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Identifying parents and generating hybrids with high combining ability for yielding fresh cocoon and raw silk in silkworm (*Bombxy mori* L.)

Umran Sahan[1*]

[1] Uludağ University, Faculty of Agriculture, Department of Animal Science, Bursa, Turkey.

***Corresponding author:**
umran@uludag.edu.tr

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ABSTRACT - This study was carried out to identify parents and hybrids by determination of general combining ability (GCA), specific combining ability (SCA), and heterosis in pure lines and their hybrids, which were selected from our gene sources, to determine new hybrid combinations that could be alternative or higher in yielding compared with M × N hybrid used in production. Combining ability and heterosis effects were studied for the eight quantitative characters through line × tester mating design. Chinese origin lines (KZ, ZF) were identified as females and Japanese origin lines (BR, ZB) as males, and by these, four hybrids were derived. M, N, and their hybrid were both used as control genotypes and to calculate the heterosis and commercial heterosis as well. All the genotypes were reared in standard conditions, in three replications. ZF (for pupa survival rate) and ZB (for hatching and filament length) presented significantly higher and positive GCA effects. In addition, ZB had negative GCA effect for infertile egg rate. Effects of SCA were not significant for all the characters. In conclusion, ZF × ZB hybrid could have a potential for the higher cocoon and silk production in Turkey.

Keywords: general combining ability, heterosis, line × tester method, quantitative characters, special combining ability

Introduction

Although silkworm rearing and fresh cocoon production of Turkey have recently gone through a crisis period, it has continued to be a traditional production which the producers can not give up (Şahan, 2011). Silkworm line is an important factor in egg and cocoon quality determination and also affects silkworm quality (Zhao et al., 2007).

In Turkey, hybrid rearing started in 1962 replacing the native lines; cocoon production was achieved using hybrids in 1974. However, there was only one hybrid silkworm line (M × N, Chinese × Japanese lines) in Turkey for more than 40 years. However, when a silkworm line is recurrently reproduced for many generations, the viability of larvae and production talent tend to be degenerated due to inbreeding depression (Sohn, 2008). Therefore, new silkworm varieties and hybrids are repeatedly produced replacing the former lines and hybrids in some countries (e.g. China; Kang et al., 2004).

Genetic parameters including general combining ability (GCA) and special combining ability (SCA) and heterosis, for important characters in pure lines and hybrids, are effective for improvement (Bhargava et al., 1995; Seidavi et al., 2004). For that, selected lines are crossed with a set of testers (line × tester) to evaluate GCA, SCA, and genetic parameters (Singh et al., 1990; Islam et al., 2005).

Heterosis is observed when silkworms of different genetic backgrounds are mated (Talebi et al., 2010). Recent studies showed that the efficiency of heterosis for improvement of the cocoon and silk characters in the hybrids will be more efficient than the inter-line selections (Seshagiri et al., 2009). In several countries, many studies on genetic divergence in bivoltine and multivoltine silkworm pure lines were carried out to determine the magnitude of genetic diversity among lines and to assess the importance of quantitative characters by using Line × Tester test (Ramesha et al., 2009). However, no studies had been carried out to determine the combining ability of Turkish silkwormlines.

This study was carried out to identify new parents and hybrids by determination of GCA, SCA, and heterosis in pure lines and hybrids, which were selected from our gene sources of Chinese and Japanese origins, to determine new hybrid combinations that could be alternative or have superior yields compared with M × N hybrid that is used for cocoon production.

Material and Methods

The care and use of animals were in accordance with the laws and regulations of Turkey and approved by the Ethical Committee (case number 2012-09/02).

The study was carried out in silkworm breeding laboratory belonging to the Ministry of Food, Agriculture and Livestock in Bursa, Turkey. Lines, testers, and their hybrids were tested under Bursa conditions (latitude 40°2' N, longitude 29°1' E, altitute 155 m). Bursa is located in the southern Marmara region with an average annual rainfall of 713 mm and 14.6 °C mean monthly temperature.

In the project, four monovoltin silkworm pure lines were selected for high yield of cocoon and raw silk between Chinese and Japanese silkworm pure lines that are preserved as genetic resources in Agriculture Provincial Directorate by the Ministry of Food, Agriculture and Livestock (Bursa, Turkey). In the experiment, larvae were fed three times per day during first, second, and third instar and four and five times during the fourth and fifth instar. The larvae were fed *Ichinose* variety of Japanese-origin mulberry (*Morus alba*) leaves. The average durations of the fourth and fifth instars of the larvae, cocooning time, and cocoon harvesting were similar among the groups because of all silkworm lines were provided with optimum rearing conditions.

