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**Evaluation of Sunflower (*Helianthus annuus* L.)
Hybrids Using Multivariate Statistical
Analysis**

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Abstract: Evaluation and characterization of sunflower hybrids using morphological, physiological and biochemical data are necessary and essential in sunflower breeding programs. The aim of this paper was to evaluate the productive possibilities of some sunflower hybrids grown in Macedonian environmental conditions. The experiment was carried out during the period 2013 and 2014 on the research field of the Faculty of Agriculture, “Goce Delchev” University – Shtip, in Ovche Pole locality, Republic of Macedonia. Total 20 sunflower hybrids were used as an experimental material. The trial was arranged as randomized complete block design (RCBD) with three replications. The average content of oil and oleic acid were 45.6 and 65.2%, respectively. The highest seed yield from all sunflower hybrids was obtained for the hybrid NLK12M144 (3,344 kg ha⁻¹) and the lowest for hybrid NLK12S126 (2,244 kg ha⁻¹). Cluster analysis classified the sunflower hybrids into four groups based on agronomic traits and seed yield. Most of the hybrids were included in cluster I and III (7 hybrids) followed by cluster IV. Using principal component analysis two main components have been extracted, with eigenvalue greater than one. They account for 72.99% of the variability in the original data. Only four hybrids had positive values for both main components (NLK12M144, NLK12S070, NLK12S125 and NLN12N011 DMR). The results from this study can be used to identify the best sunflower hybrids grown under Macedonian environmental conditions and to select the superior hybrids for future exploitation.

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Introduction

Sunflower (*Helianthus annuus* L.) is one of the most important industrial crops in the world because it is edible vegetable oil, after soybean and rapeseed (Putt, 1997; Fernandez – Martinez *et al.*, 2004; Hu *et al.*, 2010). *Helianthus annuus* L. is a diploid plant with $2n = 34$ chromosomes (Fick, 1989). Sunflower oil is widespread because of its high quality and is one of the five basic nutrients for human food (Demirer *et al.*, 2004).

The main objective in many researches is to evaluate the agronomic properties for sunflower hybrids (Killi and Altunbay, 2005; Sadghi *et al.*, 2008; Karaaslan *et al.*, 2010). Based on morphological, physiological and biochemical data, many methods were used to estimate the genetic diversity of sunflower genotypes (Dong *et al.*, 2007; Tabrizi *et al.*, 2011). Sunflower has a high potential for seed yield and oil accumulation in the seed. According to the FAO (2013), the average seed yield of sunflower in 2013 was 1,559 kg ha⁻¹ in the Republic of Macedonia.

Kaya *et al.* (2009) reported that in Turkey, Seed yield of sunflower hybrids varied between 800 kg ha⁻¹ and 4,250 kg ha⁻¹ with an average of 2,160 kg ha⁻¹. According to Ghaffari and Farrokhi (2008) seed yield of the sunflower hybrids ranged from 2,150 kg ha⁻¹ to 3,700 kg ha⁻¹. In many studies sunflower has high and stabile seed yield. According to Merren and Champolivier (1992) the most important yield components are: the number of plants per unit area, the number of seed per unit area and the mass of seed. On the other hand, oil content in sunflower seed is one of the most important agronomic traits. According to Keshta *et al.* (2008), the percentage of oil is between 38.0 and 54.4%. The values of oil content and seed yield depend on hybrid and environmental conditions in which genotypes are grown, along with interaction between them (Balalić *et al.*, 2012).

Water stress is one of the major abiotic limiting factors in sunflower seed yield. Sunflower yield can be reduced by water deficit approximately from 15 to 50% (Nagarathna *et al.*, 2012). Hence, it is necessary to protect the yield loss caused by drought in order to realize the genetic potential.

On the other hand, some biotic factors, especially diseases can cause losses in sunflower seed yield. The most frequent diseases in sunflower seed production in many countries including the Republic of Macedonia are: broomrape,

downy mildew, sunflower rust and phoma black stem (Encheva and Shindrova, 1990; Škorić, 1994).

The aim of this paper was to evaluate the productive possibilities of some sunflower hybrids grown under Macedonian environmental conditions, to identify the best genotypes and their possibility to be used in breeding programs in order to obtain high yielding sunflower hybrids.

Materials and methods

Plant material

As an experimental material 20 sunflower hybrids (NLK12M006, NLK12M008, NLK12M009, NLK12M058, NLK12M59, NLK12M063, NLK12M134, NLK12M139, NLK12M144, NLK12M148, NLK12S070, NLK12S074, NLK12S125, NLK12S126, NLN11001, NLN12N005, NLN12N007, NLN12N010 DMR, NLN12N011 DMR and NSK12001) were used. All sunflower hybrids were developed from NUSEED, global company for breeding sunflower hybrids.

