EVALUATION OF VARIATION ON SUNFLOWER SINGLE CROSSES

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ABSTRACT

Sunflower is the main crop in the rotation system in Bulgaria. Sunflower yield is strongly dependent on the cultivar. A two-year experiment with 3 repeats was carried out to study the effects of climatically difference on yield and 1000 seeds weight. 24 genotypes: 4 *A* lines, 4 *R* lines and 16 hybrids combinations were compared over two years (2013 - drought and 2014 - wet conditions). Variation was highly significant. Two-year experiment for five characteristics (as yield and 1000 seeds weight) hybrids show good adaptation of test hybrids. The *boxplot*-diagram indicated that the hybrid *A3* x *R3* was the best.

Key words: 1000 seeds weight, adaptation, genotypes, hybrid, sunflower, yield.

INTRODUCTION

Agriculture is linked with the development of heterosis in sunflower breeding and the production of a new variety resistant and adapted to the dynamically changing climatic conditions. The creation of hybrids depends on the choice of starting material. The choice of parental forms based on their high combining ability and a complex of economically valuable signs that output components possess. Between plants and environmental conditions there are complex relationships. Under the influence of environmental factors is observed modification variability within a given rate of reaction. These changes characterize the adaptive capacity of the individual genotype to conditions during their development.

Sunflower is economically important oil plant in Bulgaria. Sowing of this trench culture helps the proper crop rotation. In recent years the area planted with sunflower increases and reaches 800,000 ha with an average yield of 2.3 t/ha ("Agrarian Report" by years of MZH). Economic significance is determined by the fact that international trade in sunflower seed is very successful. Important role in seed production of hybrid sunflower varieties separated maternal line with cytoplasmic male sterility (*CMS*), i.e. creation of sterile parent used to avoid castrated stamens.

The existing genetic variability of the cultivated sunflower makes it possible to develop hybrids with a genetic potential for seed yield of over 6 t/ha and seed oil content of over 55 %. However, most often sunflower yields obtained in large-scale commercial production are in the range of 1.5-3.0 t/ha. There are multiple limiting factors preventing the realization of the high genetic potential of this crop. Their removal will enable commercial sunflower yields to stabilize at level of 4 t/ha and above (Skoric, 2012). Competitive characteristic of plants plays an important role in yield formation. They claim that if in agrophytocenosis a potential genetic productivity of individual plants is realized only 10-20 % and 50 % of general phenotypic variance is caused by genetic and ecological differences in the competitive ability, it is difficult to identify the genotype according to its phenotype which leads to the decrease in the efficiency of selection. In the practical selection which is a part of the production of hybrids with a high potential of production, as well as high adaptive potential, a strong influence belongs to the autonomy of epigenetic systems of adaptive reactions to the ecological environment they are located in, or to the dynamics of changes that happen in the environment on the location of cultivation (Kirichenko, 2005).

The aim of the study is to evaluate the genetic potential, using statistical methods, of economically valuable lines in a complex of 5 characteristics and effect of environmental factors on the results obtained in 16 hybrid combinations.

MATERIAL AND METHODS

Four sterile analogues of *B* lines (*A* lines, with *CMS Pet-1*), four *R* lines and 16 hybrids combinations (table 1) were compared over two years. The *B* and *R* lines are received by selection of varieties (*B2* and *B4*) and intraspecific (*B1*) and interspecific (*B3* and all *R*) hybridization (Hristova-Cherbadzhi, 2012, 2009; Hristova-Cherbadzi et al., 2007).

Table 1. Origin of plant material.

I. Lines:			
B1 - *H.annuus* line HA89B x H.annuus line 2607B		*R1* - *H.annuus* line HA89A x *H.neglectus*	
B2 - *H.annuus* variety Peredovik		*R2* - *H.annuus* line 2607A x *H.neglectus*	
B3 - *H.annuus* line HA89B x *H.nuttallii* ssp. *rydbergii*		*R3* - *H.annuus* line HA89A x *H.nuttallii* ssp. *rydbergii*	
B4 - *H.annuus* variety Birimirec		*R4* - *H.annuus* line HA89A x *H.pauciflorus* ssp. *subrhomboideus*	
II. Hybrids:			
A1 x *R1*	*A1* x *R2*	*A1* x *R3*	*A1* x *R4*
A2 x R1	*A2* x *R2*	*A2* x *R3*	*A2* x *R4*
A3 x *R1*	*A3* x *R2*	*A3* x *R3*	*A3* x *R4*
A4 x *R1*	*A4* x *R2*	*A4* x *R3*	*A4* x *R4*

