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Investigation on intera-specific biodiversity of 20 silkworm germplasm based on glucose, urea, uric acid, lactat D-hydrogenase, total protein and alpha amylase

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Abstract: Aim of this experiment was to investigation on the profile of biochemical markers in twenty peanut cocoon germplasm of Iran. In 5th day of 5th instar, heamolymph sampled using standard method. Sampled heamolymph transferred to laboratory. Heamolymph was obtained by cutting abdominal proleg and collected into 1.5 ml tube containing a few granules of phenylthiourea to prevent melanization. After 10 min centrifugation at 10000 rpm, the supernatant was used. Pellets were discarded also. The supernatant was transferred to new tubes and was preserved at -20 ºC until the onset of the experiments. From obtained results, it is showed that amount of glucose in twenty studied varieties included between 1.00-14.04 mg/dl. Among studied varieties, the highest level of glucose belonged to [2029] 113 (14.04 mg/dl), and some another varieties (1.00 mg/dl) remained at lower level than other varieties. Other varieties were between these two groups. Meanwhile statistical differences between studied varieties for this trait were significant (P<0.05). Based on these dendrograms, analyzed varieties were divided into two distinct groups. At cross 1.76, two clusters were formed which classified into subgroups in crosses of 1.30 and 0.80 Frequent divisions were also observed in major groups. First group included [2029] 113 variety and second group included other varieties. Among studied varieties, the highest level of total protein belonged to k-107 (6.52 mg/dl), and M2-6-18 variety (4.11 [g/dl]) remained at lower level than other varieties (P<0.05).On the basis of these dendrograms, analyzed varieties were divided into two distinct groups. At cross 3.11, two clusters were formed which classified into subgroups in cross of 0.02. Frequent divisions were also observed in major groups. First group included [151] variety. Second group included other varieties.
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Keywords: Species richness; beta-diversity; taxonomic diversity; forest

1. Introduction

Silkworm rearing has huge history in the world. Based on the obtained evidences seems to this activity started from China over the centuries and has been transferred to other countries afterward. Valuable product of this industry i.e. natural silk, according to its structural characteristics, despite lower production than other natural and synthetic fibers, has caused and provided cultural, social and economical exchanges in the history.

Biochemical metabolites and enzymes have important role in silkworm production. Chatterjee and Datta (1992) studied genetic variation of sterase enzymes in several varieties of silkworm and found sterase zymogram of fifth instar silkworm have 11 or 12 bands. Meanwhile, Lin et al (2001) also based on their studies reported significant impact of the environment conditions on silkworm biochemical parameters.

Iran already has a silkworm gene bank that stores the genetic resources for various breeding and development programs. Precise knowledge of the status of haemolymph biochemical variables in these varieties can help us for development aims. Since there are not available these data to date, this research effort will be investigate the profile of biochemical markers in twenty peanut cocoon germplasm of Iran.

2. Material and Methods

This study was conducted in Islamic Azad University, Rasht Branch, Iran during 2010-2011. Data in this study were collected from the Iran Silkworm Research Center (ISRC) which is the original trustee in sericulture researches in Iran. This center is located in Rasht.

It was applied favorite conditions for silkworm egg hatching, larvae rearing, and moth emergence. Twenty varieties were reared under

standards protocols in all rearing steps. Silkworm eggs were hatched and brushed. Disease-free eggs of the silkworm varieties were used. In order to make proper coordination in embryos growth, silkworm egg to embryos rotation stage (day 6 to 7) exposed to natural daylight and darkness at night and after being up at this stage to change the color stage of the egg they have exposure 18 hours light and 6 hours darkness. Then which completion of changing the color of the egg (more than 90%), complete darkness for three days given and on the morning of the day fourteen with the supply of light, the eggs were hatching. Silkworm rearing techniques including humidity, temperature, light, and young and mature silkworm rearing were conducted following the standard procedure of ESCAP (1993). Scientific technology of silkworm rearing was followed according to standard method. Rearing in young silkworm period was performed by chopped leaves and paraffin paper coverage and in the adult period it was performed with leaves and branches.

