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Evaluation of Resistance to Nuclear Polyhedrosis Virus in 20 Commercial Hybrids of Silkworm (Bombyx mori)【Research report】

評估20種商用家蠶雜交品系對核多角體病毒的抵抗能力【研究報告】

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Abstract

Nuclear polyhedrosis is the most-common viral disease of the silkworm Bombyx mori. Resistance to nuclear polyhedrosis virus (NPV) was evaluated in 20 new Iranian hybrids of silkworms. B. mori larvae of 3rd molt were orally inoculated with B. mori NPV (550 ppm) and reared up to the spinning stage in summer. Some biological and economic characteristics were measured in different groups. From the results obtained, hybrids 107k×124k, 107k×108k, and 101433×114 showed the highest resistance to this disease, which was significantly (P < 0.05) higher than that of the other strains. Agglomerative cluster analysis categorized different hybrids in two classes based on their degree of resistance. Furthermore, the 111×114, 103×104 and 111×116 hybrids were shown to be the most susceptible to BmNPV.

摘要

核多角體病毒是家蠶 (Bombyx mori) 最普遍的病毒疾病，本研究評估 20 種新的伊朗雜交家蠶對核多角體病毒 (NPV) 的抵抗能力。第3次蛻皮後的家蠶幼蟲，經口餵飼接種家蠶 NPV (550 ppm)，一直飼養到夏季吐絲期，並調查各處理組的一些生物學和經濟特性。根據試驗結果顯示，107k×124k、107k×108k 和 101433×114 雜交品系較其他品系對此疾病具有顯著性 (P < 0.05) 的最高抵抗能力。依抗性程度，使用 Agglomerative 叢聚分析，可將不同的雜交品系分成兩大類，其中以 111×114、103×104 和 111×116 雜交品系對 BmNPV 最為敏感。

Key words: nuclear ployhedrosis virus, Bombyx mori, resistance, hybrid

關鍵詞: 核多角體病毒、家蠶、抵抗能力、雜交

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Evaluation of Resistance to Nuclear Polyhedrosis Virus in 20 Commercial Hybrids of Silkworm (*Bombyx mori*)

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ABSTRACT

Nuclear polyhedrosis is the most-common viral disease of the silkworm *Bombyx mori*. Resistance to nuclear polyhedrosis virus (NPV) was evaluated in 20 new Iranian hybrids of silkworms. *B. mori* larvae of 3rd molt were orally inoculated with *B. mori* NPV (550 ppm) and reared up to the spinning stage in summer. Some biological and economic characteristics were measured in different groups. From the results obtained, hybrids 107k×124k, 107k×108k, and 101433×114 showed the highest resistance to this disease, which was significantly ($P < 0.05$) higher than that of the other strains. Agglomerative cluster analysis categorized different hybrids in two classes based on their degree of resistance. Furthermore, the 111×114, 103×104 and 111×116 hybrids were shown to be the most susceptible to BmNPV.

Key words: nuclear ployhedrosis virus, *Bombyx mori*, resistance, hybrid

Introduction

Disease of the silkworm, *Bombyx mori,* are the main factors seriously affecting cocoon production (Watanabe, 1986). Nuclear polyhedrosis virus (NPV) is one of the dominant diseases in almost all sericultural areas of Iran and other countries. It occurs in all larval instars but more commonly in the 4th and 5th instars during all seasons and cause 20-50% cocoon crop losses in India (Shivaprakasham and Rabindra, 1995; Biabani, 1998; Sivaprasad *et al*., 2003; Khurad *et al*., 2004). Prevention of silkworm diseases has become one of the most-important aspects in the success of commercial sericulture. In order to obtain high and stable cocoon yield it is necessary to make efforts first to decrease pathogen quantity and pathogenicity, and second to strengthen larval health by increasing their disease resistance ability (Singh *et al*., 2003).

Different parameters affect disease spread, among which environmental conditions are highly significant in the larval response to NPV (Kobayashi *et al*., 1981). Barman *et al*. (1988) reported that the seasonal effect was highly significant

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in larval resistance to NPV, with high temperatures (> 28°C) and relative humidity (> 80%) being major factors. In contrast, a comparison of differences in levels of resistance to NPV between spring- and autumn-reared individuals of *B. mori* in China showed that resistance levels did not greatly vary between seasons (Liaw and Chang, 1989).

