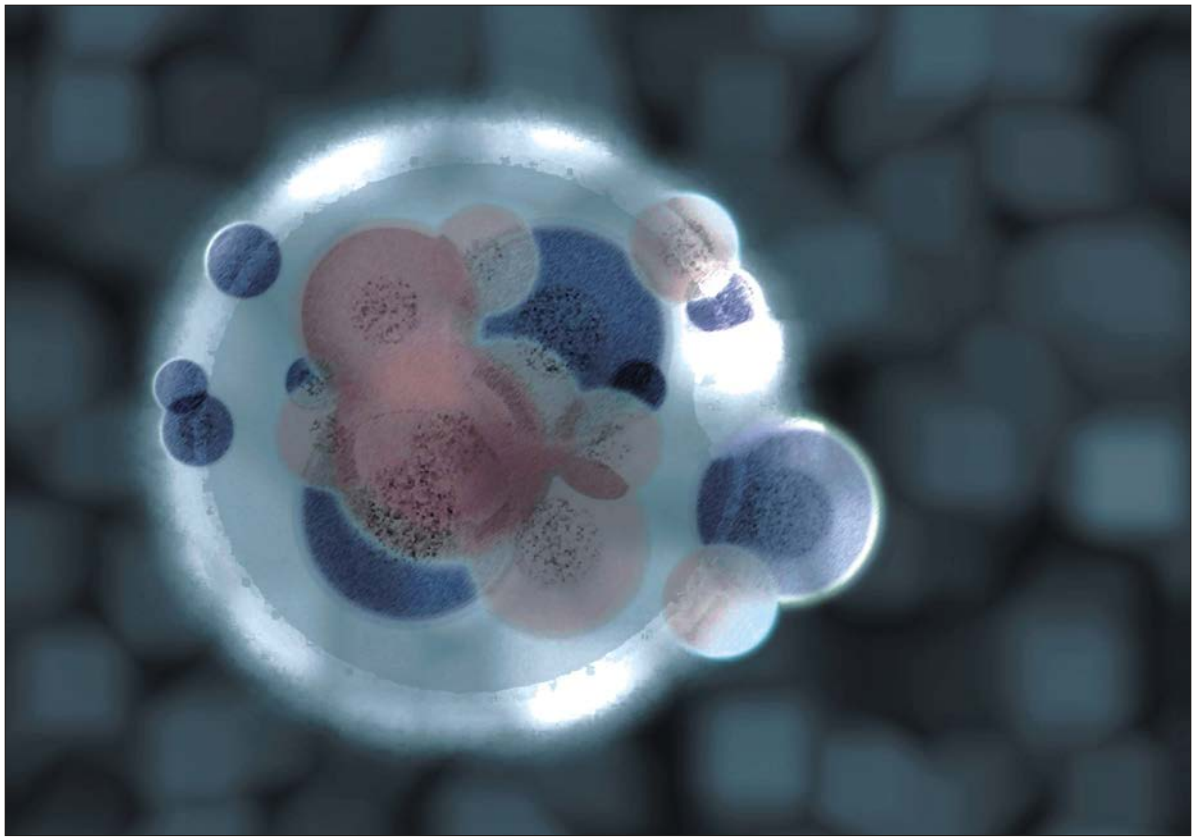




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Estimation of General and Specific Combining Ability and Heterosis in New Varieties of Silkworm, *Bombyx mori* L.

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Abstract: In this research six economical characteristics in seven lines with Chinese and Japanese origin and their hybrids were studied. The results showed that the General Combining Ability (GCA) of Japanese lines was significant for all the characteristics ($p < 0.01$), while GCA of Chinese lines for resistance characteristics were insignificant and for productive characteristics were very significant ($p < 0.0001$). In Japanese lines GCA of the number of survived larvae and pupae and also the percentage of pupa survival in line 101433 were 14.84, 34.55 and 10.65, respectively. These were higher than other groups. The highest GCA of mentioned characteristics in Chinese lines was for line Koming 1 and they were 5.86, 9.00 and 2.82, respectively. Specific Combining Ability (SCA) for productive characteristics and the number of survived larvae was considerable ($p < 0.05$) and for the number of survived pupa and the percentage of pupa survival was insignificant. SCA of resistance characteristics in the hybrid of Xihang 1 $\times$ Koming 1 were 18.41, 21.62 and 5.00, respectively which were higher than others. Chinese lines have high resistance and low genetic diversity for resistance characteristics and Japanese lines have low genetic divergence and high mean of resistance characteristics. Increasing non-additive genetical variance of cocoon characteristics show high efficiency of heterosis on above characteristics, in a way that the percentage of heterosis of productive characteristics, especially the cocoon shell weight, was higher than the percentage of heterosis obtained in resistance characteristics.

