DIALLEL CROSS ANALYSIS FOR FRUIT TRAITS IN WATERMELON

Jelica GVOZDANOVIĆ-VARGA, Mirjana VASIĆ, Dragan MILIĆ
and Janko ČERVENSKI

Institute of Field and Vegetable Crops, Novi Sad, Serbia

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Current demands of consumers and thus producers are important when targeting watermelon breeding programs, especially in programs aimed at improvement of fruit traits. A complete diallel set has been investigated for breeding values of six watermelon lines, via general and specific combining ability, relationships between general and specific combining ability, heritability and heterosis for fruit size, rind thickness, soluble solids and fruit shape. The lines P2 and P4 were good general combiners for fruit size. These lines also had high values of specific combining ability in direct and reciprocal crosses. The lines with negative general combining ability for fruit size (P1 and P5) can be used in breeding for small fruits (4-6 kg), good taste (high sugar content), desired rind thickness, desired fruit form and high fruit ratio. Relationships between general and specific combining ability indicated that the additive effect

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Corresponding author: Jelica Gvozdanović-Varga, Institute of Field and Vegetable Crops, Maksima Gorkog 30, 21000 Novi Sad, Serbia

played an important role in the expression of fruit weight, rind thickness and sugar content, while fruit shape was inherited incompletely dominantly.

Key words: diallel, GCA, SCA, heterosis, fruit traits, watermelon

INTRODUCTION

Success of watermelon production and profits accrued are determined by the choice of genotype, production method and market demand. Basic market requirements relate primarily to fruit characteristics such as fruit size, rind thickness, taste, meat color, fruit shape and color (GVOZDANOVIĆ-VARGA *et al.* 2004). Current demands of both consumers and producers are an important item in watermelon breeding programs, especially for fruit traits. These requirements are: fruit size of 4-6 kg, good taste (8-10% SS), rind thickness up to 1.5 cm, round to oval-shaped fruit, small seeds, very high meat ratio. Watermelon breeding includes the development of breeding lines, cultivars and hybrids using different crossing methods. To increase the efficiency of breeding,. it is necessary to know the genetic basis of the traits that are being improved in the available material selection. As stated by numerous authors (ČERVENSKI *et al.* 2001, 2003; MURAYA *et al.* 2006; JUMBO and CARENA, 2008; FEYZIAN *et al.* 2009; MILIĆ *et al.* 2010; 2010a), diallel crossing method is most frequently used in genetic research and breeding, because it provides maximum information about gene effects and the number, combining ability (GCA, SCA), heterosis, heritability and possible gene interactions (HALLAUER, 2007). Traits of interest in watermelon breeding are yield, biotic and abiotic stress resistance, fruit shape and size, rind pattern and nutritional value (sugar, lycopene, and citrulline). The available literature on watermelon breeding for these traits is scant and conflicting (GUSMINI and WEHNER, 2007, KUMAR 2009), especially for quantitative traits of the fruit, such as fruit size (WEHNER, 2008).

In this study we used a part of the watermelon collection of Institute of Field and Vegetable Crops in Novi Sad. Inbred lines developed from these accessions served as parents in the diallel cross. The aim of this study was to examine a possibility of using inbred lines in a full diallel set for development of new genotypes (hybrids or cultivars) with smaller fruits of improved quality, desired shape and good transportability. Breeding value of the used watermelon lines was estimated (evaluated) via GCA, SCA, GCA:SCA ratio, heterosis and heritability.

MATERIALS AND METHODS

The studied material consisted of 36 watermelon genotypes. Six of them were parents and 30 were their hybrids that made a complete diallel set. Further genotypes were used as parent components: cultivar Super sweet (P1); inbred line ML4-02 (P2) with light green fruit with pale stripes, red meat color; inbred line P3 (66/3)S3 selected from old cultivar Mramorka with light green fruit color, large seeds, luxuriant growth, light red meat color; inbred line P4 (65/3) S3 selected from old cultivar Mramorka with dark green (black) fruit color, large seeds, luxuriant growth, rosy meat color; inbred line P5 (83/3) selected from old domestic cultivar Vukovarka with dark green fruit color, no stripes, very early, red-orange meat color,

and inbred line P6 (41/3) selected from old domestic cultivar Vukovarka with dark green fruit color, no stripes, very early, intensively red meat color, small seeds.