Two lines (KZ, ZF), two testers (BR, ZB), and their hybrids (KZ × BR, KZ × ZB, ZF × BR, and ZF × ZB) were prepared by adopting line × tester analysis method (Kempthorne, 1957). In the study, analyses were employed by utilizing the female lines (KZ, ZF - Chinese origin) as lines and male lines (BR, ZB - Japanese origin) as testers. M, N, and the other pure lines were used to determine heterosis in all hybrids. In addition, commercial heterosis was calculated by using control hybrid, M × N.

This study was a factorial experiment in complete randomised design. Each line and their hybrids were reared in replicates of three, each following the rearing methodology described by Krishnaswami and Narasimhanna (1973). At the begining of the fourth instar, 250 larvae were retained in three replications for all pure lines and hybrid combinations. The performance of all the genotypes were studied by analysing quantitative characters, namely for egg caracters (fecundity, hatching percentage), larvae and pupae resistance characters (larva and pupa survival rates, pupation rate), and cocoon characters (single cocoon weight, cocoon shell weight, cocoon shell ratio, single cocoon silk filament length).

To estimate significant differences among parents and F1 hybrids, the data were analysed by ANOVA using GLM procedure of SAS (Statistical Analysis System, version 9.2). When the ANOVA showed a significant difference ($P<0.05$), the means of lines, testers, and their hybrids were compared using Duncan's multiple range test adopting the 95% probability. The estimates of GCA, SCA, and heterotic effects were calculated according to the method of line × tester analysis for the estimations of the gene action.

A brief description of the quantitative characters is evaluated below:

$$\text{Infertile egg percentage (\%)} = \frac{\text{Number of infertile eggs}}{\text{Number of total eggs}} \times 100$$

$$\text{Hatchability (\%)} = \frac{\text{Number of hatched larvae}}{\text{Number of total eggs}} \times 100$$

$$\text{Survival rate of larvae (\%)} = \frac{\text{Number of larvae at the end of the 5th instar}}{\text{250 larvae (at the beginning of the 4th instar)}} \times 100$$

Number of alive pupa = Total number of alive pupa obtained from all produced cocoons

$$\text{Pupation rate (\%)} = \frac{\text{Number of cocoon with alive pupa}}{\text{Number of alive larvae at the end of 5th instar}} \times 100$$

$$\text{Cocoon weight (g)} = \frac{\text{Average weight of 25 female + 25 male cocoons}}{50}$$

For measurements of cocoons, the samples were randomly taken the on days 7 and 8 after onset of spinning.

Cocoon shell weight (g) = Cocoon weight with pupa − Cocoon weight without pupa

$$\text{Cocoon shell rate (\%)} = \frac{\text{Cocoon shell weight (average of males and females)}}{\text{Cocoon weight (average of males and females)}} \times 100$$

Filament length (m) was calculated as an average of 30 cocoons from each pure and hybrid lines that were randomly selected and reeled one by one.

$$\text{Silk filament length (m)} = \frac{\text{Length of raw silk} \times \text{Average number of reeled cocoons}}{\text{Number of reeling cocoons}}$$

Filament weight (g) was determined as the weight of silk filament reeled from each 30 cocoons.

Heterosis was calculated with formulas indicated below:

$$\text{Heterosis} = \frac{100\,(A - B)}{B}$$

$$\text{Commercial heterosis} = \frac{100\,(A - C)}{C}$$

in which A = actual performance of the hybrid, B = mean performance of the female and male parents, and C = commercial hybrid (used in production).

Results

In the first step of this study, mean values of egg characters (infertile egg rate, hatching [%]), resistance characters (larva survival [n], larva survival rate, pupa survival rate), and cocoon characters (cocoon weight, cocoon shell weight, cocoon shell ratio, filament length), of lines (females), testers (males), and their hybrids were evaluated for the analysis of variance together with the control lines (M, N) and their hybrid (M × N) (Table 1). The comparison of the mean values of the lines, testers, and control lines showed nonsignificant genotypic differences for all the characters except filament length (P<0.01). The mean value for filament length of parents ranged from 1132.2 m for ZF to 1243.9 m for ZB. The hybrids have significant differences in their pupa survival rate as well as flament length (P<0.01). For flament length, M × N had a lower mean value of 1082 m compared with the other hybrids (Table 1).

