# ENVIRONMENTAL RESPONSE IN SUNFLOWER HYBRIDS: A MULTIVARIATE APPROACH

## Maria Duca<sup>1</sup>, Angela Port<sup>1\*</sup>, Ion Burcovschi<sup>1</sup>, Maria Joița-Păcureanu<sup>2</sup>, Mihaela Dan<sup>2</sup>

<sup>1</sup>Center of Functional Genetics, Moldova State University, 3/2 Academiei str., MD-2028, Chişinău, Republic of Moldova

<sup>2</sup>National Agricultural Research and Development Institute Fundulea, 915200 Fundulea, Călărași County, Romania <sup>\*</sup>Corresponding author. E-mail: portang@yahoo.com

#### ABSTRACT

Seed yield stability, being strong influenced of environmental factors, remains the most important objective of sunflower breeding. In present research have been assessed 8 yield related traits at 25 sunflower hybrids, in two growing season (2019, 2020), under different weather conditions in Soroca district from the northeast of the Republic of Moldova.

The principal component analysis, correlative and multicollinearity statistics highlighted their clustering according to different categories: commercial, experimental and in testing phase for hybrid performance, indicating the diverse nature of hybrids. The correlation profile of yield related traits at the commercial hybrids contrasts with that of the tested and experimental hybrids which in both evaluated years, is more heterogeneous in context of the strength and direction of correlation. The parameters *achene weight per head* and *number of achene per head*, followed by *seed yield* and *head diameter* have the highest degree of variation related to growing season. Most negative correlations are found for correlation pairs with variables *1000 seed weight* and *hectolitre mass of seeds*, which are moderate and high for the tested and experimental hybrids, and predominantly low for the commercial ones.

The commercial hybrids were more sensitive to the drought stress of the 2020 growing season, responding by a greater decrease of the majority of analyzed agronomic indices. At the same time the correlations between different traits indicated a pattern similar to previous year. Contrary, the experimental hybrids and those in the testing phase showed small changes in the values of agronomic traits that suggest a greater tolerance to drought conditions, as well as less correlations between different indices. The results have several implications for the future breeding, genotype evaluation and recommendation of sunflower hybrids for production in the local regions of growth.

Keywords: correlation, genetic diversity, principal components analysis, sunflower, yield related traits.

#### **INTRODUCTION**

Yield is one of the most economic important and complex traits of agricultural plants, regulated by genes known as quantitative trait loci (Beyene et al., 2015; Boyle et al., 2017; Rani et al., 2019).

In sunflower, seed yield is determined by indirect traits like optimal plant density, growth period, plant height, and direct traits, such as head diameter, the number of seed per head/or per plant/unit area, the mass of seed, 1000-achene-weight, filled seed per head etc. (Hladni et al. 2007; Cucereavii et al., 2018; Clapco et al., 2019).

Most sunflower breeding programs perform selection on inbred lines and then,

using combining ability, correlation, path analysis, and other univariate and multivariate approaches (Leite and Oliveira, 2015; Santos et al., 2018; Riaz et al., 2020), select optimal crosses among elite materials to produce commercial hybrids. But the progress of multiple traits selection in plant breeding remain to be complicated due to extensive pleiotropy or/and linkage disequilibrium of trait-specific genes that interact through a variety of molecular mechanisms (Furlotte and Eskin, 2015; Chahal et al., 2019).

Generally, commercial sunflower hybrids have high and stable seed yield. Single crosses are highly homogeneous, can express highest hybrid vigor and have greater seed

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yield stability in various growth environments (Pissai, 2011; Mijic et al., 2012; Kalenska et al., 2020).

However, there are frequent cases that despite the fact that regional hybrids trials are conducted every year, this characteristic of genotypes vary, leading to high unbalanced data across the years.

Sunflower is a culture of the temperate zone and is quite flexible to weather and soil conditions (Gonzáles et al., 2013; Razzaq et al., 2017; Tabără et al., 2018). However, global change of climate, especially the increasing frequency of temperature extremes, onset of droughts/floods, as well as, rapidly growing population and urbanization are major constraints against ensuring food security, requiring crop production expansion that should be economically and environmentally feasible. In this context, establishing of hybrids ecological plasticity, physiological interrelationships of in development of vegetative and generative organs depending weather on and technological factors is relevant.