Experiments have been conducted in the experimental plots of Institute of Field and Vegetable Crops in Rimski Šanĉevi near the Novi Sad in 2007. The experiment was set up in a random arrangement in three replications. The experiment unit area was 25 m2. Fifteen watermelon plants were planted in each unit: three rows 2 m apart, and plants spacing in row, 1 m. Both parents and hybrids were grown from transplants. Transplant production started by greenhouse planting on 3 April. Field transplanting was conducted on 14 of May.

The studied watermelon genotypes were analyzed for 4 fruit traits: fruit weight (FW), rind thickness (RT), sugar content (%SS) and fruit shape (L/D). Two fruits per plant were analyzed, making a total of 30 fruits per replication. Mean, minimum and maximum values and coefficient of variation were calculated for all studied traits of the parents and the F1 hybrids. Breeding value of the studied material was demonstrated via the analyses of heterosis and combining abilities in the F1 generation. Data were analyzed according to a general linear model (GLM). Last-square means, based on GLM, were used to compute combining ability via Griffing's Method 1 and Model 1 (GRIFFING 1956). All variance analyses and combining ability (GCA, SCA, reciprocal) analyses were conducted using the DIALLEL-SAS05 program developed by ZHANG and KANG (2005). Maternal (general reciprocals) and non-maternal (specific reciprocals) effects were calculated from reciprocal effects. According to ZHANG *et al* (1996, 1997), maternal effect refers to the effect of non-reproductive factors of a maternal genotype on a trait of its offspring, whereas non-maternal effect is the effect of all other non-nuclear factors on the trait. This partition will clearly identify maternal or (extranuclear) cytoplasmatic effects important for the expression of this trait. Non-maternal effect = reciprocal effect – female maternal effect + male maternal effect (ZHANG *et al* 1997). Broad-sense heritability was calculated as additive variance + dominance variance/(error variance/reps. + dominance variance + additive variance). Narrow-sense heritability was calculated as additive variance/(error variance/rep. + dominance variance + additive variance). The GCA:SCA variance ratios with a theoretical maximum of unity were computed according to BAKER (1978).This ratio was calculated to determine the relative importance of GCA and SCA effects. Mid-parent heterosis (MPH) and high-parent heterosis (HPH) were estimated according to HALLAUER and MIRANDA(1988).The critical differences (CD) for testing the significance of heterosis.

RESULTS AND DISCUSSION

The average fruit weight of the studied genotypes ranged from 5.35 to 10.42 kg (Table 2). The analysis of variance indicated that highly significant differences existed between the genotypes regarding this trait (Table 2). Most of the examined combinations (31) had a negative heterosis in relation to the parental average for fruit size. Positive effects of heterosis in relation to the averages of both parents and

the better parent ranged from 15.25 to 1.15% and were achieved in four hybrid combinations (Table 4). All of these combinations involve P2 as one of the parents. This parent line also had a highly statistically significant GCA value (Fig.1), as well as the line P4 (0.79), while GCA for the line P5 was less significant. Highest negative GCA values were found for the genotypes P1 (-1.34) and P5 (-0.25), which exhibited negative heterosis in all cross combinations (Table 4). These lines can be used for development of hybrids with small fruits. Also, in the analysis of variance of SCA, the reciprocal and non-maternal effects were statistically highly significant, while the maternal effect were at a lower level of significance (Table 2). The highly significant GCA and SCA values indicate that both the additive and non-additive genes are involved in the expression of this trait; however, the GCA:SCA ratio (0.88) indicated the predominance of additive genes in the inheritance of this trait, which was also confirmed by the high value of the additive variance. WEHNER (2008) stated that some 25 genes are involved in the inheritance of this trait, which allows the breeder to select genotypes according to the targeted model hybrid. The narrow sense heritability was intermediate ($h2n = 0,54$), and so was the broad sense heritability ($h2b = 0,60$). Studying fruit weight in different locations, GUSMINI and WEHNER (2007) obtained similar heritability values and concluded that the additive component plays an important role in watermelon breeding for fruit size. Our assessment of SCA effects in diallel crossing of the six parents indicated that statistically significant effects existed in five cross combinations (Table 3). The parents P1, P3, P4 and P5, which had negative SCA values, can be used for development of hybrids with smaller fruits, which is one of targets in watermelon breeding, and which is the result is polygenic inheritance of this trait. Statistically significant effects in reciprocal crosses were obtained with the parents P1, P2 and P4. Further fragmenting of the reciprocal sum of squares indicated that both maternal non-maternal effects were highly significant. Highly significant maternal effects were found for the parents P3 and P5 (Table 3), while highly significant non-maternal effects were obtained in the crosses of P2, P4 and P6. The significant maternal and non-maternal effects indicate that interactions between nuclear and extranuclear factors play a role in the inheritance of fruit size (ZHANG and KANG 1997).