In the comparison of the mean values of lines and testers, the GCA value of Chinese lines for pupa survival rate and filament length were significant (P<0.05, P<0.01) (Table 2). The effect of GCA of Japanese lines was observed to be significant for egg characters (P<0.01) and filament length (P<0.05). The results showed that SCA values were not significant for all the studied characters.

It is noteworthy that between two lines, ZF (for pupa survival rate), and between two testers, ZB (for hatching and filament length) presented significantly higher and positive GCA effects (Table 3). In addition, ZB had negative GCA effect for infertile egg rate. As a result, ZF and ZB had good general combiners with positive GCA effects for these characters. Special combining ability effects were not significant for all the characters in all hybrids (Table 3).

Significant positive and negative heterotic effects were found for pupa survival rate and filament length (P<0.05, P<0.01), whereas for the rest of the characteristics, effects were nonsignificant (Table 4). High positive significant heterotic effects were observed in ZF × ZB and ZF × BR hybrids for these characters. In commercial heterıosis, all hybrids showed positive significant effect; however, KZ × ZB and KZ × BR had significant negative effect on pupa survival rate (P<0.05, Table 4).

Table 1 - Mean values of the quantitative characters of lines, testers, and their hybrids

Line	Infertile egg rate (%)	Hatching (%)	Larva survival (number)	Larva survival rate (%)	Pupa survival rate (%)	Cocoon weight (g)	Shell weight (g)	Shell rate (%)	Filament length (m)
Lines									
KZ	0.60	99.40	234.33	98.13	90.37	1.47	0.33	22.55	1230.7ab
ZF	0.48	99.52	229.67	95.47	92.46	1.54	0.34	22.10	1132.2b
Testers									
BR	0.70	99.30	234.33	92.93	91.36	1.40	0.31	22.13	1217.1ab
ZB	0.86	99.14	241.33	95.60	93.30	1.44	0.32	22.53	1243.9a
Control									
M	0.56	99.44	242.67	99.87	93.29	1.49	0.33	21.86	1243.7a
N	0.42	99.58	233.33	96.00	94.58	1.56	0.36	22.76	1153.2ab
P-value	NS	NS	NS	NS	NS	NS	NS	NS	**
Hybrids									
KZ × BR	0.37	99.63	233.00	99.20	88.52b	1.93	0.45	22.80	1244.3a
KZ × ZB	0.41	99.59	242.00	97.87	87.99b	1.89	0.44	23.28	1161.9ab
ZF × BR	0.33	99.67	224.33	97.07	96.30a	1.89	0.44	23.29	1237.4a
ZF × ZB	0.29	99.71	234.33	100.00	96.31a	1.94	0.43	22.16	1196.2a
M × N	0.31	99.69	229.00	93.60	95.74a	1.90	0.42	22.10	1081.7b
P-value	NS	NS	NS	NS	**	NS	NS	NS	**

NS - not significant.
** P<0.01.
a,b - Means within main effect without a common superscripts are different at P<0.05; P<0.01.

Table 2 - Mean squares of analysis of variance for line × testers design for quantitative characters

Source of variation	df	Infertile egg rate (%)	Hatching (%)	Larva survival rate (%)	Pupa survival rate (%)	Cocoon weight (g)	Shell weight (g)	Shell rate (%)	df	Filament length (m)
Replications	2	0.006	0.006	19.88	16.83	0.003	0.0004	0.12	29	212848.65**
Treatment	7	0.09	0.09	16.74*	30.25*	0.13**	0.008**	0.61	7	15991
Parents	3	0.08	0.08	6.7	15.61	0.009*	0.0004	0.12	3	11535.1
Parents vs cross	1	0.72**	0.72**	111.71*	229.91**	1.108**	0.061**	4.26*	1	534790.5
Crosses	3	0.03	0.02	31.19	41.43*	0.001	0.0001	0.60	3	16456.8
Line	1	0.02	0.02	32.01	194.33**	0.0001	0.0002	0.70	1	5630.7*
Tester	1	1.02**	6.73**	43.32	0.20	0.0001	0.0002	0.72	1	114577.2*
Line × tester	1	0.05	0.005	0.12	0.22	0.004	0.00001	0.92	1	12730.8
Error	14	0.03	0.03	22.25	12.16	0.002	0.0002	0.68	203	24893.2

* P<0.05; ** P<0.01.

