

EVALUATION OF COMBINING ABILITY AND GENETIC COMPONENTS IN SUNFLOWER

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Evaluation of genetic material involved in breeding proces is of great importance in order to choose the most valuable genotypes with ability to efficiently combine desirable genes in progeny. The objective of this study was to utilize diallel method in order to estimate mode of inheritance, heterosis effect, combining abilities and components of genetic variance for important properties in sunflower and to identify those genotypes that can make progress in sunflower breeding. Present research had been carried out during 2012 and 2013 growing seasons. Evaluation included 6 sunflower genotypes and their 15 combinations which were crossed according to incomplete diallel. For seed yield/plant dominance of better parent (d+) was expressed as a mode of inheritance, while regarding thousand seed weight the most common modes of inheritance were dominance (d+) and partial dominance of better parent (pd+). The most common mode of inheritance regarding oil content was superdominance (sd+). Empirical estimates of combining abilities of genotypes involved in breeding process is main startegy in evaluation of breeding value. Regarding seed yield/plant significant and positive heterosis effect (28.9%) was calculated in combination Harkovski x Rodnik (P3xP5), while the highest heterosis effect for oil content (12.8%) was determined in combination Harkovski x Amaian (P3xP6). Combining ability analysis indicated that both genetic components, additive and non-additive, were important in expression of investigated traits. Components of genetic variance indicated

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prevalence of dominant component (H) compared to additive (D) and higher concentration of dominant genes (u) compared to recessive (v), in all investigated traits.

Key words: sunflower, mode of inheritance, heterosis, combining abilities, genetic components

INTRODUCTION

The main objective of sunflower (*Helianthus annuus* L.) breeding is to increase seed and oil yield (GJORGJEVA *et al.*, 2015). Yield as a complex trait requires the most effort in breeding process because due to its polygenic character and great influence of the environment it is characterized by low heritability (NASREEN *et al.* 2011). Successful selection of genotypes with a high genetic potential for seed and oil yield requires first of all the possession of the appropriate genetic variability in the initial material. In order to achieve the ultimate goal it is necessary to have information on the mode of inheritance and the manifestation of heterosis effect for trait of interest as well as to identify superior genotypes based on breeding value, i.e. combining abilities.

Knowledge of the genetic basis of combining abilities is one of the most important tasks in modern genetics (MARINKOVIĆ *et al.*, 2003). ŽIVANOVIĆ *et al.* (2006) suggest that the correct assessment of combining ability of genotypes can be made by crossing each other, and the most reliable method is diallel crossing. Diallel crossing method is proposed by the zoologist-geneticist DR. SCHMIDT'S (1919) and the first time was applied in plants by SPRAGUE and TATUM (1942). Knowledge about combining abilities of the genotypes involved in breeding program as well as fundamental understanding of the genetic determinants accelerates the breeding process and enhance probability of achieving the desired goal. Combining abilities are divided into general (GCA) and specific (SCA) combining abilities. Average performance of genotype in combinations with other genotypes refers to GCA, while SCA refers to performance of certain combination. Combining abilities provide information about nature of gene effects involved in expression of certain trait as higher GCA indicate greater role of additive effects, while SCA is referred to non-additive gene effects. Combining abilities are widely used in breeding programs in order to identify superior genotypes that can enhance progress of sunflower breeding (GOKSOY *et al.*, 2002; KAYA and ATAKISI, 2004; GVOZDENOVIĆ *et al.*, 2005; MASOOD *et al.*, 2005; MARJANOVIĆ-JEROMELA *et al.*, 2007; VUYLSTEKE and EEUWIJK, 2008; IBRAHIM *et al.*, 2010; KARASU *et al.*, 2010; KANG *et al.*, 2013; ZEINAB and HELAL, 2014; MASNY *et al.*, 2016).

Hence, this research was planned in order to estimate the mode of inheritance, heterosis effect, combining abilities and components of genetic variance for important properties in sunflower and to identify those genotypes that can make progress in breeding process.

