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An analysis of oval cocoons strains of Iranian silkworm (*Bombyx mori* L.) germplasm

Najme Najafi[1], Alireza Seidavi[2*], Seyed Ziaeddin Mirhoseini[3], Shahabodin Gharahveysi[4], Anuradha H. Jingade[5]

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[1]MSc Graduated, Department of Animal Science, Qaemshahr Branch, Islamic Azad University, Qaemshahr, Iran
[2]Professor, Department of Animal Science, Rasht Branch, Islamic Azad University, Rasht, Iran
[3]Professor, Department of Animal Science, University of Guilan, Rasht, Iran
[4]Assistant Professor, Department of Animal Science, Qaemshahr Branch, Islamic Azad University, Qaemshahr, Iran
[5]Researcher, Central Sericultural Germplasm Resources Centre, Central Silk Board, Thally Road, Hosur, Tamil Nadu-635 109, India
[*]Corresponding author E-mail: alirezaseidavi@iaurasht.ac.ir

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Introduction: Silkworm (*Bombyx mori*) is an important economic insect which is used as experimental material for the evolution of economically important silkworm breeds. Breeds/strains thus evolved are maintained in germplasm stations for breeders and researchers to use (Chiang 1980). Silkworm germplasm resources have a very important role not only for use in sericulture and silkworm breeding, but also for experimentation in genetics and molecular biology research. Based on several parameters like nutrition, genetics, and biochemistry, silkworms have been classified into different inbred strains (ESCAP 1993). However, few studies are reported for classification of silkworm based on larval growth potential. The Islamic Republic of Iran has valuable silkworm genetic resources which differ in cocoon shape. The present study was carried out to evaluate and identify oval cocoon bivoltine breeds suitable for rearing as germplasm resources under climatic conditions in Iran.

Material and method: The present study was conducted in the Iran Silkworm Research Center (ISRC) and Islamic Azad University, Qaemshahr Branch, Iran. Fifty-four oval cocoon strains maintained by the Iran Silk Research Station (ISRC) were analyzed for the estimation of evaluation index to study their genetic divergence. Silkworm rearing was carried out as a single batch rearing system. Disease free laying of these strains were incubated in a controlled environmental chamber. When the eggs attained the head pigmentation stage on the 8th day of development, they were shaded with a black gobo for 48 hr to shield them from light, so as to obtain uniform hatching on the 10th day. Neonates were brushed and reared separately on fresh mulberry (*Morus alba*) leaves. Young 1st to 3rd instar larvae were reared at 27–28°C and 85-90% relative humidity. Late age larvae viz., 4th and 5th instars, were reared at 24-26°C and 70-80% relative humidity. The larvae were fed mulberry leaves *ad libitum* three times a day. 250 larvae each from all batches of all strains were retained for completion of the life cycle. Feeding and other conditions of larval rearing were conducted following standard procedures. Larval growth weight was recorded on day 1, 3, and 5 of

the 5th instar (3 replicates each), using a precision digital balance. Evaluation index and sub-ordinate function values were calculated for performance indices based on changes in larval weight at the last larval instar. The Evaluation index and sub-ordinate function values for all traits were calculated separately and average index value was obtained. Silkworm strains were ranked based on the average of evaluation index method and sub-ordinate function method. Hierarchical agglomerative clustering was done by using NTSYS-pc, version 2.02e based on complete, single, UPGMA, UPGMC, and FLEXI approaches, and SAS-pc based on WARD and average approaches. However, the method of average linkage between groups (Romesburg 1984) under UPGMA (Unweighted Pair-Group Method using Arithmetic average) was considered as the major and final protocol for data conclusions and the resulting clusters were expressed as dendrograms.

Results and discussion: ANOVA based on a completely randomized design indicated significant variations among the silkworm strains ($P<0.01$). On the fifth day, strains 104×110, Shown, 102[Shown], 124-16-9[116] and Shaki A×D had significantly higher larval weights ranging from 4.153–3.965 g ($P<0.01$). Larval growth parameters revealed higher evaluation index and higher sub-ordinate function values for strains 104×110 (197.272 and 2.857), 1001 (193.268 and 2.780), BH-4 (186.365 and 2.647), 124-16-9[116] (183.023 and 2.602), and Shown (180.744 and 2.556). In the present study, by adopting quantitative approaches, 54 silkworm oval strains with different geographical distribution were analyzed. Based on these results it was observed that strains of the same origin did not group together, indicating they have different biological background. Main groups were divided into various sub-groups. Some oval strains were grouped together and were placed far away from other silkworm strains, indicating they might be suitable for future crossing, maintenance of parental strains, and hybridization with peanut cocoon strains so as to maximize heterosis and to avoid inbreeding depression. Because of the low effective population size and each female mating only with one male (thus, all her offspring were full-sibs), the inbreeding rate for this study was very high. This could cause more differentiation among these strains. The grouping methods allowed us to subdivide observations into several subgroups in such a way that we obtained homogeneity inside the subgroups and heterogeneity between the subgroups. Present results confirmed and complemented the results of previous studies about the importance of evaluation and classification of Iranian silkworm strains based on economical and biological characters (Zhao *et al*. 2007; Zanatta *et al*. 2009). Silkworm oval strains could be clustered into different groups according to the geographic areas initially observed. Present results confirmed and complemented the results of previous studies about the importance of evaluation and classification of Iranian silkworm strains based on economical and biological characters (Sohn 2003). During selection of two parents for hybridization, some characters should be matched, including high silk yield, adversity-resistance, good combining ability, and excellent silk quality, so that hybrids have good characters of both parents. Effective utilization of selected germplasm plays an important role in saving time in the synthesis of new hybrids. Critical assessment of variability present in the breeding material is a pre-requisite for paving the way of combining most of the desirable traits present in different genotypes into a single hybrid combination. Generally, hybrids from distant background have high heterosis. The study of diversity is important for selection of useful races, use of the heterosis advantage and generating new races. The selection of high yielding breeds with wider adaptation and stable performance are important goals in breeding programs.

Key words: *Bombyx mori*; Commercial exploitation; Evaluation index; Genetic diversity; Larval growth; Sub ordinate function

Introduction

The historical and commercial importance of the domesticated silkworm (*Bombyx mori* L.) has led to its promotion as a laboratory model. Many silkworm breeds/strains have been developed globally through selection or cross breeding. Nearly 4000 strains are available in germplasm collections (Nagaraju 2002;

Kumaresan *et al*. 2004a; 2004b) which are suitable for rearing in different agro climatic regions. Silkworm breeding programs are based on the development and selection of outstanding hybrids from inbred lines (Tazima 1984). Silkworm germplasm resources have a very important role not only for use in sericulture and silkworm breeding, but also for experimentation in genetics and molecular biology research (Petkov *et al*. 2006). Based on several parameters like nutrition, genetics, and biochemistry, silkworms have been classified into different inbred strains (Seidavi *et al*. 2008; Seidavi *et al*. 2009; Seidavi 2009; Seidavi 2010a; Seidavi 2010b; Seidavi 2010c; Seidavi 2011a; Seidavi 2011b; Seidavi 2011c). However, few studies are reported for classification of silkworm based on larval growth potential, an important character for agricultural use.

High genetic diversity is present among the silkworm breeds and considered to be the result of adaptation (Murakami 1994). Genetic variation within populations and within species has a highly adaptive role in enabling an organism to respond to varying environmental stress (Dobzhansky 1970, Azizpour *et al*. 2020). This variation can be used to measure the genetic diversity for efficient conservation of germplasm. Development and selection of inbred lines for economic interest is mainly based on the morphological parameters. A high level of genetic diversity helps in the selection of parental stocks. The Iran Silkworm Research Station (ISRC) maintains valuable silkworm genetic resources. Intrinsic genetic diversity of peanut-shaped cocoon strains of the Iran silkworm germplasm bank has been reported (Salehi Nezhad *et al*. 2009; Salehi Nezhad *et al*. 2010a; Salehi Nezhad *et al*. 2010b; Salehi Nezhad *et al*. 2010c)**.**

The present study was carried out to evaluate and identify oval cocoon bivoltine breeds suitable for rearing as germplasm resources under climatic conditions in Iran. Fifty-four oval cocoon strains present in this station were evaluated for larval growth rate at 3 times during the fifth larval instar based on evaluation index and sub-ordinate function statistical methods. A primary aim was to ascertain the genetic potential of various silkworm lines/strains for commercial exploitation. There is not data about larval characteristics of oval cocoons strains of Iranian silkworm germplasm. The information generated will be useful for future breeding programmes and for the proper utilization of the available lines.