Rind thickness in the studied genotypes ranged from 0.9 to 2.1 cm (Table 1). The maximum value for this feature was realized in the combination P2 x P4 (2.1 cm), while the fruits of the combination P1 x P5 had the thinnest rind (0.9 cm). Studying the traits of the watermelon fruit, GUSMINI *et al*. (2004) divided watermelons into three groups based on the rind thickness. The first group were genotypes with the rind thickness >19 mm, the second from 10 to 19 mm and the third <10 mm. According to this division, our hybrids were distributed in all three groups. ANOVA showed highly significant differences between the genotypes for this trait. Most of the studied combinations had a negative heterosis value of -42.67% for HPH (Table 4). Positive heterosis values were found in six combinations and they ranged from 18.89% to 14.75% (HPH). These values were achieved in the combinations that included the following parents: P2, P4 and P3.

Table 1. Mean values and variability of watermelon traits in the 6 x 6 diallel

Genotype	FW (kg)	RT (cm)	%SS	L/D
P1	5.48	1.17	10.40	1.11
P2	8.52	1.70	10.80	1.13
P3	9.46	1.80	9.00	1.16
P4	9.52	1.83	8.80	1.31
P5	8.76	1.57	9.60	1.15
P6	8.87	1.70	8.80	1.18
1H (P1 x P2)	6.90	1.50	9.6	1.11
2H (P1 x P3)	6.08	1.23	9.8	1.28
3H (P1 x P4)	6.86	1.10	9.7	1.15
4H (P1 x P5)	5.35	0.90	9.8	1.10
5H (P1 x P6)	6.37	1.00	9.9	1.12
6H (P2 x P1)	6.81	1.30	10.2	1.12
7H (P2 x P3)	9.75	1.60	10.0	1.13
8H (P2 x P4)	10.42	2.10	9.9	1.22
9H (P2 x P5)	6.41	1.17	10.4	1.44
10H (P2 x P6)	8.88	1.50	10.4	1.16
11 H (P3 x P1)	5.50	1.57	9.4	1.24
12 H (P3 x P2)	6.37	1.20	10.3	1.09
13 H (P3 x P4)	8.31	1.23	9.8	1.08
14 H (P3 x P5)	6.47	1.27	9.7	1.15
15 H (P3 x P6)	7.76	1.27	8.9	1.14
16 H (P4 x P1)	7.26	1.60	9.4	1.13
17 H (P4 x P2)	9.02	1.30	9.7	1.16
18 H (P4 x P3)	9.27	1.57	9.0	1.13
19 H (P4 x P5)	7.93	1.77	9.3	1.17
20 H (P4 x P6)	8.39	1.37	9.1	1.18
21 H (P5 x P1)	6.76	1.27	9.9	1.14
22 H (P5 x P2)	9.45	1.53	9.9	1.18
23 H (P5 x P3)	7.48	1.37	10.2	1.15
24 H (P5 x P4)	7.67	1.67	9.7	1.13
25 H (P5 x P6)	6.18	1.23	9.3	1.36
26 H (P6 x P1)	6.20	1.20	9.6	1.12
27 H (P6 x P2)	8.10	1.37	9.5	1.23
28 H (P6 x P3)	7.30	1.20	9.4	1.11
29 H (P6 x P4)	6.54	1.23	9.2	1.22
30 H (P6 x P5)	8.63	1.20	9.0	1.10
Min		5.35	0.9	8.8	1.08
Max		10.42	2.1	10.8	1.44
Average		7.48	1.36	9.67	1.17
CV (%)		11.13	10.92	3.27	3.59
LSD-	0.05	1.40	0.25	0.51	0.21
	0.01	1.87	0.33	0.68	0.32

WF - fruit weight; *RT* - rind thickness; *%SS* - soluble solids; *L/D* - fruit shape

These parents had highly significant GCA values (Fig.1), while P6 had a statistically significant negative GCA value. Highly significant SCA values were found in F1 genotypes whose parents were P2, P3, P4 and P5. Statistically significant GCA and