MATERIALS AND METHODS

The experiment for the present study was carried out at the Institute of Field and Vegetable Crops from Novi Sad during 2012 and 2013 growing seasons. Initial germplasm for the experiment was consisted of 6 sunflower genotypes: Azovsky, Kazachy, Harkovski, Lider, Rodnik and Amaian. During first year of experiment genotypes were crossed according to incomplete diallel (without reciprocals) and their 15 combinations were made. Crosses were made by hand emasculation. In second year of experiment total of 21 genotypes (6 parental genotypes and their 15 crosses) were sown in three replicates at the experimental field Rimski Šančevi of the Institute of Field and Vegetable Crops from Novi Sad. Basic plot size was 10 m²,

with four 3.6 m long rows and 70x30 cm plant spacing. Harvest was done by hand and the data were recorded on 10 plants from middle rows in each replicate. Seed yield/plant (SYP) and thousand seed weight (TSW) were recorded in laboratory on a technical scale with an accuracy of 0.01 g. Oil content (OC) was determined by nuclear magnetic resonance (NMR) in pure seed and expressed in percentage (%). GCA of parents and SCA of F_1 were tested according to diallel method II, model I (GRIFFING, 1956). The assumption of this method is that there are no differences in reciprocal crosses.

Mathematical model for analysis of combining abilities is as follows:

$$Y_{ij} = m + g_i + g_j + s_{ij} + 1/bc \sum \sum e_{ijkl}$$

Where: Y_{ij} – average value of $i \times j$ genotype

m – average value of population

g_i – GCA of i parent

g_j – GCA of j parent

s_{ij} – SCA

$1/bc \sum \sum e_{ijkl}$ – mean error

Better parent heterosis effect (heterobeltiosis) was calculated by comparing mean values of F_1 generation with respect to the average value of better parent and significance was determined using t-test.

$$h = \frac{F_1 - BP}{BP} \times 100 (\%)$$

h – heterosis effect

Bp – average value of better parent for analyzed trait

Analysis of components of genetic variance was performed according to the method suggested by JINKS (1954), HAYMAN (1954), and MATHER and JINKS (1971).

RESULTS AND DISCUSSION

Efficient progress in breeding highly depends on genotypes involved and their ability to efficiently combine desirable genes in order to improve traits of interest. Understanding the nature and mode of gene effects is essential for improvement in breeding process.

The results of the mode of inheritance of SYP in F_1 generation differed between crossing combinations (Table 1). In most cases dominance of better parent ($d+$) was manifested as a mode of inheritance, while superdominance ($sd+$) was manifested only in one combination. In addition to these modes, in the inheritance of SYP also were expressed partial dominance of better parent ($pd+$), intermediary (i) and dominance of poorer parent ($d-$). Regarding TSW as a mode of inheritance in most cases partial dominance of better parent ($pd+$) and dominance of better parent ($d+$) were expressed. Dominance of poorer parent ($d-$) and intermediary (i) were expressed in two F_1 generations. Contrary to SYP, negative superdominance ($sd-$) as mode of inheritance for TSW was expressed in one F_1 generation. Contrary to our results in earlier studies several authors found that positive superdominance prevailed as a mode of inheritance in TSW

(NAIK *et al.*, 1999; JOCIĆ, 2002; AHMAD *et al.*, 2012). Comparing the mean values of OC in the F₁ generation compared to the average of the parents in most cases superdominance (sd+) was expressed as a mode of inheritance. In earlier studies PUSTAVOJT (1963) came to the conclusion that the oil content in the F₁ generation inherits intermediate, while in later researches heterosis prevailed in expression for this trait (SCHUSTER, 1964; LECLERCQ, 1968; STOYANOV, 1971; ŠKORIĆ, 1975).

