EVALUATION OF SUNFLOWER (*Helianthus annuus* L.) VARIETIES USING MULTIVARIATE STATISTICAL ANALYSIS

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SUMMARY

Collecting, evaluation and characterization of sunflower varieties is necessary and essential in sunflower breeding programs. The aim of this paper was to evaluate the productive possibilities of some sunflower varieties grown in Macedonia.

The experiment was carried out during the period 2013 and 2014 on the research fields of Faculty of Agriculture, "Goce Delchev" University in Ovche Pole locality, Republic of Macedonia. Total 20 sunflower varieties were used as experimental material and the experimental work was conducted in a randomized complete block system. During the vegetation symptoms of charcoal rot, sunflower rust and phoma black stem were observed in the field. The highest seed yield from all sunflower varieties was obtained for genotype NLK12M144 (3 344 kg/ha) and the lowest for variety NLK12S126 (2 244 kg/ha). Cluster analysis classified the sunflower varieties into four groups based on agronomic traits and seed yield. The largest number of genotypes were included in cluster I and III (7 genotypes) followed by cluster IV. Using principle component analysis were separate two main components with eigenvalue greater than one accounting for 72.99 % of the total variation. Only four genotypes had positively values to both main components (NLK12M144, NLK12S070, NLK12S125 and NLN12N011 DMR).

Key words: sunflower, seed yield, agronomic traits, principle component analysis, cluster analysis

INTODUCTION

Sunflower (*Helianthus annuus* L.) is one of the most important industrial crops in the world because it's edible vegetable oil, after soybean and rapeseed (Putt, 1997; Fernandez – Martinez at al., 2004; Hu et al., 2010). *Helianthus annuus* L. is a diploid plant with 2n=34 chromosomes (Fick, 1989). Sunflower oil is widespread because 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 commercial sunflower cultivars (Killi and Altunday, 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). Sunflower has a high potential for seed yield and oil accumulation in the seed. According to the FAO (2013), in Republic of Macedonia the average grain yield of sunflower in 2013 was 1 559 kg/ha. Arshad et al. (2007) and Sepehr et al. (2008), reported sunflower seed yield between 1 500 kg/ha - 4 500 kg/ha. In many studies this cultivar has high and stabile seed yield. According to Merren and Champolivier (1992) the most important yield components are: number of plants per unit area, number of seed per unit area and mass of grains. 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 % - 54.4%. The values of oil content and seed yield depend of genotype and environmental conditions in which genotypes are grown (Marinkovic et al., 2003).

Among the abiotic factors, during the sunflower growing period, drought stress is limiting factor. Sunflower yield can be reduced by water deficit approximately 15 - 50 % (Nagarathna et al., 2012) and this factor is one of the major limitations in other crop yield.

On the other side, some biotic factors, especially diseases can influence significant to yield losses. In many studies the aims were target to achieve resistance cultivars to various diseases. Therefore, one of the most important task in sunflower breeding programs is to the develop resistance hybrids. The most frequent diseases in sunflower seed production in many counties including Republic of Macedonia are: broomrape, downy mildew, sunflower rust and phoma black stem (Encheva and Shindrova, 1990; Škorić, 1994; Kaya et al., 2004).

The aim of this paper was to evaluate the productive possibilities of some sunflower varieties grown in Macedonia.

MATERIALS AND METHODS

Plant material

As an experimental material were used 20 sunflower varieties (NLK12M006, NLK12M008, NLK12M009, NLK12M058, NLK12M59, NLK12M063, NLK12M134, NLK12M139, NLK12M144, NLK12M148, NLK12S070, NLK12S074, NLK12S125, NLK12S126, NLN11001, NLN12N005, NLN12N007, NLN12N010 DMR, NLN12N011 DMR and NSK12001).

Experimental design

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

The experiment was conducted in a randomized complete block system with three replicates. 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 0.30 m within the rows and 0.60 m between the rows. In 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 in the second year on 15 April. The standard growing measures were applied during the vegetation. Before sowing, the fertilizer 33 % SAN was broadcast and incorporated at a rate of 200 kg/ha. The experiment was treated with herbicide *Goal* in the second half of April and with insecticide *Ahilus* in the early May. During the vegetation no additional irrigation was applied. The harvest was made by hand at harvestable maturity. During the growing stage, the plant healthy was evaluated. The most significant diseases were observed: charcoal rot, sunflower rust and phoma black stem.