Experimental design

The experiment was carried out during the period of 2013 and 2014 in the research field of the Faculty of Agriculture, “Goce Delchev” University – Shtip, in Ovche Pole locality, Republic of Macedonia. Ovche Pole locality is characterized by an altitude between 200–400 m above sea level, longitude 41°49'21.9" and latitude 21°59'03.9".

The trial was arranged as randomized complete block design (RCBD) with three replications. Each experimental plot was 6 m long, consisted of 4 rows, with 24 plants in one row. The seeds were sown by the sowing machine at a spacing of 0.25 m within the rows and 0.60 m between the rows. In the first year of setting the experiment (2013), the previous crop was wheat and in the second experimental year (2014) barley. The sowing occurred on 19 April in the first testing year and on 15 April in the second year. The standard growing measures were applied during the vegetation. Before sowing, the fertilizer 33% SAN was broadcasted and incorporated at a rate of 200 kg ha⁻¹. The experiment was treated with herbicide *Goal* in the second half of April and with insecticide *Ahilus* in the early May. Further irrigation was not used during the vegetation. At maturity, the head samples for yield were harvested.

Data collection

Morphological and agronomic traits were measured: 1,000 seed weight (g), seed length and width (mm), oil content (%), oleic acid content (%) and seed yield (kg ha⁻¹). Data were collected after the harvest. For obtaining thousand seed weight, 100 seeds taken by random from each sample, were weighted in an electronic balance with an accuracy of 0.001 g and then multiplied by 10, to give weight of 1,000 seed. For determining the seed length and width about 20 randomly selected seeds of each sample were used and the measurements were done by micrometer gauge. Oil and oleic acid content were determined utilizing nuclear magnetic resonance (NMR). Seed yield was measured separately from each plot (moisture 11%) and expressed per kg ha⁻¹.

Trial environmental conditions

According to the classification of Filipovski *et al.* (1996), Ovche Pole has continental and sub-Mediterranean climate. Figures 1 and 2 present the weather characteristics of Ovche Pole for the period in which study was conducted (2013 and 2014), as well as data for the long-term period (2001–2012).

Generally, the average monthly temperature in the first experimental year (2013) in all months was higher than in the second year except in July. The average monthly temperatures in 2013 were also higher in comparison to the long term period except in June, July and October. Figure 1 shows that in the second experimental year (2014), the average air temperatures were lower in all months compared to the average monthly temperatures from the long term

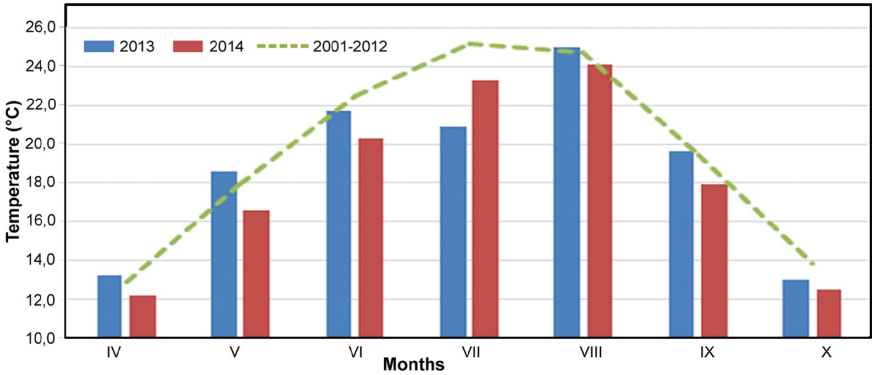


Figure 1: Average monthly temperatures (°C) for the period of study and long term period in Ovche Pole locality.

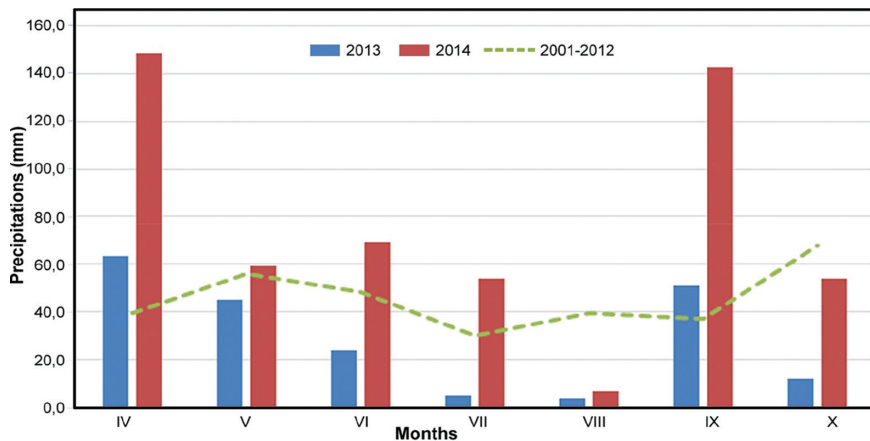


Figure 2: Monthly amount of precipitations (mm) for the period of study and long term period in Ovche Pole locality.

period. There were no significant differences between the average monthly temperatures in the first and in the second experimental year (Figure 1).