Key words: Silkworm, heterosis, general and specific combining ability

INTRODUCTION

The primary goal of silkworm breeding programs is to bring together the desirable genes in appropriate combinations in order to improve the genetic performance for maximizing the yield and productivity per unit area^[1]. Therefore, production of new strain and hybrid with high general and specific combining ability and high hybrid vigour can improve the sericultural yield.

Combining ability analysis is the most widely used biometrical tool in determining promising parents and hybrids and detecting relative magnitude of genetic variability^[1,2].

Also many researchers reported the presence of positive heterosis of a few characters in new silkworm hybrids and suggested that the function and structure of additive gene action is responsible for this phenomenon^[3-7]. Datta and Pershad^[8] observed that additive genes play more important role in the inheritance of some economical characters such as fecundity, larval

duration, pupation rate cocoon shell ratio and filament length and subsequently many documents confirm this result^[3,9-11].

Furthermore several researchers reported that the cross between female of polyvoltine with bivoltine's male causes the enhancement of heterosis^[12]. Mladenov^[13] had reported the structure of heterosis phenomenon in intra and inter crosses of lines. In the other hand it has been demonstrated that SCA in double cross hybrids is more than single cross hybrids^[14]. Another research determined that the combination of double cross rarely show complete heterosis for more than two characteristics^[15].

During last decade quite a good number of promising bivoltine breeds/hybrids were evolved at Iran Sericultural Research Center^[16,17]. The quantitative and qualitative characters of some of these newly evolved hybrids were well documented^[18,19]. Therefore, present study estimated general and specific combining ability and manifestation of heterosis in newly evolved varieties of silkworm in Iran to introduce the commercial sericulture.

MATERIALS AND METHODS

Seven new silkworm lines including Xihang 1, Xihang 2, Xihang 3 and 101433 of Japanese origin and Koming 1, Koming 2 and Y with Chinese origin were used. Also twelve hybrids obtained from crosses between Japanese female and Chinese male (incomplete diallele cross) including $X_1 \times K_1$, $X_1 \times K_2$, $X_1 \times Y$, $X_2 \times K_1$, $X_2 \times K_2$, $X_2 \times Y$, $X_3 \times K_1$, $X_3 \times K_2$, $X_3 \times Y$, 101433 $\times K_1$, 101433 $\times K_2$ and 101433 $\times Y$ were used.

This study as a factorial experiment in Complete Randomized Design with five replications for each group was conducted. To estimate the single cocoon parameters in each replication 25 female and male cocoons were recorded. Data were analyzed using SAS software in GLM^[20]. The comparison of the means was done using Duncan's Multiple Range Test in 5% level. Each replication consists of 250 IV instar larvae and in total 27500 larvae were used.

Feeding and other conditions of larval rearing were conducted following the standard procedure^[21]. Six measured characteristics include characteristics, which determine larval and pupal resistance (the number of survived larvae and pupae and the percentage of pupal survival) and productivity characteristics (single cocoon weight, single cocoon shell weight and the percentage of single cocoon shell).

For statistical trial of combining ability parameters bottom statistical model was utilized:

$$y_{ijkl} = \mu_i + jGCA_{ij} + cGCA_{ik} + SCA_{ijk} + e_{ijkl}$$

Where:

- y_{ijkl} = the record of lth replication obtained from cross between jth Japanese line and kth Chinese line for ith characteristic
- μ_i = the mean of ith characteristic
- $jGCA_{ij}$ = the effect of GCA of jth Japanese line for ith characteristic
- $cGCA_{ik}$ = the effect of GCA of kth Chinese line for ith characteristic
- SCA_{ijk} = the effect of SCA of hybrid obtained from cross between jth Japanese line with kth Chinese line for ith characteristic
- e_{ijkl} = the effects of residue factors

In the above model all the effects were random and to estimate the variance parameters the model followed VARCOMP procedure (REML) of SAS software.