SCA values indicated that this trait was controled by additive genes, as confirmed by the GCA:SCA ratio for rind thickness of 0.92 (Table 2), which was in accordance with the results of FERREIRA *et a.l* (2002). The high heritability of this trait ($h2n =$ 0,68; $h2b = 0,74$) results from high values of σ2g, i.e., from low values of σ2s i σ2r. Highly significant reciprocal, maternal and non-maternal effects were achieved in a large number of cross combinations (Table 3).
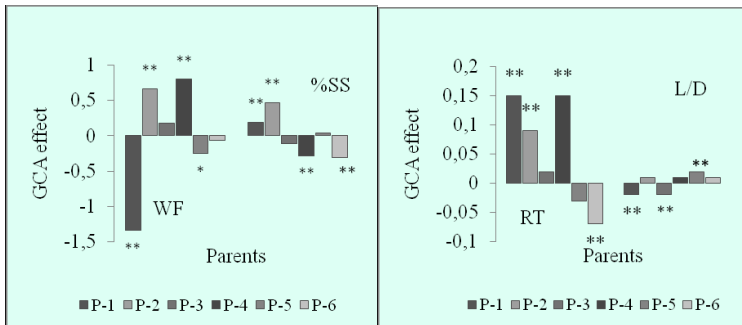

Fig. 1 Estimates of general combining ability (GCA) effects for the studied fruit traits *WF* - fruit weight; *RT* - rind thickness*; %SS* - soluble solids*; L/D* - fruit shape. The symbols * and ** indicate that GCA effects are significant at 5% and 1%, respectively.

Tabela 2. Combining ability analysis, GCA:SCA ratio and heritability assessment of the studied watermelon traits

Source	df	Mean Square			
		WF (kg)	RT (cm)	%SS	L/D
Rep	2	1.51	0.13	0.66	0.001
Genotype	35	5.60**	0.21**	0.73**	0.018**
Error	70	0.74	0.002	0.01	0.001
GCA	5	21.43**	0.434**	3.20**	0.010**
SCA	15	2.71**	0.162**	0.32**	0.022**
REC	15	3.22**	0.168**	0.31**	0.015**
MAT	5	3.40*	0.145**	0.42**	0.006**
NMAT	10	3.11**	0.179**	0.26**	0.020**
GCA/ SCA		0.88	0.92	0.92	0.47
h^2_n		0.54	0.68	0.64	0.51
h^2_b		0.60	0.74	0.69	0.77

WF - fruit weight; *RT* - rind thickness*; %SS* - soluble solids*; L/D* **-** fruit shape

*, ** significantly different at 5% and 1% probability levels, respectively

Sugar content in the watermelon edible part is a sum of total soluble substances, and it is a major component that determined fruit taste. In the studied genotypes, sugar content ranged from 8.8 to 10.8%, but these values were registered in the parental genotypes (Table 1). The analysis of variance showed that highly significant differences existed between the genotypes. Heterosis for this trait was very low and it ranged from 10.11% (MPH) to 8.89% (HPH) (Table 4). The highest heterosis was obtained in the combination P3 x P4. Both of these parents had highly significant negative GCA values (Fig.1). Positive GCA values were found in P1 and P2. The analisys of combining ability found highly significant GCA, SCA, reciprocal, and maternal and non-maternal effects (Table 2). The mean square for GCA was significantly higher than the mean square for SCA, indicating a relatively greater importance of additive gene effects compared with non-additive ones. Significantly high GCA effects in their investigations were found by FERREIRA *et a.l* 2002, SOUZA *et al*. 2002 and EL-MEGHAWRY *et al*. 2002 who found a higher contribution of additive gene effects. Further to this point, the GCA:SCA ratio was very high, amounting to 0.92. The narrow sense heritability was relatively high ($h2n$ = 0,64) as well as $h2b$ which was 0.69. SCA effect was highly significant in three combinations (P1 x P2, P2 x P6, P3 x P5), but it was positive only in the third combination (Table 3). Statistically significant reciprocal effects were achieved in combinations with P2, which had a significant maternal effect as well as negative non-maternal effect. Significant negative non-maternal effects were found in the hybrid combinations P6 x P3, P3 x P2 and P4 x P2, while the cross- P4 x P3 had a significant non-maternal effect (Table 3).