Experiences are displayed on the black-earth without fertilization and one hoeing. Experimental hybrids are included in the competitive variety trials ordered in the scheme by the principle of randomized block method, in triplicate, with reporting land area - 20 m^2. The results are represented by boxplot-diagrams in R multiplier for five quantitative traits - yield (kg/dka), 1000 seeds weight (*g*), seed oil (%), diameter head (*cm*) and plant height (*cm*). A two-year experiment with 3 repeats was carried out to study the effects of climatically difference on yield and 1000-seeds weight. *Correlation* and *cluster analysis* were realized. *Correlation analysis* (R Core Team, 2014) is to calculate the correlation coefficient between the values of characteristics yield and weight of 1000 seeds for two consecutive years (2013 - drought and 2014 - wet conditions) and subsequent verification of its statistical significance to evaluate the influence of environmental factors on the values in hybrids and parents. *Bootstrap*-intervals are represented by a histogram, which is designated the primary value of the correlation coefficient as a vertical line, QQ-diagram (Canty and Ripley, 2015; Davison and Hinkley, 1997), visualizing the location of the correlation coefficient and limits of 95 % confidence interval. For *cluster analysis* (R Core Team, 2014) was used the *hclust* function.

RESULTS AND DISCUSSION

Correlation analysis. Zero hypothesis H_0 was that the correlation coefficient is zero, i.e. the value of characteristics were not correlated. The results from correlation analysis are presented on table 2.

For five characteristics hybrids show *very good adaptation* to changing environmental conditions and correlations are high significant- p <0.001, i.e. different climatic conditions in both years (drought and wet conditions) no significantly impact on the appearance of traits. A statistically significant correlation coefficient indicates that the values change synchronously over the years and hybrids. The exception is the trait "diameter head" which also exists statistically proven strong correlation between its values, but it is with hybrids evaluation 0.01 <p <0.05.

This is an evaluation of the total tolerance of the hybrids by traits, i.e. combinations are tolerant to environmental changes. Similar findings were reported for the parental lines, which is the result of their good uniformity. The change in climatic conditions affected most of the "diameter head" in hybrids.

Table 2. *Correlation analysis* of repeatability in years for five quantitative traits.

Quantitative traits	Estimates of correlation coefficient	t-distribution	Degrees of freedom	Lower limit	Upper limit	p-value
yield (hybrids)	0.9683	26.297	46	0.944	0.982	< 2.2e-16
yield (parents)	0.983	13.119	6	0.906	0.997	1.21e-05
1000 seeds weight (hybrids)	0.966	25.27	46	0.94	0.98	< 2.2e-16
1000 seeds weight (parents)	0.998	50.4	6	0.993	0.999	4.096e-09
Seed oil (hybrids)	0.998	66.74	14	0.995	0.999	< 2.2e-16
Seed oil (parents)	0.962	8.73	6	0.803	0.993	0.0001
Diameter head (hybrids)	0.53	2.34	14	0.047	0.812	0.034
Diameter head (parents)	0.979	11.61	6	0.882	0.996	2.449e-05
Plant height (hybrids)	0.834	5.66	14	0.577	0.94	5.883e-05
Plant height (parents)	0.978	11.61	6	0.881	0.995	2.453e-05

***Bootstrap*-intervals for correlation between observations of two traits in years**

Confidence interval on 0.9525 - 0.9855 at hybrids and 0.9288 - 1 at parents for characteristics yield (Fig.1, A and B), and on 0.9485 - 0.9826 at hybrids and 0.9907 - 1 at parents for characteristics 1000 seeds weight (Fig.1, C and D) is observed.

In figure 1A thick vertical line corresponds to the experimental value of 0.968 correlation coefficient between the values for 2013 and 2014 for hybrids for trait 'yield' and in figure 1B value

0.983 of the correlation of parental lines. Since the QQ-diagram (Fig. 1B) can be seen that the distribution of the correlation coefficient is significantly different from normal.

