abcde	147.083 bcde	0.699 e	1.514 a
O	80.000 abc	0.573 abc	1.777 de	1.404 abcde	1.317 ab	0.024 abcd	0.624 ab	0.164 abcd	1.754 a	214.000 abc	1.068 cde	0.972 abc
P	80.000 abc	0.708 a	1.454 e	1.910 a	1.007 abc	0.029 a	0.381 abcde	0.179 abc	1.426 abcd	235.666 a	1.859 ab	0.555 cd
Q	70.000 bcd	0.307 de	3.382 b	0.718 f	0.546 c	0.004 gh	0.251 cdef	0.031 de	0.825 f	97.722 e	0.839 de	0.555 cd
R	96.666 a	0.725 a	1.418 e	1.562 abc	0.817 bc	0.031 a	0.464 abc	0.198 abc	1.672 ab	231.000 ab	1.917 a	0.522 cd

Same letters within column are insignificant at p=0.05.

CONCLUSIONS

Having knowledge about germplasm diversity and genetic relationships among breeding materials could be an invaluable tool in crop improvement strategies. A number of methods are currently available for analysis of genetic diversity in germplasm accessions, breeding lines and populations. These methods have relied on pedigree data, morphological data, agronomic performance data, biochemical data, and more recently molecular (DNA-based) makers data. Principal components analysis can be considered as a multivariate powerful technique for data reduction that removes interrelationships among components and effective in finding structures of data sets, genotypes grouping and estimation of genetic diversity of breeding materials. The results of this study indicated that used sunflower F_1 hybrids could be differentiated based on observed characters.

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