However, in terms of the amount of precipitation significant differences exist (Figure 2). In the first experimental year (2013), the monthly amount of precipitations in all months was lower compared to the second experimental year (2014). In the second year, significant amounts of monthly precipitations were recorded in April (148.2 mm) and September (142.3 mm). In this year (2014), higher quantity of precipitations was registered in all months except in August and October in comparison to the monthly precipitations in long term period.

Before seedling, soil samples for agrochemical analysis were taken from the experimental plot. Soil from experimental area contained good delivery with readily available phosphorus (24.19 mg/100 g soil), rich supply of readily available potassium (74.1 mg/100 g soil), low humus content (1.94%) and low salinity (7.65). The content of total nitrogen was 0.98 mg/g. The fertilizer used in the experiment area was in accordance to the results of soil analysis.

Statistical data analysis

Descriptive statistics (Stat Soft, 8.0) was performed for each property. The obtained results for the seed yield were statistically processed with an analysis of variance (ANOVA) using statistical programs JMP version 5.0 1a (2002). Mean values were compared using LSD test at 5% probability level. Cluster analysis

(CA) and principal component analysis (PCA) were performed with statistical package programs SPSS Statistics 19 (2010). Cluster analysis was performed to divide the hybrids into similar group based on their agronomic traits and seed yield. Principal component analysis was used to identify and explained the variability in the data set.

Results and discussion

According to Smith *et al.*, 1991) the evaluation and characterization of morphological traits are the first and basic step in description of germplasm. In some crop species like, garlic (Panthee *et al.*, 2006), melon (Lotti *et al.*, 2008) and sunflower (Kholghi *et al.*, 2011), descriptive statistics has been used for determining the genetic variation. The mean values for agronomic traits for the period of study are given in Table 1.

Table 1: Mean values for agronomic traits during the period of study.

	1,000 seed weight (g)	Seed length (mm)	Seed width (mm)	Oil content (%)	Oleic acid content (%)
Average	75.97	11.14	6.22	45.6	65.2
Min.	56.67	9.06	4.07	38.1	36.4
Max.	87.83	13.45	8.51	50.3	89.0
σ	9.18	0.15	0.45	1.56	3.89
CV (%)	12.08	5.10	7.28	3.73	6.40

In our study the obtained minimum and maximum values for 1,000 seed weight were 56.67 g and 87.83 g, respectively, with 75.97 g average during the both experimental years. The similar results were reported by Ghaffari and Farrokhi (2008). In their research the weight of 1,000 seed varied between 59.25 g and 84.25 g. According to Mijić *et al.* (2009) the values for 1,000 seed weight ranged from 54.8 g to 66.7 g and those results were lower than the values from our experiment.

Some investigators (Singh and Goswami, 1996; Sahebeh *et al.*, 2011; Duca et and Glijin, 2013) have measured the seed length and width to determine the seed size. The seed length is a trait which has great variability but usually it ranges from 6 mm to 25 mm, while the seed width ranges between 3–13 mm (Fick, 1978). According to Encheva *et al.* (2012), the values for seed length were between 9.98 mm and 11.10 mm. In our research the average values for the seed length and width were 11.14 mm and 6.22 mm, respectively. The highest values for those traits were 13.45 mm for seed length and 8.51 mm for seed width.

Škorić *et al.* (1996) reported that oil content in sunflower hybrids ranges from 38.1 to 49.2% and depends on genetic potential and environmental conditions (Hladni *et al.*, 2006). In our experiment the average value for oil content during the period of study was 45.6% and was within the values reported by Škorić *et al.* (1996). In Makanda *et al.* (2012) research, the oil content was between 36.6 and 44.6%.

In our experiment the average value for oleic acid content for both years was 65.2%. Pacureanu – Joita *et al.* (2005) reported higher values for oleic acid content, compared with ours, ranging from 78.7% to 87.4%.

In our study the highest coefficient of variation was obtained for 1,000 seed weight (12.08%).