Material and Methods

The present study was conducted in the Iran Silkworm Research Center (ISRC) and Islamic Azad University, Qaemshahr Branch, Iran. Fifty-four oval silkworm strains were used (Table 1).

Silkworm rearing was carried out as a single batch rearing system. Feeding and other conditions of larval rearing were conducted following standard procedures (ESCAP 1993). Disease free laying of these strains were incubated in a controlled environmental chamber. When the eggs attained the head pigmentation stage on the 8^{th} day of development, they were shaded with a black gobo for 48 hr to shield them from light, so as to obtain uniform hatching on the 10^{th} day.

Neonates were brushed and reared separately on fresh mulberry (*Morus alba*) leaves. Young 1^{st} to 3^{rd} instar larvae were reared at 27–28°C and 85-90% relative humidity. Late age larvae viz., 4^{th} and 5^{th} instars, were reared at 24-26°C and 70-80% relative humidity. The larvae were fed mulberry leaves *ad libitum* three times a day. After completion of the third moult, 250 larvae each from all batches of all strains were retained for completion of the life cycle. Larval growth weight was recorded on day 1, 3, and 5 of the 5^{th} instar (3 replicates each), using a precision digital balance.

Table 1- List of 54 oval silkworm strains

Sl. No.	Silkworm Strains	Sl. No.	Silkworm Strains	Sl. No.	Silkworm Strains
1	6/4-6/6	19	110×104(152)	37	Mos.Black-Black(2)
2	104	20	32×110	38	823
3	124-K	21	110×32	39	1640
4	120-K	22	18-1	40	102(Shown)
5	108-K	23	1538-8-2(114)	41	W2-13-9(108)
6	W2-11-19-2(110)	24	1538-14-9(112)	42	1001
7	W2-11-19-3	25	4-4	43	W1-2-7
8	1002-4-C-5	26	32	44	CS120(N19)
9	1002-E-8-3	27	Tokaee-202	45	Koming-2-5
10	Guilan-Orange	28	106	46	W2-13-4
11	Khorasan-Orange	29	17	47	Y-5
12	Shown	30	Shaki A×D	48	127-17
13	T1-P	31	124-16-9(116)	49	Lemon Khorasan
14	T5-P	32	Mose.Black-Plain(2)	50	Lemon Haratee
15	CS120(7409)	33	726(118)	51	White Haratee
16	BH-4	34	1627-14-4-3	52	Yellow Haratee
17	BH-3	35	Koming-1(154)	53	Pink Khorasan
18	104×110	36	1627-14-2-8	54	Baghdadi

Evaluation index and sub-ordinate function values were calculated for performance indices based on changes in larval weight at the last larval instar. The Evaluation index and sub-ordinate function values for all traits were calculated separately and average index value was obtained. Silkworm strains were ranked based on the average of evaluation index method and sub-ordinate function method. Evaluation index values (EI) for silkworm strain performance were calculated using the following formula: EI= [(A-B)/C] ×10+50 (MANO *et al*. 1993, RAO *et al*. 2004), whereby A is the mean of the particular trait in a strain; B is the overall mean of the particular trait in all strains; C is the standard deviation of a trait in all strains; and 50 is used as the constant.

The sub-ordinate function was calculated by utilizing the following formula: Xu = (Xi-Xmin)/(Xmax-Xmin) (Gower 1971; Rao *et al*. 2003) where, Xu is the sub-ordinate function; Xi is the measurement of the trait of the tested strain; Xmin is the minimum value of the trait among all the tested strains; and Xmax is the maximum value of the trait among all the tested strains.

Data were homogenized before statistical analysis. Hierarchical agglomerative clustering was done by using NTSYS-pc, version 2.02e (Rohlf 1998) based on complete, single, UPGMA, UPGMC, and FLEXI approaches, and SAS-pc (SAS 1997) based on WARD and average approaches. However, the method of average linkage between groups (Romesburg 1984) under UPGMA (Unweighted Pair-Group Method using Arithmetic average) (Sneath and Sokal 1973) was considered as the major and final protocol for data conclusions and the resulting clusters were expressed as dendrograms. UPGMA uses the average distance among all the equal genotypes for the formation of each group (Cruz and Regazzi 2001; Zanatta *et al*. 2009). The clustering was based on the squared Euclidean distance. The average linkage between two groups was considered as the average of distance between all pairs of cases with one number from each group. Hierarchical clustering analysis was carried out by considering all studied parameters together.

For analyzing the data from the CRD model, the GLM approach and SAS software were used. The DNMRT method was used for average compares.

Results and Discussion

The silkworm strains studied showed varied performance based on larval growth potential measured by weights at 3 time points during the fifth instar. The larval growth of strains 104 X 110, Shown, 124-16-9[116], 1640 and 104 had significantly higher weights than all the others, ranging from 1.115–1.032 g, on the

first day of the fifth instar. On the third day of the fifth instar, strains BH-4, 1001, Lemon Haratee, 104×110, 102[Shown] had significantly higher larval weight ranging from 3.716–3.422 g, and on the fifth day strains 104×110, Shown, 102[Shown], 124-16-9[116] and Shaki A×D had significantly higher larval weights ranging from 4.153–3.965 g ($P<0.01$) (Table 2).

The evaluation index (EI) with respect to 1st day, V instar larval weight was 67.291 in strain 104×110, 65.136 in strain Shown, 62.981 in strain 124-16-9[116], 61.827 in strain 1640 and 60.903 in strain 104. EI with respect to 3rd day, V instar larval weight was 69.906 in strain BH-4, 66.588 in strain 1001, 64.394 in strain Lemon Haratee, 61.666 in strain 104×110, 61.637 in 102(Shown). EI with respect to last day, V instar larval weight was 68.315 in strain 104×110), 66.470 in strain 1001, 64.841 in strain 102[Shown], 64.841 in the strain 124-16-9[116], and 64.841 in the strain Shaki A×D (Table 3). Overall higher EI values were observed in 104×110 (197.272), 1001 (193.268), BH-4 (186.365), 124-16-9[116] (183.023), and Shown (180.744).

Table 2- Mean (± standard deviation) performance of larval weight during different days of the fifth instar for 54 oval silkworm strains