Utilized statistical model for heterosis trial was as follows:

$$H_{ijk} = \mu_i + V_{ij} + e_{ijk}$$

Where:

- H_{ijk} = percentage of heterosis of kth replication of jth hybrid for ith characteristic
- μ_i = the mean of heterosis percentage for ith characteristic
- V_{ij} = the effect of jth hybrid on the percentage of heterosis of ith characteristic
- e_{ijk} = the effects of residual factors

To estimate the parameters of specific (SCA_{ij}) and general combining ability (GCA_i) and heterosis (H_{ij}) following equations were used:

$$GCA_i = M_i - \mu$$

$$SCA_{ij} = M_{ij} - (GCA_i + GCA_j + \mu)$$

$$H_{ij} = \frac{M_{ij} - \left(\frac{M_i + M_j}{2} \right)}{\left(\frac{M_i + M_j}{2} \right)} \times 100$$

Where:

- μ = the mean of all of the hybrids
- M_i = the mean of hybrids which obtained from ith line
- M_{ij} = the mean of hybrid which obtained from cross between I and j lines

RESULTS AND DISCUSSION

The highest mean of cocoon weight (g), cocoon shell weight (g) and the percentage of cocoon shell (percent) were, respectively for lines Koming 1 (1.477), Y (0.336) and Y (23.18) and the least mean of above characteristics were for lines Xihang 2 (1.153), Xihang 2 (0.243) and Koming 2 (18.89). Line Koming 2 (214.8, 193.8 and 90.19, respectively) had the highest mean of the number of leaving larvae and pupa and the percentage of pupal survival (percent) and the lowest mean of these characteristics are, respectively for lines Xihang 1 (146.8), Xihang 1 (113.2) and Xihang 3 (74.29). The results show that the amount of resistance of Chinese lines to environmental conditions is more than Japanese lines. Also Koming 2 and Xihang 1, respectively had the highest and the lowest resistance between the studied varieties (Table 1).

Variance analysis of studied characteristics for combining ability (Table 2) shows that the effect of Japanese lines GCA was significant for all of the results ($p < 0.1$). The effect of GCA of Chinese lines was

Table 1: The average of six economical parameters in different lines of silkworm

Parameters	Cocoon weight (g)	Cocoon shell weight (g)	Shell ratio (%)	Survived larvae (No.)	Survived pupae (No.)	Survival rate (%)
Xihang 1	1.356b	0.303b	22.39b	146.8b	113.2c	77.75c
Xihang 2	1.153c	0.243e	21.21c	187.6a	151.0b	80.35bc
Xihang 3	1.355b	0.274d	20.10d	202.0a	152.0b	74.29c
Koming 1	1.477a	0.287c	19.70d	214.2a	188.2a	87.98ab
Koming 2	1.328b	0.248e	18.89e	214.8a	193.8a	90.19a
Y	1.463a	0.336a	23.18a	206.6a	164.6ab	79.50bc

There is no significant difference between the numbers that are shown with the same letter(s) in columns

Table 2: Analysis of variance (MS) for GCA and SCA in different groups of silkworm

Parameters	Productive characteristics				Tolerance characteristics			
	df	Cocoon weight	Cocoon shell weight	Shell ratio	df	Surv. larvae	Surv. pupae	Survival rate
jGCA	3	2.13****	0.120****	56.87****	3	2430.47**	13871.98****	1607.32****
cGCA	2	1.15****	0.140****	112.71****	2	687.41	1890.75	219.60
SCA	6	0.74****	0.150****	231.57****	6	1177.68*	2140.60	169.75
Error	3066	0.07	0.004	9.54	43	413.04	1078.35	156.36

* Significant at level 5%, ** Significant at level 1%, *** Significant at level 0.1%, **** Significant at level 0.01%,
jGCA: General combining ability in Japanese line, cGCA: General combining ability in Chinese line, SCA: Specific combining ability in silkworm hybrids

Table 3: Estimation of GCA and SCA for economical characters of different silkworm breed

