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Investigation on Intera-Specific Biodiversity of 51 Peanut Cocoon Strains of Iran Silkworm (*Bombyx mor*i) Germplasm Based on Reproductive Traits

[1]M. Salehi Nezhad, [2]S.Z. Mirhosseini, [1]S. Gharahveysi, [3]M. Mavvajpour and [4]A.R. Seidavi
[1]Department of Animal Science, Islamic Azad University, Ghaemshahr Branch, Ghaemshahr, Iran
[2]Department of Animal Science, Faculty of Agriculture, Guilan University, Iran
[3]Iran Silkworm Research Center, Rasht, Iran
[4]Animal Science Department, Islamic Azad University, Rasht Branch, Rasht, Iran

Abstract: This study was conducted in ordered to analyze of phylogenetic relationships and determination of relationship amounts in all peanut cocoon strains of Islamic Republic of Iran germplasm based on reproductive characteristics. Hierarchical agglomerative clustering was done by using NTSYS-pc, version 2.02e based on complete, single, UPGMA, UPGMC, FLEXI approaches and SAS-pc based on WARD and average approaches. However, method of average linkage between groups under UPGMA (Unweighted Pair-Group Method using Arithmetic average) was considered as major and final protocol for data conclusion and the resulting clusters were expressed as dendrograms. Various methods generated similar dendograms. This study reveals the phylogenetic relationship of peanut cocoon strains of Iran germplasm. Based on data from studied characters, we constructed dendrograms that resolved the 51 silkworm strains into 2 major clusters. However, the strains of the same origin did not grouped together, demonstrating they can have different biological and development performance. First cluster divided into one sub-group included five strains. However, second cluster divided into two sub-groups. Other strains were grouped together and far from other silkworm strains, indicating they might be suitable for future crossings, maintenance of parental strains and hybridizations with oval cocoon strains so as to maximize heterosis and to avoid depression inbreeding.

Key words: Cluster, *Bombyx mori*, germplasm, peanut strain, fecundity

INTRODUCTION

Today, many farmers are active in sericulture and natural silk production in different regions of the world. Farmers are not rearing silkworm pure lines, so they are rearing silkworm hybrids instead on pure lines and these hybrids are produced via the crosses between pure lines. Thus, research on silkworm pure lines performance and its application to silk production has a significant role to play in improving the quantity and quality of silk produced commercially. Most knowledge of silkworm genetics has relied on external morphological characteristics, although some physiological and biochemical evidence has been available (Yi, 1990; Li *et al*., 2005).

A classification is an orderly arrangement of varieties in a hierarchical series. The kind of relationships revealed in a classification depends on the criteria or characters used in its construction. If characters are quantified and statistically assessed on the basis of overall similarity, the relationships are called phenetic. As Mohammadi and Prasanna (2003) stated accurate assessment of the levels and patterns of genetic diversity can be invaluable in breeding for diverse applications including analysis of genetic variability in cultivars (Smith, 1984; Cox *et al*., 1986), identifying diverse parental combinations to create segregating progenies with maximum genetic variability for further selection and introgressing desirable genes from diverse germplasm into the available genetic base (Thompson *et al*., 1998; Mohammadi and Prasanna, 2003).

Gepts (2006) reviewed plant genetic resources conservation and utilization and presented the accomplishments and future of a societal insurance policy. Also, there are some reports regarding classification of germplasm resources of agriculture and animal gene pool based on various traits especially reproductive characters (Wu *et al*., 2005; Reif *et al*., 2005; Upadhyaya *et al*., 2006; Kumaresan *et al*., 2007).

Previously, 32 grouped as silkworm pure lines and reported these genotypes classified into seven clusters. They suggested those genotypes have long distances must be selected as parents for hybridization crosses and production of commercial

Corresponding Author: Alireza Seidavi, Animal Science Department, Islamic Azad University, Rasht Branch, Rasht, Iran

silkworm eggs. Also, Rao *et al*. (1991) analyzed 15 silkworm pure lines and detected 5 genetic clusters. They reported crosses between long distances will improve economic traits.