The ability of larvae to resist infection is very markedly reduced when they were kept at a low temperature (5ºC for 24 h) or kept without food (for 24 hours at 25ºC), although 1st and 2nd instar larvae resist starvation well (Wu, 1983a).

The quantity and quality of the diet are two of the most important factors in larval susceptibility to baculoviruses diseases (Matsubara *et al.*, 1989). Larval susceptibility decreased as the level of leaf powder in an artificial diet increased (Wu, 1983b).

Larval susceptibility to viral disease greatly differed in various hybrids, and that was a turning point in silkworm breeding programs. Many researchers reported inter-breed/strain differences in susceptibility to *Bombyx mori* NPV (BmNPV), but very few studies have been carried out on the development of resistance of insects to pathogens (Furuta, 1995; Ghosh *et al.,* 2000; Seydavi *et al.*, 2003; Singh *et al.*, 2003; Sivaprasad *et al.*, 2003). Most of these resistances in silkworm hybrids have physiological and genetic origins that are controlled by polygenes (Li *et al.*, 1994; Chen *et al.*, 2003). Studies of 11 Chinese and 11 Japanese lines of silkworms showed that the Japanese varieties had greater free proline contents and greater resistances to NPV. There was a positive correlation (0.93) between the free proline content and resistance (Li *et al.*, 1994). The proteinase activity and anti-NPV activity of the gut juice were correlated with the amount of protein in the diet. Also Watanabe *et al.* (1990) reported that the anti-NPV activity of the gut juice was mainly dependent upon the level of proteinase activity.

Silkworm hybrids are produced in efforts to improve the yield of silkworm rearing, whereas these genetic characterristics greatly differ among groups of hybrids. So, the identification and evaluation of the resistance and susceptibility of hybrids to nuclear polyhedrosis virus in different seasons are very important as selection indices for silkworm breeding.

Identification of silkworm lines and commercial hybrids tolerant to BmNPV and other diseases is very useful and was conducted in China by Liu (1984), by Furuta (1995) in Japan, and recently by Sivaprasad *et al.* (2003) in India.

In this research, we evaluated the resistance to NPV of new silkworm hybrids, which evolved in the Iran Sericultural Research Center (ISRC) from pure lines (Gholami and Merat, 2002; Gholami *et al.*, 2002; Mirhoseini and Gholami, 2002) in order to select the most-resistant hybrids for commercial production.

Materials and Methods

BmNPV Inoculum Preparation

Larvae infected with Grasseri disease were collected from a field in north of Iran and their hemolymph was extracted into Eppendorf microtubes. The BmNPV in the larval hemolymph was confirmed by light microscopy for polyhedra. BmNPV polyhedra were purified as described by Sugimori *et al.* (1990). Samples were centrifuged at 10,000 rpm for 10 min to pelletize the BmNPV polyhedra. The polyhedra were then suspended in distilled water and quantified by hemocytometry.

Silkworm Hybrids and Rearing

Twenty hybrids of silkworm, which were produced in the Iran Sericultural

Research Center (Rasht, Iran), were screened to determine their resistance or susceptibility to BmNPV and their performance under disease stress. These hybrids were produced by crossing lines with Chinese and Japanese origins which have been purified through the years and which are kept in Iran Sericultural Research Center.

The larvae were reared in decontaminated rearing house using disinfected rearing appliances. The feeding, cleaning, and sanitation schedules followed these of Krishnaswami (1978).

In this study, larvae were reared in summer, and for each group of hybrids 1000 larvae were utilized in five replications. The silkworm larvae of the 3rd molt (4th instar) were orally inoculated with BmNPV-diluted polyhedra at a 550 ppm concentration by spraying on to mulberry leaves. These larvae were reared under standard conditions. The mortality due to NPV was recorded during the larval and pupal stages. The cocoon weight, cocoon shell weight, cocoon shell ratio and other parameters were determined using standard sericultural techniques described by Lim *et al*. (1990). Also, the resistance index was calculated as a product of the number of best cocoons to best cocoons weight.

