

## Evaluation of Combining Ability in Ornamental Sunflower for Floral and Morphological Traits

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### Abstract

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Ornamental sunflowers are widely cultivated for use as cut flowers and/or as garden plants. The objective of the study was to investigate breeding values of new F<sub>1</sub> ornamental sunflower hybrids using incomplete diallel crossing of four ornamental inbred lines: Heliopa, Talia, Iskra and Neoplanta. Six traits important for ornamental market use were studied. Additive gene effects prevailed in most tested traits except for lateral floral diameter and duration of lateral flowering, which exhibited non-additive effects. The results indicated that the Talia × Neoplanta hybrid combination was the most promising for cut flowers due to long and strong branches, relatively big lateral flowers while Heliopa × Iskra and Heliopa × Talia hybrids were suitable for use as garden plants due to strong plant habit, long flowering time and desirable plant height. The results obtained from this study will be helpful for further ornamental sunflower breeding.

**Keywords:** heterosis; hybrids; inbred lines; cut flowers; garden plants

The commonly cultivated sunflower (*Helianthus annuus* L.) includes a number of varieties that are known as ornamental. Ornamental sunflowers are widely cultivated for use as cut flowers, potted plants, or in the garden (KAYA *et al.* 2012). Therefore, the breeding of ornamental sunflower depends on its use. A common characteristic of all ornamental sunflowers is breeding for desirable plant architecture and morphology, flower performances, duration of flowering, and other characteristics (ATLAGIĆ *et al.* 2005).

While the inheritance of floral colour and morphology has been observed by many researchers (FICK 1976; SHARYPINA *et al.* 2008; CVEJIĆ *et al.* 2016), there are few studies of other traits important for the ornamental use of sunflower. Research of SLOAN and HARKNESS (2006) indicated that a desirable length

of sunflower cut flower is 60–90 cm, preferred for floral arrangements. Plants used for this purpose must have a stem which is strong but not thick, a short vegetation period, resistance to long transport and a long vase life. The ideal flower diameter is 8–15 cm (SLOAN & HARKNESS 2006). The other direction of ornamental sunflower breeding is that intended for garden production. Plants for this purpose are characterized by strong plant habit, branching and the height ranging from 50 to 170 cm, depending on whether they are intended for use as a hedge or to be combined with other flowers (KAYA *et al.* 2012).

Both varieties and hybrids are used in commercial production. The use of hybrids has more advantages compared to the use of varieties because of the exploitation of heterosis, enabling higher crop uniformity, better vigour, wider range of floral colours, simple

breeding methods for gene introduction for disease resistance, easier harvesting and providing uniform seed moisture and storage suitability (CVEJIĆ & JOČIĆ 2010). The heterotic performance of a hybrid combination depends on its combining ability. The combining ability analysis is an important tool for the selection of desirable hybrid combinations, and obtaining information about the nature and magnitude of gene effects controlling quantitative traits. There is extensive literature on sunflower hybrids used as an oil crop, but it is scarce on the topic of hybrids used as ornamental flower.

The main objective of this study was to investigate breeding values of new  $F_1$  hybrids for ornamental use as a cut flower and/or garden plant using diallel crossing of four ornamental inbred lines. The objective was to estimate heterosis and the combining ability of these materials for flowering time, lateral floral diameter, number of branches, length of branches, duration of lateral flowering and plant height.

## MATERIAL AND METHODS

Four genetically diverse inbred lines of ornamental sunflower were used for crossings; Heliopa (H), Iskra (I), Talia (T), and Neoplanta (N). All lines are branching and differ in flower colour. They had been developed at the Institute of Field and Vegetable Crops in Novi Sad.

The research was performed during two growing seasons – 2013 and 2014, at a breeding nursery of the Institute of Field and Vegetable Crops, Novi Sad, Serbia. In the first season inbred lines were planted and crossed by a diallel method without reciprocal crosses (incomplete diallel method). Mutual crossings were done using the conventional hybridisation method. Before flowering, sunflower heads were isolated by covering them with paper bags. The lines used as a female parent were emasculated by removing stamens every morning until the end of flowering. All stamens were removed during flowering. Lines used as males were planted earlier to ensure the presence of pollen prior to female flowering. Pollen was deposited on emasculated plants three to four times. Pollen was applied to the emasculated flower heads with a soft brush. In the second season, the seeds of four ornamental sunflower parental lines and their six  $F_1$  hybrids were planted. The experimental plot was organized in a randomized complete block design with three replications. Each plot consisted of four rows, with row-to-row spacing 0.70 m and

plant-to-plant spacing 0.3 m (4.5 m long rows). All necessary agricultural practices typical of sunflower production were performed.

Six traits important for ornamental market use were studied: flowering time, lateral floral diameter, number of branches, length of branches, duration of lateral flowering and plant height. Data were recorded according to a descriptor list (UPOV 2000).