Utilization of silkworm gene bank abilities and new and high reproductive pure lines is necessary in order to reproductive improvement for silkworm egg producers. We must recognize genetical relationships of all germplasm pure lines based on reproductive characteristics and determine the distances between these pure lines for scientific organization of future breeding projects.

Iran Silkworm Research Center (ISRC), Rasht, Iran is only repository centre exclusively established for conserving the silkworm genetic resources (*Bomby mori* L.) which is being maintaining 51 silkworm genetic resources collected from different geographical regions possessing large amount of genetic variability, which could be exploited well for breeding programs. To date, phylogenetic relationships of the Iranian germplasm of silkworm *Bombyx mori* based on reproductive characters have also been inadequately studied.

Therefore, this study was conduct in order to analyze of phylogenetic relationships and determination of relationship amounts in all peanut cocoon strains of Islamic Republic of Iran germplasm based on reproductive characteristics.

MATERIALS AND METHODS

This study was conducted in Iran Silkworm Research Center (ISRC) and Islamic Azad University, Ghaemshahr Branch, Iran during 2008-2009. The experiments were carried out on 51 *Bombyx mori* strains maintained at the Iran Silkworm Research Center germplasm bank and data were analyzed for studied characteristics. For the experiments, 250 silkworm larvae from each strain were raised in rearing trays under controlled environment and hygienic conditions. All strains received the same nutritional and rearing treatments (ESCAP, 1993). After pupae development, obtained male and female moths mated and female moths laid egg individually for each replication.

Fifty one silkworm strains were used in the present study. These strains included (1):107-K, (2):119-K, (3):113-K, (4):105, (5):31, (6):51, (7):103, (8):BH-2, (9):B2-09, (10):1003-4, (11):1003-5, (12):1005, (13):M2-6-22-2, (14):M2-6-18(109), (15):M-1-2(5), (16):M2-6-22(107), (17):M2-6-18.3, (18):307-300-2, (19):202A-204B, (20):I 20, (21):101433-9-5, (22):101433-1-4, (23):101433-6-6, (24):1126 (111), (25):113 (2029), (26):151 (103×M-1-1), (27):Xihang 2.3, (28):Xihang 3.3, (29):153 (Xihang-1), (30):5118×10133-2-2, (31):5118×10133-3-3, (32):Black-White, (33):101×F6, (34):F6×101, (35):Kinshu, (36):M-1-1×31, (37):31×M-1-1, (38):M-1-1×103, (39):103 Poly Marking, (40):Shaki, (41):101, (42):T1-J, (43):T5-M, (44):236, (45):1524, (46):1433-15, (47):1433-9, (48):7409, (49):N19, (50):White Larvae-Yellow Cocoon and (51):Black Larvae-White Cocoon. Studied quantitative characteristics included hatchability percentage (%), number of laid eggs, number of fertilized eggs, number of un-fertilized eggs and number of un-hatched eggs.

The grouping methods allowed us to subdivide observations into several subgroups in such a way that we obtained homogeneity inside the subgroups and heterogeneity among the subgroups. Hierarchical agglomerative clustering was done by using NTSYS-pc, computer package version 2.02E (Rohlf, 1998) based on complete, single, UPGMA, UPGMC, FLEXI approaches and SAS-pc (SAS, 1997) based on WARD and average approaches. However, method of average linkage between groups (Romesburg, 1984) under UPGMA (Unweighted Pair-Group Method using Arithmetic average) was considered as major and final protocol for data conclusion (Sneath and Sokal, 1973) and the resulting clusters were expressed as dendrograms. This method employed for grouping, 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 is 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.