	Silkworms	Cocoon weight	Cocoon shell weight	Shell ratio	Survived larvae	Survived pupae	Survival rate
jGCA	Xihang 1	0.0573a	0.0126a	-0.093b	-15.90c	-38.85c	-13.43c
	Xihang 2	-0.0687c	-0.0168b	-0.057b	-2.81cb	9.03b	6.10ab
	Xihang 3	-0.0392b	-0.0114b	-0.198b	3.82ab	-3.36b	-2.41b
	101433	0.0322a	0.0107a	0.283a	14.84a	34.55a	10.65a
cGCA	Koming 1	0.0137a	-0.0016b	-0.201b	5.86	9.00	2.82
	Koming 2	-0.0370b	-0.0091c	-0.029b	2.12	5.58	2.32
	Y	0.3090a	0.0137a	0.354a	-8.92	16.29	-5.75
	101433	0.0322a	0.0107a	0.283a	14.84a	34.55a	10.65a
SCA	Xihang 1×Koming 1	-0.0134a-c	-0.0067cd	-0.166c	6.68a	5.20	1.84
	Xihang 1×Koming 2	0.0186b-d	0.0128a-c	0.735b	18.41a	21.62	5.00
	Xihang 1×Y	-0.0054ab	-0.0073ab	-0.753c	-24.15b	-25.11	-6.24
	Xihang 2×Koming 1	-0.0443g	-0.0187e	-0.577c	2.19a	11.32	3.41
	Xihang 2×Koming 2	0.0594f	0.021d	0.456b	-13.07a	-27.26	-8.19
	Xihang 2×Y	0.00017f	0.0192a-c	1.239a	16.17a	19.71	4.83
	Xihang 3×Koming 1	0.0245d-f	0.0151d	0.642b	-4.24a	-13.19	4.82
	Xihang 3×Koming 2	-0.0674g	-0.035f	-1.508d	-3.76a	1.73	1.21
	Xihang 3×Y	0.0299c-e	0.0137a-c	0.664b	8.93a	13.80	4.52
	101433×Koming 1	0.0466a	0.0164a	0.309b	-4.66a	-8.00	-2.78
	101433×Koming 2	-0.0115ef	-0.0024d	0.062b	-1.52a	2.22	0.84
	101433×Y	-0.0427c-e	-0.0172b-d	-0.445b	7.12a	7.49	2.54

There is no significant difference between the numbers that are shown with the same letter(s) in each column. Each group of data without any letter has got no significant differences jGCA: General Combining Ability in Japanese line cGCA: General Combining Ability in Chinese line
SCA: Specific combining ability in silkworm hybrids

inconsiderable for resistance characteristics and for the productive characteristics was very significant ($p < 0.0001$).

SCA was significant for the number of survived larvae ($p < 0.05$) and insignificant for the number of survived pupae and the percentage of pupal survival. SCA of productive characteristics was very considerable ($p < 0.0001$). As a result the cocoon characteristics are under both additive and non-additive genetical effects, while the effect of non-additive genetical effects on resistance characteristics seems very negligible. Significance of GCA of the larval and pupal resistance characteristics in Japanese lines (although the mean of these characteristics is low) is representative of the additive effects on genetical control of these characteristics. Therefore it could be expected that with the selection of Japanese lines with the better resistance characteristics as a parental breeds for combination, the resistance of the hybrids will considerably increase. In

contrast, in Chinese lines (although they have higher resistance characteristics), the resistance characteristics have lower additive genetical variance and it is expected that in the resulting hybrids considerable improvement does not occur.

Parents possessing high GCA are generally considered for population development and for initiation of pedigree breeding, as it is heritable and can be fixed. Parents with high GCA produce high heterosis as GCA consists of additive effects and additive×additive type of interactions. SCA consists of non-additive effects, dominant effects and other interactions^[22]. SCA is not heritable and therefore it cannot be utilized in pure line breeding. Hybrids with high SCA are useful for commercial exploitation^[1].

Probably Chinese lines through the years have been affected by natural selection and with the elimination of individuals with weak resistance characteristics through

Table 4: Components of variance for GCA and SCA in Japanese and Chinese lines of silkworm

Parameters	Cocoon weight	Cocoon shell weight	Shell ratio	Survived larvae	Survived pupae	Survival rate
σ_{JGCA}^2	0.00240	0.000000	0.00	84.56	779.56	98.30
σ_{cGCA}^2	0.00051	0.000028	0.00	0.00	15.44	6.89
σ_{SCA}^2	0.00250	0.000540	0.66	165.41	765.18	6.70
$\frac{\sigma_{JGCA}^2}{\sigma_{SCA}^2}$	0.99000	0.000000	0.00	0.51	2.94	14.66
$\frac{\sigma_{cGCA}^2}{\sigma_{SCA}^2}$	0.21000	0.050000	0.00	0.00	0.06	1.03

the generations, genetical resistance of above lines has increased and their genetical diversity has decreased. In contrast Japanese lines have not tolerate considerable selection and have a related low resistance and high genetical diversity. Previously there have been such reports about other countries lines, too^[10,23].