In connection, the present research is focused on comparison of performance of three sunflower hybrids categories by revealing the variation of correlation pattern of seed yield and its components in two growing seasons, different in terms of weather conditions. The results provide useful informations for the future breeding, genotype evaluation and recommendation of sunflower hybrids for production in the local regions of growth.

### MATERIAL AND METHODS

### Plant material

A group of 25 sunflower (*Helianthus* annuus L.), single cross hybrids, created by "AMG-Agroselect Comerț" SRL seed company, were studied in two contrasting years (2019 and 2020). Eight hybrids, including Codru, Dacia, Talmaz, Zimbru, Doina, Nistru, Cezar and Oscar represent hybrids with high yield, approved in the Republic of Moldova (commercial hybrids); four genotypes: US235CLP, US237SU, US2472CLP, US2137SU are in testing phase for hybrid performance by the public national institution *State Commission for Crops Variety Testing* (conventionally noted as the SCCVT tested hybrids) and thirteen genotypes: 413, 415, 454, 457, 618, 1583, 1625, 1686, 1718, 1719,1721, 1722 and 1727 represent hybrids tested in comparative trials in the experimental field (experimental hybrids).

Hybrid seeds were sown manually on April 21 (2019) and April 24 (2020) in a randomized complete block design with four replications, on plots with an area of  $33.6 \text{ m}^2$ , 2-3 seeds in pits at 0.20 m from each other within rows. All the recommended agronomic practices (Vronschih et al., 2002), including soil fertilization (70 kg/ha of  $N_{16}P_{16}K_{16}$ ), were carried out during the crop season. Weed and pest controls were applied according to the crop's needs, being used the herbicide Avangard Duo SC (3.0 l/ha) and the insecticide Force (1.5 g-12.0 kg/ha). The precursor was straw cereals. The fields cultivated with the studied sunflower hybrids have the same locations in both 2019 and 2020 (Soroca district, Republic of Moldova).

# **Climatic conditions**

Average monthly temperatures and precipitations for the study period in comparison with multiannual average are shown in Figure 1 (A and B).

Generally, there were no significant differences between the average monthly temperatures in the 2019 and in 2020 experimental year.

However, in terms of the amount of precipitation significant differences exist. In 2020 there are more pronounced water stress, especially in critical periods for sunflower development. The monthly amount of precipitation in April-September was lower compared to 2019. For instance, in April the precipitation rate reached 9.6 mm compared to 39 mm, which is the multiannual average. In the first decade of June there was insufficient rainfall of about 13.5 mm and days with low temperatures at night. The first half of the month of July was very dry, with rainfall, which in total did not exceed 10 mm.

Thus, for 2020 was characteristic the drought as a common abiotic stress that

considerably limits the crop production in the Republic of Moldova.



*Figure 1.* Average monthly of temperatures (A) and precipitations (B) for the period of study in comparison with multiannual average (Soroca district, Republic of Moldova)

#### **Data recording**

Data was recorded for ten plants (n=10, 4 repetitions), selected from central rows, for eight quantitative traits: Plant Height (PH, cm), Number of Leaves (NL), Head Diameter (HD, cm), Number of Achene per Head (APH), Achene Weight per Head (AWH, g), 1000-Seed Weight (SW, g), Hectolitre Mass of Seeds (HMS kg/hl) and Seed yield (SY, kg/ha). Data were recorded during vegetative stage (PH and NL) and after being harvested (HD, APH, AWH, SW, HMS, SY).

The average height of the sunflower plants was measured using a graduated wood ruler from soil level to head insertion. The average number of leaves per plant was determined by counting the leaves per plant. The diameter of sunflower head was determined measuring diameter of bv the the inflorescence receptacle with a graduated ruler. The average number of seeds per head was assessed by counting all the full seeds from the sunflower receptacle. The weight of the achene per head was evaluated by weighing them on the laboratory balance, with an accuracy of 0.001 g. After drying in the air, 1000 seed weight was determined by counting and weighing them on the analytical balance. The hectoliter mass was determined by weighing two samples of a volume of one

liter of seeds. Plants were harvested individually, after physiological maturity stage (R9) (Schneiter and Miller, 1981), and seed yield (kg/ha) was evaluated separately from each plot (moisture 11%).