RESULTS AND DISCUSSION

Obtained results are summarized in Fig. 1-7 and Table 1. Various methods generated similar dendograms. The cluster analysis revealed a clear division into some groups and sub-groups (Fig. 1-7). Figure 1 shows cluster analysis of silkworm strains under WARD approach. Meanwhile, Fig. 2 presents cluster analysis of silkworm strains under average approach. Also, Fig. 3

Table 1: Proximity comparison of used methods for clustering analysis in the study using NTSYSpc

Method	UPGMA	Complete	FLEXI	UPGMC	Single
UPGMA	1				
Complete	0.408	1			
FLEXI	0.775	0.408	1		
UPGMC	0.286	0.306	0.327	1	
Single	0.245	0.184	0.224	0.142	1

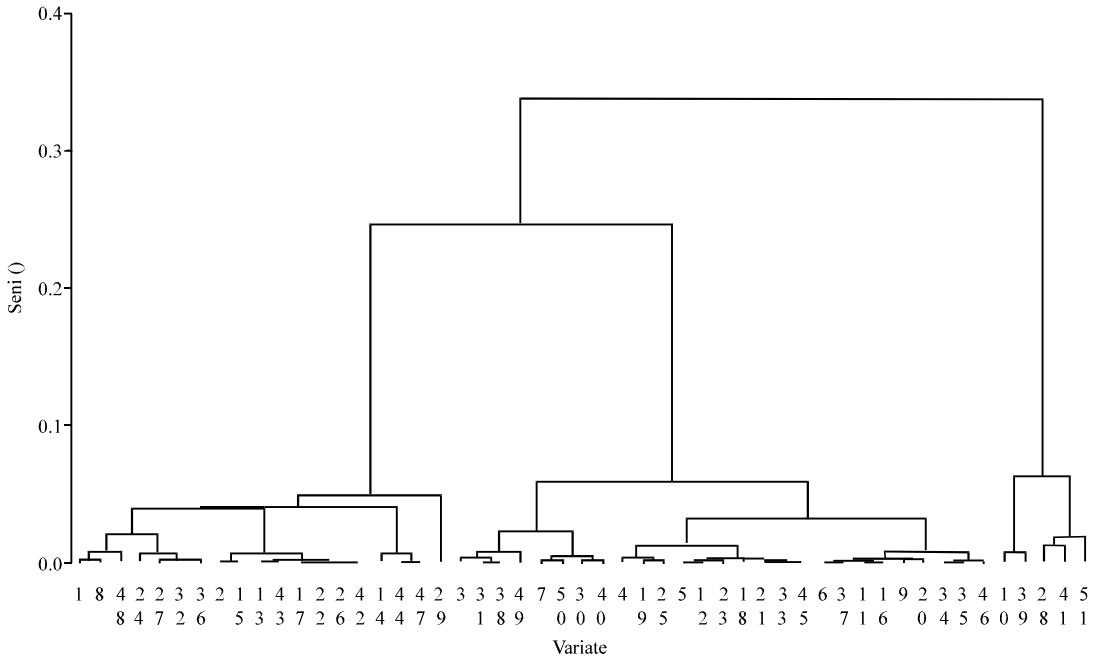

Fig. 1: Cluster analysis based on all 5 studied reproductive traits for 51 silkworm strains according to the grouping from WARD method using SAS (1997)

