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AGRO-MORPHOLOGICAL TRAITS OF INBRED SUNFLOWER LINES AND THEIR GENETIC ASSESSMENT

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SUMMARY

As plant breeding is a very complex and sophisticated process, it is of paramount importance to understand the nature and magnitude of interdependence between specific plant features. Using PCA and correlation analysis, this study examined the variability in features of two types of inbred sunflower lines and determined its connection with their seed yields. The experiment was conducted as a randomized complete block design with four replications in Rimski Šančevi (the experimental field of the Institute of Field and Vegetable Crops in Novi Sad) in 2018 and 2019. It examined a total of nine inbred sunflower lines created at the Institute of Field and Vegetable Crops in Novi Sad. The results obtained suggest a high degree of variability in the examined traits of inbred sunflower lines considered, whereas the significance indicated by the *F* test was confirmed by the Duncan test performed. A high degree of variability was also found between the different types of inbred lines considered. A positive correlation was found between all four traits observed in the inbred sunflower lines considered, with different levels of significance. A statistically significant positive increase in the head diameter was followed by an increase in the weight of 1000 seeds, which generated a higher seed yield per unit area. According to the arrangement of different types of inbred lines considered on the PCA biplot, it can be concluded that the linoleic inbred lines G7^L and G8^L had the highest average values of the 1000-seed weight, head diameter and seed yield throughout the course of both experimental years. The useful data obtained by PCA analysis can facilitate successful selection programs aimed at developing sunflower genotypes that possess high stability and seed yields.

Key words: *Helianthus annuus* L., inbred lines, sunflower, yield, correlations, PCA

Abbreviations: HD - head diameter; M1000S – the weight of 1000 seeds; PCA - principal component analysis; SL - seedling length; SY - seed yield

INTRODUCTION

The sunflower seed yield (*Helianthus annuus* L.) is a complex polygenic trait influenced by both physiological and morphological traits, as well as environmental factors (Zia-Ullah et al., 2013; Razzaq et al., 2017). Genotype adaptability is an extremely important trait that represents the result of responses to the variability in abiotic and biotic environmental factors (González et al., 2013; Kalenska et al., 2020). Achieving the broad-spectrum crop resilience is more urgent today than ever due to growing trends in the severity and frequency of various biotic and abiotic stresses caused by climate change (Tirnaz & Batley, 2019; Kerchev et al., 2020). Plants respond to stress exposure by changing the expression of a wide range of genes (Balan et al., 2017; Liu & He, 2020). When plants are physiologically prepared, they remember the experiences gained and thus are better prepared for an event that is

repeated by rapid or strong induction of reacting genes (Mladenov et al., 2021). The task of creating plant genotypes with the high yield potential by optimizing cultivation conditions compromises their adaptation to the external environment. One of the biggest challenges of sunflower breeders is the creation of new hybrids that enable higher seed yields in wider growing areas (Cvejić et al., 2019). In sunflower breeding programs, indigenous species of sunflower genera, the so-called wild relatives of sunflower, are becoming increasingly important as a source of adaptive traits to stressful environmental conditions (Seiler et al. 2017). Homozygous inbred lines are used as parent components for the production of sunflower hybrids (Cvejić, 2011). The actual seed yield represents the sunflower line's productivity when all production processes are followed, cultural practices are timely applied, and climatic conditions are met. The quality of inbred lines is crucial for the improvement and success of breeding programs. It is also of paramount importance to understand the nature and magnitude of interdependence between specific plant features (Radić et al., 2021). In addition to their yields, inbred lines should be distinguished according to a number of other desirable traits (Čuk et al., 2020). Nichal et al. (2015) argue that the correlation coefficient can be used to measure the interdependence between different plant traits and determine the character of the component on which the selection can be based. Principal component analysis (PCA) is one of the statistical tools for estimating genetic variability. PCA results are of great use for the identification of genotypes that possess high values of certain traits, thus facilitating planning and implementing future breeding programs (Mustafa et al., 2015; Venujayakanth et al., 2017). The purpose of this study is to examine, using PCA and correlation analysis, the variability in features of two types of inbred sunflower lines and determine its connection with their seed yields.