#### Statistical analysis

The primary data were subjected to descriptive the statistics analysis and further the average of variables was analysed by Principal component analysis, multicollinearity statistics. pairwise of correlations and Mantel test using XLSTAT (https://www.xlstat.com/). software The Mantel test (Legendre et al., 2015) was applied to evaluate association of proximity matrices generated by the differences at the level of genotypes, or at the level of yield related traits in case of all investigated hybrids and separately on categories.

#### **RESULTS AND DISCUSSION**

Economically important seed yield and contributing traits are often underestimated due to the lack of a preliminary complex research of relationships correlation among them, resulting in instability and unexpected or unintended outcomes of selective breeding. Multivariate analysis is an efficient tool to identify several correlated variables and quantify the variability among genotypes, providing information on relative contributions of desired traits to total divergence and understanding of their genetic basis (Felipe et al., 2021).

In this study, twenty-five sunflower hybrids were subjected to bivariate and multivariate analysis to highlight:

- the most variable morphological and agronomic traits under growth conditions, particularly drought-induced stress;

- the expected divergence of relationships among yield related traits at different categories of hybrids;

- change of the traits correlation profile

related to growing season for each of these categories of hybrids;

- the genotypes with a higher yield performance.

#### Principle component analysis (PCA)

The PCA results of the original data recorded for eight biometric traits in the 2019 growing season (PCA 2019) and those from 2020 (PCA 2020) were analyzed comparatively in order to ascertain the parameters with higher degree of variation depending of the environmental factors.

Thus, PCA 2019 model have displayed eight principal components (PC1-8), of which the first three explain 73% of the total variance of variables or observations (Table 1).

Commonste		2019		2020				
Components	PC1	PC2	PC3	PC1	PC2	PC3		
Eigenvalue	2.70	1.76	1.38	2.27	2.11	1.57		
Variability (%)	33.76	21.96	17.24	28.43	26.38	19.64		
Cumulative %	33.76	55.72	72.97	28.43	54.81	74.45		
Traits	Correlations between variables and factors							
Seed yield (kg/ha)	0.19	-0.79	-0.16	0.22	0.86	0.13		
1000 Seed weight (g)	-0.12	-0.23	0.81	-0.11	0.31	0.72		
Hectolitre mass of seeds (kg/hl)	-0.10	-0.35	-0.73	0.40	0.71	-0.44		
Number of leaves	0.79	-0.35	-0.01	-0.09	-0.41	-0.57		
Plant height (cm)	0.59	-0.32	-0.11	0.48	0.38	-0.57		
Head diameter (cm)	0.75	-0.32	0.36	0.55	-0.62	-0.12		
Achene weight per head (g)	0.82	0.46	-0.04	0.84	-0.28	0.34		
Number of achene per head	0.66	0.64	-0.16	0.90	-0.09	0.26		

Table 1. Principal components analysis based on quantitative traits in studied sunflower hybrids

Cumulatively, PC1 and PC2 exhibit the highest informational contents (56%) that explain the variance of the original values. Similar results have been obtained in the case of the PCA for the parameters registered at sunflower in 2020 growing season.

According the PCA 2019, the traits NF, HD, AWH and SY are strongly correlated with PC1 and PC2, respectively. The APH correlates at a moderate level with the first two main components. Two parameters SW and HMS are attributed to the linear variables combination of PC3, while PH does not show relevant associations with first three latent variables (Table 1).

It should be noted that some parameters negatively correlate with components, for instance, SY (r=-0.79) and HMS (r=-0.73)

with PC2 and PC3, respectively.

The meteorological conditions of 2020 influenced the growth and development of sunflower plants, a fact revealed by the changes in the distribution of variables on the factorial planes. Thus, PC1 contain only two parameters AWH (r=0.84) and APH (r=0.90). SY (r=0.86) is included in the PC2 linear combination together with HMS (r=0.71) and HD (r=-0.62). The latent factor PC3 is also modified, that in addition to SW (r=-0.57)contains the initial variable PH (r=0.84), but not and HMS. Interestingly, the indices SY and MHL correlate positive in the ACP 2020. predictive model includes This other parameters - HD and PH, that are associated negatively moderate with PC2 and PC3, respectively.

The variables that represent well a factorial axis  $(cos^2 \ greater \ than \ 0.5)$  and at the same time have a contribution more than 12.5% (average value of the expected contribution) on each component are illustrated in Figure 2.