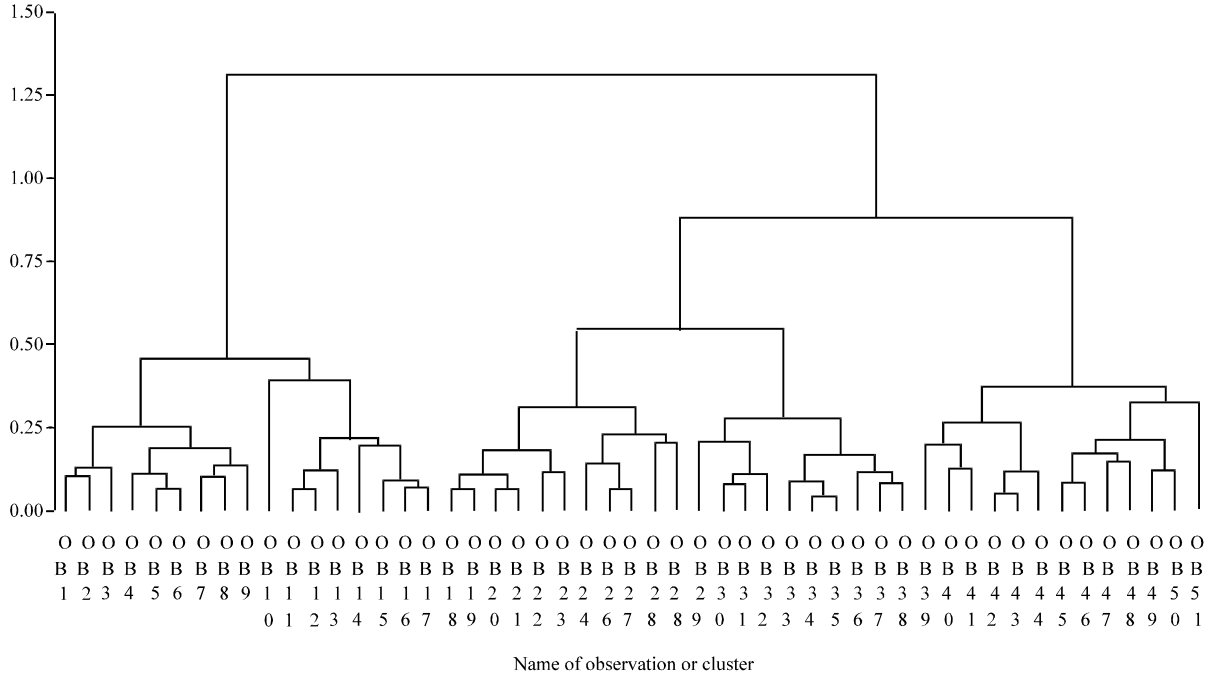

Fig. 2: Cluster analysis based on all 5 studied reproductive traits for 51 silkworm strains according to the grouping from average method using SAS (1997)

shows cluster analysis of silkworm strains under complete approach. Meanwhile, Fig. 4 shows cluster analysis of silkworm strains under single approach. Figure 5 shows cluster analysis of silkworm strains under UPGMC approach. Figure 6 shows cluster analysis of silkworm strains under FLEXI approach.