In figure 1C thick vertical line corresponds to the experimental value of 0.966 correlation coefficient between the values for two years for hybrids for trait '1000 seeds weight' and in figure 1D value 0.998 of the correlation of parental lines. Since the QQ-diagram (Fig. 1D) can be seen that the distribution of the correlation coefficient is significantly different from normal.

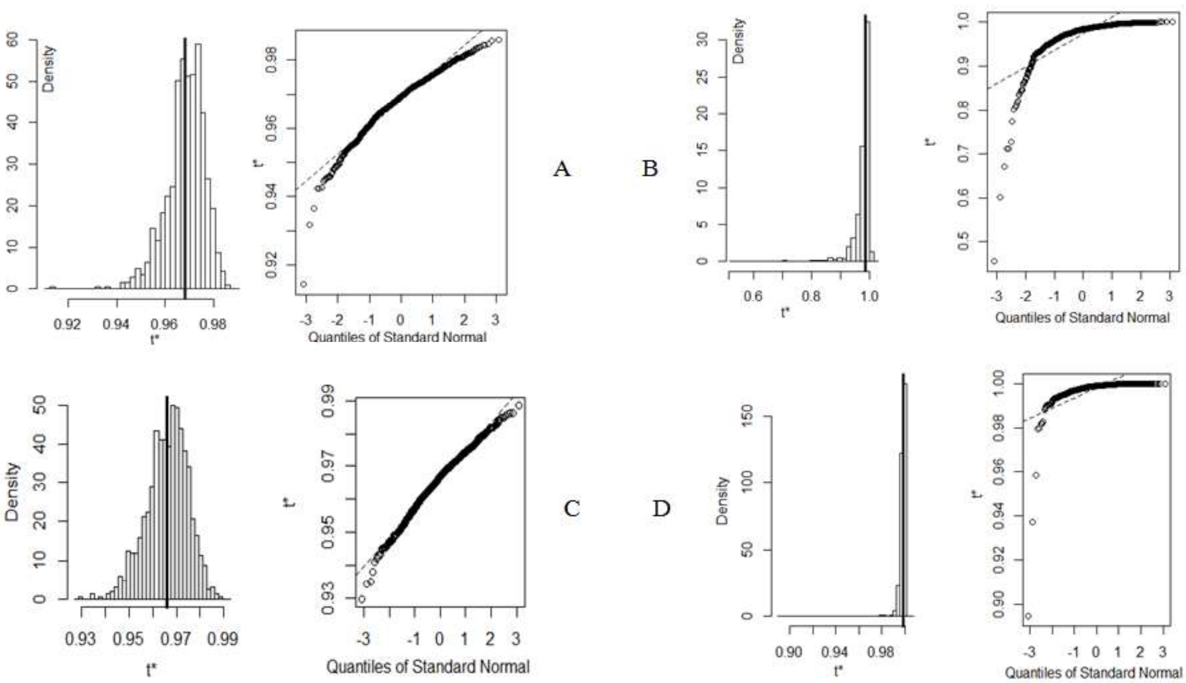

Fig.1. Histogram of the distribution of the bootstrap-values of the correlation coefficient for the characteristic 'yield' in hybrids (A) and in parental lines (B) and the characteristic '1000 seeds weight' in hybrids (C) and in parental lines (D) and their corresponding QQ-diagram.

Relationship does not have significantly change over the years, i.e. studied materials are tolerant to environmental changes with very good adaptability of hybrids, resulting in the statistically significant correlation coefficient. The results obtained from the displayed field experience vary depending on the year and the hybrid combination:

- The average *yield* of hybrids (from 347 kg/dka - in 2013 and 316 kg/dka - 2014 for cross *A2* x *R1* to 503 kg/dka - in 2013 and 509 kg/dka - 2014 for cross *A3* x *R3*);

- For the average *weight of 1000 seeds* in hybrids (from 70.1 g - 2013 and 70.3 g - 2014 for cross *A2* x *R2* to 88.9 g - 2013 and 90.3 g - 2014 for cross *A3* x *R3*).

Selection aim is to create new highly productive hybrids to realize their potential in different agro-ecological conditions. To achieve this, both parent lines of hybrid combinations must have high combining ability. The increase seed yield of new varieties remains one of the most important directions in the selection of the sunflower.