Sl. No.	Silkworm Strain	Larval Weight at 1st Day of 5th Instar (g)	Larval Weight at 3rd Day of 5th Instar (g)	Larval Weight at Last Day of 5th Instar (g)
1	6/4-6/6	0.010 ± 0.395^{p}	$1.669^{u}\pm0.096$	$2.177^{q}\pm0.065$
2	104	0.038 ± 1.032^{cde}	$3.408^{bc}\pm0.057$	$3.722^{ef}\pm0.019$
3	124-K	0.129 ± 0.906^{ef}	$3.017^{mn}\pm0.283$	$3.473^{ef}\pm0.082$
4	120-K	0.040 ± 0.018^{ef}	$2.944^{mn}\pm0.207$	$3.357^{f-n}\pm0.194$
5	108-K	0.056 ± 0.866^{ef}	$3.018^{mn}\pm0.071$	$3.671^{ef}\pm0.091$
6	W2-11-19-2(110)	0.099 ± 0.844^{kl}	$2.830^{mn}\pm0.393$	$3.424^{ef}\pm0.352$
7	W2-11-19-3	0.031 ± 1.009^{ef}	$3.204^{fg}\pm0.075$	$3.487^{ef}\pm0.130$
8	1002-4-C-5	$0.958^{ef}\pm0.033$	$2.768^{n-q}\pm0.263$	$3.571^{ef}\pm0.160$
9	1002-E-8-3	$0.919^{ef}\pm0.036$	$3.168^{g-m}\pm0.193$	$3.836^{cde}\pm0.309$
10	Guilan-Orange	$0.723^{lmn}\pm0.053$	$2.473^{srt}\pm0.218$	$3.050^{op}\pm0.308$
11	Khorasan-Orange	$0.536^{o}\pm0.401$	$2.470^{rst}\pm0.043$	$3.140^{op}\pm0.088$
12	Shown	$1.087^{a}\pm0.036$	$3.241^{fg}\pm0.198$	$3.812^{cde}\pm0.102$
13	T1-P	$0.985^{ef}\pm0.042$	$2.784^{mn}\pm0.147$	$3.817^{cde}\pm0.316$
14	T5-P	$0.973^{ef}\pm0.013$	$3.213^{fg}\pm0.111$	$3.782^{ef}\pm0.155$
15	CS120(7409)	$0.678^{n}\pm0.027$	$2.394^{st}\pm0.056$	$2.867^{op}\pm0.194$
16	BH-4	0.087 ± 1.021^{ef}	$3.716^{a}\pm0.250$	$3.714^{ef}\pm0.161$
17	BH-3	0.068 ± 0.654^{n}	$2.543^{p-s}\pm0.198$	$3.107^{op}\pm0.025$
18	104×110	$1.115^{a}\pm0.068$	$3.423^{bc}\pm0.260$	$4.153^{a}\pm0.144$
19	110×104(152)	$0.942^{ef}\pm0.021$	$2.999^{mn}\pm0.027$	$3.343^{f-n}\pm0.188$
20	32×110	$0.873^{ef}\pm0.041$	$3.080^{mn}\pm0.235$	$3.605^{ef}\pm0.131$
21	110×32	$0.785^{n-l}\pm0.118$	$3.003^{mn}\pm0.189$	$3.443^{ef}\pm0.184$
22	18-1	$0.870^{ef}\pm0.009$	$2.993^{mn}\pm0.065$	$3.486^{ef}\pm0.160$
23	1538-8-2(114)	$0.867^{ef}\pm0.050$	$2.998^{mn}\pm0.045$	$3.413^{ef}\pm0.154$
24	1538-14-9(112)	$0.838^{kl}\pm0.022$	$2.825^{mn}\pm0.099$	$3.500^{ef}\pm0.051$
25	4-4	$0.89^{ef}\pm0.086$	$2.856^{mn}\pm0.195$	$3.302^{n-j}\pm0.113$
26	32	$0.848^{kl}\pm0.119$	$3.309^{fg}\pm0151$	$3.493^{ef}\pm0.610$
27	Tokaee-202	$0.943^{ef}\pm0.025$	$3.108^{mn}\pm0.283$	$3.773^{ef}\pm0.112$
28	106	$0.909^{ef}\pm0.021$	$3.327^{bc}\pm0.145$	$3.418^{ef}\pm0.020$
29	17	$0.941^{ef}\pm0.042$	$3.419^{mn}\pm0.069$	$3.808^{cde}\pm0.170$
30	Shaki A×D	$0.969^{ef}\pm0.064$	$3.334^{bc}\pm0.100$	$3.965^{bc}\pm0.069$
31	124-16-9(116)	$1.059^{abc}\pm0.058$	$3.197^{fg}\pm0.100$	$4.021^{abc}\pm0.171$
32	Mose.Black-Plain(2)	$0.86^{f-k}\pm0.048$	$2.936^{mn}\pm0.168$	$3.559^{ef}\pm0.196$
33	726(118)	$0.836^{kl}\pm0.063$	$2.956^{mn}\pm0.179$	$3.453^{ef}\pm0.163$
34	1627-14-4-3	$0.881^{ef}\pm0.024$	$2.991^{mn}\pm0.066$	$3.492^{ef}\pm0.142$
35	Koming-1(154)	$0.961^{ef}\pm0.004$	$3.237^{fg}\pm0.193$	$3.754^{ef}\pm0.153$
36	1627-14-2-8	$0.938^{ef}\pm0.062$	$3.188^{fg}\pm0.221$	$3.765^{ef}\pm0.097$

37	Mos.Black-Black(2)	0.918^{ef}±0.047	2.942^{mn}±0.218	3.291^{j-o}±0.124
38	823	0.898^{ef}±0.164	3.284^{fg}±0.491	3.547^{ef}±0.610
39	1640	1.044^{bc}±0.061	3.404^{bc}±0.153	3.760^{ef}±0.753
40	102(Shown)	0.927^{ef}±0.028	3.422^{bc}±0.075	4.250^{ef}±0.209
41	W2-13-9(108)	1.000^{ef}±0.011	3.256^{fg}±0.129	3.777^{ef}±0.186
42	1001	1.023^{ef}±0.056	3.598^{ab}±0.108	4.85^{ab}±0.264
43	W1-2-7	0.907^{ef}±0.072	3.247^{fg}±0.146	3.557^{ef}±0.199
44	CS120(N19)	0.887^{ef}±0.076	2.871^{mn}±0.266	3.011^{op}±0.075
45	Koming-2-5	0.850^{kl}±0.064	2.755^{n-q}±0.192	2.980^{nop}±0.323
46	W2-13-4	0.857^{f-k}±0.013	2.902^{mn}±0.075	3.056^{op}±0.094
47	Y-5	0.849^{kl}±0.020	2.919^{mn}±0.141	3.393^{ef}±0.080
48	127-17	0.930^{ef}±0.050	3.049^{mn}±0.094	3.579^{ef}±0.240
49	Lemon Khorasan	0.696^{mn}±0.047	2.249^{t}±0.073	2.792^{p}±0.172
50	Lemon Haratee	0.929^{ef}±0.028	3.520^{abc}±0.396	3.550^{ef}±0.085
51	White Haratee	0.738^{n-l}±0.030	2.693^{p-s}±0.111	2.499^{op}±0.031
52	Yellow Haratee	0.987^{ef}±0.056	3.087^{mn}±0.244	3.563^{ef}±0.207
53	Pink Khorasan	0.850^{kl}±0.066	2.821^{mn}±0.092	3.057^{op}±0.147
54	Baghdadi	0.832^{kl}±0.003	2.657^{qrs}±0.204	3.027^{op}±0.167

Means in each column followed by the same letters are not significantly different at α=0.01،

Table 3- Evaluation index and sub-ordinate function values for 54 oval silkworm strains