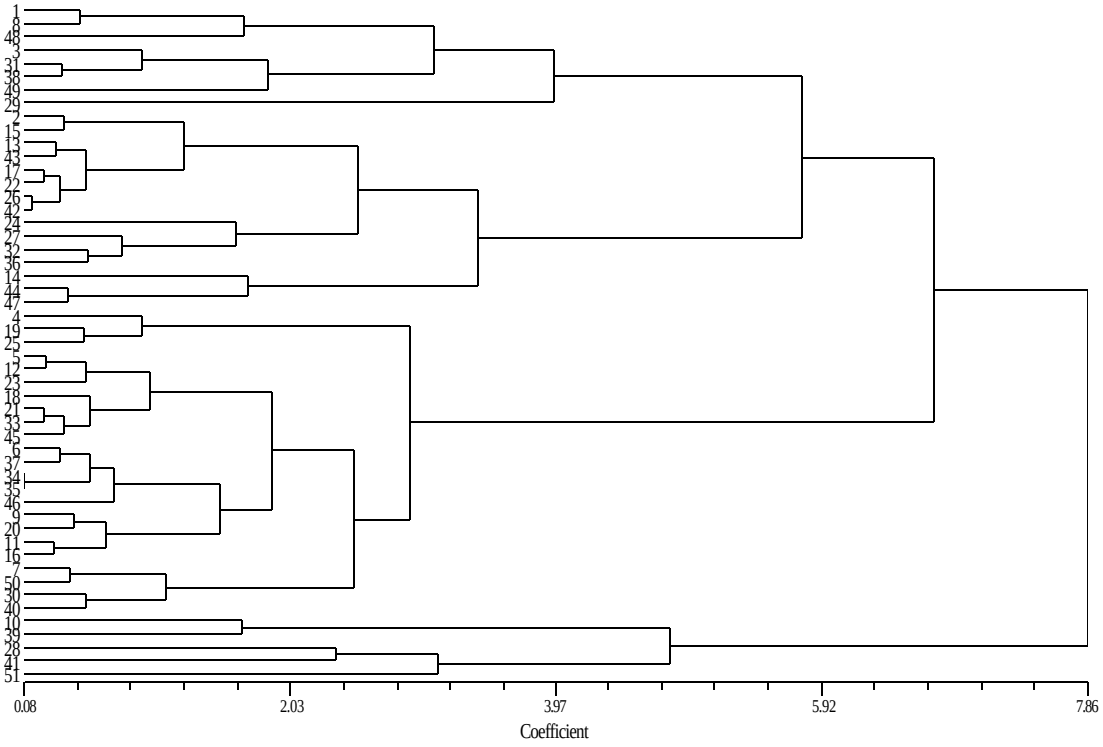

Fig. 3: Cluster analysis based on all 5 studied reproductive traits for 51 silkworm strains according to the grouping from complet method using NTSYS

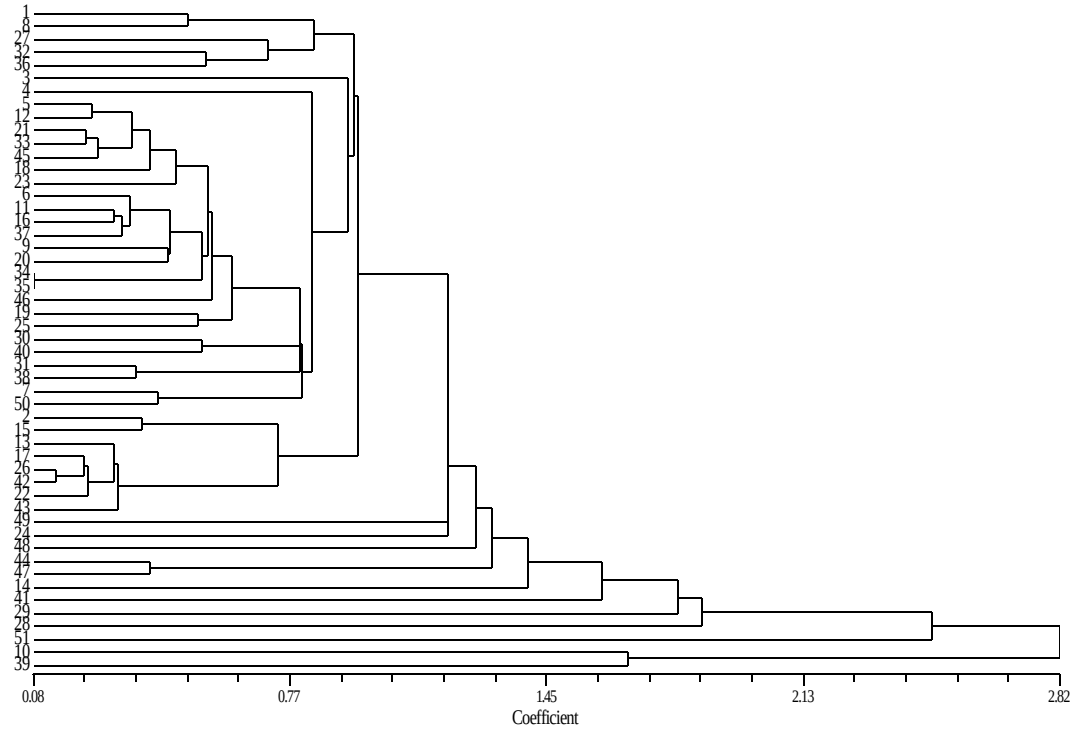

Fig. 4: Cluster analysis based on all 5 studied reproductive traits for 51 silkworm strains according to the grouping from single method using NTSYS