The original measurements with the largest contribution in differentiating hybrids grown in 2019 are: AWH, NL, HD and APH (PC1), followed by SY (PC2), SW and HMS

(PC3). In the case of the ACP 2020 model, the indices APH, AWH and HD (PC1), followed by SY, HMS (PC2), SW and PH (PC3) are highlighted by the percentage contribution in identification of the new principal variables.

It should be mentioned that the traits PH and NL do not show significance in the discrimination of hybrids cultivated in 2019 and 2020, respectively.



Note: Abbreviations are given in Material and methods section.

Figure 2. Contribution of the yield related parameters (%) at studied sunflower hybrids

The pairwise association test of original values for all 25 investigated hybrids, in both revealed 2019 and 2020, statistically significant correlations (p<0.05) for the agronomic traits AWH:APH (r=0.8-0.9, p=0.0001) and HD:AWH (r=0.4-0.5. p=0.014). The positive and significant correlation coefficient explains the relationship between the parameters. Over all, it is logical to conclude that since these characters had high correlation and also high direct effect, thus direct selection for these characters should be a major concern for plant breeders.

At the same time, statistically significant correlative associations were identified that differentiate the hybrids cultivated in 2019 from those in 2020. Thus, the average correlations between NL:PH (r=0.51,

p=0.009) and HD:NL (r=0.66, p=0.0004) were attested in the case of hybrids cultivated in 2019. Other correlative associations of moderate intensity, being formed by the pairs of variables HMS:SY (r=0.60, p=0.002); HMS:PH (r=0.55, p=0.004); HD:APH (r=0.42, p=0.036) are identified in the case of hybrids cultivated in 2020, the year where the water deficit was more pronounced.

analysis, The results of the PCA which showed a reduction in the number of original variables from eight to three with more than 70% cumulative variance and those of the bivariate association test, suggest on impact of productivity indices in hybrids differentiation due to multiple correlations, finding argued linear bv multicollinearity statistics (Table 2).

Table 2. Variance Inflation Factor (%) of productivity parameters at studied sunflower genotypes

Growing season	SY	SW	HMS	NL	PH	HD	AWH	APH
2019	1.78	1.75	1.62	3.23	1.60	2.87	7.29	6.64
2020	2.66	1.98	2.92	1.38	1.58	1.77	4.80	4.80

According to this statistical test, the variables AWH and APH are multicollinear, showing VIF (Variance Inflation Factor) values of approximately 5-7% (Olivoto et al., 2017), regardless of the year of data recording.

At the same time, have been ascertained that the VIF values of the NF and HD parameters are higher for 2019 hybrids and in the case of 2020 hybrids, those of SY and HMS, which explains the linear interrelationships between these parameters that were revealed at the entire group of studied hybrids.

A conclusion that emerges from the results of the PCA model, of the paired association test and multicollinearity statistics is that, the estimated productivity indices and the relationship correlation between them are quantitatively changeable with respect to the growth season.

The traits AWH, APH have the highest degree of variation, respectively of differentiation at level of entire group of 25 genotypes, and additionally, for the 2019 hybrids are relevant NF and HD, but SY and HMS for the 2020 sunflower hybrids.

In contrast of APH and AWH variables that positive correlated among them and with main component in PCA, clearly indicating that direct selection of a characteristic would increase the other, the rest of high discriminant variables, like SY, HMS, HD, NF vary in strength and direction of correlation, both in association with the main component and between them. Therefore, further to these conclusions, if the breeding program aims to increase a desired yield component, it will affect differently the others components, resulting various growth and development responses from year to year, which is not an appropriate purpose for selection process. An interesting question arises regarding the identification of the causes of these findings, it is due only to different growing season or, and to the various level of stability of hybrid performance of the genotypes studied in this research.

### **Biplot display**

From Figure 3 (A and B) that illustrates projection of the eight biometric traits in association with the 25 studied hybrids, into the obtained PCA space, can be observed the clustering trend of hybrids with respect to the known categories (commercial, experimental and SCCVT tested hybrids), more pronounced in the case of data recorded in 2020. Also, there are some hybrids in the three analyzed categories, which differ more strongly, from average of group, according to the investigated original variables.