Sl. No.	Silkworm Strain	EI of larval wt. on different days of fifth instar			Xu of larval wt. on different days of fifth instar		
		First	Third	Last	First	Third	Last
1	6/4-6/6	11.877	12.333	14.692	0.000	0.000	0.000
2	104	60.903	61.244	56.619	0.885	0.850	0.782
3	124-K	51.206	50.246	49.862	0.710	0.659	0.656
4	120-K	59.826	48.193	46.714	0.865	0.623	0.597
5	108-K	48.127	50.274	55.235	0.654	0.659	0.756
6	W2-11-19-2(110)	46.434	44.987	48.532	0.624	0.567	0.631
7	W2-11-19-3	59.133	55.506	50.242	0.853	0.750	0.663
8	1002-4-C-5	57.286	43.243	52.521	0.819	0.537	0.705
9	1002-E-8-3	52.206	54.493	59.712	0.728	0.732	0.840
10	Guilan-Orange	37.121	34.946	38.383	0.456	0.393	0.442
11	Khorasan-Orange	22.729	34.861	40.825	0.196	0.391	0.487
12	Shown	65.136	56.547	59.061	0.961	0.768	0.827
13	T1-P	57.286	43.693	59.197	0.819	0.545	0.830
14	T5-P	56.362	55.759	58.247	0.803	0.754	0.812
15	CS120(7409)	33.658	32.724	33.417	0.393	0.354	0.349
16	BH-4	60.057	69.906	56.402	0.869	1.000	0.778
17	BH-3	31.811	36.915	39.930	0.360	0.427	0.471
18	104×110	67.291	61.666	68.315	1.000	0.857	1.000
19	110×104(152)	53.976	49.740	46.334	0.760	0.650	0.590
20	32×110	48.666	52.018	53.444	0.664	0.689	0.723
21	110×32	41.893	49.853	49.048	0.542	0.652	0.641
22	18-1	48.435	49.571	50.215	0.660	0.647	0.662
23	1538-8-2(114)	48.204	49.712	48.234	0.656	0.649	0.626
24	1538-14-9(112)	45.972	44.846	50.594	0.615	0.565	0.670
25	4-4	49.974	45.718	45.221	0.688	0.580	0.569
26	32	46.742	58.459	50.405	0.629	0.801	0.666
27	Tokaee-202	54.053	52.806	58.003	0.761	0.703	0.808
28	106	51.437	58.965	48.369	0.714	0.810	0.628
29	17	53.900	53.959	58.953	0.758	0.723	0.825
30	Shaki A×D	56.054	59.162	63.213	0.797	0.813	0.905
31	124-16-9(116)	62.981	55.309	64.733	0.922	0.746	0.933
32	Mose.Black-Plain(2)	47.665	47.968	52.196	0.646	0.619	0.699
33	726(118)	45.818	48.531	49.319	0.613	0.629	0.646

34	1627-14-4-3	49.282	49.515	50.377	0.675	0.646	0.665
35	Koming-1(154)	55.439	56.434	57.487	0.786	0.766	0.798
36	1627-14-2-8	53.669	55.056	57.786	0.754	0.742	0.804
37	Mos.Black-Black(2)	52.129	48.137	44.923	0.726	0.622	0.564
38	823	50.590	57.756	51.870	0.699	0.789	0.693
39	1640	61.827	61.131	57.650	0.901	0.848	0.801
40	102(Shown)	52.822	61.637	64.841	0.739	0.856	0.935
41	W2-13-9(108)	58.440	56.968	58.111	0.840	0.775	0.810
42	1001	60.211	66.588	66.470	0.872	0.942	0.966
43	W1-2-7	51.283	56.715	52.684	0.711	0.771	0.709
44	CS120(N19)	49.743	46.140	37.325	0.683	0.587	0.422
45	Koming-2-5	46.896	42.877	36.483	0.632	0.531	0.406
46	W2-13-4	47.435	47.012	38.546	0.642	0.602	0.445
47	Y-5	46.819	47.490	47.691	0.631	0.611	0.615
48	127-17	53.053	51.146	52.738	0.743	0.674	0.710
49	Lemon Khorasan	35.043	28.646	31.382	0.418	0.283	0.311
50	Lemon Haratee	52.976	64.394	51.951	0.742	0.904	0.695
51	White Haratee	38.276	41.134	36.863	0.476	0.500	0.413
52	Yellow Haratee	57.440	52.215	52.304	0.822	0.693	0.701
53	Pink Khorasan	46.896	44.734	38.573	0.632	0.563	0.445
54	Baghdadi	45.510	40.121	37.759	0.607	0.483	0.430

EI: Evaluation index; Xu: Sub-ordinate function value

Table 4- Ranking of 54 oval silkworm strains based on the mean of evaluation index and sub-ordinate function method for larval weight

Sl. No.	Silkworm Strain	Evaluation Index Method		Sub-Ordinate Function Method	
		Value	Rank	Value	Rank
1	6/4-6/6	38.902	54	0.000	54
2	104	178.766	8	2.516	8
3	124-K	151.314	30	2.024	30
4	120-K	154.733	26	2.085	26
5	108-K	153.637	28	2.069	28
6	W2-11-19-2(110)	139.953	42	1.822	42
7	W2-11-19-3	164.881	17	2.266	18
8	1002-4-C-5	153.050	29	2.062	29
9	1002-E-8-3	166.412	16	2.300	15
10	Guilan-Orange	110.450	49	1.290	49
11	Khorasan-Orange	98.416	52	1.074	52
12	Shown	180.744	5	2.556	5
13	T1-P	160.176	22	2.194	20
14	T5-P	170.368	11	2.369	11
15	CS120(7409)	99.799	51	1.096	51
16	BH-4	186.365	3	2.647	3
17	BH-3	108.655	50	1.257	50
18	104×110	197.272	1	2.857	1
19	110×104(152)	150.051	31	2.000	31
20	32×110	154.128	27	2.076	27
21	110×32	140.793	41	1.834	41
22	18-1	148.221	33	1.969	33
23	1538-8-2(114)	146.150	35	1.930	35
24	1538-14-9(112)	141.413	39	1.850	39
25	4-4	140.914	40	1.837	40
26	32	155.606	25	2.096	25
27	Tokaee-202	164.862	18	2.272	17
28	106	158.771	23	2.152	23
29	17	166.811	14	2.307	14
30	Shaki A×D	178.430	9	2.515	9
31	124-16-9(116)	183.023	4	2.602	4
32	Mose.Black-Plain(2)	147.829	34	1.964	34

33	726(118)	143.668	37	1.887	37
34	1627-14-4-3	149.174	32	1.986	32
35	Koming-1(154)	169.360	12	2.350	12
36	1627-14-2-8	166.510	15	2.300	16
37	Mos.Black-Black(2)	145.189	36	1.912	36
38	823	160.216	21	2.181	22
39	1640	180.608	6	2.550	6
40	102(Shown)	179.301	7	2.530	7
41	W2-13-9(108)	173.520	10	2.425	10
42	1001	193.268	2	2.780	2
43	W1-2-7	160.682	20	2.190	21
44	CS120(N19)	133.208	43	1.693	43
45	Koming-2-5	126.256	46	1.569	46
46	W2-13-4	132.992	44	1.689	44
47	Y-5	142.000	38	1.857	38
48	127-17	156.938	24	2.127	24
49	Lemon Khorasan	95.071	53	1.013	53
50	Lemon Haratee	169.321	13	2.341	13
51	White Haratee	116.273	48	1.390	48
52	Yellow Haratee	161.959	19	2.216	19
53	Pink Khorasan	130.202	45	1.640	45
54	Baghdadi	123.390	47	1.520	47

Higher sub-ordinate function (Xu) values of 1.000 (104×110), 0.961 (Shown), 0.922 (124-16-9[116]), 0.901 (1640), and 0.885(104) were obtained for larval weight at first day of V instar. Xu values of 1.000 (BH-4), 0.942 (1001), 0.904 (Lemon Haratee), 0.857 (104×110), and 0.856 (102(Shown)) were obtained for larval weight at 3rd day of V instar. Xu values of 1.000 (104×110), 0.966 (1001), 0.935 (102(Shown)), 0.933 (1003-5124-16-9[116]), and (Shaki A×D) were obtained for larval weight at last day of 5th instar (Table 3). Overall higher Xu values were shown by 104×110 (2.857), 1001 (2.780), BH-4 (2.647), 124-16-9[116] (2.602), and Shown (2.556).

Based on the overall ranking of EI and Xu values, the strains 104×110,1001, BH-4, 124-16-9[116], and Shown ranked from 1 to 5, respectively (Table 4).

Hierarchical agglomerative clustering was carried out/performed based on complete, single, UPGMA, UPGMC, FLEXI, WARD and average approaches. All these approaches yielded similar dendrograms. The dendrograms obtained from hierarchical cluster analysis of Iranian silkworm oval strains is presented in Figures 1-7.

Based on these results it was observed that strains of the same origin did not group together, indicating they have different biological background. Main groups were divided into various sub-groups. Some oval strains were grouped together and were placed far away from other silkworm strains, indicating they might be suitable for future crossing, maintenance of parental strains, and hybridization with peanut cocoon strains so as to maximize heterosis and to avoid inbreeding depression. Because of the low effective population size and each female mating only with one male (thus, all her offspring were full-sibs), the inbreeding rate for this study was very high. This could cause more differentiation among these strains (Falconer 1989; Mirhosseini *et al*. 2007).