Data collection

Different morphological and agronomic traits were measured that included: 1 000 seed weight (g), grain length and width (mm), oil content (%), oleic acid content (%) and seed yield (kg/ha). Data were collected after harvest.

Trial environmental conditions

According to the classification of Filipovski et al. (1996), Ovche Pole locality is under continental and sub-Mediterranean weather type. Figures 1 and 2 present the weather characteristics of Ovche Pole for the period in which testing were conducted, 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. Average monthly temperatures in 2013 were also higher in comparison with the long term period except in June, July and October. Figure 1 show that in the second experimental year (2014), in all months the average air temperatures were lower compared to average monthly temperatures from the long term period. There were no significant differences between the average monthly temperatures in first and in the second experimental year (Figure 1).

However, significant differences exist in terms of the amount of precipitation (Figure 2). In the first experimental year (2013), the monthly amount of precipitations in all months was lower compared with 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 doses of precipitation were registered in all months except in August and October in comparison with monthly precipitation in long term period.

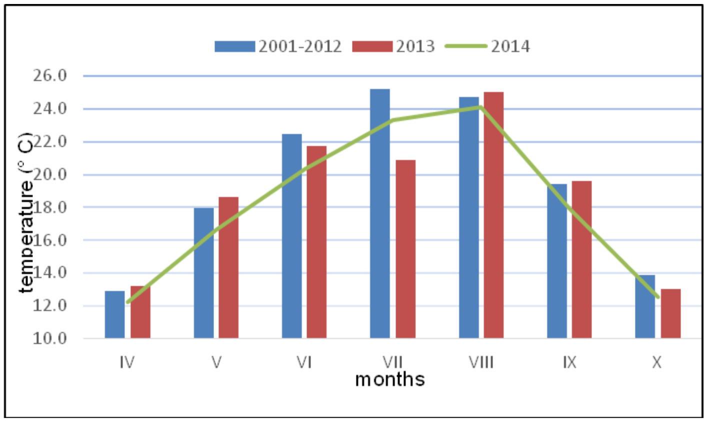


Figure 1. Average monthly temperatures (°C) for period of study and long term period in Ovche Pole locality

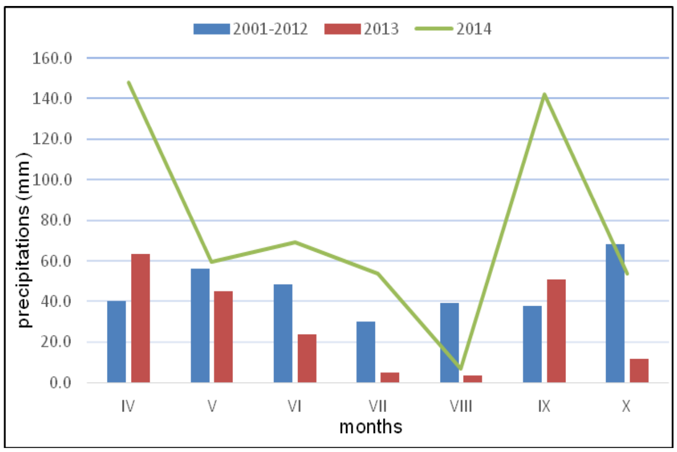


Figure 2. Monthly precipitations (mm) for period of study and long term period in Ovche Pole locality

Statistical data analysis

The seed yield was collected separately from each experimental plot. The yield was calculated per hectare. Weight of 1 000 seed was measured by counting 100 seeds in the laboratory and multiplying the obtained result by 10. To determine the oil content and content of oleic acid, nuclear magnetic resonance was used.

Descriptive statistics (Stat Soft, 8.0) was performed for each property. The obtained results for seed yield were statistically processed with analysis of variance (ANOVA) using statistical programs JMP version 5.0 1a (2002). Means values were compared using LSD test at 5% probability level. Cluster analysis (CA) and principle component analysis (PCA) were performed with statistical package programs SPSS Statistics 19 (2010).