MATERIAL AND METHODS

A total of nine inbred sunflower lines, created at the Institute of Field and Vegetable Crops in Novi Sad, were enrolled in this study: three linoleic (oil) inbred lines (G^L) and six sulfonylurea-tolerant (G^S) inbred lines. The experiment was conducted as a randomized complete block design with four replications in Rimski Šančevi (the experimental field of the Institute of Field and Vegetable Crops in Novi Sad) in 2018 and 2019 (Fig. 1). The size of the basic plot was 34 m^2 ($16.2 \times 2.1 \text{ m}$). Sowing was performed in four rows at an inter-row spacing of 70 cm and an intra-row spacing of 23 cm. During the vegetation period, all the necessary cultural practices were applied to create optimal conditions for the development of plants, using wheat as a pre-crop.

Two middle rows (excluding marginal plants) were used to measure the seed yields (SY), which were converted into kg ha^{-1} , and head diameters (HD) of the sunflower lines considered (the HD was measured with a millimeter strip when the inbred lines were in full bloom). After harvest, the weight of 1000 (M1000S) seeds was determined using the standard ISTA method: 8 x 100 randomly selected seeds were counted with seed counters and the average measurement was multiplied by 10 (ISTA, 2018). Upon calculating the weight of 1000 seeds, the seeds were sown in a soil substrate under controlled greenhouse conditions, involving the day/night light regime (12/12h), a light level of about $300 \mu\text{mol m}^{-2} \text{s}^{-1}$, a temperature of $25^\circ\text{C} \pm 1^\circ\text{C}$, a relative air humidity of about 70%, and an irrigation norm of 3 mm/m^2 per day. The seedling lengths (SL) of 20 plants were measured 10 days after sowing with a millimeter ruler (plants from each experimental replication were carefully removed from the soil substrate by random selection). The following statistical methods were used in the paper: the analysis of variance, Duncan's multiple interval test, correlation analysis and principal component analysis. All statistical analyzes were performed using Statistica 13.2. and the SPSS statistical software. The data used in the PCA analysis were standardized by calculating their z-scores. The Pearson correlation coefficients of the values measured were calculated to determine the interdependence between the inbred sunflower line traits.



Figure 1. A graphical display of the location of Rimski Šančevi

RESULTS AND DISCUSSION

Plant breeding programs aimed at improving yields are essentially based on agro-morphological and physiological plant traits that are easy to measure while exhibiting cause-and-effect relationship with seed yields (Chambo et al., 2017). Moreover, Radić et al. (2021) suggest that a thorough understanding of the relationship between agronomic and technological features is required for a successful sunflower breeding and selection effort. Table 1. shows the results obtained for the traits of inbred sunflower lines considered. The average weight of 1000 seeds was significantly higher in 2019 (63.45 g) than that measured in 2018 (57.62 g). The inbred line G7^L (77.37 g) had the greatest weight of 1000 seeds in 2019, whereas the smallest weight of 1000 seeds was measured in the inbred line G4^S (40.68 g) in 2018. Significantly higher seed yields were produced in 2019 (1,821 kg ha⁻¹) than in 2018 (1,694 kg ha⁻¹). The inbred line G7^L (2,996 kg ha⁻¹) produced the highest seed yield in 2019, whereas the lowest yield was produced by the inbred line G4^S (933 kg ha⁻¹) in 2018. The average seedling length was 2 cm higher in 2018 than in 2019, which was not statistically significant (p 0.128). The longest seedling length was measured in the inbred line G8^L (28.8 cm) in 2018, whereas the inbred line G5^S (10.4 cm) was found to have the smallest seedling length in 2019. The average value of head diameter was 0.4 cm higher in 2019 than that measured in 2018, which was not statistically significant (p 0.052). The largest head diameter was measured in the inbred line G7^L (19.3 cm) in 2018, whereas the inbred line G4^S (14.8 cm) was found to have the smallest head diameter the same year. The linoleic inbred lines (G7^L, G8^L, G9^L) achieved higher average values for all the traits tested in both experimental years. The weight of 1000 seeds in 2018 was 8.92 g greater than that measured in 2019. The seedling length of both types of inbred lines considered averaged 22 cm in 2018, whereas the seedling length of linoleic inbred lines averaged 1 cm in 2019. Furthermore, the linoleic inbred lines had higher head diameter values in both 2018 (by 1 cm) and 2019 (by 2 cm). The greatest differences between the inbred sunflower lines considered were found relative to their overall yields: the linoleic inbred lines produced 766 kg ha⁻¹ more yields than the sulfonylurea-tolerant inbred lines in 2018, and as much as 1,069 kg ha⁻¹ more yields in 2019. The mean daily temperatures and soil moisture levels in the period of seed filling had a significant effect on the actual seed yields (Škorić, 2012; Kaya, 2016; Miklič et al., 2020). The seed yield parameter was found to have the highest coefficient of variation (CV = 36.4%), followed by the seedling length (CV = 31.1%), the weight of 1000 seeds (CV = 15.66%), and the head diameter (CV = 8.93%).