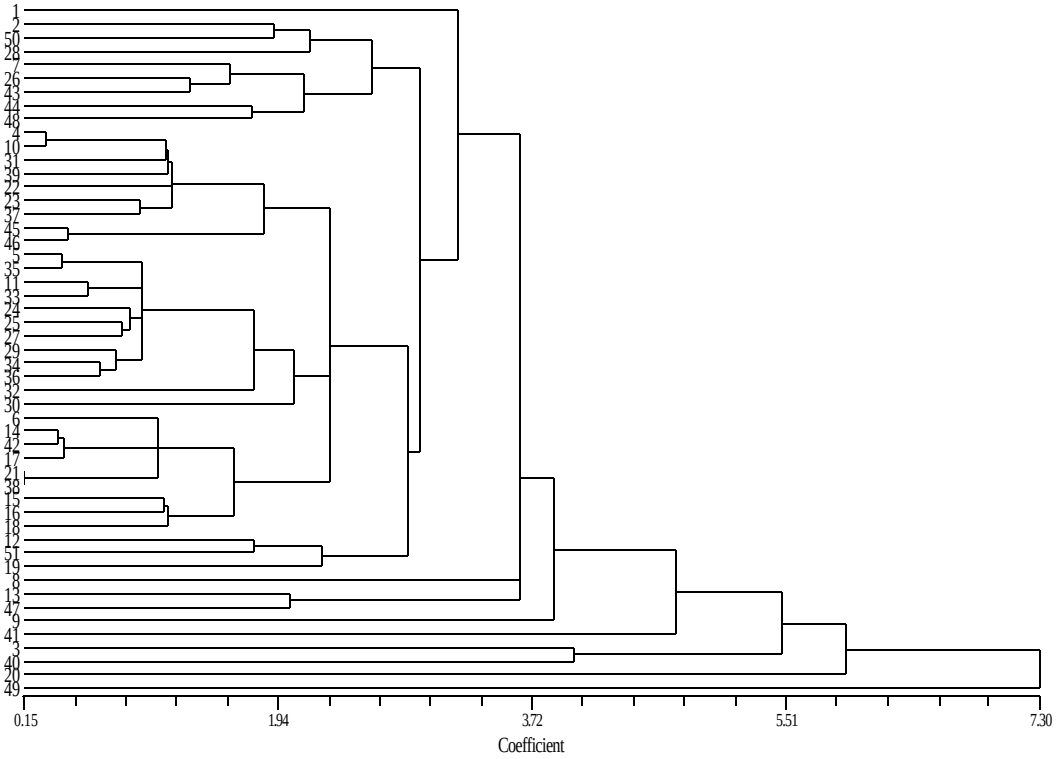

Fig. 5: Cluster analysis based on all 5 studied reproductive traits for 51 silkworm strains according to the grouping from UPGMC method using NTSYS