Table 1. Average values and mode of inheritance in F₁ generation in sunflower

Genotypes	SYP (g)	TSW (g)	OC (%)
P1	98.29	65.86	44.35
P2	68.41	67.22	45.43
P3	46.52	50.47	46.11
P4	75.49	52.62	49.78
P5	66.66	56.15	49.17
P6	54.81	52.77	45.41
P1xP2	62.51 ^d	65.17 ^d	50.37 ^{sd+}
P1xP3	85.91 ^{pd+}	61.38 ^{pd+}	51.38 ^{sd+}
P1xP4	91.71 ^{pd+}	62.73 ^{pd+}	49.47 ^{d+}
P1xP5	81.69 ⁱ	56.73 ^d	51.87 ^{sd+}
P1xP6	85.52 ^{pd+}	56.00 ⁱ	50.41 ^{sd+}
P2xP3	83.69 ^{d+}	64.72 ^{pd+}	49.83 ^{sd+}
P2xP4	63.61 ^d	56.95 ⁱ	49.35 ^{d+}
P2xP5	75.11 ^{d+}	59.26 ^{pd}	48.25 ^{pd+}
P2xP6	62.76 ⁱ	44.90 ^{sd}	48.84 ^{sd+}
P3xP4	89.60 ^{d+}	59.25 ^{d+}	50.79 ^{d+}
P3xP5	85.91 ^{d+}	58.80 ^{d+}	50.77 ^{d+}
P3xP6	64.16 ^{d+}	49.49 ^d	52.00 ^{sd+}
P4xP5	95.83 ^{sd+}	57.57 ^{d+}	53.13 ^{sd+}
P4xP6	56.16 ^d	47.13 ^d	51.74 ^{d+}
P5xP6	73.81 ^{d+}	50.91 ^d	51.94 ^{sd+}
LSD (0.05)	19.81	7.26	2.54
LSD (0.01)	26.47	9.71	3.41

P1 – Azovsky, P2 – Kazachy, P3 – Harkovski, P4 – Lider, P5 – Rodnik, P6 – Amaian.

The main objective of sunflower breeding is to develop highly productive hybrids. This aim can be realized by using intra-allelic and inter-allelic interaction, i.e. heterosis. Heterosis can be successfully attained by crossing genetically divergent genotypes. Heterosis effects in addition to better parent from this study are presented on figure 1.

Analysis showed that cross combinations exhibited different effect of superiority in addition to better parent based on inherited genetic background. Regarding SYP significant and positive heterosis effect (28.9%) was calculated in combination P3xP5, while positive heterosis effect but without statistical significance was expressed in 6 other combinations. Negative heterosis effect for SYP was expressed in 8 combinations. Concerning TSW positive heterosis without significant effect was calculated in just 3 combinations, while in other combinations negative heterosis effects were calculated. Contrary to this, in expression of oil content in F₁

generation positive and significant heterosis effect was manifested in 9 combinations. The highest heterosis effect for OC (12.8%) was determined in combination P3xP6. Negative heterosis effect, without significance, for OC was expressed in only 3 combinations. Analysis of heterosis effect showed that regarding SYP only one combination showed statistical superiority in addition to better parent, while regarding TSW none of the combinations were statistically better. This result indicates the need for more divergent genetic material in order to develop genotypes that will enable more efficient progress in breeding program, regarding SYP and TSW. Conversely, heterosis estimates for OC showed that most of the combinations were statistically superior in addition to better parent indicating valuable genetic variability that can be efficiently exploited in order to increase level of OC in seed (Fig. 1).

Analysis of variance of combining ability showed statistically highly significant differences in both, general (GCA) and specific (SCA), combining abilities between parents used in this experiment. Obtained results showed that both, additive and non-additive, genetic components were important in the inheritance of investigated traits (Table 2).

Table 2. Analysis of variance of general (GCA) and specific (SCA) combining abilities in sunflower

SYP	Df	SS	MS	F	F (0.05)	F (0.01)
GCA	5	2718.59	543.72	11.31**	2.45	3.51
SCA	15	2664.86	177.66	3.70**	1.94	2.55
Error	40	1922.97	48.07			
TSW						
GCA	5	508.76	101.75	15.73**	2.45	3.51
SCA	15	291.81	19.45	3.01**	1.94	2.55
Error	40	258.70	6.47			
OC						
GCA	5	27.61	5.52	7.01**	2.45	3.51
SCA	15	90.41	6.03	7.65**	1.94	2.55
Error	40	31.51	0.79			