Table 1. The seedling lengths, head diameters, seed yields and 1000-seed weight of the inbred sunflower lines considered

G	Weight of 1000 seeds (g)			Seedling length (cm)			Head diameter (cm)			Seed yield (kg ha ⁻¹)		
	2018	2019	A±Sd	2018	2019	A±Sd	2018	2019	A±Sd	2018	2019	A±Sd
G1 ^S	62.0	69.4	65.7±4 ^b	19.6	24.6	22.1±6.6 ^{ab}	17.9	17	17.5±0.8 ^b	2027	2006	2017±15.8 ^c
G2 ^S	55.4	67.4	61.4±6.4 ^c	20.4	22	21.2±4.9 ^{ab}	17.5	18.1	17.8±1.1 ^b	1982	1865	1924±66.8 ^d
G3 ^S	48.6	58.1	53.3±5.1 ^f	19.8	26.8	23.3±8.1 ^{ab}	16.6	18	17.3±1.1 ^b	995	1466	1231±252 ^g

G4^S	40.7	44.5	42.6±2.1 ^g	20.6	13.8	17.2±10 ^b	14.8	15.3	15.1±1.1 ^c	933	953	943±18 ^h
G5^S	64.6	66.7	66.6±1.3 ^b	27.2	10.4	18.8±9.3 ^b	17.5	17.3	17.4±0.9 ^b	1444	1305	1375±78.1 ^f
G6^S	56.6	55.9	56.3±1.1 ^e	24.6	19.6	22.1±5 ^{ab}	15.3	15.9	15.6±1 ^c	1251	1196	1224±37.8 ^g
G7^L	71.1	77.4	74.2±3.4 ^d	20.4	18.2	19.3±2.4 ^b	19.3	19	19.2±0.8 ^a	2578	2996	2787±225.4 ^a
G8^L	60.2	72.6	66.4±6.6 ^b	28.8	23.4	26.1±4.1 ^a	18.7	18.8	18.8±0.4 ^a	2638	2801	2720±89.79 ^b
G9^L	59.5	59.3	59.4±1.2 ^d	17.4	21.4	19.4±4.1 ^b	16.8	18.7	17.8±1.3 ^b	1399	1805	1602±217.7 ^c

Legend: G - genotype; Sd - standard deviation; A - average

The results of the analysis of variance performed show a high degree of variability in the examined traits of inbred sunflower lines considered, whereas the significance indicated by the F test was confirmed by the Duncan test (Tab. 2). Čuk et al. (2020) report that there is a high degree of variability in the quantitative traits of inbred sunflower lines created at the Institute of Field and Vegetable Crops Novi Sad. The results of this research are consistent with those of Ćirić et al. (2013), Radić et al. (2013), Hladni et al. (2014), Babec et al. (2020), Dar et al. (2021) and Krstić et al. (2021a).