Thus, according to contributions (Figure 2) of the original variables correlated with the main components, four from the eight commercial hybrids, Doina (the lowest values of SY), Cezar (comparatively higher values for all five original variables highlighted by the ACP), Nistru (maxim values for HD, NF and large for AWH, SY) and Zimbru (the lowest values of HD, NL, APH and AWH) are distinguished from the other hybrids investigated (Figure 3A). From the second of hybrids are category differentiated US235CLP, by low values of SY, NL, HD and higher of APH and AWH and genotype US237SU, which on the contrary, has lower values of APH and AWH. Among the experimental hybrids that differ from the group average by the variables with the highest variance are 415 (maximum SY and NL values), 1625 and 457 (lower values of APH and AWH, and higher values of NL), 454 and 1727 (the lower values of NL and HD).

In case of data recorded in 2020, the categories of hybrids analyzed are also spatially separated on biplot by the variables of PC1 and PC2, but the water-deficit changed the profile of hybrids. Thus, the hybrids with the lowest values of SY, HMS, APH, AWH and HD are mostly the commercial ones (Figure 3B).

However, the analysis of observations distribution, separately by hybrids categories, showed that in PCA2020, like in PCA2019, the hybrid Zimbru were highlighted by the lowest values of HD, APH and AWH, but Cezar by the comparatively higher values of HD, AWH and APH. Another commercial hybrid, Talmaz, is differentiated by minimum values for SY and HMS. In case of genotypes in testing phase for hybrid performance, two can be noticed by lower values of HMS (US235CLP) and SY (US237SU).

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Contrary results to those found in the group of experimental hybrids in 2019 are revealed in 2020 for genotype 1625 (higher values of APH and AWH) and for hybrid 457 (higher values of SY). Another experimental hybrid - 1718 is distinguished by higher values of HMS associated with low values of HD, APH and AWH. Similarly, the low

values of HD, APH and AWH also differentiate the 1686 hybrid.

The mode of the observations grouping demonstrates specific features of genotypes at the level of quantitative traits that ensure agricultural performance and that are changed differentiated related to the environmental factors.



*Figure 3.* Biplot representation of the relation between hybrids, and between traits and hybrids in 2019 (A) and 2020 (B) Note: *The labels in bold correspond for observation which the squared cosine on F1 and F2 is greater than 0.5.* 

Correlation analysis between agronomic traits, showed a number of significant correlations, in both studied years. The response of hybrids evaluated according to the intensity and type of correlative associations between eight quantitative agricultural parameters, in two different years, demonstrates changes in plant metabolism phenotypically manifested by the lack or presence of new physiological correlations. Thus, the correlative association profile between studied traits in different categories of hybrids is not similar. For example, the commercial hybrids cultivated in 2019, unlike the experimental ones and in the testing phase, are characterized by multiple positive correlations between investigated parameters, the number of which decreases in 2020 (Figure 4).

Also, the profile of linear correlations at the hybrids in the testing phase and experimental ones changes from one year to another, but in contrast to those revealed at the commercial hybrids, it showed a higher number of statistically significant negative correlations (Figure 4).

-1 -0.9 -0.8	-0.7 -0.6	-0.5 -0.4	-0.3 -0.2	-0.1 0	0.1 0.	2 0.3 (	0.4 0.5	0.6 0.7	0.8 0.9	1
Traits,			C	Commerci	al hybrid	s			Traits,	
2019	SY	SW	HMS	NL	PH	HD	AWH	APH	2020	
SY		-0.22	0.74	0.25	0.33	-0.08	-0.35	-0.12	SY	
SW	-0.64		-0.27	-0.38	0.03	-0.43	-0.49	-0.44	SW	
HMS	0.63	-0.47		0.44	0.55	0.43	0.06	0.28	HMS	
NL	0.77	-0.61	0.63		0.77	0.20	-0.02	-0.18	NL	
PH	0.09	-0.18	0.20	0.57		0.36	-0.04	-0.10	PH	
HD	0.64	-0.55	0.49	0,90	0.71		0.84	0.85	HD	
AWH	0.38	-0.42	0.56	0.83	0.86	0.88		0.92	AWH	
APH	0.29	-0.28	0.81	0.47	0.42	0.52	0.60		APH	