*P<0.05, **P<0.01

Considering that GCA provide information for additive and SCA represent non-additive gene actions, GCA/SCA ratio indicated that additive gene action had greater importance in the inheritance of SYP and TSW. These findings indicate that efficient selection can improve investigated traits in sunflower. As opposed to our results AHMAD *et al.* (2012) published that SCA component for investigated traits was higher indicating prevalence of non-additive gene effects. Using additive-dominant model for assessment of gene actions for TSW (JOCIĆ, 2002) concluded that in addition to additive and dominant gene actions epistasis exist. In agreement with our results PUTT (1966) and SINDAGI *et al.* (1979) reported that additive gene effects are more important in expression of seed yield in sunflower. Previous findings of several researchers pointed greater importance of non-additive component in the inheritance of seed yield/plant in sunflower (HLADNI *et al.*, 2012; JOCIĆ *et al.*, 2012; ANDARKHOR *et al.*, 2012). Contrary to results for SYP and TSW, non-additive gene actions were substantial in OC expression. The GCA/SCA ratio was lower than 1 meaning that non-additive component was more important than additive.

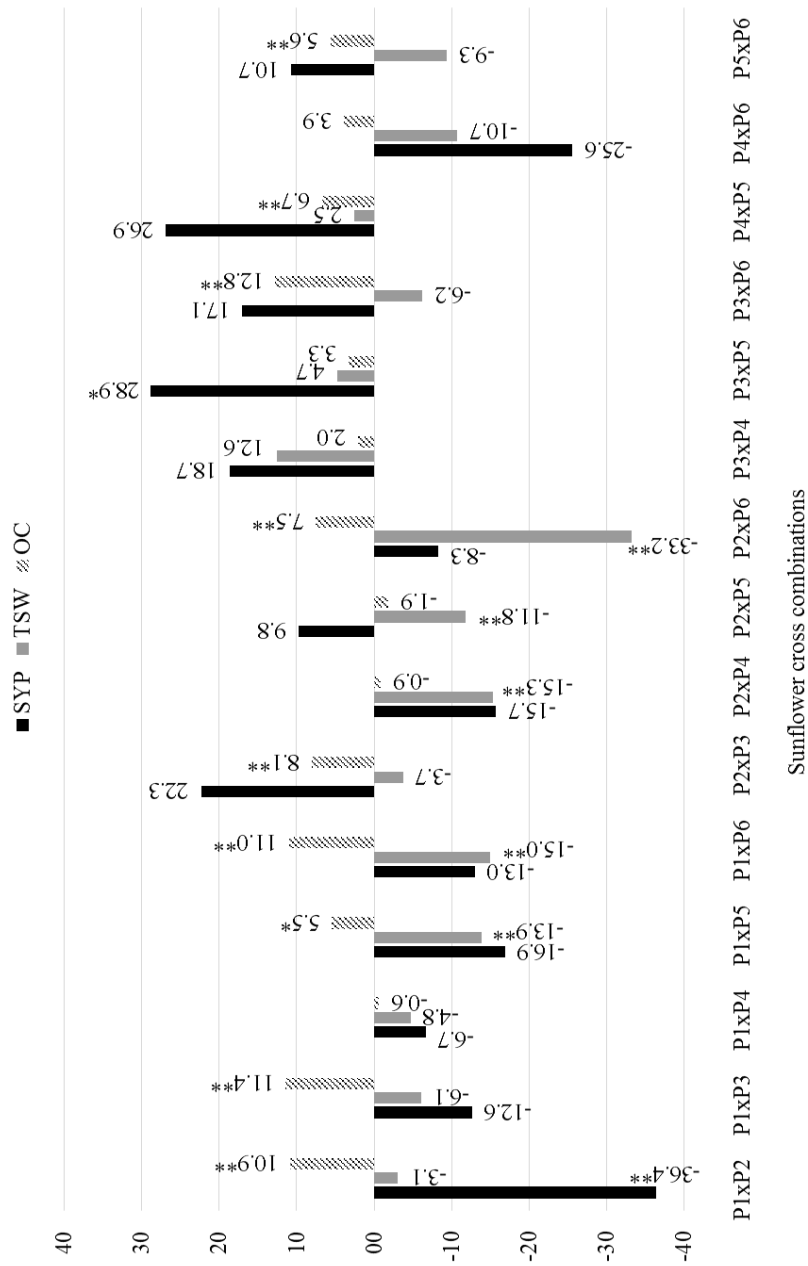


Figure 1. Heterosis effect (%) in addition to better parent

Earlier reports of several authors are in agreement with our results indicating that non-additive genetic component was more important in expression of oil content (PARAMESWARI *et al.*, 2004; HLADNI *et al.*, 2006; MACHIKOWA *et al.*, 2011). Nonetheless, few other researchers reported higher importance of additive component in the expression of oil content (ROJAS and FERNADNEZ-MARTINEZ, 1998; MIJIC *et al.*, 2008; CHANDRA *et al.*, 2011).

Testing combining abilities of existing material is of great importance in order to select those genotypes that will allow further progress in breeding. Desirable combining ability refers to ability of genotype to transfer preferable genes and provide superior progeny when combined with another genotype. By comparison of GCA values between parents the highest and statistically significant GCA value for SYP (13.62) and TSW (4.37) was calculated for the P1 genotype (Table 3). This