All data were statistically analysed using Statistical 12 (StatSoft, DELL, USA). The differences between mean values were tested using Duncan's multiple range tests at a 0.05 level of probability. Entries marked with the same letters were not significantly different.

The heterosis was estimated in relation to mid-parent values for all studied traits. Mid-parent heterosis was calculated using the formula presented by MATHER and JINKS (1971):

$$\text{Mid-parent heterosis (\%)} = ((F_1 - MP)/MP) \times 100$$

where:

$F_1$  – hybrid mean performance

MP =  $(P_1 + P_2)/2$  in which  $P_1$  and  $P_2$  are the mean performances of parental lines

The analysis of combining ability was estimated using GRIFFING (1956) method 2, model 1. Mathematical model 1 for the analysis of combining ability was as follows:

$$X_{ij} = \mu + gi + gj + S_{ij} + e$$

where:

$X_{ij}$  – mean value of two line crosses

$\mu$  – mean value of the total population

$gi, gj$  – effect of general combining ability (GCA) of parents  $i, j$

$S_{ij}$  – effect of specific combining ability (SCA) of crosses  $i \times j$

$e$  – error due to environmental conditions onto parents  $i, j$

Estimation of SCA of crosses ( $S_{ij}$ ) was done:

$$S_{ij} = X_{ij} - 1/p + 2((Ti + ii)(Tj + jj)) + 2GT/(p + 1)(p + 2)$$

where:

$p$  – number of parents

$Ti + ii$  – total of  $i$  row + mean value of parent  $i$

$Tj + jj$  – total of  $j$  row + mean value of parent  $j$

GT – sum of individual values of parents and crosses

The standard error (SE) of the estimated SCA was obtained by

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$$SE = ((2p/p+2)Me)^{1/2}$$

where:

Me – mean square of error divided by the number of replications

## RESULTS AND DISCUSSION

**Estimates of heterosis.** The mean values of parental lines and six F<sub>1</sub> hybrids of ornamental sunflower were evaluated in field trials for the studied traits (Table 1). There were great variations among inbred lines concerning flowering time, number and length of branches. Between some inbred lines there were no noticeable differences in the lateral floral diameter, duration of lateral flowering and plant height, but most crossings between these lines exhibited a heterotic effect for these traits. The estimates of heterosis over the mid-parent values were significant or highly significant for flowering time, lateral floral diameter, length of branches and plant height. For the number of branches and duration of lateral flowering, mid-parent heterosis was detected only in some hybrids. The Heliopa × Iskra hybrid combination exhibited heterosis for all studied traits. In the Talia × Neoplanta crossing, all traits showed higher values than the better parent, except for the number of branches and duration of lateral flowering.

Flowering time in all F<sub>1</sub> hybrids was longer than in both parents, thus showing the heterotic effect. It is a favourable trait when sunflower is used as a garden plant, because of the possibility of combining lines with different flowering time which provides a long flowering period in gardens and green spaces. Combining the early and late flowering lines in cut flower production provides permanent harvesting and market supply. According to SLOAN and HARKNESS (2006), one of the characteristics with the strongest influence on the marketing of ornamental sunflowers as cut flowers is the flower diameter. In our study, hybrids generally showed positive heterosis for this trait, although parental lines did not differ. Lateral floral diameter should be larger to make it more appealing to the producers. Hybrid combinations Heliopa × Iskra, Heliopa × Talia and Talia × Neoplanta had the highest value of lateral floral diameter, which made them suitable for both garden and cut flower use. Moreover, the length of branches plays an important role in sunflower use as a cut flower. According to ARMITAGE and LAUSHMAN (2003) sunflower stems should be cut as long as possible.

Table 1. Mean values and mid-parent heterosis (MP) of four ornamental sunflower parents and their F<sub>1</sub> hybrids