Traits,		SCCVT tested hybrids										
2019	SY	SW	HMS	NL	PH	HD	AWH	APH	2020			
SY		0.93	-0.30	-0.81	0.84	-0.13	-0.94	-0.47	SY			
SW	0.49		0.06	-0.55	0.98	-0.48	-0.80	-0.38	SW			
HMS	0.09	0.24		0.75	0.26	-0.90	0.53	0.20	HMS			
NL	0.44	0.54	-0.67		-0.38	-0.43	0.84	0.65	NL			
PH	0.60	0.76	0.75	-0.02		-0.65	-0.67	-0.31	PH			
HD	0.94	0.63	-0.14	0.71	0.50		-0.11	-0.04	HD			
AWH	-0.43	-0.99	-0.10	-0.64	-0.65	-0.61		0.28	AWH			
APH	-0.43	-0.98	-0.06	-0.67	-0.63	-0.63	1.00		APH			

Traits,		Traits,							
2019	SY	SW	HMS	NL	PH	HD	AWH	APH	2020
SY		0.35	0.31	-0.28	-0.26	-0.45	-0.12	-0.18	SY
SW	0.16		-0.53	0.34	-0.41	-0.24	0.55	0.25	SW
HMS	-0.12	-0.74		-0.57	0.39	-0.31	-0.58	-0.44	HMS
NF	0.39	0.02	-0.13		-0.13	0.10	0.57	0.46	NF
PH	0.34	-0.35	0.58	0.52		0.23	-0.02	-0.16	PH
HD	0.12	0.51	-0.45	0.38	-0.21		0.41	0.63	HD
AWH	-0.05	-0.05	0.34	-0.14	0.14	0.17		0.85	AWH
APH	-0.40	-0.17	0.47	-0.29	0.03	0.01	0.85		APH

Values in bold are significant at  $p \le 0.05$  level (2-tailed).

*Figure 4*. Correlation map (Pearson's coefficients of correlation) between 8 agro-morphological traits recorded in 2019 (below the diagonal) and in 2020 (above the diagonal) at the commercial, tested and experimental hybrids

According to the result of the association test applied separately on variables corresponding of hybrids categories, a strong positive correlation between APH:AWH is identified at the experimental hybrids in both years analyzed and only at SCCVT tested hybrids grown in 2019 and the commercial ones from 2020. Most negative correlations are found in the case of the association pairs with parameters SW or HMS, that are moderate and strong at SCCVT tested and experimental hybrids, and predominantly weak at commercial ones.

However, it should be mentioned that for commercial hybrids grown in 2020 was observed a higher number of negative correlations, which, although they are of low intensity, it involve several parameters, not only those associated with HMS.

The correlation profile of commercial hybrids contrasts with those of SCCVT tested and experimental hybrids, which in both experimental years is more heterogeneous in context of the intensity and direction of correlation. Thus, the commercial hybrids from 2019 exhibit statistically positive correlations ( $p \le 0.05$ ) for the pairs with the variable AWH (HD:AWH, r=0.88; NL:AWH, r=0.83; PH:AWH, r=0.86), HD (HD:NL, r=0.90; PH:HD, r=0.71), including for SY:NF (r=0.77) and MHL:APH (r=0.81). At this group of hybrids, in the vegetative growth conditions of 2020, the number of correlations slightly decreases, from 7 to 5 (SY:MHL, r=0.74; HD:AWH, r=0.84; HD:APH, r=0.85; AWH:APH, r=0.92; NL:PH, r=0.77).

The SCCVT tested hybrids are characterized in 2019 by three strong correlations of traits, statistically significant (AWH:APH, r=1.00; SW:AWH, r=-0.99 and SW:APH, r = -0.9). It should be mentioned that at this group of genotypes, in 2020, there are two high positive correlations that involve SW (SW:PH, r=0.98; SW:REC, r=0.93) and one, high negative between AWH and SY (r=-0.94). At the experimental hybrids in 2019 are revealed three high correlations (HD:SY, r=0.94; AWH:APH, r=0.85; SW:MHL, r=0.74) and one of moderate intensity (MHL:NP, r=0.58), all being positive.

In 2020, three positive correlations are found (AWH:NL, r=0.57; HD:APH, r=0.63; AWH:APH, r=0.85) and two negative (HMS:NL, r=-0.57; HMS:AWH, r =-0.58), all of moderate intensity, except AWH: APH, which is an high correlation, attested in both years studied.

In conclusion, the share of correlated yield related traits is different at the three categories of hybrids, fact that determines their discrimination, by genotype and phenotypic response (hybrid performance) to environmental factors.

All eight productivity indices at the commercial and SCCVT tested hybrids are multicolliniar ( $R^2=1$ ) both in 2019 and 2020, that differentiates them from experimental hybrids at which these variables have different strength of the correlation in the same year and from one year to another (Table 3).