Seed yield is determined by the number and weight of fertile seeds per head (Robinson, 1983). The sunflower head has the ability to compensate for seed damage by increasing the weight of the individual seed Seiler (1997). According Charlet and Miller (1993) 10 % reduction in the number of flowers meet the 20 % reduction in the number of fertile seeds, 18 % of the total weight of the seed per head to 22 % of the total volume of the seed. Seed yield is a complex character that results from the influence of a large number of traits, which can exert effects individually as well as jointly. The genetic basis of this character is polygenic in nature. Sunflower seed yield is a product of interactions between the genotype and environmental factors that take place throughout the growing season (Skoric, 2012). 1000-seed weight varies depending on genotype and environmental factors, too. The prevailing mode of inheritance of 1000 seed weight is partial dominance, although complete dominance and positive heterosis occur frequently as well.

Yan (2002) declared that typically environment (E) explains most (up to 80 % or higher) of total yield variations, and genotypes (G) and GE are usually smaller. Partitioning of variances revealed the significance of the environmental variance, compared to the genotype and GE interaction variances. It shows that in spite of the same location over the years there were big differences between environments due to different precipitation, temperature and 1-time irrigation in different years (Pourdad and Moghaddam, 2013).

Study the effects of abiotic conditions on plant growth and development, and yield of sunflower is an important prerequisite for the creation of many high-yield crops. Interaction of genotype and environment is important moment to the realization of the genetic potential of many crops, and stability in their production. The potential of sunflower is very high but average yield of sunflower is low and depending on weather conditions. One of reasons is inappropriate selection of varietal composition - parents.

Boxplot-diagram

Boxplot-diagrams for hybrids visually represent variations of maternal (*cms* sterile) and father (fertile) lines regarding on the relevant traits. On the graphs (Fig.2), due to high significant correlation coefficient for the two years, shows the variation of the characteristics. For each trait is evaluated and visualized the genetic potential of parental components. The main range of the relevant parent is defined and different colored rectangle. The solid line within the rectangle is the median, and it is perpendicular to the variation range for the each trait.

The obtained results indicate variation of the five traits in individual lines in different degrees. On the graph shows that the largest potential for trait "yield" means the lines *A1*, *R3* and *R4* for "weight" - *A1*, *A4*, *R2* and *R3*, for "oil" - a *A2* and *R2*, a "diameter" - a *A4* and *R1* and "height" - a A3 and R3. The highest values are around that range maternal *A3* and paternal *R3* line. Combining them to produce hybrid (*A3* x *R3*) is successful and leads to the highest expression of the trait "yield" - 506 kg/dka, due to the overlapping of their main interval of variation (IQR). This is subject to verification by the following analyzes. The lowest values ranging around maternal *A2* and paternal *R1*. Combining these two lines of hybrid (*A2* × *R1*) can be the lowest value for the same trait. In the case in hybrid yield is 332 kg/dka, the lowest in comparison with other results for the remaining hybrid combinations. Some lines for trait "oil", "diameter" and "height" have values close to the median. These lines have very low genetic potential for the trait. For example, the variation of the trait "oil" at *A3* and *A4* is highly asymmetric, which can lead to narrowing of the variation, but this feature may not be an indication of heterosis. Similar asymmetry was observed in the indicators "diameter" of the lines *A1* and *R4*, and "height" of line *A2*.

"height" (E).