	Flowering time		Lateral floral diameter		Number of branches		Length of branches		Duration of lateral flowering		Plant height	
	mean (days)	heterosis (MP %)	mean (cm)	heterosis (MP %)	mean (n)	heterosis (MP %)	mean (cm)	heterosis (MP %)	mean (days)	heterosis (MP %)	mean (cm)	heterosis (MP %)
H	73.67 <sup>c</sup>		10.44 <sup>a</sup>		18.87 <sup>ac</sup>		44.99 <sup>e</sup>		15.67 <sup>d</sup>		112.67 <sup>b</sup>	
I	51.33 <sup>e</sup>		10.61 <sup>a</sup>		13.70 <sup>b</sup>		54.51 <sup>b</sup>		20.40 <sup>b</sup>		82.60 <sup>a</sup>	
T	49.67 <sup>d</sup>		13.10 <sup>b</sup>		9.13 <sup>e</sup>		18.83 <sup>c</sup>		21.10 <sup>bc</sup>		85.97 <sup>a</sup>	
N	60.33 <sup>f</sup>		10.85 <sup>a</sup>		20.70 <sup>cd</sup>		38.49 <sup>d</sup>		25.30 <sup>a</sup>		144.63 <sup>c</sup>	
H × I	79.33 <sup>g</sup>	26.93 <sup>**</sup>	14.62 <sup>c</sup>	38.85 <sup>**</sup>	21.37 <sup>d</sup>	31.22 <sup>*</sup>	60.36 <sup>a</sup>	21.33 <sup>**</sup>	25.67 <sup>a</sup>	42.33 <sup>**</sup>	156.27 <sup>f</sup>	60.05 <sup>**</sup>
H × T	74.67 <sup>c</sup>	21.08 <sup>**</sup>	15.25 <sup>cd</sup>	29.58 <sup>**</sup>	13.40 <sup>b</sup>	-4.29	50.53 <sup>b</sup>	58.34 <sup>**</sup>	25.13 <sup>a</sup>	36.72 <sup>**</sup>	112.53 <sup>b</sup>	13.31 <sup>**</sup>
H × N	71.33 <sup>b</sup>	6.47 <sup>*</sup>	13.10 <sup>b</sup>	23.06 <sup>**</sup>	18.20 <sup>a</sup>	-8.00	60.61 <sup>a</sup>	45.20 <sup>**</sup>	21.97 <sup>c</sup>	7.24	161.87 <sup>g</sup>	25.82 <sup>**</sup>
I × T	69.33 <sup>a</sup>	37.29 <sup>**</sup>	14.69 <sup>c</sup>	23.89 <sup>**</sup>	11.80 <sup>b</sup>	3.36	51.91 <sup>b</sup>	41.56	20.63 <sup>bc</sup>	-0.56	121.40 <sup>d</sup>	44.04 <sup>**</sup>
I × N	71.33 <sup>b</sup>	27.76 <sup>**</sup>	13.45 <sup>b</sup>	25.33 <sup>**</sup>	16.70 <sup>a</sup>	-2.91	60.92 <sup>a</sup>	31.01 <sup>**</sup>	25.13 <sup>a</sup>	9.99	137.03 <sup>e</sup>	20.61 <sup>**</sup>
T × N	69.33 <sup>a</sup>	26.06 <sup>**</sup>	15.68 <sup>d</sup>	30.95 <sup>**</sup>	17.73 <sup>a</sup>	18.88	59.74 <sup>a</sup>	108.42 <sup>*</sup>	25.63 <sup>a</sup>	10.49 <sup>*</sup>	146.87 <sup>c</sup>	27.38 <sup>**</sup>

<sup>a-f</sup>Values between parents and hybrids, followed by the same letters do not differ significantly at a 0.05 probability level (Duncan's test); <sup>\*</sup> significance of mid-parent heterosis at the 1% and 5% level according to Student's *t*-distribution; H – Heliopa; I – Iskra; T – Talia; N – Neoplanta

Table 2. Estimation of mean squares for general combining ability (GCA) and specific combining ability (SCA) of four sunflower parents and their  $F_1$  hybrids for flowering time (FT), lateral floral diameter (LFD), number of branches (NB), length of branches (LB), duration of lateral flowering (DLF) and plant height (PH)

Parameters	FT	LFD	NB	LB	DLF	PH
GCA	115.28**	2.60**	37.21**	208.40**	10.36**	1121.36**
SCA	91.38**	4.45**	5.40**	160.29**	11.17**	601.49**
Error	0.29	0.09	0.53	2.25	0.22	1.47
GCA/SCA	1.26	0.58	6.89	1.30	0.93	1.86

\*\*significant at  $P = 0.01$  probability level

In the post-harvest period stems can be re-cut to a desired length (DOLE & WILKINS 2005). In this research, the length of branches varied between 50 and 60 cm in  $F_1$  hybrids. The positive heterosis for the length of branches in Heliopa  $\times$  Talia and Heliopa  $\times$  Neoplanta hybrids indicated that they were suitable for use as a cut flower. Plant height is one of the most frequently investigated morphological characteristics and from an ornamental point of view it provides suitability for covering hedges and walls. Significant differences in plant height were found between parent lines and  $F_1$  hybrids. Heterosis was detected in all hybrid combinations. Duration of lateral flowering in hybrid lines varied

between 20 and 25 days. This is a desirable trait in both gardens and the production of cut flowers. According to the results, hybrids achieved heterosis in Heliopa  $\times$  Talia and Iskra  $\times$  Neoplanta, prolonging the flowering period compared to the parents.