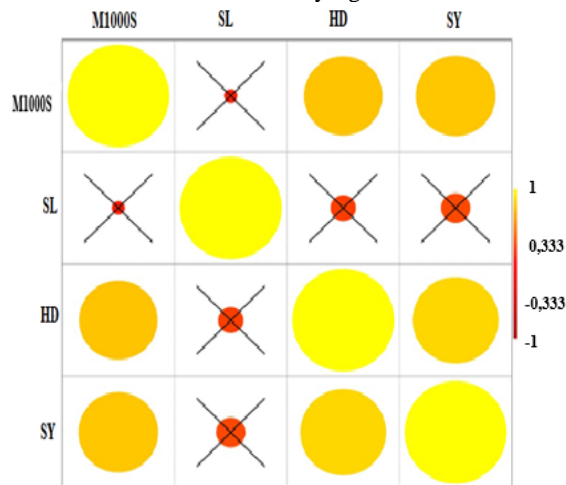
Table 2. The analysis of variance (ANOVA) results obtained and influence of different experimental factors on the sunflower traits examined

Source	Traits	df	F	p
Genotype	M1000S	8	1213.178**	0.000
	SL	8	1.500	0.179
	HD	8	17.241**	0.000
	SY	8	7030.122**	0.000
Years	M1000S	1	1105.720**	0.000
	SL	1	2.387	0.128
	HD	1	3.933	0.052
	SY	1	590.066**	0.000
Genotype x Years	M1000S	8	87.983**	0.000
	SL	8	4.239**	0.001
	HD	8	1.824	0.093
	SY	8	242.076**	0.000

Legend: p<0.05* - statistically significant differences; p<0.01** - statistically highly significant differences

Radić et al. (2013), Calamai et al. (2018) and Krstić et al. (2021a) argue that there are statistically highly significant differences between the sunflower genotypes relative to their weight of 1000 seeds. Incomplete or inadequate fertilization (Miklić et al., 2011), diseases (Kaya & Day, 2008) and unfavorable weather conditions during grain filling (Angeloni et al., 2021) can greatly affect the seed size and/or 1000-seed weight of sunflowers. There are also statistically highly significant differences between the sunflower genotypes relative to their head diameters (Ćirić et al., 2013; Babec et al., 2020; Dar et al., 2021). Variations in the sunflower head diameter largely depend on the photosynthetic activity, which depends on the composition of plants per unit area (Saleem et al., 2019). Ebrahimian et al. (2019) state that seasons do not exert a significant impact on the seed yield and 1000-seed weight of sunflowers, which is at variance with the findings of this research. Hladni et al. (2014) argues that there is a significantly high degree of variability in seed yields between inbred sunflower lines. The external environment has a great influence on the variability in sunflower seed yields (Radić et al., 2020), as well as the genotype itself (Kvashin et al., 2018). In addition to genetic factors (the genotype), sunflower seed yields are also conditioned by cultural practices applied and environmental conditions. Nichal et al. (2015) and Kaya et al. (2018) reported highly significant variations in the seedling length between the sunflower genotypes considered, which is at variance with the findings of this research. The results of Miladinov et al. (2014), who researched the treatment of inbred sunflower seeds of different lines with biostimulators before sowing, are consistent with the results obtained in the present study. They also reported that there were no statistically significant differences in the seedling length between the sunflower genotypes.

Table 3. The Pearson correlations of sunflower line traits examined: the weight of 1000 seeds, seedling length, head diameter and seed yield in the period 2018-2019 (at the location of Rimski Šančevi). Positive correlations are shown in yellow and negative correlations are represented in red. The color intensity and circle sizes are relatively proportional to the correlation computed. The correlations which were not statistically significant are marked with “X”.