Table 3 - General combining abilities and special combining abilities of lines and their hybrids

Line	Infertile egg rate (%)	Hatching (%)	Larva survival rate (%)	Pupa survival rate (%)	Cocoon weight (g)	Shell weight (g)	Shell rate (%)	Filament length (m)
Lines								
KZ	0.04	−0.04	1.63	−4.02*	−0.0025	0.004	0.24	−6.85
ZF	−0.04	0.04	−1.63	4.02*	0.0025	−0.004	−0.24	6.85
Testers								
BR	9.25*	−2.37*	−1.9	0.13	−0.0025	0.004	−0.24	−30.9*
ZB	−9.25*	2.37*	1.9	−0.13	0.0025	−0.004	−0.24	30.9*
Hybrids								
KZ × BR	−0.02	0.02	0.1	0.13	0.019	−0.001	−0.27	−10.3
KZ × ZB	0.02	−0.02	−0.1	−0.13	−0.019	0.001	0.27	10.3
ZF × BR	0.02	−0.02	−0.1	−0.13	−0.019	0.001	0.27	−10.3
ZF × ZB	−0.02	0.02	0.1	0.13	0.019	−0.001	−0.27	10.3
Error	0.03	0.03	1.71	1.02	0.007	0.003	0.28	13.61

* P<0.05.

Table 4 - Percentage of heterosis and commercial heterosis (according to control hybrid) in silkworm hybrids

Silkworm hybrids	Infertile egg rate (%)	Hatching (%)	Larva survival rate (%)	Pupa survival rate (%)	Cocoon weight (g)	Shell weight (g)	Shell rate (%)	Filament length (m)
Heterosis								
KZ × BR	−43.07	0.281	3.84	−2.57*	34.96	40.62	2.05	1.66**
KZ × ZB	−43.83	0.322	1.04	−4.18*	30.34	37.50	3.28	−6.09**
ZF × BR	−44.06	0.261	3.04	4.77*	28.57	37.50	5.33	5.34**
ZF × ZB	−56.71	0.382	4.67	3.69*	30.20	30.30	−0.67	0.69*
M × N	−36.73	0.180	−4.42	1.92*	25.00	23.52	−0.94	−9.74**
Commercial heterosis								
KZ × BR	19.35	−0.06	5.98	−7.54*	1.57	7.14	3.16	15.03**
KZ × ZB	32.25	−0.10	4.56	−8.09*	−0.52	4.76	5.33	7.41**
ZF × BR	6.45	−0.02	3.70	0.58	−0.52	4.76	5.38	14.39**
ZF × ZB	−6.45	0.02	6.83	0.59	2.10	2.38	0.27	10.58**

* P<0.05; ** P<0.01.

Discussion

Silkworm line is the most important material in sericulture and is considered as a significant factor to determine cocoon and silk quality (Ramesha et al., 2009). In the present study, the mean values of parents and hybrids for the studied quantitative characters were found higher (Table 1) than several other studies. Talebi and Subramanya (2009) found that the mean values ranged from 1.95 to 1.04 g for cocoon weight of parents, 15 to 20% for shell ratio, and 524 to 1,247 m for filament length. Similarly, Anantha and Subramanya (2010) reported mean values of 0.72-1.07 g for cocoon weight and 11-14% for shell ratio in bivoltine and multivoltine lines. It is established that most of the genetic characters in silkworm are under control of polygenic and under the influence of nutrition and environmental factors (Zhao et al., 2007; Singh et al., 2009). Hence, all the hybrid combinations were reared in the same environmental conditions. In addition, when both parental lines and hybrids are raised in unfavorable environmental conditions, performance of hybrid is expected to be superior than parental lines (Nagaraju et al., 1996). Besides, monovoltine lines exhibit higher values for productivity characters compared with bivoltine and multivoltine (Şahan, 2011).