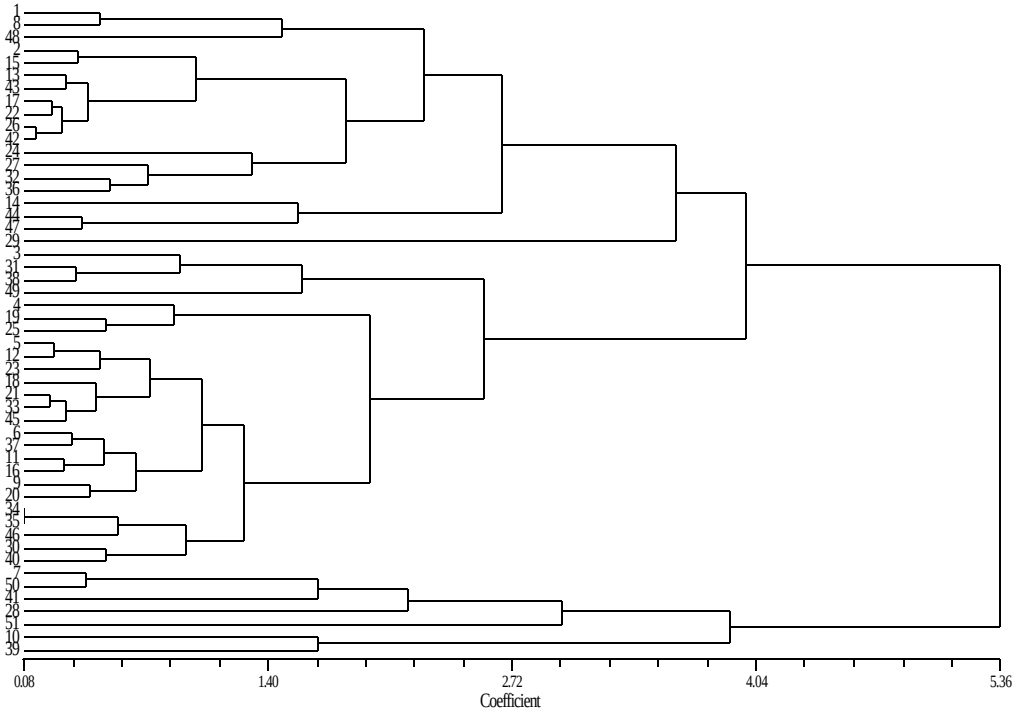

Fig. 6: Cluster analysis based on all 5 studied reproductive traits for 51 silkworm strains according to the grouping from FLEXI method using NTSYS

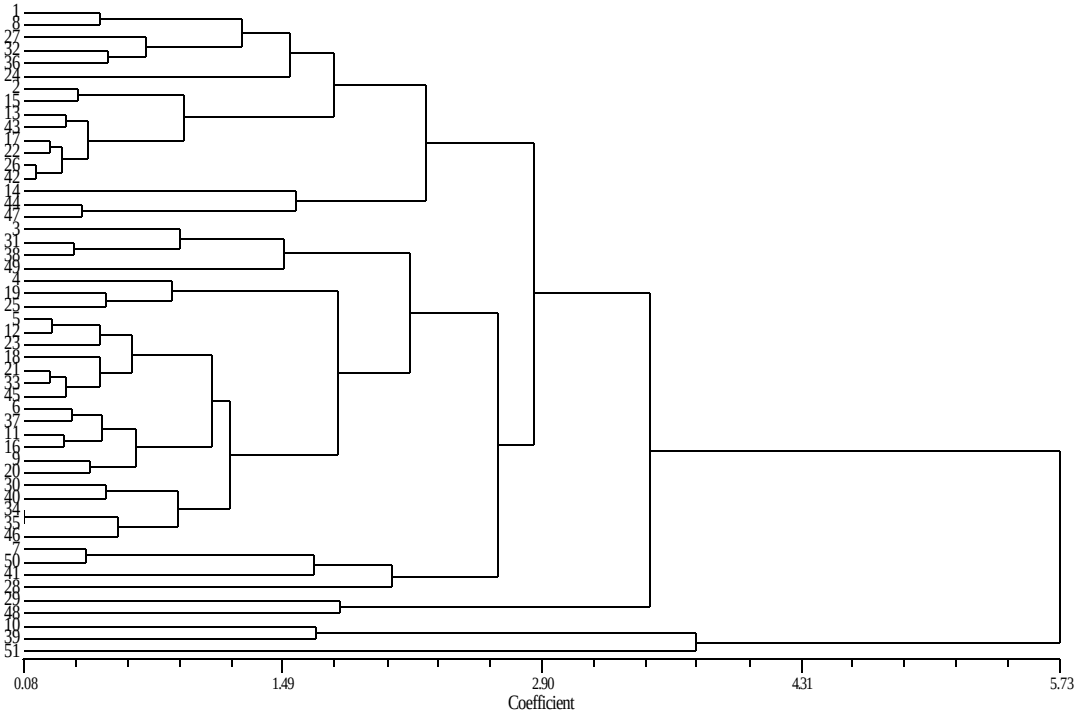

Fig. 7: Cluster analysis based on all 5 studied reproductive traits for 51 silkworm strains according to the grouping from UPGMA (Unweighted Pair Group Method Average) method using NTSYS

Figure 7 shows cluster analysis of silkworm strains under UPGMA approach. This study reveals the phylogenetic relationship of peanut cocoon strains of Iran germplasm. Based on data from studied characters, we constructed dendrograms that resolved the 51 silkworm strains into 2 major clusters. However, the strains of the same origin did not grouped together, demonstrating they can have different biological and development performance. First cluster divided into one sub-group included five strains. However, second cluster divided into two sub-groups. Other strains were grouped together and far from other silkworm strains, indicating they might be suitable for future crossings, maintenance of parental strains and hybridizations with oval cocoon strains so as to maximize heterosis and to avoid depression inbreeding.