Watermelon *fruit is either round*, elliptic and elongated (cylindrical) (WEHNER, 2008a). The numerical expression (the L/D ratio) for these value ranges from 1 for the round shape to more than 2 for elongated one (GUSMINI, 2005a). F1 crosses had the L/D values from 1.08 to 1.44 (Table 2), i.e., their fruits were round to round/elliptical. Despite the low variations in the L/D ratio, statistically highly significant GCA, SCA, reciprocal, and maternal and non-maternal effects were obtained (Table 2). The GCA:SCA ratio amounted to 0.47, while $h2n$ was 0.51. Fruit shape is determined with a single *O* gene which is incompletely dominant, so that genotypes that are homozygously dominant are elongated and those homozygously recessive are round (WEHNER, 2001; GUSMINI 2003). The achieved heterosis as compared with the parents' mean was 26.32%, while HPH was 25.22%, in the cross P2 x P5 (Table 4). The parents P1 and P3 had high negative GCA values, while P5 had a positive GCA value (Fig.1). These parents also had highly significant SCA values, as well as their reciprocals (Table 3).

Table 3. Assessment of SCA , reciprocal, maternal and non-maternal effects for the studied fruit traits

Parent	WT (kg)	RT (cm)	%SS	L/D
S_{12}	-0.06	0.06	-0.45**	-0.03*
S_{13}	-0.66*	0.13*	-0.14	0.13**
S_{15}	0.08	-0.14*	-0.05	-0.04**
S_{23}	-0.37	-0.12*	0.15	-0.05**
S_{24}	0.66*	0.06	-0.04	0.01
S_{25}	-0.10	-0.12*	-0.01	0.12**
S_{26}	0.73	-0.10	-0.41*	0.06*
S_{34}	0.21	-0.17**	0.11	-0.07**
S_{35}	-0.55*	-0.08	0.37**	-0.01
S_{36}	-0.73**	-0.47**	0.32	-0.04*
S_{45}	-0.34	0.20**	0.04	-0.05**
S_{46}	-1.21*	-0.31**	0.38	-0.09**
S_{56}	-2.40**	-0.30**	-0.07	0.09**
R_{12}	0.05	0.10	-0.30**	-0.01
R_{13}	0.28	-0.17*	0.23*	0.02
R_{14}	-0.18	-0.25**	0.15	0.01
R_{15}	-0.72*	-0.18**	-0.03	-0.01
R_{23}	1.68**	0.20**	-0.13	0.02
R_{24}	0.70*	0.40**	0.10	0.03*
R_{25}	-1.53**	-0.18**	0.23*	0.13**
R_{26}	0.42	0.07	0.42**	-0.04*
R_{34}	-0.48	-0.17*	0.38**	-0.03*
R_{46}	0.93*	0.07	-0.05	-0.02
R_{56}	-0.35	0.02	0.17	0.13**
M_1	-0.08	-0.10**	0.03	0.01
M_2	0.20	0.03*	0.15**	0.03**
M_3	-0.46**	-0.04	-0.03	-0.01
M_4	0.17	0.02	-0.15**	-0.01
M_5	0.38**	0.07*	0.07	-0.01
M_6	-0.22	-0.01	-0.07	-0.02*
N_{12}	0.33	0.26**	-0.18	0.01
N_{13}	-0.09	-0.10*	0.17	0.01
N_{14}	0.07	-0.13*	-0.03	0.01
N_{23}	1.03**	0.10*	-0.32**	0.01
N_{24}	0.67*	0.36**	-0.20*	0.00
N_{25}	-1.36**	-0.18**	0.15	0.10**
N_{26}	-0.01	-0.01	0.19	-0.08**
N_{34}	0.14	-0.11*	0.27*	-0.02
N_{36}	0.47	0.05	-0.26*	0.01
N_{45}	0.34	0.09*	0.02	0.02*
N_{46}	0.54*	0.03	0.02	-0.09**
N_{56}	0.95**	-0.06	0.01	0.08**

WF - fruit weight; *RT* - rind thickness*; %SS* - soluble solids*; L/D* **-** fruit shape