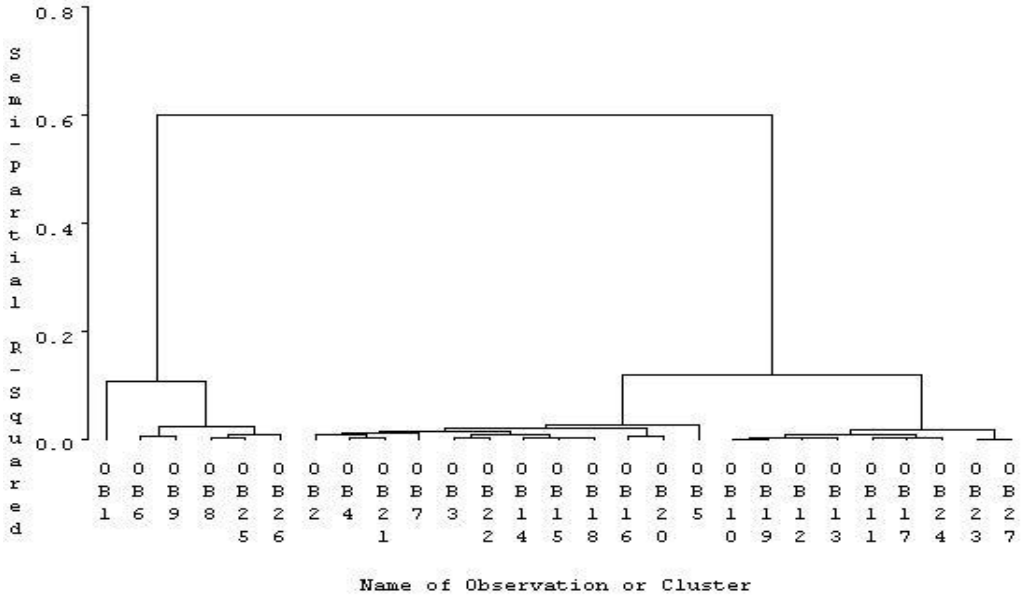

Figure 1- Cluster analysis based on larval weights at different days for 54 oval silkworm strains as per WARD's method using SAS

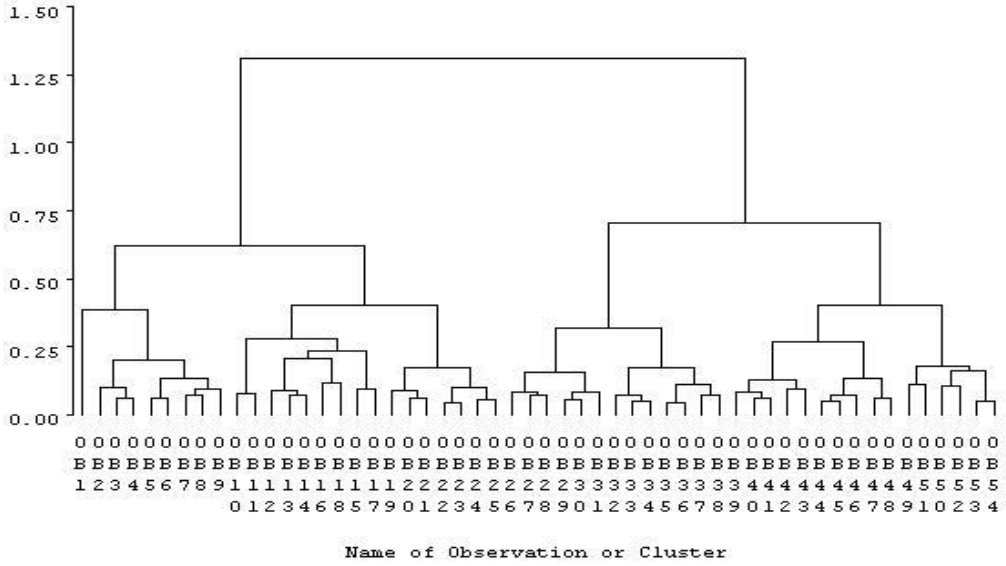

Figure 2- Cluster analysis based on larval weights at different days for 54 oval silkworm strains according to grouping from the average method using SAS.

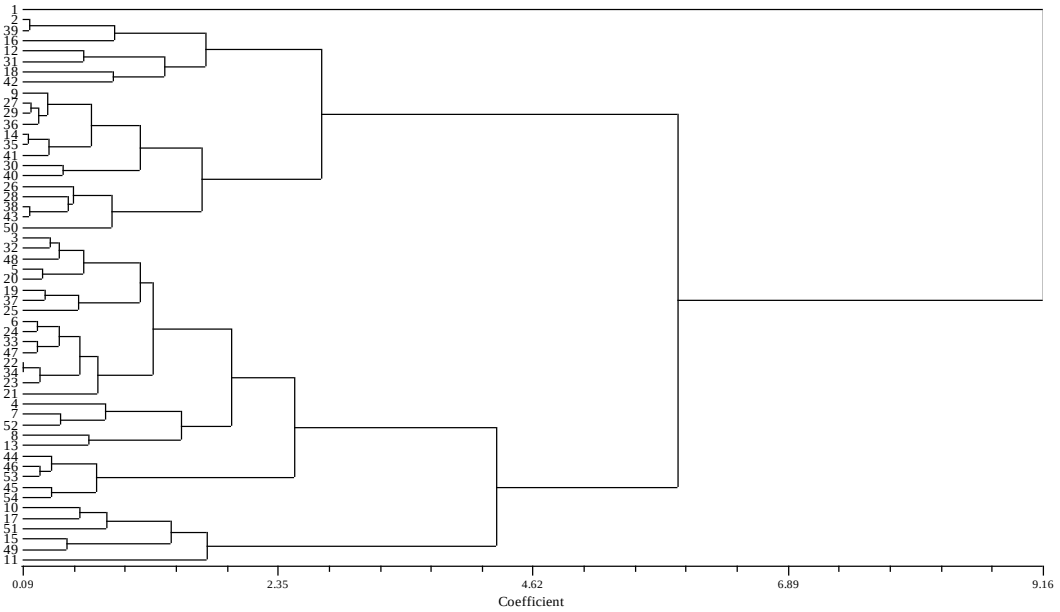

Figure 3- Cluster analysis based on larval weights at different days for 54 oval silkworm strains according to grouping from the complete method using NTSYS

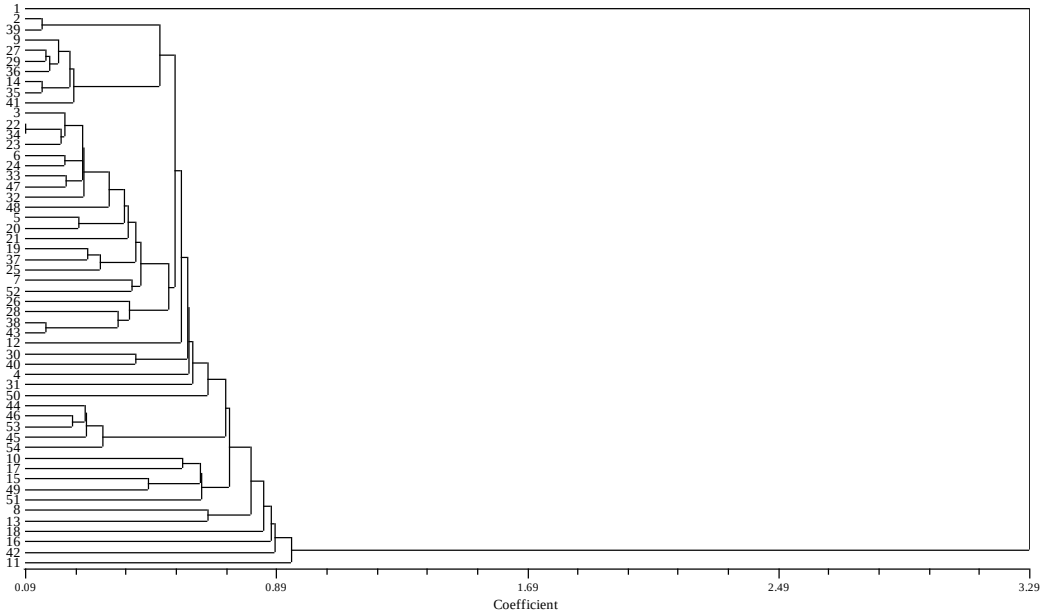

Figure 4- Cluster analysis based on larval weights at different days for 54 oval silkworm strains according to grouping from the single method using NTSYS