Growing season	Multicollinearity indices	SY	SW	HMS	NL	РН	HD	AWH	APH
2019	$R^2$	0.58	0.78	0.91	0.79	0.85	0.64	0.87	0.91
	Tolerance	0.42	0.22	0.09	0.21	0.15	0.36	0.13	0.09
	VIF	2	4	10	5	7	3	8	11
2020	R <sup>2</sup>	0.55	0.79	0.85	0.56	0.82	0.84	0.95	0.95
	Tolerance	0.45	0.20	0.15	0.44	0.18	0.16	0.05	0.05
	VIF	2	5	7	2	6	6	21	21

Table 3. Multicollinearity statistics of yield related traits at the experimental hybrids

It is noted that the seed yield of studied hybrids is the parameter with the least linear relationships, being practically unchanged in these two years. The most variable parameters depending on the year, in the context of collinearity, are AWH, APH, HD, NL and HMS. Since the results of multicollinearity statistic of eight agronomic traits recorded in two growing season have different values at the three categories of hybrids studied, it is interesting to apply the Mantel test to found the overall relationship between the distance matrices of variables and observations to evaluate the response pattern of these type of hybrids to the environmental changeable conditions (2019 versus 2020).

#### Mantel test

The analysis of the divergence between 25 hybrids at the level of 8 yield related indices, as well as at the level of some of them, highlighted the lack of correlation (Figure 5), or low correlations, as is the case of the matrices generated by the data of all productivity indices (r=0.20, p=0.002) or only three of them: SY, SW, HMS (r=0.45, p=0.0001).

Also, low values of the correlation coefficients were revealed when comparing the degree of dissimilarity between productivity indices recorded in 2019 versus 2020 (r=0.43, p=0.002).



*Figure 5.* Mantel correlogram (Pearson dissimilarity) for 25 genotypes based on eight yield related traits (A), only on three of them SY, SW, HMS (B) and Mantel correlogram generated by yield indices for all studied sunflower hybrids (C)

The Mantel test applied on separate groups of genotypes brings more information in discriminating hybrids in response to environmental factors (Figure 6). Thus, the correlation coefficient resulted (r=0.85, p=0.0153) from the association of dissimilarity matrices between hybrids. based of 8 productivity indices, denote a profile of intra-group variation of tested genotypes very similar in both years of cultivation. Also, in the case of experimental hybrids there is a moderate level (r=0.60, p=0.0001) of maintaining in 2020 of the productivity profile ascertained in 2019.

Much more heterogeneous in their response to the environmental factors are commercial hybrids, fact that reveal genetic variability between them. MARIA DUCA ET AL.: ENVIRONMENTAL RESPONSE IN SUNFLOWER HYBRIDS: A MULTIVARIATE APPROACH



*Figure 6.* Mantel correlogram (Pearson dissimilarity) obtained on separate categories of hybrids in base of eight yield related traits (A) and Mantel correlogram generated by all yield indices for the hybrids categories (B)

Interesting are the results of comparing the dissimilarity matrices between yield related traits that indicate a correlation of moderate intensity (r=0.61, p=0.001), statistically significant only in the case of commercial hybrids. This suggests the same degree of variables variation or the same variation trend, from one year to another, at more than half of the studied yield related traits.

The lack of positive Mantel correlations between the dissimilarity matrices of the productivity indices registered in 2019 and 2020 at the experimental and SCCVT tested hybrids indicates less interrelated changes of the agronomic traits under the action of various factors.

The results evidences that the hybrids genetic diversity resulted in different behaviour in response to the environmental conditions.

The data discussed in this work are in compliance with the reported by Santos et al. (2018), which showed the suitability of multivariate analysis (PCA) in assessing of the yield potential of different sunflower genetic classes based on several variables (plant height, lower stem diameter, upper stem diameter, head diameter, achene

weight). They found high positive correlations of the upper stem diameter and lower stem diameter with plant height, as well as of head diameter and head weight with plant height, but and lack of relationships among lower stem diameter and achene weight. Another recent study (Riaz et al., 2020) regarding the variability and identification of superior sunflower genotypes by multivariate analysis revealed significant variation for all evaluated quantitative traits (days to maturity, plant height, stem diameter, head diameter, number of leaves, achene per head, achene yield per plant, 100 - achene weight, filled achene percentage) excepting oil contents. This statement is compliant with PCA that accounted for first four main components only 62.63% of the total variation. From all the ten traits, among plant height and number of leaves a high correlation was established. The authors conclude on the usefulness of the obtained results for breeders to develop high yielding sunflower hybrids.