In Japanese lines the highest GCA of resistance characteristics (the number of leaving larvae and pupae and the percentage of pupal survival) and the percentage of cocoon shell are belong to 101433 (14.84, 34.55, 10.65 and 0.283, respectively) and cocoon weight and cocoon shell weight for line X_1 (0.0573 and 0.0126, respectively) (Table 3). The lowest GCA of resistance characteristics was for line X_1 (-15.9, -38.85 and -13.43, respectively), of the cocoon weight and the cocoon shell weight are for X_2 (-0.0687 and -0.0168, respectively) and of cocoon shell percentage belong to X_3 (-0.198).

The results of previous studies showed that 101433 varieties have weak resistance and high production ability. With respect to high GCA of resistance characteristics and also positive combining ability of productive characteristics, this line is an appropriate choice for combination. The mean and GCA of resistance characteristics is low in line X_1 , while for the productive characteristics this is convert. In Chinese lines GCA of resistance characteristics of line K_1 is higher (5.86, 9.00 and 2.82) and in line Y was lower (-8.92, -16.29 and -5.75). The combining ability of productive characteristics (cocoon weight, cocoon shell weight and percentage of cocoon shell weight) in line Y were higher (0.0309, 0.0137 and 0.354), while the mentioned parameter was lower in lines K_2 (-0.037), K_2 (-0.0091) and K_1 (0.201), respectively. The conflict of GCA of resistance characteristics and cocoon characteristics represents the negative genetic correlation between them (Table 2). The mean and GCA of resistance characteristics in line Y was low and the mean and GCA of productive characteristics in above line was high. As shown, the amount and diversity of GCA in resistance characteristics between Japanese varieties was

more than Chinese lines. These results were reported previously^[24-26] and recent research is the confirmation of those.

SCA of the number of survived larvae and pupae and the percentage of pupal survival rate in hybrid $X_1 \times K_1$ (18.41, 21.62 and 5.00, respectively), of cocoon weight and cocoon shell weight in $X_2 \times K_2$ (0.021 and 0.0594, respectively) and of cocoon shell percentage and cocoon shell weight in $Y \times X_2$ (1.239) was higher. SCA of number of survived larvae and pupae and the percentage of pupal survival in hybrid $Y \times X_1$ (-24.15), $X_2 \times K_2$ (-27.26) and $K_2 \times X_2$ (-8.19) and the productive characteristics in hybrid $X_3 \times K_2$ (-0.0674, -0.035 and -1.508) was lower. The amount of SCA of hybrids with genetical differences at maternal lines (with respect to their sign and GCA) has direct correlation. Generally one of maternal bases at higher crosses with respect to SCA has had negative GCA. Which shows sometimes a weak cross with respect to GCA, the results due to positive SCA will have good efficiency.

In conclusion, $K_2 \times X_1$ for resistance characteristics and hybrid $K_2 \times X_2$ for productive characteristics show the highest SCA and therefore it is expected that their progenies have better means in above characteristics.

The results show that the variance of GCA in resistance characteristics (which represents additive genetical variance) is much higher in Japanese lines compared to Chinese lines (Table 4). As a result in Chinese lines the non-additive genetical variance has a main role in diversity of resistance characteristics, while in Japanese lines the additive genetical variance for the number of survived larvae and pupae and the percentage of pupal survival were respectively, 0.5, 3 and 15 times higher. The part of additive and non-additive genetical variance from the total variance of cocoon weight in Japanese lines was almost equal but in Chinese lines the cocoon weight was more affected by non-additive genetical effects. Also due to the results of this research the cocoon shell weight and the percentage of cocoon shell weight are very much affected with non-additive genetical effects.