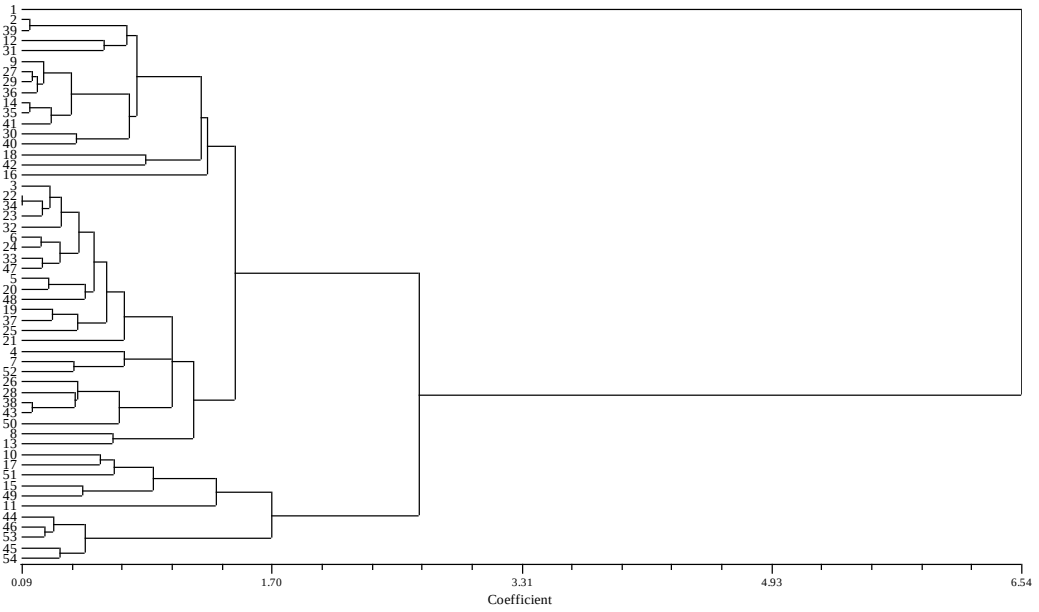

Figure 5- Cluster analysis based on larval weights at different days for 54 oval silkworm strains according to grouping from the UPGMC method using NTSYS

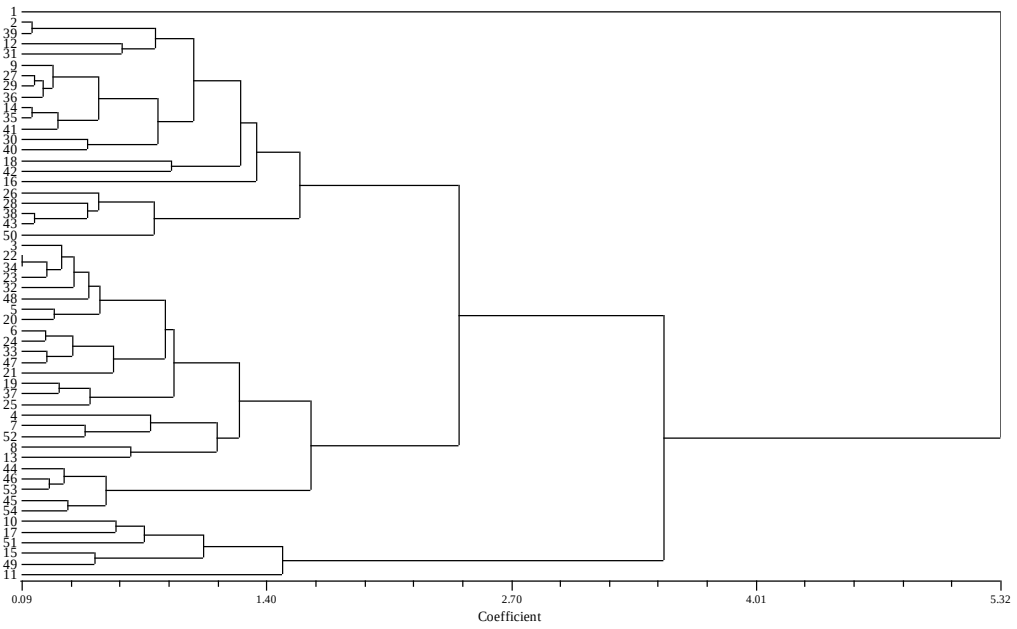

Figure 6- Cluster analysis based on larval weights at different days for 54 oval silkworm strains according to grouping from the FLEXI method using NTSYS

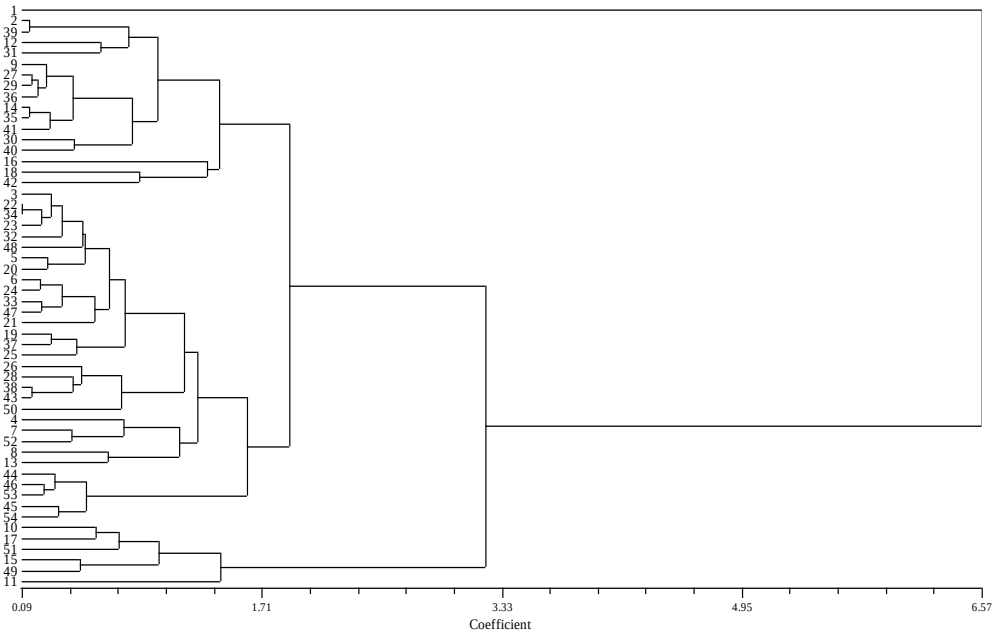

Figure 7- Cluster analysis based on larval weights at different days for 54 oval silkworm strains according to grouping from the UPGMA (Unweighted Pair Group Method Average) method using NTSYS

The grouping methods allowed us to subdivide observations into several subgroups in such a way that we obtained homogeneity inside the subgroups and heterogeneity between the subgroups.