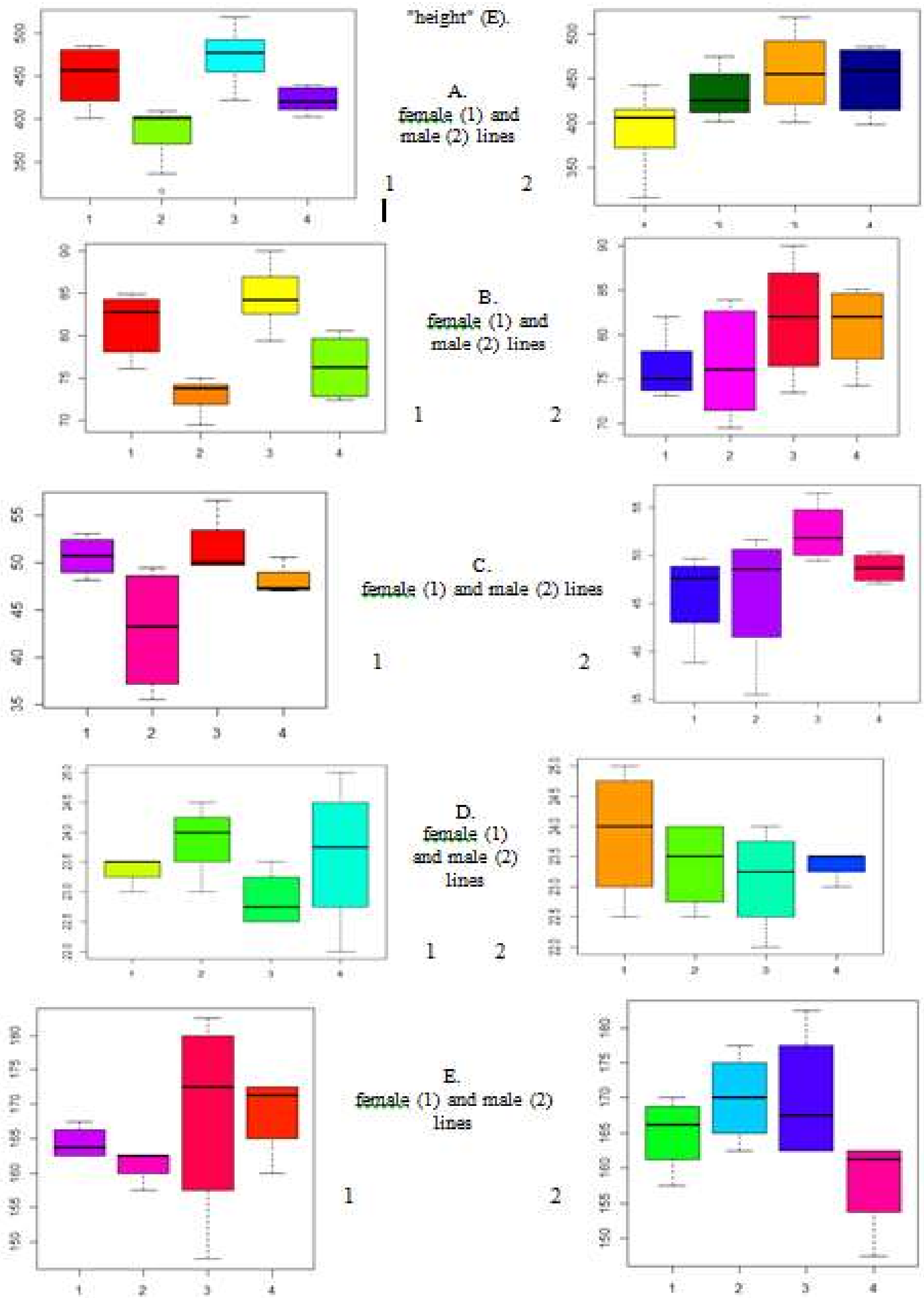

Fig.2. Variation of the characteristics “yield” (A), "1000-seeds weight" (B), "oil" (C), "diameter"

Combining statistical results facilitates breeder in his choice of starting materials in the creation of new hybrids. For example the trait "diameter" in lines *A3* and *R3* values around that range are the lowest, i.e. median or middle observation in the corresponding variation order is the smallest. These same two lines are the highest values for the "oil" and "weight" in hybrid combination (*A3* x *R3*) is the highest manifestation of these traits, i.e. hybrid with the smallest diameter of the heel is the highest yield and the weight of 1000 seeds.

Hybrid combining ***A3* × *R3*** is the highest manifestation of traits "oil" and "weight" even the smallest "diameter" and is the most promise, combining the most important economic business qualities.

Cluster analysis

Interspecific hybridization or detection of desirable genes in wild species of the genus *Helianthus* and their insertion into cultivated sunflower genotypes occupies a special place in sunflower production. Heterosis in the sunflower hybrids is highly linked to the genetic distance between the parental lines. With great economic importance for the sunflower is cytoplasmic male sterility - with the including of *CMS* in sunflower (Leclercq, 1969) *Rf* and the identification of genes (Enns et al, 1970; Kinman, 1970) and the creation of lines *R*. Carrying these genes it is possible to use a heterosis breeding for increasing the yield of hybrid seed. Of the hybrid seeds obtained after crossing the two parental forms - *A* (sterile analogues of sterile lines) and *R* (fertility restorer) lines are obtained 100 % fertile F_1 hybrid plants (Putt, 1997). *CMS* system for the production of sunflower hybrid seeds, for first time was used in 1972 (Fick and Miller, 1997).