As indicated earlier, our final analysis and conclusion has been done on the basis of the average linkage between groups or UPGMA, since as others researchers have shown (Chatterjee and Datta, 1992), UPGMA yields more accurate results for classification purposes than other hierarchical methods. Thus, the present study also presents the result of other clustering approaches.

Proximity of used methods for clustering analysis in the study compared using NTSYSpc. From obtained results, it is showed FLEXI and UPGMA had the highest proximity (0.775). Then both complete and UPGMA and also complete and FLEXI had the highest proximity (0.408), respectively. Meanwhile single and complete had the lowest proximity (0.184).

Hierarchical agglomerative clustering was done based on complete, single, UPGMA, UPGMC, FLEXI, WARD and average approaches. All these approaches yielded optimal some cluster solution after 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 number of influential variables criteria. Also, Kenezovi *et al*. (2005) stated 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 wellseparated groups.

Garson (2009) stated hierarchical clustering is appropriate for smaller samples (typically<250). When n is large, the algorithm will be very slow to reach a solution and indeed, may hang one's computer. To accomplish

hierarchical clustering, the researcher must specify how similarity or distance is defined and how clusters are aggregated (or divided). Hierarchical clustering generates all possible clusters of sizes 1...K, but is used only for relatively small samples. In hierarchical clustering, the clusters are nested rather than being mutually exclusive, as is the usual case. That is, in hierarchical clustering, larger clusters created at later stages may contain smaller clusters created at earlier stages of agglomeration. Forward clustering, also called agglomerative clustering: Small clusters are formed by using a high similarity index cut-off (ex., .9). Then this cut-off is relaxed to establish broader and broader clusters in stages until all cases are in a single cluster at some low similarity index cut-off. The merging of clusters is visualized using a tree format (Garson, 2009).

As Peck (2005) stated a main advantage of this approach to identifying subgroups within evaluation data is that people rarely have only one trait at a time; instead, they are individually complex among complex populations. Using cluster analysis to identify underlying groups of observations within the data capitalizes on this heterogeneity and sorts it out to make it useful in the context of a subgroup analysis (Peck, 2005).

Bombyx mori strains have been reared in different regions of the world and different strains have evolved because of changes in their phenotype and genotype over time (Mirhosseini *et al*., 2007). Based on one hypothesis, all the strains during a long period have been differentiated from a monovoltine Chinese variety (Chatterjee and Datta, 1992; Mirhosseini *et al*., 2007).

Li *et al*. (2007) performed ISSR amplification to analyze the genetic relationship among different silkworm strains maintained at Sericultural Research Institute (SRI-CAAS) of China. They identified the monovoltine, bivoltine and polyvoltine strains, which clustered separately (Li *et al*., 2007; Velu *et al*., 2008).

As Chatterjee and Datta (1992) presented further 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 attributes. Moreover, as Chatterjee and Datta (1992) presented based on such observations, attempts were made (Chiang, 1980; Gamo, 1983) to assess genetical distances between different groups of silkworm races in tropical and temperate regions. 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 Datta, 1992).

On other hand, as Li *et al*. (2005) presented Lepidoptera contains more than 140000 species. It is interesting to understand their origin and the cause of their diversity. The evolutionary relationships among different species had been studied using the conserved gene, for example, 16S RNA (Li *et al*., 2005).

Chattarijee and Datta (1992) utilized the biochemical markers to classify 54 silkworm strains with different geographical origins. They also obtained similar results on some strains with different origin in one group and also strains with the same origin in different groups (Chattarijee and Datta, 1992; Etebari *et al*., 2005).

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