Statistical Analysis

Collected data were subjected to statistical analysis of variance test to find out the low significant difference between each set of treated groups. For all analyses of variance, Duncan's multiple range test in the SAS software was used (SAS, 1997).

Agglomerative hierarchical clustering was done using Ntsys software. The method of average linkage between groups (Romesburg, 1984) was applied under the UPGMA (Unweighted Pair-Group Method using Arithmetic average). The clustering was based on the squared Eucliden distance. The average linkage between two groups is considered the average of distance between all pairs of cases with one number from each group. Hierarchical cluster analysis was carried out by considering the five survival parameters together. Also two productive characteristics were used in the hierarchical cluster analysis.

Results and Discussion

The results obtained from this research imply that there are considerable differences among the main commercial hybrids in terms of the contamination stress conditions to nuclear polyhedrosis virus. The results of comparisons among means of quantitative variables are summarized in Tables 1 and 2.

The number of surviving larvae which had reached the cocoon spinning stage is shown in Table 1. It is obvious that hybrids 101433×114 with 167.8 surviving larvae and 107k×108k with 166.8 larvae are both in the *a* statistical group and hybrid 107k×124k with a mean of 162.8 surviving larvae is in the next group. Also hybrid 111×116 with a mean of 61.6 surviving larvae, in *g* group, had the smallest population of surviving larvae.

The mortality and survival rates of larvae are very important factors for selecting resistant hybrids. Sivaprasad *et al*. (2003) categorized the responses of silkworms to BmNPV stress into apparent tolerance, real tolerance and susceptibility. The degree of tolerance was estimated from the mortality recorded during the larval and pupal stages. Sivaprasad *et al*. (2003) defined apparent tolerance as inoculated larvae completing the larval period and forming cocoons but not pupating into pupae. If larvae completed their larval period, pupated into pupae and moths emerged from the cocoons, it was defined as the real tolerance group.

Results of this research indicated

Table 1. Survival characteristics of different hybrids of silkworms to NPV stress

Hybrid	No. of surviving larvae	No. of pupae	Pupal survival rate (%)	No. of cocoons			
				Best	Middle	Poor	Total
113K×108K	134.8 cde[1)]	120.4 a-f	89.33 a	84.8 b-e	36.8 b-e	2.8 a	129.6 cd
113K×114K	151.4 abc	137.8 a-d	90.67 a	97.2 abc	40.2 a-d	6.0 abc	147.4 abc
113×118	112.8 ef	80.8 hij	70.67 def	71.8 def	27.4 def	9.6 bcd	110.8 def
113K×120K	134.6 cde	108.4 efg	80.43 a-d	71.2 def	50.8 ab	8.2 bcd	132.4 bcd
113K×124K	130.4 cde	111.0 d-g	84.36 ab	83.8 cde	35.6 b-e	6.2 bcd	128.0 cd
107K×108K	166.8 a	139.2 abc	83.27 abc	108.0 ab	43.0 a-d	9.4 bcd	163.6 a
107K×114K	156.4 abc	135.0 a-e	86.33 ab	81.6 cde	54.2 a	8.2 bcd	150.0 abc
107K×120K	137.8 b-e	119.4 a-f	86.67 ab	81.4 cde	39.8 a-d	4.6 ab	131.8 cd
107K×124K	162.8 ab	145.6 a	88.69 a	97.8 abc	47.6 abc	7.0 bcd	157.6 ab
101433×110	137.0 b-e	116.0 b-f	84.59 ab	79.4 cde	34.4 cde	8.4 bcd	129.6 cd
101433×112	143.0 a-d	104.2 fgh	72.89 c-f	95.0 a-d	34.0 cde	9.6 bcd	140.8 abc
101433×114	167.8 a	139.8 ab	83.25 abc	113.6 a	34.8 cde	6.6 bcd	161.4 a
101433×116	110.4 ef	85.2 g-j	75.81 b-e	77.4 c-f	24.0 ef	6.6 bcd	109.2 def
101433×118	117.2 def	94.4 f-i	80.21 a-d	79.0 cde	23.6 ef	7.4 bcd	113.6 de
31×32	143.6 a-d	111.4 c-g	77.47 b-e	98.4 abc	29.4 def	8.2 bcd	139.8 abc
103×104	91.2 f	62.0 j	67.45 ef	65.2 efg	17.6 f	4.8 abc	89.4 ef
109×110	114.2 ef	84.4 g-j	72.21 def	55.6 fg	36.6 b-e	10.0 cd	108.2 def
111×112	113.2 ef	75.4 ij	63.99 f	81.8 cde	16.2 f	9.6 bcd	110.4 def
111×114	91.2 f	32.2 k	36.34 h	47.0 gh	17.4 f	20.0 e	87.8 f
111×116	61.6 g	30.6 k	46.20 g	30.6 h	16.6 f	11.2 d	60.0 g