Table 5: Analysis of variance (MS) for percentage of Heterosis (H) in silkworm hybrids

	Productive characteristics				Tolerance characteristics			
S.O.V	df	Cocoon weight	Cocoon shell weight	Shell ratio	df	Survived larvae	Survived pupae	Survival rate
H	11	191.10***	426.28	71.17	11	400.76	1542.99	896.03*
Error	30	41.95	221.43	86.16	31	758.50	890.08	405.64

* Significant at level 5%

*** Significant at level 0.1%

Table 6: Percentage of heterosis for Survival and productive characters in different hybrids

Silkworm hybrids	Cocoon weight	Cocoon shell weight	Shell ratio	Survived larvae	Survived pupae	Survival rate
Xihang 1×Koming 1	19.84bc	25.69	4.61	19.52	3.68	-11.86ab
Xihang 1×Koming 2	26.14ab	39.17	10.67	23.28	9.23	-10.64ab
Xihang 1×Y	32.14a	38.62	5.39	-5.32	-29.66	-29.27b
Xihang 2×Koming 1	16.42b-d	21.02	3.64	10.79	24.91	12.90a
Xihang 2×Koming 2	26.62ab	44.33	13.33	-0.18	-8.62	-8.72ab
Xihang 2×Y	23.105b	37.24	11.16	12.26	21.43	8.60ab
Xihang 3×Koming 1	16.10b-d	31.30	13.84	3.02	-5.55	-7.70ab
Xihang 3×Koming 2	7.35d	8.88	0.51	7.35	21.00	13.72a
Xihang 3×Y	15.68b-d	22.86	7.29	10.00	31.28	19.54a

There is no significant difference between the numbers that are shown with the same letter(s) in columns

The difference between hybrids in heterosis percentage for cocoon weight ($p < 0.001$) and the percentage of pupae survival ($p < 0.05$) was significant and for the rest of the characteristics was insignificant (Table 5). The percentage of heterosis of cocoon weight, the cocoon weight shell, the percentage of cocoon shell and the number of survived larvae in the hybrids from $X_1 \times Y$ (32.14), $X_2 \times K_2$ (44.33), $X_3 \times K_1$ (13.84) and $X_1 \times K_2$ (23.28) and the characteristics of the number of survived pupae (31.28) and the percentage of pupal survival (19.54) in the hybrid of $X_3 \times Y$ were higher. The percentage of resistance characteristics of hybrid $X_1 \times Y$ (-5.32, -29.66 and -29.27, respectively) and the productive characteristics in the hybrid $X_3 \times K_2$ (7.35, 8.88 and 0.51, respectively) were lower. The comparison between arrangement of SCA and heterosis for studied characteristics showed that these arrangements are almost similar. In the hybrids of $X_2 \times K_1$, $X_3 \times K_2$ and $X_3 \times Y$ the number of survived pupae and the percentage of cocoon shell and in the rest of the hybrids the cocoon shell weight and the pupal survival percentage, respectively showed the highest and the lowest percentage of heterosis (Table 6). In most of the varieties, the percentage of heterosis in the productive characteristics was higher than the percentage of heterosis for the survival of the larvae and the pupae. Between the cocoon characteristics, cocoon shell weight and cocoon shell percentage had the highest and the lowest amount of heterosis, respectively. This represents the high portion of non-additive effects in genetical control of this characteristic. The percentage of the heterosis varied among the resistance characteristics in the studied hybrids and in some of them was negative. High percentage of heterosis in productive characteristics could be illuminated with respect to the additive and non-additive genetical variance of cocoon characteristics (Table 4). As it is shown non-additive genetical effects have a high portion the genetical

variance of all the cocoon characteristics. In contrast, non-additive genetical variance has a smaller role in expression of phenotype of resistance characteristics and it is expected that the mentioned characteristics (especially the percentage of pupal survival) are less affected by heterotic effects^[18].

The cocoon characteristics are important economical characteristics of silkworm and due to their high heredity, the efficiency of direct selection of them is very high. The results of recent research shows that the efficiency of using the heterosis in the improvement of the mean of cocoon characteristics in the hybrids especially for the cocoon shell weight will be manifold than the interstrain selections. This clarifies the undeniable role of heterosis in the technology of silkworm egg production. As a result, the better hybrid must be determined from adding the amounts of the heterosis of the characteristics related to cocoon and resistance and with using other information like GCA and SCA, evolvement of appropriate maternal bases to produce commercial silkworm eggs could be conducted. While the aim of breeding is to enhance the hybrid egg of silkworm and keeping high production level, the crossing of lines X_3 and Y is recommended. SCA and heterosis of resistance characteristics in hybrid of $X_3 \times Y$ is high. In addition the amount of mentioned parameters for the productive characteristics in above combination is in a proper level. Therefore, the combined silkworm egg obtained from the crossing of mentioned lines in contaminated rearing environments and inappropriate conditions will have a good efficiency.

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