In 5th day of 5th instar, heamolymph sampled using standard method. Sampled heamolymph transferred to laboratory. Heamolymph was obtained by cutting abdominal proleg and collected into 1.5 ml tube containing a few granules of phenylthiourea to prevent melanization. After 10 min centrifugation at 10000 rpm, the supernatant was used. Pellets was discarded also. The supernatant was transferred to new tubes and was preserved at -20 ºC until the onset of the experiments.

Twenty silkworm varieties were used in the present study. These varieties included 31, 1005, 113 (2029), 153 (Xihang-1), 5118×10133-3-3, F6×101, 1433-15, 1126 (111), 113-K, 1003-4, M2-6-18 (109), M2-6-22 (107), 151 (103×M-1-1), Xihang 2/3, M-1-1×103, 7409, 107-K, 103, T5-M, and 101433-1-4.

Data recorded for this study were glucose, urea, uric acid, lactat D-hydrogenase, total protein and alpha amylase. These parameters were measured using commercial and experimental kits (Thomas, 1998). Furthermore data above 70% or below 30%, undergone inverse sin transformation ($Z=\text{Arcsin } P_{ij}^{1/2}$) and data between 0-1, undergone square transformation ($P^{1/2}$). The data were subjected to analysis of variance (ANOVA) to determine if the differences found between treatments and the differences between treatments were significant. For analysis of variance, Tukey's studentized range (HSD) test in a complete randomized design was used at $\alpha=0.05$.

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, version 2.02e (Rohlf, 1998) based on UPGMA (Unweighted Pair-Group Method using Arithmetic average) approach and the resulting clusters were expressed as dendrograms.

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3. Results and Discussion

Obtained results are summarized in Table 1 and Figures 1-7.

Glucose

From obtained results, it is showed that amount of glucose in twenty studied varieties included between 1.00-14.04 mg/dl. Among studied varieties, the highest level of glucose belonged to [2029] 113 (14.04 mg/dl), and some another varieties (1.00 mg/dl) remained at lower level than other varieties. Other varieties were between these two groups. Meanwhile statistical differences between studied varieties for this trait were significant ($P<0.05$).

Figure 1 obtained from hierarchical analysis of these varieties, represents phylogeny classification of six studied varieties based on glucose parameter. On the basis of these dendrograms, analyzed varieties were divided into two distinct groups. At cross 1.76, two clusters were formed which classified into subgroups in crosses of 1.30 and 0.80 Frequent divisions were also observed in major groups. First group included [2029] 113 variety and second group included other varieties.

Urea

From obtained results, it is showed that amount of urea in twenty studied varieties included between 6.02-8.02 mg/dl. Among studied varieties, the highest level of urea belonged to 101433-1-4, 1005, M-1-1×103 and 103 (6.02 mg/dl), and some another varieties (8.02 mg/dl) remained at lower level than other varieties. Other varieties were between these two groups. Meanwhile statistical differences between studied varieties for this trait were significant ($P<0.05$).

Figure 2 obtained from hierarchical analysis of these varieties, represents phylogeny classification of six studied varieties based on urea parameter. At cross 1.80, two Clusters were formed which classified into subgroups. First group included White Haratee and Pink Khorasan varieties and second group included other varieties.

Uric Acid

From obtained results, it is showed that amount of uric acid in twenty studied varieties included between 0.20-2.80 mg/dl. Among studied varieties, the highest level of uric acid belonged to

[2029] 113 (2.80 mg/dl), and M-1-1×103 (0.20 mg/dl) remained at lower level than other varieties. Other varieties were between these two groups. Meanwhile statistical differences between studied varieties for this trait were significant (P<0.05).

Figure 3 obtained from hierarchical analysis of these varieties, represents phylogeny classification of six studied varieties based on uric acid parameter. On the basis of these dendrograms, analyzed varieties were divided into two distinct groups. At cross 4.19, two clusters were formed which classified into subgroups in crosses of 1.30 and 0.10 Frequent divisions were also observed in major groups. First group included [2029] 113 variety. Second group was included 31, 153 and (Xihang-1) varieties.