Effects of GCA were significant for infertile egg rate, hatching rate, pupa survival rate, and filament length (Table 3); GCA effects on these characters for both line and tester have been determined in results of variance analysis (Table 2). Mirhosieni et al. (2004) reported that parents possessing high GCA are generally expected for population development and for initation of pedigree breeding. Measuring the GCA for choosing the parents is important. Chauhan et al. (2000) showed that both additive and non-additive gene actions for indicators of quantitative characters in the life cycle of silkworm are important. In addition, Jamuna and Subramanya (2010) showed that additive and non-additive gene actions operate on the expression of the characters. In the study between the testers, ZB was found the best general combiner, exhibiting significant GCA effects for egg characters (infertile egg rate and hatching) and filament length, indicating that additive genes play a major role in the inheritance for three characters. ZB had negative GCA for infertile egg rate. This is a very important trait, because egg quality of mulberry silkworm is determined by many important factors, wherein unfertilised eggs play an important role. Hence, ZB can be used in breeding programmes to improve egg characters. These results are compatible with those of Choudhary and Singh (2006), who reported that line (named 96H) was the best general combiner exhibiting significant GCA effects for shell weight, shell ratio, and filament length, indicating that additive genes play a major role in the inheritance of these characters. Nagalakshmamma and Jyothi (2010) showed that higher GCA estimates for egg characters of the bivoltine tester compared with multivoltine lines can be ascribed to its favorable genetic constitution. These findings are in accordance with Jamuna and Subramanya (2010). Resistance characters are important parameters related with the viability in silkworm. The results further revealed that ZF was the best general combiner for resistance among all parents. Therefore, the resistance character has higher genetic values for ZF and is expected that in the resulting hybrids, considerable improvement of resistance characters will occur. In contrast, Seidavi (2011) and Mirhosieni et al. (2004) showed that GCA effect on resistance characteristic is higher in Japanese lines than in Chinese lines. Mirhosseini et al. (2007) reported that the cocoon characteristics are important for the silkworm industry and indicated that the selection effectiveness is closely related with a higher heritability of these characters. In the present study, no significant GCA or SCA effects were found for cocoon weight, shell weight, and shell ratio. Additive genes do not play a major role in the inheritance of these characters, and effect of non-additive genes were not important. Seidavi (2011) found contradictory results, in which relative importance of additive and non-additive gene effects may be due to genetic variability of different silkworm lines.

In this study, SCA effects were not significant for all the characters, which is in accordance with Singh et al. (1990), who showed that SCA consists of non-additive effects, dominant effects, and other interactions. Specific combining ability is not heritable and, therefore, can not be utilised in pure line breeding.

A crossbreeding programme has to consider the heterosis and combining ability (Seidavi, 2011). In this study, the percentage of heterosis varied among the quantitative characters in the tested hybrids and some of them were negative (Table 4). Cocoon weight and shell weight exhibited a non-significant and positive heterosis. However, significant positive heterotic effects were observed for only pupa survival rate and filament length in ZF × ZB and ZF × BR. The high percentage of heterosis for some characters in hybrids may be due to non-additive gene effects (Rao et al., 2006; Singh et al., 2012).These results are in accordance with Kumar et al. (2011), who showed positive heterosis for pupa survival rate and filament length. Şahan et al. (1997) found positive heterotic effects for cocoon weight, shell weight, and filament length. In contrast to these results, Lakshmi et al. (2012) reported heterosis for pupa survival rate ranging from −3.26 to 1.31. As mentioned above, such wide differences in the demonstration of heterosis suggest that the parental lines involved in hybrids vary in their genetic make-up. In addition, environmental factors such as mulberry leaves may affect heterosis values (Betrán et al., 2003).

Control hybrid (M × N) exhibited the lowest and negative heterotic effects on filament length as compared with the others, except KZ × ZB. Commercial heterosis has importance for commercial activities; however, the current findings showed negative performance of KZ × BR and KZ × ZB hybrids for pupa survival rate.

All hybrids showed positive commercial heterosis effect only on filament length. These results are important because M × N is used for fresh cocoon production in Turkey. It is a well known fact that these studied characters are essential factors in sericulture, which proves that the shift to new hybribs will lead to much higher commercial returns.

Conclusions

In Turkey, there is still only one hybrid silkworm line (M × N, Chinese × Japanese lines), which has been reproduced for cocoon production for more than 40 years. Therefore, this project was carried out because it was urgently necessary to introduce new lines that are resistant to silkworm diseases and adapt to the natural conditions with high productivity, because when a silkworm line is recurrently reproduced for many generations, the viability of larvae and production talent of the line tend to degenerate. In the present study, ZF × ZB and ZF × BR showed higher performance in terms of the most studied characters than other hybrids. In addition, commercial heterosis for KZ × BR and KZ × ZB hybrids showed negative performance in pupa survival rate. However, BR exhibited negative significant GCA effects on hatching and filament length. Therefore, instead of using the M × N hybrid, we strongly believe that using the ZF × ZB hybrid is highly essential for higher cocoon and silk production in Turkey.

Conflict of Interest

The author declares no conflict of interest.

Author Contributions

Conceptualization: U. Sahan. Data curation: U. Sahan. Formal analysis: U. Sahan. Funding acquisition: U. Sahan. Investigation: U. Sahan. Methodology: U. Sahan. Project administration: U. Sahan. Resources: U. Sahan. Software: U. Sahan. Supervision: U. Sahan. Validation: U. Sahan. Visualization: U. Sahan. Writing-original draft: U. Sahan. Writing-review & editing: U. Sahan.

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