**Estimates of general and specific combining abilities.** The results of the diallel crossing of four different ornamental inbred lines and their  $F_1$  hybrids indicate that important traits for growing ornamental sunflower are controlled by the additive and non-additive gene action of equal importance. This is in accordance with DIVITA *et al.* (2012), who concluded that quantitative ornamental traits were controlled by additive and dominant genetic effects and affected by the environment. Our results illustrated that the estimated GCA was higher than SCA in terms of flowering time, number of branches, length of branches and plant height. These results suggested that the additive gene effects appeared to be relatively more important for the inheritance of these characteristics than the non-additive gene effects (Table 2). Additive gene effect was prevalent to the number of branches and length of branches, which was in agreement with ŠEČEROV-FISER (1999) and DIVITA *et al.* (2012) within their investigation of ornamental sunflower. Generally these traits were not studied much, due to the absence of lateral branches in oil sunflower hybrids. Additive gene effect was important to plant height, which was in accordance

Table 3. Estimates of specific combining ability (SCA) effects of sunflower  $F_1$  hybrids on flowering time (FT), lateral floral diameter (LFD), number of branches (NB), length of branches (LB), duration of lateral flowering (DLF) and plant height (PH)

Hybrids	FT	LFD	NB	LB	DLF	PH
H $\times$ I	8.133**	2.098**	4.146**	3.138	4.720**	34.406**
H $\times$ T	5.133**	1.426**	-1.143	7.034**	3.959**	-4.800**
H $\times$ N	-1.533*	0.589	-1.816*	7.379**	-0.830	14.150**
I $\times$ T	8.133**	0.841*	-0.504	4.961*	-1.897**	16.750**
I $\times$ N	6.800**	0.917*	-1.077	4.236*	0.981	2.000
T $\times$ N	6.467**	1.842**	2.634**	16.783**	1.253*	16.353**
SE ( <i>Sij</i> )	0.342	0.185	0.459	0.948	0.296	0.768
SE ( <i>Sii-Sjj</i> )	0.441	0.239	0.593	1.224	0.382	0.991
SE ( <i>Sij-Sik</i> )	0.698	0.377	0.937	1.935	0.604	1.567
SE ( <i>Sij-Skl</i> )	0.624	0.338	0.838	1.731	0.541	1.401
LSD 1%	1.797	0.971	2.413	4.982	1.556	4.034
LSD 5%	1.312	0.709	1.762	3.637	1.136	2.945

SE – standard error; \*, \*\*significant at  $P = 0.05$  and  $0.01$  probability level; LSD – least significant difference; H – Heliopa; I – Iskra; T – Talia; N – Neoplanta;

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with the results obtained in other ornamental plants, such as gladiolus (KUMAR *et al.* 2008) and zinnia for plant height (LOU *et al.* 2010). Our findings were also supported by the findings of GOKSOY *et al.* (2000) showing similar results for plant height in oil sunflower, while ČIRIĆ *et al.* (2013) suggested opposite results for plant height in oil sunflower. On the other hand, the non-additive gene effect was more important for lateral floral diameter and duration of lateral flowering since the estimated values of SCA were found to be higher than the values of GCA. This is in accordance with findings of DIVITA *et al.* (2012), who stated that the non-additive effect determines the size of heads of branched hybrids. However, findings of MACHIKOWA *et al.* (2011) suggested that the additive gene effects were found to be more important for the standard oil sunflower head diameter.

Two hybrid combinations Heliopa × Iskra and Talia × Neoplanta expressed highly positive SCA effects on flowering time, lateral floral diameter, number of branches and plant height (Table 3). The goals of ornamental sunflower breeding are different for cut flowers and for garden plants. Cut flowers require positive SCA effects on lateral floral diameter and length of branches. Among six hybrid combinations, Talia × Neoplanta was the most promising for cut flowers due to long and strong branches, and relatively big lateral flowers. This combination had longer flowering time and higher number of branches and the plant height had the required properties for cutting. Desirable traits of the garden plants are strong plant habit, long flowering time and the required plant height. It depends on whether they are intended for use as a hedge or combined with other flowers (KAYA *et al.* 2012), which requires dwarf or tall plants, higher number of branches and long duration of lateral flowering. The Heliopa × Iskra hybrid exhibited high positive SCA effects on these traits suitable for use as a hedge plant. However, Heliopa × Talia exhibited a high negative SCA effect on plant height, which can be combined with other flowers preferably tall.

In conclusion, it can be seen that heterosis and combining ability estimates have been widely used in the prediction of the best hybrid combinations. The results indicated that the Talia × Neoplanta combination was the most promising for use as cut flowers, due to long and strong branches and relatively big lateral flowers, while Heliopa × Iskra and Heliopa × Talia were suitable for use as garden plants. New combinations of traits give more opportunities for

the selection of desirable hybrids suitable for the ornamental market.

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