Lactat D-Hydrogenase (LDH)

From obtained results, it is showed that amount of lactat D-hydrogenase (LDH) in twenty studied varieties included between 1.00-17.05 [mg/dl]. Among studied varieties, the highest level of lactat D-hydrogenase (LDH) belonged to4-1-101433 (17.05 [IU/L]) and 4-1003variety (1.00 [mg/dl]) remained at lower level than other varieties. Other varieties were between these two groups. Meanwhile statistical differences between studied varieties for this trait were significant (P<0.05).

Figure 4 obtained from hierarchical analysis of these varieties, represents phylogeny classification of six studied varieties based on glucose parameter. On the basis of these dendrograms, analyzed varieties were divided into three distinct groups. At cross 1.61, two clusters were formed which classified into subgroups in crosses of 1.10. Frequent divisions were also observed in major groups. First group included [111] 1126, 151 variety.

Total Protein

From obtained results, it is showed that amount of total protein in twenty studied varieties included between 4.11-6.52 [g/dl]. Among studied varieties, the highest level of total protein belonged to k-107 (6.52 mg/dl), and M2-6-18 variety (4.11 [g/dl]) remained at lower level than other varieties. Other varieties were between these two groups. Meanwhile statistical differences between studied varieties for this trait were significant (P<0.05).

Figure 5 obtained from hierarchical analysis of these varieties, represents phylogeny classification of six studied varieties based on total protein parameter. On the basis of these dendrograms, analyzed varieties were divided into two distinct groups. At cross 3.11, two clusters were formed which classified into subgroups in cross of 0.02. Frequent divisions were also observed in major groups. First group included [151] variety. Second group included other varieties.

Alpha Amylase

From obtained results, it is showed that amount of alpha amylase in twenty studied varieties included between 1.50-16.05 [IU/L]. Among studied varieties, the highest level of alpha amylase belonged to [7409] (16.05 [IU/L]), and T5-M and4-1-101433 varieties (1.50 [IU/L]) remained at lower level than other varieties. Other varieties were between these two groups. Meanwhile statistical differences between studied varieties for this trait were significant (P<0.05).

Figure 6 obtained from hierarchical analysis of these varieties, represents phylogeny classification of six studied varieties based on glucose parameter. Based on these dendrograms, analyzed varieties were divided into three distinct groups. At cross 2.36, two clusters were formed which classified into subgroups in crosses of 1.50. Frequent divisions were also observed in major groups. First group included 4-1003 and k-107 varieties and second group included other varieties.

There are similar reports regarding silkworm-rearing technologies. For example, Zhu (1990) investigated the relationship between cocoon spining conditions and physical characteristics of cocoon. In addition, Hsieh et al (1995) studied the amount of silkworm resistance against heat. Meanwhile, Tzenov (1996) reporeted the effects of temperature during the fifth larval instar on dry matter intake and the biological and reproductive traits. Also, Sathyanarayana et al (1995) studied the effects of microclimate conditions during larvae molting on economic traits of silkworm. On the other hand, Basavaraju et al (1996) investigated the effects of temperature on amylase activity in the silkworm. Meanwhile, Elena (2002) reported effects of changes in microclimate and nutritional conditions on the incidence of stress and disease conditions in the silkworm.

Table 1- Mean comparison (±SEM) of biochemical parameters in heamolymph of twenty studied silkworm varieties