The *B* and *R* lines, using in this research are received by selection of varieties (*B2* and *B4*) and intraspecific (*B1*) and interspecific (*B3* and all *R*) hybridization (Hristova-Cherbadzhi, 2012, 2009, 2007; Hristova-Cherbadzi and Christov, 2008; Hristova-Cherbadzi et al., 2007). The sterile analogues of *B* lines (*A* lines) were with *CMS Pet-1*.

The results from cluster analysis are presented on figures 3 and 4. Each cluster dendrogram shows the diversity (remoteness) of the materials by grouping parents (Fig.3A) or hybrids (Fig.4A) for 5 traits (Fig.3B, 4B).

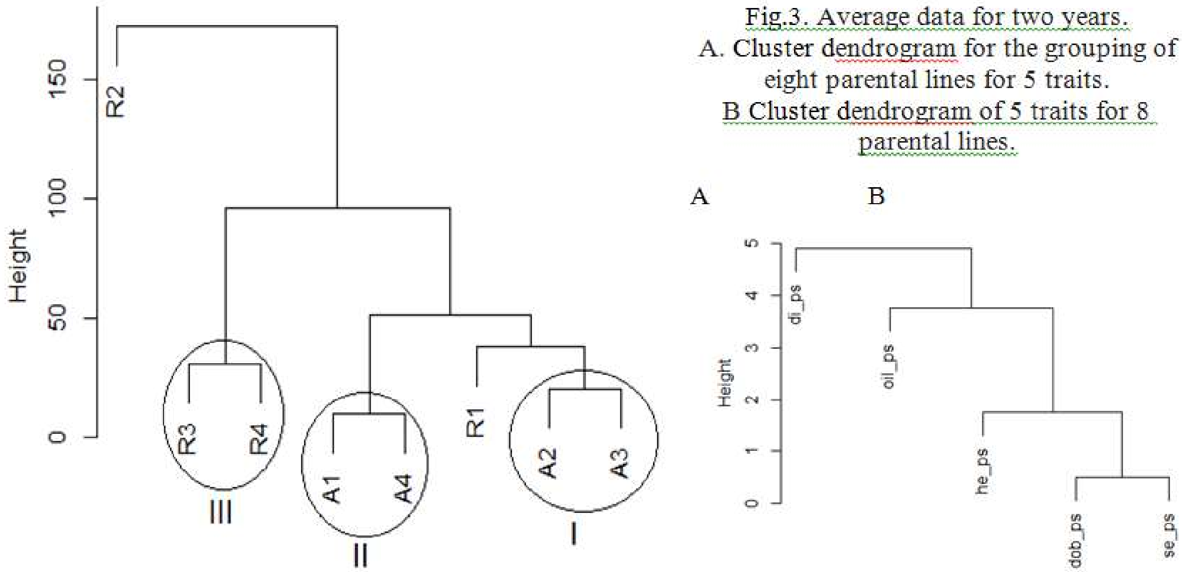

Fig.3. Average data for two years.
A. Cluster dendrogram for the grouping of eight parental lines for 5 traits.
B Cluster dendrogram of 5 traits for 8 parental lines.

Figure 3A shows a design space on the 5 characteristics, where lines *A2* and *A3* are the closest (group I). Next to them is a line *R1*. The lines *A1* and *A4* are in the next near group (II). Group III includes lines *R3* and *R4*. Line *R2* is most remote from all other lines.

Line *B2* is selected from variety Peredovik and line *B3* - after interspecific hybridization *H.annuus* line HA89B x *H.nuttallii* ssp. *rydbergii*. Many years ago line HA89B is selected from variety Peredovik (from J. Miller, Fargo, ND, USA), too.