The average values for seed yield of examined sunflower hybrids for the period of study are given in Table 2. The highest seed yield from all examined sunflower hybrids was obtained for the hybrid NLK12M144 (3,344 kg ha⁻¹) and

Table 2: Average values for seed yield of examined sunflower hybrids for the period of study.

	Sunflower hybrids	Average (kg ha ⁻¹)	Group	Rang
1	NLK12M006	2,908	a_d	7
2	NLK12M008	2,870	a_d	8
3	NLK12M009	2,762	b_e	9
4	NLK12M058	2,575	b_e	15
5	NLK12M059	2,577	b_e	14
6	NLK12M063	2,485	c_e	16
7	NLK12M134	3,024	abc	3
8	NLK12M139	2,991	abc	4
9	NLK12M144	3,344	a	1
10	NLK12M148	2,376	de	17
11	NLK12S070	2,644	b_e	13
12	NLK12S074	2,264	e	19
13	NLK12S125	2,748	b_e	10
14	NLK12S126	2,244	e	20
15	NLN11001	2,737	b_e	11
16	NLN12N005	3,047	ab	2
17	NLN12N007	2,914	a_d	6
18	NLN12N010 DMR	2,924	abc	5
19	NLN12N011 DMR	2,282	e	18
20	NSK12001	2,707	b_e	12
Average		2,721		
LSD _{0,05}		546.01		
CV (%)		12.16		

the lowest for the hybrid NLK12S126 (2,244 kg ha⁻¹). The average seed yield for all genotypes during the both years was 2,721 kg ha⁻¹. Much higher values for the seed yield compared with ours were reported by Hladni et al. (2012) and Makanda et al. (2012). The coefficient of variation for seed yield in our study was 12.16% (Table 2). Seed yield and harvest index had the highest variation in the research of Kholghi et al. (2011), 42.07% and 48.36%, respectively.

Based on LSD test, all tested hybrids differed significantly on seed yield and they were divided into groups (Table 2). The existence of differences among the tested hybrids, give us the opportunity in the future, to select parental forms and used them in breeding programs, in order to improve the seed yield. Hybrids marked with the same later (Table 2) did not significantly differ for seed yield at the 5% level.

Multivariate statistical techniques are widely used tools in analysis of genetic diversity. Most commonly used are cluster analysis (CA) and principal component analysis (PCA), (Mohammadi and Prasanna, 2003). Multivariate analysis has been used for evaluation of genetic diversity in many crops such as barley (Cross, 1992), wheat (Hailu *et al.*, 2006), sorghum (Ayana and Becele, 1999) and sunflower (Kholghi *et al.*, 2011).

Cluster analysis indicates the extent of genetic diversity in the material that could be reflected towards the parental lines that is of practical use in plant breeding (Sultana and Ghafoor, 2008). Based on agronomic traits and seed yield, all sunflower hybrids by cluster analysis were divided into four groups (Figure 3). The most of the hybrids were included in cluster I and III (7 hybrids) followed by cluster IV. The hybrid NLK12M144, which has the highest value for seed yield, was separated. Hybrids from cluster IV (NLK12M063, NLK12M148, NLK12S074, NLK12S126 and NLN12N011 DMR) were actually the hybrids with the lowest seed yield. Clustering of 36 genotypes into four groups was performed in Kholghi et al. (2011) study. Hierarchical clustering using Ward' method, for accessions from the 18 States of USA into four clusters based on the 13 morphological traits was reported by Nooryazdan et al. (2010). According to Murthy and Arunachalam (1966) selection of hybrids for parent must be based not only on geographical diversity, but actually more on the genetic drift.

In many researches principal component analysis (PCA) has been used to obtain a small number of linear combinations which account for most of the variability in data used (Kholghi *et al.*, 2011; Tabrizi *et al.*, 2011). In Arshad *et al.* (2010) study the principal component analysis could help for identification of the best sunflower hybrids.

In this study, using this analysis two main components have been extracted with eigenvalue higher than one. They account for 72.99% of the total variability (Table 3). The first main component (PC1) explaining 42.23% and the second main component (PC2) accounted 30.76% of the total variation.