No	Variety	Parameters					
		Glucose	Urea	Uric Acid	Lactat D-Hydrogenase (LDH)	Total Protein	Alpha Amylase
		[mg/dl]	[mg/dl]	[mg/dl]	[IU/L]	[g/dl]	[IU/L]
1	31	4.01±0.01^{h}	7.02±0.02^{b}	0.30±0.00^{h}	3.01±0.58^{hi}	5.01±0.02^{e}	5.017±0.02^{fgh}
2	1005	4.01±0.01^{h}	8.02±0.03^{a}	0.30±0.00^{a}	9.53±0.29^{cd}	5.36±0.03^{c}	10.03±0.03^{c}
3	113 (2029)	14.04±0.05^{a}	7.02±0.02^{b}	2.80±0.01^{a}	13.04±0.04^{b}	4.86±0.03^{f}	4.51±0.87^{gh}
4	153 (Xihang-1)	1.00±0.00^{k}	7.02±0.02^{b}	0.30±0.00^{h}	13.04±0.04^{b}	4.31±0.01^{j}	5.51±0.29^{efgh}
5	5118×10133-3-3	10.03±0.03^{c}	7.02±0.02^{b}	0.60±0.00^{c}	7.52±1.44^{def}	5.16±0.03^{d}	8.52±0.29^{cd}
6	F6×101	8.02±0.03^{d}	6.02±0.02^{c}	0.45±0.03^{e}	5.01±0.58^{fgh}	5.16±0.03^{d}	6.52±0.29^{ef}
7	1433-15	2.00±0.01^{i}	7.02±0.02^{b}	0.40±0.00^{f}	8.52±1.44^{de}	5.56±0.03^{b}	6.52±0.29^{ef}
8	1126 (111)	5.01±0.02^{g}	7.02±0.02^{b}	0.35±0.03^{g}	3.51±0.29^{h}	4.76±0.03^{g}	7.02±0.02^{de}
9	113-K	5.51±0.29^{f}	7.02±0.02^{b}	0.30±0.00^{h}	9.53±0.29^{cd}	4.56±0.03^{h}	8.52±0.29^{cd}
10	1003-4	2.00±0.01^{i}	7.02±0.02^{b}	0.25±0.03^{i}	1.00±0.00^{i}	4.41±0.01^{i}	6.52±0.29^{cd}
11	M2-6-18 (109)	1.00±0.00^{k}	6.02±0.02^{c}	0.30±0.00^{h}	5.01±0.02^{fgh}	4.11±0.01^{k}	7.02±0.02^{de}
12	M2-6-22 (107)	4.01±0.01^{h}	7.02±0.02^{b}	0.30±0.00^{h}	9.53±2.02^{cd}	5.31±0.02^{c}	12.54±0.29^{b}
13	151 (103×M-1-1)	1.00±0.00^{k}	6.02±0.02^{c}	0.20±0.00^{j}	13.04±0.04^{b}	5.16±0.03^{d}	4.51±0.29^{gh}
14	Xihang 2/3	1.00±0.00^{k}	6.52±0.29^{c}	0.30±0.00^{h}	11.53±0.87^{bc}	4.91±0.02^{f}	8.52±0.29^{cd}
15	M-1-1×103	1.00±0.00^{k}	8.02±0.03^{a}	0.25±0.03^{i}	8.52±0.29^{de}	4.86±0.03^{f}	6.02±0.02^{efg}
16	7409	10.03±0.03^{c}	7.02±0.02^{b}	0.50±0.00^{d}	7.02±0.58^{defg}	5.01±0.02^{e}	16.05±0.05^{a}
17	107-K	7.02±0.02^{e}	8.02±0.03^{a}	0.50±0.00^{d}	4.51±0.87^{gh}	6.52±0.02^{a}	4.01±0.58^{h}
18	103	10.03±0.03^{c}	8.02±0.03^{a}	0.60±0.00^{c}	7.02±0.58^{defg}	4.56±0.03^{h}	8.52±2.02^{cd}
19	T5-M	11.53±0.29^{b}	7.02±0.02^{b}	0.70±0.00^{b}	6.52±0.87^{efg}	4.71±0.02^{g}	1.50±0.29^{i}
20	101433-1-4	1.50±0.29^{j}	8.02±0.03^{a}	0.30±0.00^{h}	17.05±0.58^{a}	4.31±0.01^{j}	1.50±0.29^{i}

*Means in each row followed by the same letters are not significantly different at α=0.05

Glucose [mg/dl]

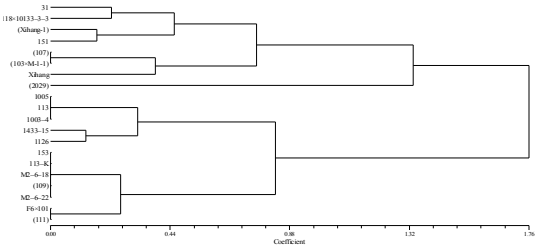

Figure 1 Cluster analysis of 20 studied silkworm varieties based on heamolymph glucose

Urea [mg/dl]