*, ** significantly different at 5% and 1% probability levels, respectively

Table 4. MP and HP heterosis (%) for the studied fruit traits

Cross	WT (kg)		RT (cm)		%SS		L/D	
	MP	HP	MP	HP	MP	HP	MP	HP
12	-1.42	-19.01	4.89	-11.76	-9.43	-11.11	-0.89	-1.77
13	-18.60	-28.63	-16.89	-31.66	1.03	-5.77	12.78	10.34
14	-8.53	-19.48	-26.66	-39.89	1.04	-6.73	-4.96	-12.21
15	-24.86	-38.93	-34.31	-42.67	-2.00	-5.77	-2.65	-4.35
16	-11.28	-25.23	-30.06	-41.17	3.12	-4.81	-2.18	-5.08
21	-2.75	-20.07	-9.09	-23.53	-3.77	-5.56	0.00	-0.88
23	8.45	3.06	6.08	-11.11	1.01	-7.41	-1.31	-2.59
24	15.52	9.45	18.98	14.75	1.02	-8.33	0.00	-6.87
25	-25.81	-26.82	-28.44	-31.18	1.96	-3.70	26.32	25.22
26	1.15	0.11	-11.76	-11.76	6.12	-3.70	0.43	-1.69
31	-26.37	-41.86	5.72	-12.78	-3.09	-9.62	9.25	6.90
32	-29.14	-33.08	-31.42	-33.33	4.04	-4.63	-4.80	-3.54
34	-11.78	-12.71	-32.04	-32.79	10.11	8.89	-12.55	-17.56
35	-28.98	-31.60	-22.08	-29.44	4.30	1.04	-0.43	-0.86
36	-15.28	-17.97	-27.42	-29.44	0.00	-1.11	-2.56	-3.39
41	-3.20	-23.73	6.66	-12.57	-2.08	-9.62	-6.61	-13.74
42	0.00	-5.25	-26.13	-28.96	-1.02	-10.19	-4.92	-11.45
43	-1.59	-2.62	-13.25	-14.21	1.12	0.00	-8.50	-13.74
45	-13.23	-16.70	4.11	-3.28	1.09	-3.12	-4.88	-10.69
46	-8.30	-11.86	-22.15	-25.14	3.41	3.41	-5.22	-9.92
51	-5.06	-22.83	-7.30	-19.11	-1.00	-4.81	0.88	-0.87
52	9.37	7.87	-6.13	-10.00	-2.94	-8.33	3.51	2.61
53	-17.89	-20.93	-15.95	-23.89	9.68	6.25	-0.43	-0.86
54	-16.08	-19.32	-1.76	-8.74	5.43	1.04	-8.13	-13.74
56	-29.85	-30.32	-24.53	-27.65	1.09	-3.12	16.74	15.25
61	-13.65	-30.10	-16.08	-29.41	0.00	-7.69	-2.18	-5.08
62	-6.90	-8.68	-4.19	-19.41	-3.06	-12.04	6.49	4.24
63	-20.30	-22.83	-31.42	-33.33	5.62	4.44	-5.13	-5.93
64	-28.52	-31.30	-30.11	-32.79	4.55	4.55	-2.01	-6.87
65	-2.04	-2.70	-26.38	-29.41	-2.17	-6.25	-5.58	-6.78
mean	-10.95	-17.63	-15.41	-22.86	1.17	-3.79	-0.32	-3.20
Min	-29.85	-41.86	-34.31	-42.67	-9.43	-12.04	-12.55	-7.56
Max	15.25	9.45	18.98	14.75	10.11	8.89	26.32	25.22
$CD_{(0,01)}$	0.304	0.373	0.054	0.067	0.112	0.137	0.015	0.018

MP - mid-parent heterosis; *HP* - high-parent heterosis; *WF* - fruit weight; *RT* - rind thickness*; %SS* - soluble solids*; L/D* **-** fruit shape; *CD* - critical difference

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DIALELNA ANALIZA OSOBINA PLODA KOD LUBENICA

Jelica GVOZDANOVIĆ - VARGA, Mirjana VASIĆ, Dragan MILIĆ
i Janko ĈERVENSKI

Institut za ratarstvo i povrtarstvo, Novi Sad, Srbija

I z v o d

Pozitivne vrednosti GCA za krupnoću ploda su imali dva roditelja, P2 i P4, koji se mogu koristiti kako u direktim tako i u reciproĉnim ukrštanjima, za stvaranje hibrida veće krupnoće. Visoko negativne vrednosti GCA su imali P1 i P5, te se ove linije mogu koristiti za stvaranje hibrida manje krupnoće ploda (od 4-6 kg), dobrog ukusa (9,8 %SS), željene debljine kore, odgovarajućeg oblika i visokog randmana ploda. GCA/SCA odnosom ustanovljeno je da adiditivni efekat ima važnu ulogu u ekspresiji mase ploda, debljine kore i %SS. Ova istraživanja ukazuju da je za stvaranje hibrida lubenica, zavisno od postavljenog modela, neophodno korišćenje divergentnih linija za ispitivane osobine ploda, a posebno za masu (veličinu) ploda.