RESULTS AND DISCUSSION

During the period of vegetation, symptoms of charcoal rot, sunflower rust and phoma black stem were observed in the field. Symptoms caused by sunflower rust (*Puccinia helianthi* Schwein.) were observed only on leafs. The damages were without significant importance. More significant were damages caused by other observed fungi.

Numbers of minor sclerotia (230 – 710 μm) were observed around 30 cm above the ground in the pith of the stem (Figure 3). Diseased plants were higher without developed head around 9 cm in diameter and without seeds. The fungus was identified as *Macrophomina phaseolina* (Tassi) Goidonich.

Another symptom which was present in the field was “phoma black stem” symptom caused by the fungus *Phoma macdonaldi* Borema. The steam of the diseased plants was covered with black spots on the lower and middle parts of the stem, especially at points of petiole attachment (Figure 4). The stem was hardly injured below the spots, but the medulla turns brown to black in colour and dies.

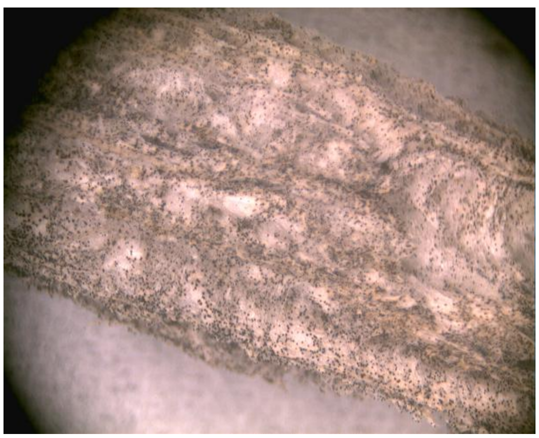


Figure 3. Sclerotia of *Macrophomina Phaseolina* on sunflower stem pith

Figure 4. Black spots at points of petiole attachment caused by *Phoma macdonaldi*

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. In Table 1 are given the values for average, minimum, maximum, standard deviation and coefficient of variation at all tested traits.

In our study the highest coefficient of variation was obtained for 1 000 seed weight (12.08 %). Nooryazdan et al. (2010) reported also the highest coefficient of variation for this trait, but also for head diameter and plant height.

Table 1. Mean, minimum, maximum, standard deviation and coefficient of variation for examined traits for sunflower varieties for the period of study

	1 000 seed weight (g)	Grain length (mm)	Grain 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

According to Encheva et al. (2012), the average values for grain length was between 9.98 mm to 11.10 mm. In our study, rang for this trait was bigger, from 9.06 mm to 13.45 mm.

The obtained minimum and maximum values for 1 000 seed weight were 56.67 and 87.83 g. respectively. The similar results were reported by Makada et al. (2012). According to these researches the content of oil was between 36.6 % - 44.6 %. The data for this trait in our study were a little bit higher (38.1 % - 50.3 %).

Division of sunflower varieties in 8 groups (Table 2), based on LSD test for average values for seed yield, give us the opportunity in the future, in order to create new hybrids, to select parental genotypes belonging to different groups. This means that if we do cross between genotypes' belonging to the different groups, much more like is to get better progeny with desirable trait from parent's trait.

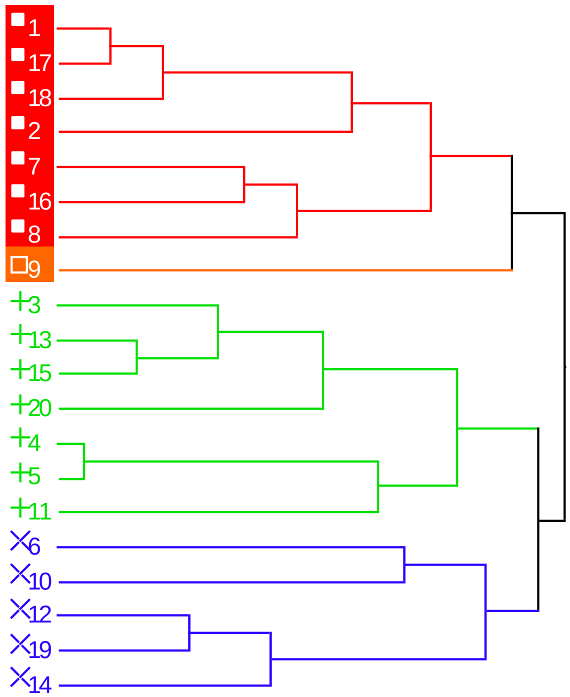
The highest seed yield from all sunflower varieties was obtained for genotype NLK12M144 (3 344 kg/ha) and the lowest for variety NLK12S126 (2 244 kg/ha). The average seed yield for all genotypes during the both years was 2 721 kg/ha. The coefficient of variation for seed yield in our study was 12.16 % (Table 2). Seed yield and harvest index had the highest variation in Kholghi et al. (2011) research, 42.07 % and 48.36 %, respectively. Much higher values for seed yield compared with ours were reported by Hladni et al. (2012) and Makada et al. (2012).