Effective utilization of selected germplasm plays an important role in saving time in the synthesis of new hybrids (Rao *et al*. 2006). Critical assessment of variability present in the breeding material is a pre-requisite for paving the way of combining most of the desirable traits present in different genotypes into a single hybrid combination. Generally, hybrids from distant background have high heterosis. The study of diversity is important for selection of useful races, use of the heterosis advantage and generating new races. The selection of high yielding breeds with wider adaptation and stable performance are important goals in breeding programs. Nassar and Huehn (1987), and Kang (1988) proposed parametric procedures based on the ranking of breeds in each environment with similar ranking across different environments and classified as stable. Results obtained in the present study corroborates these earlier findings. Ramesha *et al* (2009) evaluated various silkworm strains and stated selection of suitable parents and information on the nature and magnitude of gene action involving traits of economic importance determine the success of any crop. They believed critical assessment of variability present in the breeding material is a pre-requisite to enable combining most of the desirable traits present in different genotypes into a single hybrid combination. However, the individual performance of parental breeds is not always a good reflection of the combining ability, and its analysis therefore helps the breeders to understand the nature of gene action to identify prospective parents/hybrids (Ramesha *et al*. 2009). During selection of two parents for hybridization, some characters should be matched, including high silk yield, adversity-resistance, good combining ability, and excellent silk quality, so that hybrids have good characters of both parents (Zhao *et al*. 2007). Zhao *et al*. (2007) believe phenotype is the joint product of genotype and environment. According to the breeding goal, we should combine good characters of the parents by setting up a specific breeding environment and carrying out breeding experiments. Genetical relationships between yield attributes and other genetical markers were shown by Hirata (1974) and Gamo and Ohtsuka (1980). The genetic markers included both biochemical

and physiological characters. Studies to assess genetical distance between different groups of silkworm races in tropical and temperate regions were reported (Chiang 1980; Gamo 1983). The tropical races of Southeast Asia were shown to have a higher number of gene substitutions than the Chinese, European and Japanese races (Gamo 1983; Chatterjee and Data 1992).

Hierarchical agglomerative clustering yielded an optimal cluster solution. Using Ward's cluster algorithm, there was a notable discrepancy in the size and shape of the clusters. Nevertheless, as Knezovi *et al*. (2005) stated, evaluation of the results using criteria proposed by Franco *et al*. (1997) showed that all methods have similar efficiency, on the basis of the number of influential variables criteria. Franco *et al*. (1998) developed a nonhierarchical clustering method for classification using both continuous and categorical variables, called the Modified Location model (MLM). Using the sequential Ward after Gower– MLM clustering strategy, they concluded that posterior use of MLM can improve the composition of the clusters obtained by Ward's method and produce compact and well separated groups. Results of the present study are based on the average linkage between groups or UPGMA which is supported by the work of other researchers (Peters and Martinelli 1989; Chatterjee and Data 1992) which states that UPGMA yields more accurate results than other hierarchical methods for classification purposes.

In the present study, by adopting quantitative approaches, 54 silkworm oval strains with different geographical distribution were analyzed. Silkworm oval strains could be clustered into different groups according to the geographic areas initially observed. Present results confirmed and complemented the results of previous studies about the importance of evaluation and classification of Iranian silkworm strains based on economical and biological characters.

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References

Azizpour N, Khelatabadi AH, Moradi MH and Mohammadi H, 2020. Genome-wide association study based on gene-set enrichment analysis associated with milk yield in Holstein cattle. Journal of Animal Science Researches 30(1): 79-92

Chatterjee SN, and Datta RK, 1992. Heirarchiacal clustering of 54 races and strains of the mulberry silkworm, *Bombyx mori* L.: Significance of biochemical parameters. Theoritical and Appllied Genetics 85: 394-402.

Chiang Y, 1980. Origin and differentiation of the silkworm, *Bombyx mori* L. Monogram Publication by The Sericultural Society of Zhejiang.

Cruz CD and Regazzi AJ, 2001. Modelos biométricos aplicados ao melhoramento genético. 2nd ed, Imprensa Universitária, 390p.

Dobzhansky T, 1970. Genetics of the Evolutionary process. New York: Columbia University Press. p. 71-93.

ESCAP, 1993. Principle and Techniques of Silkworm Breeding. United Nations, New York.

Falconer DS, 1989. Introduction to quantitative genetics, 3rd ed., Longman, Scientific and Technical, New York.

Franco J, Crossa J, Villasenor J, Taba S and Eberhart SA, 1997. Classifying Mexican maize accessions using hierarchical and density search methods. Crop Science 37: 972-980.

Gamo T, 1983. Biochemical genetics and its application to the breeding of silkworm. Japan Agricultural Research Quarterly 16: 264-273.

Gamo T and Ohtsuka Y, 1980. Phylogenetic studies on the racial differentiation of the silkworm, *Bombyx mori*, on the basis of polymorphic genes in haemolymph proteins. Bulletin Sericulture Experiments Statation, Japan 28: 15-50.

Gower JC, 1971. A general coefficient of similarity and some of its properties. Biometrics 27: 857-871.

Hirata Y, 1974. Relations between the amylase activity of the larval digestive juice and several quantitative characters in strains of the silkworm, *Bombyx mori.* Journal of Sericultural Science 43: 384-390.

Kang MS, 1988. A rank-sum method for selecting high yielding, stable corn genotypes. Cereal Research Communication 16: 113-115

Knezovi Z, Gunjaca J, Satovic Z and Kolak I, 2005. Comparison of Different Methods for Classification of Gene Bank Accessions. Agricultural Crop Science 70(3): 87-91.

Kumaresan P, Mohan B, Koundinya PR, Sinha RK and Thangavelu K, 2004a. Silkworm germplasm conservation- A perspective. Indian Silk 44: 4-9.

Kumaresan P, Sinha RK, Mohan B and Thangavelu K, 2004b. Conservation of multivoltine silkworm (*Bombyx mori* L.) germplasm in India: An overview. International Journal of Industrial Entomology 9: 1-13.

Mano Y, Nirmal Kumar S, Basavaraja HK, Mal Reddy N and Datta RK, 1993. A new method to select promising silkworm breeds/combinations. Indian Silk 31: 53-59.

Mirhosseini SZ, Dalirsefat SB and Pourkheirandish M, 2007. Genetic Characterization of Iranian Native *Bombyx mori* Strains Using Amplified Fragment Length Polymorphism Markers. Journal of Economical Entomology 100: 939-945.

Murakami A, 1994. Growth phenomena in *Bombyx mori* L. with a special reference to genetic factors responsible for growth acceleration and moultinism, Indian Journal of Sericulture 33: 12-14.

Nagaraju J, 2002. Application of genetic principles for improving silk production. Current Science 83: 409-414.

Nassar A and Huehn A, 1987. Studies on estimation of phenoptypic stability: Tests of significant for non-parametric measures of phenotypic stability. Biometrics 43: 45-53

Peters, J. P., and J. A. Martinelli (1989). Hierarchical cluster analysis as a tool to manage variation in germplasm collection. The Appllied Genetics 78: 42-48.

Petkov NL, Tzenov PI, Petkov ZM, Natcheva YS, and Vasileva YB, 2006. Silkworm, *Bombyx mori* L. germplasm resources in Bulgaria, Publish Seic Set-Eco, 288, ISBN 10: 954-749-065-6

Ramesha C, Seshagiri SV and Rao CGP, 2009. Evaluation and Identification of Superior Polyvoltine Crossbreeds of Mulberry Silkworm, *Bombyx mori* L. Journal of Entomology 6(4): 179-188.

Rao CGP, Chandrashekaraiah R, Ramesh C, Ibrahim Basha K, Eshagiri SV and Nagaraju H 2004. Evaluation of polyvoltine hybrids based on silk productivity in silkworm, *Bombyx mori*. International Journal Industrial Entomology 8:181-187.

Rao CGP, Seshagiri SV, Ramesh C, Basha A, Ibrahim K, Nagaraju H and Chandrashekaraiah C, 2006. Evaluation of genetic potential of the polyvoltine silkworm (*Bombyx mori* L.) germplasm and identification of parents for breeding program. Journal of Zhejiang University Sericulture B: 7(3): 215-220.

Rohlf FJ, 1998. NTSYSpc: numerical taxonomy and multivariate system, version 2.02e. Exeter Software, New York.