result implicate that parental genotype P1 could be utilized in sunflower breeding process for efficient improvement of analyzed traits. Parental genotype P2 had significant but lower GCA value (3.39) for TSW. Regarding specific combining abilities for SYP the highest positive value was calculated for combination P2xP3 (17.59), while for TSW none of the combinations had significantly positive value.

Table 3. General (diagonal) and specific (above diagonal) combining abilities in sunflower
Seed yield/plant (SYP)

Parents	P1	P2	P3	P4	P5	P6
P1	13.62**	-21.39	-0.04	-1.83	-9.65	10.48
P2		-5.83	17.59*	-10.48	3.22	1.92
P3			-3.78	13.45	11.96	1.26
P4				3.81	14.29	-14.33
P5					1.61	5.52
P6						-9.43

Parents	P1	P2	P3	P4	P5	P6
P1	4.37**	0.44	0.59	2.63	-4.20	0.23
P2		3.39*	5.06	-2.17	-0.70	-9.89
P3			-0.54	4.06	2.78	-1.36
P4				-1.24	2.25	-3.03
P5					-0.40	-0.09
P6						-5.57

Parents	P1	P2	P3	P4	P5	P6
P1	-0.57	2.60*	2.37*	-0.39	1.96	1.57
P2		-1.19	1.41	-0.08	-1.04	0.62
P3			0.04	0.32	0.25	2.55*
P4				0.89	1.76	1.43
P5					0.95*	1.58
P6						-0.12

*P<0.05, **P<0.01, P1 – Azovsky, P2 – Kazachy, P3 – Harkovski, P4 – Lider, P5 – Rodnik, P6 – Amaian

Bearing in mind that sunflower is mostly grown for eddible oil, improving oil content is one of the main goal of sunflower breeding. Significant and positive GCA effect was recorded for parental genotype P5 (0.95) which indicate the possibility to use this genotype in breeding program for the improvement of oil content (Table 3). Positive GCA effect was also recorded for

parental genotypes P3 (0.04) and P4 (0.89), but without significance. Considering SCA effects for oil content positive and significant values were found in crosses P1xP2 (2.60), P1xP3 (2.37) and P3xP6 (2.55). Significant SCA values showed that poor general combiners in some crosses can manifest improvement in desirable direction which is due to concentration of favourable genes and their possibility to efficiently combine.

For success in breeding it is important to know the components of genetic variability of traits of interest in order to know which approach to use in the selection process to achieve the best results. Calculated values of components of genetic variance for investigated traits of sunflower are presented in table 4.