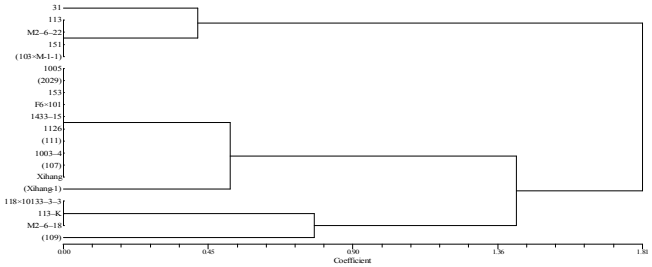

Figure 2 Cluster analysis of 20 studied silkworm varieties based on heamolymph urea

Uric Acid [mg/dl]

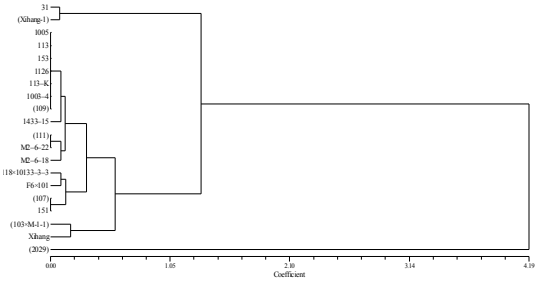

Figure 3 Cluster analysis of 20 studied silkworm varieties based on heamolymph uric acid

Lactat D-Hydrogenase (LDH) [IU/L]

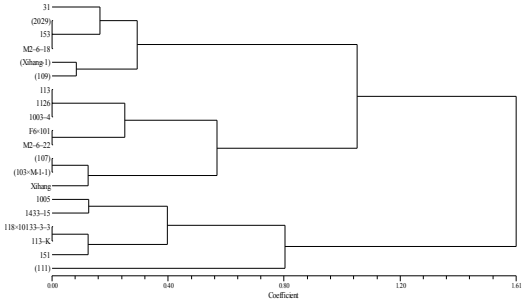

Figure 4 Cluster analysis of 20 studied silkworm varieties based on heamolymph lactate D- Hydrogenase

Total Protein [g/dl]

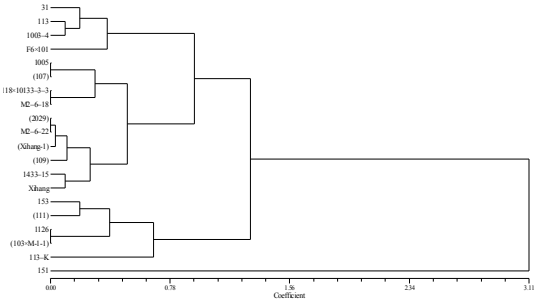

Figure 5 Cluster analysis of 20 studied silkworm varieties based on heamolymph total protein

Alpha Amylase (IU/L)

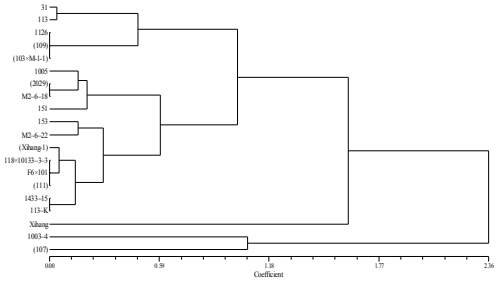

Figure 6 Cluster analysis of 20 studied silkworm varieties based on heamolymph alpha amylase

Glucose [mg/dl], Urea [mg/dl], Uric Acid [mg/dl], Lactat D-Hydrogenase (LDH) [IU/L], Total Protein [g/dl] and Alpha Amylase (IU/L)

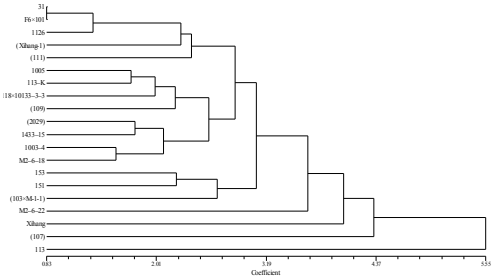

Figure 7 Cluster analysis of 20 studied silkworm varieties based on all studied heamolymph biochemical parameters

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