Romesburg HC, 1984. Cluster Analysis for Researchers. Lifetime Learning Publications, Belmont, CA.

Salehi Nezhad M, Mirhosseini SZ, Gharahveysi S, Mavvajpour M, and Seidavi AR, 2009. Analysis of Genetic Divergence for Classification of Morphological and Larval Gain Characteristics of Peanut Cocoon Silkworm (*Bombyx mori* L.) Germplasm. American-Eurasian Journal of Agriculture and Environmental Science 6(5): 600-608.

Salehi Nezhad M, Mirhosseini SZ, Gharahveysi S, Mavvajpour M, and Seidavi AR, 2010a. Comparative study on the larval development duration of 51 different peanut cocoon strains of Iran silkworm *Bombyx mori* (Lepidoptera: Bombycidae) gene bank. Asian Journal of Animal and Veterinary Advances 5: 234-245.

Salehi Nezhad M, Mirhosseini SZ, Gharahveysi S, Mavvajpour M and Seidavi AR, 2010b. Investigation on intera-specific biodiversity of 51 peanut cocoon strains of Iran silkworm (*Bombyx mori*) germplasm based on reproductive traits. Biotechnology 9: 149-156.

Salehi Nezhad M, Mirhosseini SZ, Gharahveysi S, Mavvajpour M and Seidavi AR, 2010c. Evaluation of 37 economically important traits from 51 strains of the silkworm *Bombyx mori* and their relationships. Journal of Food Agriculture and Environment 8: 924-929.

SAS, 1997. SAS/STAT User Guide for Personal Computers, Cary, NC: SAS Institute.

Seidavi AR, 2009. Determination and Comparison of Nutritional Indices in Commercial Silkworm Hybrids during Various Instars. Asian Journal of Animal and Veterinary Advances 4: 104-113.

Seidavi AR, 2010a. Estimation of genetics parameters and selection effect on genetic and phenotype trends in silkworm commercial pure lines. Asian Journal of Animal and Veterinary Advances 5(1): 1-12.

Seidavi AR, 2010b. Investigation on effect of individual selection based on cocoon weight on additive genetic value and selection index value in six commercial silkworm pure lines. World Journal of Zoology 5: 7-14.

Seidavi AR, 2010c. Relationship between Season and Efficiency of Individual Selection in Six Peanut and Oval Lines of Silkworm. International Journal of Engineering Science and Technology 2: 211-214.

Seidavi AR, 2011a. Analysis of Biochemical Divergence for Classification of six native silkworm based on Total Cholesterol, Triglycerides, HDL Cholesterol, LDL Cholesterol, Total Lipase and Lipids. African Journal of Microbiology Research 5: 2912-2919.

Seidavi AR 2011b. Analysis of combining ability for some parameters in Iranian lines of silkworm *Bombyx mori* L. (Lepidoptera: Bombycidae). Annals of Biological Research 2: 158-163.

Seidavi AR, 2011c. Evaluation of the genetic potential of six native strains of silkworm, *Bombyx mori* L. African Journal of Agricultural Research 6: 4816-4823.

Seidavi AR, Bizhannia AR, Ghanipoor M and Qotbi AAA, 2008. Investigation on improvement possibility of resistance, production and reproduction traits in 3P, 2P and P generations in three Japanese pure lines of silkworm *Bombyx mori* L., using individual selection in 3P generation. Asian Journal of Animal and Veterinary Advances 3: 443-447.

Seidavi AR, Mirhosseini SZ, Mavvajpour M, Ghanipoor M, Bizhannia AR, Qotbi AAA and Chamani M. 2009. Additive genetic variations and selection index changes of economic traits of the silkworm commercial pure lines against parent selection pressure. American-Eurasian Journal of Agriculture and Environmental Science 6: 460-465.

Sneath PHA and Sokal RR, 1973. Numerical taxonomy. Freeman, San Francisco, CA.

Sohn KW, 2003. Conservation Status of Sericulture Germplasm Resources in the World. II. Conservation Status of Silkworm (*Bombyx mori*) Genetic Resources in the World. FAO Press. 168 pp.

Tazima Y, 1984. Silkworm Moth, Evolution of Domesticated Animals, Longman, New York, NY, USA, 1984

Zanatta DB, Bravo JP, Barbosa JF, Munhoz REF and Fernandez MA, 2009. Evaluation of Economically Important Traits from Sixteen Parental Strains of the Silkworm *Bombyx mori* L (Lepidoptera: Bombycidae). Neotropical Entomology 38: 327-331.

Zhao Y, Chen K and He S, 2007. Key principles for breeding spring-and-autumn using silkworm varieties: from our experience of breeding 873×874. Caspian Journal of Environmental Science 5: 57-61.

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تجزیه و تحلیل سویه‌های با پیله تخم‌مرغی شکل بانک ژن کرم ابریشم ایران

نجمه نجفی[۱]، علیرضا صیداوی[۲]*، سیدضیاءالدین میرحسینی[۳]، شهاب‌الدین قره‌ویسی[۴] و آنورادها اچ جینگاده[۵]

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[۱]دانش‌آموخته کارشناسی ارشد گروه علوم دامی، واحد قائم‌شهر، دانشگاه آزاد اسلامی، قائم‌شهر، ایران

[۲]استاد گروه علوم دامی، واحد رشت، دانشگاه آزاد اسلامی، رشت، ایران

[۳]استاد گروه علوم دامی، دانشگاه گیلان، رشت، ایران

[۴] استادیار گروه علوم دامی، واحد قائم‌شهر، دانشگاه آزاد اسلامی، قائم‌شهر، ایران

[۵]محقق مرکز منابع بانک ژن نوغانداری، اتحادیه مرکزی ابریشم، تامیل نادو، هند

*مسئول مکاتبه: Email: alirezaseidavi@iaurasht.ac.ir

چکیده

زمینه مطالعاتی: کرم ابریشم (*Bombyx mori*) یک حشره مهم اقتصادی است که به عنوان ماده آزمایشی برای تکامل نژادهای کرم ابریشم مهم اقتصادی مهم مورد استفاده قرار می‌گیرد. **هدف**: نژادها/سویه‌هایی که بدین ترتیب تکامل یافته‌اند در بانک ژن برای اصلاحگران و محققان حفظ و نگهداری می‌شوند. جمهوری اسلامی ایران از منابع ژنتیکی کرم ابریشم با ارزشی برخوردار است که از نظر شکل پیله متفاوت هستند. **روش کار**: پنجاه و چهار سویه دارای پیله تخم‌مرغی شکل، نگهداری شده توسط مرکز تحقیقات کرم ابریشم ایران (ISRC)، با محاسبه شاخص ارزیابی جهت بررسی تنوع ژنتیکی آنها مورد تجزیه و تحلیل قرار گرفتند. خوشه بندی سلسله مراتبی با استفاده از نرم‌افزار NTSYS-pc انجام شد. **نتایج**: بررسی پارامترهای رشد لاروها نشان داد که شاخص ارزیابی و تابع عملکرد، برای سویه‌های ۱۱۰×۱۰۴ (۱۹۷/۲۷۲ و ۲/۸۵۷) ، ۱۰۰۱ (۱۹۳/۲۶۸ و ۲/۷۸۰ 186.365) BH-4 (۱۸۶/۳۶۵ و ۲/۶۴۷)، ۹-۱۶-۱۲۴ [۱۱۶] (۱۸۳/۰۲۳ و ۲/۶۰۲)، و Shown (۱۸۰/۷۴۴ و ۲/۵۵۶) به‌طور معنی‌داری بیشتر است (P<۰/۰۱). **نتیجه‌گیری نهایی**: تجزیه و تحلیل ANOVA تغییرات قابل توجهی در بین سویه‌های کرم ابریشم را نشان داد.

واژگان کلیدی: رشد لارو؛ تنوع ژنتیکی؛ شاخص ارزیابی؛ تابع عملکرد؛ بهره‌برداری تجاری؛ *بومبیکس موری*