The result is very interesting because the lines *A3* and *R3* are genetically distant, but at the same time received hybrid combination had strong positive heterosis for traits "1000 seeds weight" and "yield". These two lines (*A3* and *R3*) are received by interspecific hybridization. They are unique in that, that they are obtained after selection of the initial cross *H. annuus* (line HA89*B/A*) x *H. nuttallii* ssp. *rydbergii*. Until 2007 (Hristova-Cherbadzi and Christov, 2008) successful hybridization with this wild perennial deploid subspecies had not yet been reported. The subspecies *subrhomboideus* of the perennial hexaploid species *Helianthus pauciflorus* (*rigidus*), that was crossed with the cultivated sunflower, was studied less, too. Genes that controlled such characters as resistance to *Plasmopara helianthi*, *Phomopsis helianthi*, *Phoma macdonaldii* and *Orobanche cumana*, *Rf* gene for *CMS Pet-1*, suitable type of branching for *R* lines, high oil content (53.43 % for line *R3*) and high combining ability (line *A3*) were transferred.

Differences in expression of the characteristics “yield” and "1000-seeds weight” are the small (Fig.3B). To them next is a "height". In remove of these is the "oil", and finally "diameter".

Figure 4A shows a design space on the 5 characteristics for hybrid combinations. The 16 hybrids can group in three near close groups, too. Only one hybrid stay single, remove from other.

The closeness between the characteristics at hybrids keeps like this at the lines, but it has one difference - places of "oil" and "height" are exchanged. Here the “oil” is closer to the characteristics "yield" and "1000-seeds weight.

Maintaining the relationship between the closeness of the characteristics in parental lines and hybrids is confirming the genetic factor.

The use of statistical analysis in evaluation of the genetic properties of genotypes of *CMS* system together with the use of the selection method - remote hybridization, is practicable and can be further developed.

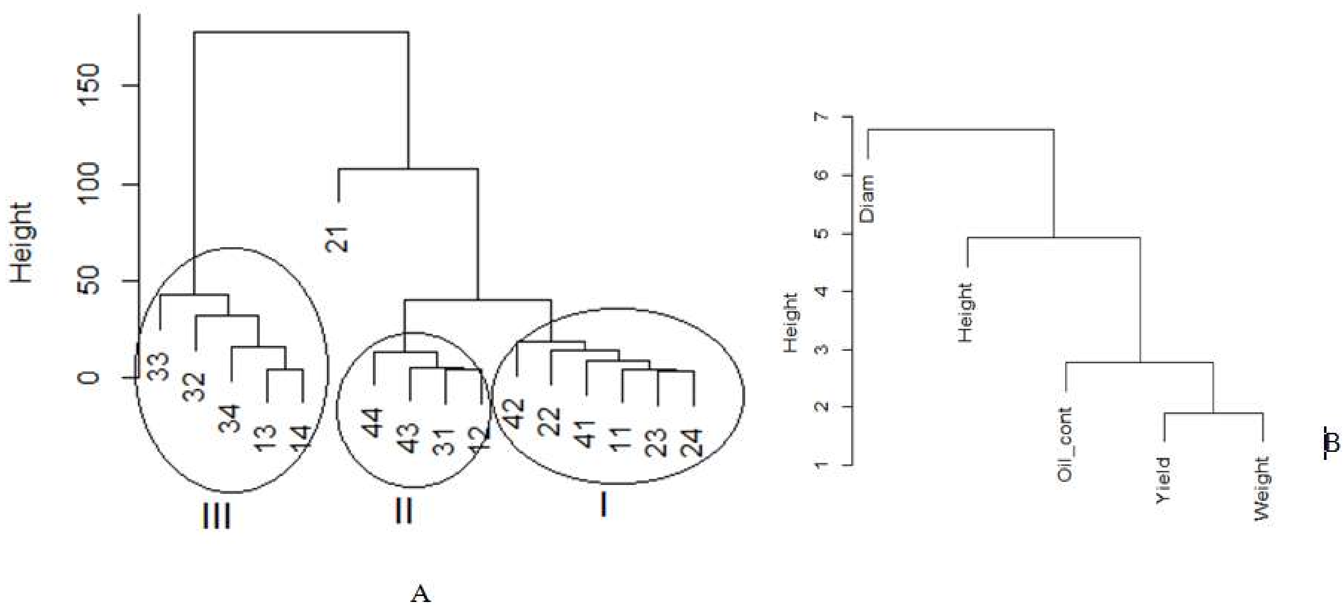

Fig.4. Average data for two years.
A. Cluster dendrogram for the grouping of 16 hybrids for 5 traits.
B. Cluster dendrogram of 5 traits for 16 hybrids.

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