Major differences among performance sunflower hybrids for yield and its components, have also been previously reported in various applications of multivariate and univariate analysis (Petcu

et al., 2001; Kholghi et al., 2011; Leite and Oliveira, 2015; Ruzdik et al., 2015). For instance, for thirty-seven sunflower hybrids were found positive correlations among number of days to flower initiation, days to maturity and plant height, but head diameter had negative association with all the traits except 100 seeds weight. Significant is the result acording to which the seed yield had negative correlation with oil contents, being suggested to break either through conventional or novel breeding techniques to breed high yielding hybrids with maximum oil contents (Bot et al., 2010). In other investigations on sunflower hybrids, on the contrary, a high degree of correlation between numerous agronomic traits was highlighted including seed yield, oil yield, seed numbers per head, oil content and harvest index (Tabrizi, 2009).

The correlation between the different characteristics targeted in breeding programs attests the probability of indirect selection focused on genetic gains. At the same time, from the evidence presented in this research and from the numerous investigations carried out over several years, it is obvious the complexity of the response in different environmental conditions, even after a long process of hybrid performance improvements.

Finally, an important fact to consider is that, considering of climate change in recent years, it would still be necessary to conduct more systemic investigations of the stability potential of agronomic performance, simultaneously with obtaining of favorable allelic combinations that provide hardiness and good adaptation to adverse soil and climate conditions.

# CONCLUSIONS

Phenotypic and genotypic correlations within varieties are of value to indicate the degree to which various characters are associated with economic productivity. The PCA revealed that the first three principal components explain 73% of the total variance of variables and observations. The traits with the largest contribution in differentiating hybrids grown in 2019 are: AWH, NL, HD and APH (PC1), followed by SY (PC2), SW and HMS (PC3). In the case of hybrids from 2020 growing season, the indices APH, AWH and HD (PC1), followed by SY, HMS (PC2), SW and PH (PC3) are highlighted by the percentage contribution in their agronomic performance characterization. The traits PH and NL do not show significance in the discrimination of hybrids cultivated in 2019 and 2020, respectively.

The investigation of twenty-five sunflower hybrids by principal component analysis, correlative and multicollinearity statistics revealed their clustering with respect to different categories: commercial, experimental and being in testing phase for hybrid performance, more pronounced for 2020 growing season (droughty), demonstrating variability of genotypes at the level of yield related traits, manifested in different behaviour of hybrids to the changing of environmental conditions.

The correlation profile of yield related traits at the commercial hybrids contrasts with those of tested and experimental hybrids that in both evaluated years are more heterogeneous in context of the strength and direction of correlation.

Specific for the experimental hybrids, in the context of inter-correlative relationships is that AWH, APH, HD, NL and HMS are the most variable parameters depending on the year in contrast of the seed yield with the least linear relationships, being practically unchanged in these two years.

The commercial hybrids are characterized by multiple positive correlations of the investigated parameters unlike the experimental ones and in the testing phase, that are distinguished by higher number of statistically significant negative correlations. Most negative correlations are found in the case of the association pairs with SW or HMS, which are moderate and high at tested and experimental hybrids, and predominantly low at the commercial ones.

The traits AWH and APH followed by REC and HD have the highest degree of variation related to growing season.

The commercial hybrids, in contrast of the experimental and tested ones, have been

found more sensitive to the drought of 2020 growing season, responding by a greater decrease of values at most agronomic indices, being maintained a near similar level of variation in their pattern of correlations. The experimental hybrids and those in the testing phase showed small changes in the values of agronomic traits that suggest a greater tolerance to drought conditions, as well as less correlations between different indices.

Among commercial hybrids four of them Cezar, Codru, Dacia and Talmaz showed a good yield potential associated with relative better stability of agronomic indices from year to another. Related to the 17 experimental and from testing phase hybrids, genotypes US235CLP, US237SU, 415, 1625, 457, 454 were proven to be the most suitable for local cultivation area, in drought years.

The direct relationship between valuable economic traits and yield may be a useful selection criterion for determining candidate parents for sunflower drought tolerance breeding.

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