Correlation is a statistical method that determines the degree of linear dependence between two variables (Stevanović et al., 2012). Table 3. show that all four traits measured in 9 inbred sunflower lines considered were positively correlated, with different levels of significance. The seedling length trait does not have a significant positive correlation with any of the traits examined. The head diameter was found to have a highly significant positive correlation with the weight of 1000 seeds ($r = 0.668$) and seed yield ($r = 0.724$). The seed yield and weight of 1000 seeds also had a highly significant positive correlation ($r = 0.779$), which is the strongest correlation between the traits examined. In the structure of sunflower seed production, the head diameter is an important trait which determines the number of blooms and consequently the number of grains per head, both of which are required for high hybrid seed yields (Tahir et al. 2002; Hladni, 2010). Therefore, the head diameter may be a good criterion in sunflower selection programs (Borleanu & Bonea, 2020). However, a number of authors such as Jocković et al. (2012) and Ćuk et al. (2020) report that head diameters and seed yields are not positively correlated in a statistically significant manner, which may be a consequence of different types of inbred lines. Škorić (2016) believes that increasing the head diameter above the optimal measures for the genotype can bring a reduced weight of 1000 seeds. Theoretical studies and practical experience have shown that high yields per unit area are usually associated with the number of plants per unit area, number of seeds per plant (depending on the degree of fertilization), head diameter (Hladni et al., 2014; Singh & Chander, 2018; Zeinalzadeh-Tabrizi et al., 2019; Riaz et al., 2019; Keipp et al., 2020; Naik & Ghodke, 2021) and the weight of 1000 seeds (Radić et al., 2013; Jalil et al. 2014; Nichal et al., 2015; Ieremenko & Kalitka, 2016; Kvashin et al., 2018; Baraiya et al., 2018; Kalenska et al., 2020; Radić et al., 2021; Naik & Ghodke, 2021). The authors mentioned above also argue that increases in the weight of 1000 seeds and head diameter significantly increase seed yields, which is consistent with the results obtained herein. A statistically significant positive interdependence between the head diameter and weight of 1000 seeds was confirmed by Kholghi et al. (2011), Ćuk et al. (2020) and Naik & Ghodke (2021). The head diameter does not have a direct impact on seed yields, but has a direct impact on the number of seeds per head (Follmann et al., 2019), which is at variance with the findings of this research. Nichal et al. (2015) point out that seedling lengths have a positive effect on seed yields, which is also at variance with the results of this study.

Correlation reflects the linear dependence between two variables, but two variables can also be strongly dependent without linear connection. Therefore, a multivariate analysis such as PCA is considered very useful for a full understanding of the interrelationship between large numbers of variables (Krstić et al., 2021). Table 4. shows that the first two PCA axes are statistically significant (values greater than 1), as reported by Hussain et al. (2017), which is in accordance with the findings of this paper.

Table 4. The significance of PCA axes and percentage of original PCA axis variability obtained

PC	Eigenvalue	% Variance	Cumulative
1	2.67883	66.971	66.971
2	1.02867	23.334	90.305

As shown in Table 5., the PCA 1 axis most faithfully reflects the seed yield, followed by the head diameter and weight of 1000 seeds. The PCA 2 reflects the seedling length.

Table 5. Factor load of different traits of inbred sunflower lines considered

Trait	PC 1	PC 2
M1000S	0.54451	-0.25047
SL	0.21918	0.9632
HD	0.56952	-0.087189
SY	0.57543	-0.043568

Principal component analysis (PCA) is an analysis in which a large amount of data is reduced and the correlation of new variables is expressed to interpret the data more accurately (Jolliffe & Cadima, 2016). The PCA biplot obtained shows that the inbred sunflower lines considered are diffusely distributed, represented by colors (years), symbol shapes (types of inbred lines), and different lengths and directions of feature propagation (Fig. 2).