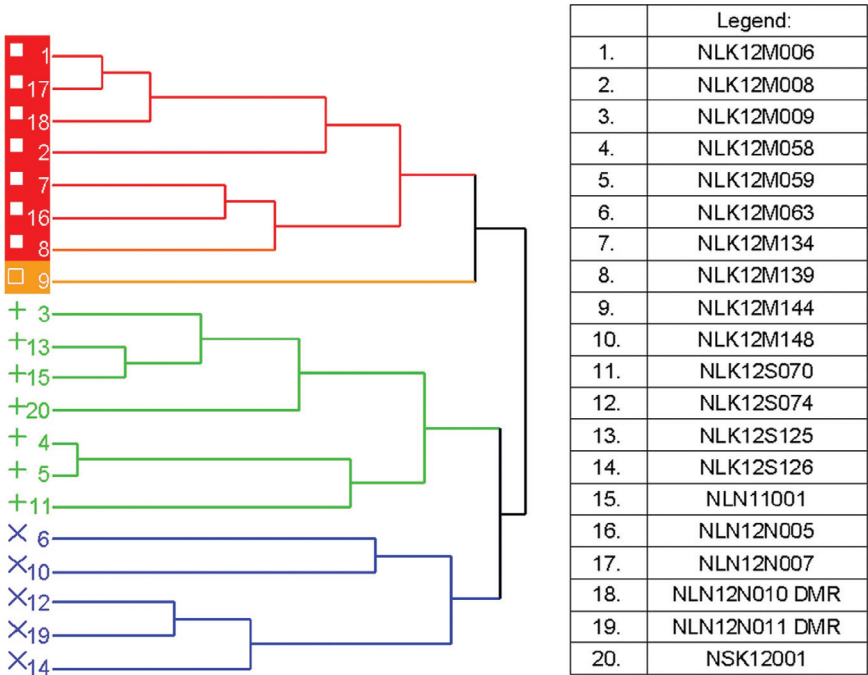


Figure 3: Cluster analysis for the examined sunflower hybrids based on agronomic traits and seed yield.

Table 3: Principal component analysis of sunflower hybrids.

Main components	Eigenvalue	Percent of variance (%)	Cumulative percentage (%)
PC1	4.53	42.23	42.23
PC2	1.85	30.76	72.99

The weights of two main components associated with analyzed agronomic traits are given in Table 4. First main component (PC1) was positively associated with 1,000 seed weight and seed width. Negative correlation was obtained for oil content.

The second main component (PC2) was positively correlated with the content of oleic acid and seed yield and negatively associated with the seed length and oil content. This means that selecting the hybrid for higher seed yield with higher oil content is difficult because in both main components negative coefficients with oil content were obtained.

Table 4: Weights of agronomic traits to main components of sunflower hybrids grown in Ovche Pole locality.

Agronomic traits	PC1	PC2
1,000 seed weight	0.58	−0.01
Seed length	0.28	−0.49
Seed width	0.53	0.04
Oil content	−0.47	−0.32
Oleic acid content	−0.22	0.60
Seed yield	0.19	0.55

Table 5: Main components values of the analyzed sunflower hybrids grown in Ovche Pole locality.

Sunflower hybrids	PC1	PC2
NLK12M006	−0.06	0.21
NLK12M008	−0.43	0.01
NLK12M009	−0.26	0.32
NLK12M058	−0.43	0.06
NLK12M059	−0.90	0.04
NLK12M063	−0.71	0.70
NLK12M134	−0.22	−0.42
NLK12M139	−1.93	−0.56
NLK12M144	2.93	1.57
NLK12M148	−0.58	1.33
NLK12S070	1.67	0.60
NLK12S074	−0.48	1.91
NLK12S125	2.18	0.97
NLK12S126	2.73	−2.15
NLN11001	0.14	−1.15
NLN12N005	2.69	−3.95
NLN12N007	1.09	−0.50
NLN12N010 DMR	−0.48	−1.42
NLN12N011 DMR	1.59	0.39
NSK12001	−2.69	0.87

Of the all examined hybrids only four have positive values for both main components (Table 5). Those hybrids were: NLK12M144, NLK12S070, NLK12S125 and NLN12N011 DMR. This means that those hybrids can be used in future exploitations and can represent parents' genotypes for some desirable trait.

Conclusion

The results from agronomic traits and seed yield showed variability in the analyzed sunflower hybrids. Different average values almost for all analyzed traits were obtained. Based on the significance of the differences in the average values, the used hybrids were divided into groups.

The highest seed yield from all sunflower hybrids was obtained for the hybrid NLK12M144 (3,344 kg ha⁻¹) and the lowest for the hybrid NLK12S126 (2,244 kg ha⁻¹).

Based on agronomic traits and seed yield, all sunflower genotypes on the basis of cluster analysis were divided into four clusters.

Using principal component analysis, two main components with eigenvalue greater than one, have been extracted. They accounted for 72.99% of the variability in the original data. Of the all examined hybrids only four have positive values for both main components (NLK12M144, NLK12S070, NLK12S125 and NLN12N011 DMR). Those hybrids can be used in future like as parent genotypes for some desirable traits.

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