Table 4. Components of genetic variance in sunflower

Components	SYP	TSW	OC
D	535.98	46.71	4.17
H ₁	698.17	77.28	15.64
H ₂	473.63	47.57	14.02
F	544.60	19.96	3.49
E	48.07	6.47	0.79
u	0.78	0.81	0.66
v	0.22	0.19	0.34
H ₂ /4H ₁	0.17	0.15	0.22
$\sqrt{H_1/D}$	1.14	1.29	1.94
K _D /K _R	2.60	1.40	1.55

The analysis of components of genetic variance revealed that dominant component (H₁) was greater than the additive (D) in all investigated traits. This implies that most of the genetic variation in the inheritance of SYP, TSW and OC is rely upon the non-additive component (Table 4). In accordance with this, calculated F value also shows that dominant genes prevailed in relation to recessive ones. Calculated frequency of dominant (u) and recessive (v) genes confirmed that dominant genes were prevalent. Moreover, calculated value of the H₂/4H₁ ratio indicated unequal representation of dominant and recessive genes in parents. From the K_D/K_R ratio, which is greater than 1, is also evident that the dominant genes prevailed in respect to the recessive ones. Average degree of dominance $\sqrt{H_1/D}$ was greater than 1 which indicated that superdominance was the case in expression of SYP, TSW and OC, traits.

CONCLUSIONS

Identification of superior genotypes using empirical estimates of GCA and SCA originated by diallel method is fundamental strategy in evaluation of breeding value of genotypes involved in breeding program. Considering the estimates of mode of inheritance and heterosis effect compared to better parent analysis showed that for efficient progress in SYP and TSW there is need for more superior genotypes that can enhance progress of sunflower breeding, while regarding OC analysis indicated existance of valuable genetic background that can be easily exploited with the aim of increasing oil content.

Combining ability analysis indicated that both genetic components, additive and non-additive, were important in expression of investigated traits. GCA effects showed that parental genotype P1 can be useful for improving SYP and TSW, while parental genotype P5 is distinguished as a best general combiner for improving OC. Analysis of SCA effects pointed out the possibility of efficient combination of desirable genes in some crosses as a result of non-additive gene effects. Components of genetic variance indicated prevalence of dominant component compared to additive and higher concentration of dominant genes compared to recessive in all investigated traits.

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**GENETSKA EVALUACIJA KOD SUNCOKRETA (*Helianthus annuus* L.)
PRIMENOM DIALELA**

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Izvod

Procena genetičkog materijala koji je uključen u oplemenjivački program je od velike važnosti kako bi se izabrali najvredniji genotipovi sa sposobnošću da efikasno kombinuju poželjne gene u potomstvu. Cilj ove studije je bio da se primenom dialel metode procenile način nasleđivanja, efekat heterozisa, kombinacione sposobnosti i komponente genetičke varijanse važnih osobina suncokreta i identifikuju genotipovi koji mogu da unaprede program oplemenjivanja. Da bi se sprovedo istraživanje eksperiment je sproveden tokom dve godine. Evaluacija je obuhvatila 6 genotipova suncokreta i njihovih 15 kombinacija koje su ukrštene prema nepotpunom dialelu. Analize su pokazale da što se tiče prinosa semena po biljci u većini slučajeva se ispoljila dominacija boljeg roditelja (d+) kao način nasleđivanja, dok su kod mase hiljadu semena najčešći načini nasleđivanja bili dominacija (d+) i parcijalna dominacija boljeg roditelja (pd+). Najčešći način nasleđivanja sadržaja ulja je bila superdominacija (SD+). Empirijske procene kombinacionih sposobnosti genotipova koji su uključeni u proces oplemenjivanja je glavna strategija u proceni oplemenjivačke vrednosti. Što se tiče prinosa semena po biljci značajan i pozitivan heterozisni efekat (28.9%) je izračunat u kombinaciji P3xP5, a najviši heterozisni efekat za sadržaj ulja (12,8%) je utvrđen u kombinaciji P3xP6. Analiza kombinacionih sposobnosti pokazala je da su obe genetičke komponente, aditivna i ne-aditivna, važne u ekspresiji ispitivanih osobina. Komponente genetičke varijanse ukazuju na prevlađivanje dominantne komponente (H) u odnosu na aditivnu (D) i veću koncentraciju dominantnih gena (u) u poređenju sa recesivnim (v), u svim ispitivanih osobinama.

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