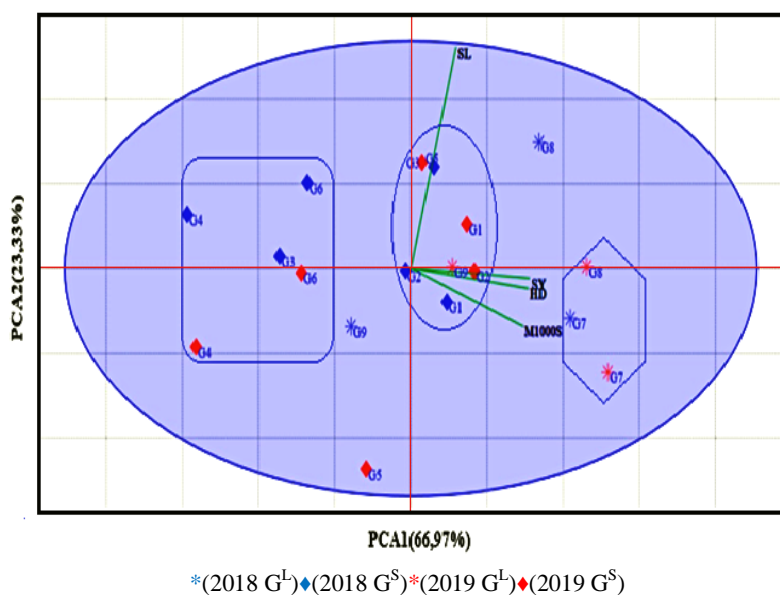


Figure 2. A PCA biplot for the correlations between the examined traits of inbred sunflower lines considered (where red and blue denote years and symbol shapes denote types of inbred lines and different lengths and directions of feature propagation)

The PCA biplot created explains a total of 90.30% of the phenotypic variability determined: the PCA 1 axis describing 66.97% and the PCA 2 axis describing 23.33%. The PCA 1 and PCA 2 distances of each trait observed show the influence of these traits on the variability in the genotypes considered. A statistically highly significant positive interdependence between the seed yield, head diameter and 1000-seed weight can be explained according to the sharpness of angles which form the axes indicating these traits. The seedling length trait forms obtuse angles with all the traits examined, which was also confirmed by the Pearson correlation coefficients calculated.

The inbred sunflower lines with high values of the head diameter, 1000-seed weight and seed yield have the highest PCA 1 values. The linoleic types of inbred lines belong to this group marked with a hexagon (G7^L in both experimental years, whereas G8^L in 2019). These inbred lines are most often used in breeding programs to obtain hybrids with a yield of over 2000 kg ha⁻¹ (Radanović et al., 2018; Ćuk et al., 2020). The group marked with a square is characterized by a low weight of 1000 seeds, head diameter and seed yield. This group includes the inbred lines resistant to sulfonylurea (G4^S and G6^S in both experimental years, whereas G3^S in 2018). The last group marked with a circle is distinguished by high values of the seedling length. This group mostly includes the inbred lines resistant to sulfonylurea and one line of the linoleic type (G1^S and G2^S in both experimental years, G3^S and G9^L in 2019, and G5^S in 2018). The inbred lines that are not framed by different geometric shapes do not stand out according to the traits examined, i.e. they do not have extremely high or low values of these traits (which is consistent with the results of Ćuk et al. (2020)). The PCA results determined the degree of variability in different traits of inbred sunflower

lines considered, which could be used in hybrid selection programs to improve the head diameter and seed yield of sunflower hybrids (Nazir et al., 2013). Therefore, it can be concluded that the seed yield of inbred sunflower lines considered is simultaneously related to their head diameter and weight of 1000 seeds, whereas no relationship was found between their seed yields and seedling lengths.

CONCLUSION

The results obtained indicate a high degree of variability in the examined traits of inbred sunflower lines considered. Upon comparison of the PCA graphs made and Pearson's coefficients calculated, the PCA graphs were found to provide a more in-depth analysis of the interdependence between the examined traits of inbred sunflower lines considered. The PCA results could facilitate planning successful selection programs aimed at developing sunflower genotypes which possess high stability and seed yields. A statistically highly significant positive increase in the head diameter was followed by an increase in the weight of 1000 seeds, which produced a higher seed yield per unit area. According to the diffuse distribution of different types of inbred lines considered on the PCA biplot, the linoleic inbred lines G7^L and G8^L were found to have the highest average values of the weight of 1000 seeds, head diameter and seed yield in both experimental years. This may be accounted for by the fact that the introduction of sulfonylurea tolerance genes impaired a certain number of genes responsible for the traits examined. However, the extent of this impairment is yet to be established in further research.

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Conflict of interest: The authors declare that they have no conflict of interest.

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