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

	Sunflower varieties	Average (kg/ha)	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		

Multivariate statistical techniques are widely used tools in analysis of genetic diversity. Most commonly useful 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).

Based on agronomic traits and seed yield, all sunflower genotypes by cluster analysis were divided into four groups (Figure 5). The largest number of genotypes were included in cluster I and III (7 genotypes) followed by cluster IV. The genotype NLK12M144, which has the highest value for seed yield, was separate. Similar results were reported by Teklewold et al. (2000) and Kholghi et al. (2011). According to Murthy and Arunachalam (1996) selection of genotypes for parent must be based not only on geographical diversity actually more on genetic drift. The genotypes from cluster IV (NLK12M063, NLK12M148, NLK12S074, NLK12S126 and NLN12N011 DMR) were actually the genotypes with lowest seed yield.



Legend:	
1.	NLK12M006
2.	NLK12M008
3.	NLK12M009
4.	NLK12M058
5.	NLK12M059
6.	NLK12M063
7.	NLK12M134
8.	NLK12M139
9.	NLK12M144
10.	NLK12M148
11.	NLK12S070
12.	NLK12S074
13.	NLK12S125
14.	NLK12S126
15.	NLN11001
16.	NLN12N005
17.	NLN12N007
18.	NLN12N010 DMR
19.	NLN12N011 DMR
20.	NSK12001

Figure 5. Cluster analysis for the examined sunflower varieties based on agronomic traits and seed yield

Principle component analysis (PCA) is usually used to identify the most significant variables in the data. With this analysis were separate two main components with eigenvalue greater than one accounted for 72.99 % of the total variation (Table 3). The first main component (PC1) explaining 42.23 % and the second main component (PC2) accounted 30.76 % of the total variation.

Table 3. Principle component analysis of sunflower varieties

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

In Table 4 are given the two main components associated with analyzed agronomic traits. First main component (PC1) was positively associated with 1 000 seed weight and grain width. The negative correlation was obtained for oil content.

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

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

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

From all examined genotypes only four have positively values to both main components (Table 5). Those varieties were: NLK12M144, NLK12S070, NLK12S125 and NLN12N011 DMR. This means that those varieties can be used in future like a parent genotypes for some desirable trait.

Table 5. Main components values of the analyzed sunflower varieties grown in Ocvhe Pole locality

Sunflower varieties	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

CONCLUSION

The results from agronomic traits and seed yield showed variability in the analyzed sunflower varieties. For sunflower genotypes were obtained different average values almost for all analyzed traits. Based on the significance of differences in average values, the genotypes were divided into groups. This polymorphism provides an opportunity in future breeding programs, different genotypes to be used as parents in hybridization. In such a case, the probability to get a genotype that would have better properties than the parents is greater.

During the vegetation, symptoms of charcoal rot, sunflower rust and phoma black stem were observed in the field.

The highest seed yield from all sunflower varieties was obtained for genotype NLK12M144 (3 344 kg/ha) and the lowest for variety NLK12S126 (2 244 kg/ha).

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

Using principle component analysis were separate two main components with eigenvalue greater than one accounted for 72.99 % of the total variation. From all examined genotypes only four have positively values to both main components (NLK12M144, NLK12S070, NLK12S125, and NLN12N011 DMR). This means that those genotypes can be used in future like a parent genotypes for some desirable trait.

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