1) Numbers in the same column with the same letters do not significantly differ at the $p < 0.05$ level.

that hybrid 107k×124k with a mean of 145.6 surviving pupae had the maximum resistance under these conditions and was in the *a* statistical group, followed by hybrid 101433×114 with a mean of 139.8 surviving pupae. On the other hand, hybrids of 111×114 and 111×116 with respective means of 32.2 and 30.6 surviving pupae had the least number of live pupae and were in the last group (Table 1).

Also from the percentage of pupal survival, it can be seen that as expected, hybrids 113×114 with a mean of 90.67%, 113k×108k with one of 89.33%, and 107k×124k with one of 88.69% were all in the *a* group, which represents the highest resistance among the hybrids tested. Hybrid 111×114 with a 36.34% pupal survival rate showed the lowest resistance to this pathogen and was in the *k* group. The highest numbers of obtained cocoons were in hybrids 107k×108k and 101433×114 with 163.6 and 161.4 cocoons, respectively. These results with high survival of larvae and pupae of these hybrids were as expected. Hybrids 111×114 with 87.8 cocoons (*f* group) and 111×116 with 60 cocoons (*g* group) had the lowest cocoon production (Table 1).

Susceptibility to disease in different life stages of insects is variable (Wu, 1983a). Susceptibility to NPV infection markedly decreases during the pupal period (Mikhailov *et al.*, 1992). Wu (1983a) showed that resistance increases about 10-fold with each molt, except that newly moulted 5th instar larvae were as susceptible as 4th instar larvae when reared under aseptic conditions on an artificial diet.

The results of this research suggest

Table 2. Best cocoon characteristics of different hybrids under NPV stress

Hybrids	Best cocoon weight (g)	Best cocoon shell weight (g)	Best cocoon shell ratio (%)
113K×108K	1.69 j [1)]	0.34 j	20.0 I
113K×114K	1.88 hi	0.37 i	20.1 hi
113×118	2.04 b-f	0.44 def	22.5 bcd
113K×120K	1.60 k	0.30 k	19.1 j
113K×124K	1.77 j	0.36 i	20.3 ghi
107K×108K	2.01 c-f	0.41 gh	20.2 ghi
107K×114K	2.07 bcd	0.42 fgh	20.4 ghi
107K×120K	1.77 j	0.35 ij	20.0 i
107K×124K	2.02 c-f	0.42 fgh	20.9 fgh
101433×110	1.92 gh	0.40 h	21.0 fg
101433×112	1.96 e-h	0.43 efg	21.8 de
101433×114	2.03 c-f	0.46 b-e	22.5 bcd
101433×116	2.18 a	0.48 abc	22.0 cde
101433×118	2.10 abc	0.45 cde	21.4 ef
31×32	2.13 ab	0.48 ab	22.4 bcd
103×104	1.94 fgh	0.44 def	22.8 bc
109×110	1.79 ij	0.36 ij	20.2 ghi
111×112	2.06 b-e	0.50 a	23.7 a
111×114	1.91 gh	0.47 bcd	22.9 ab
111×116	1.99 d-g	0.46 b-e	22.2 bcd

[1)] Numbers in the same column with the same letters do not significantly differ at the $p < 0.05$ level.

that hybrid 101433×114 with a mean of 113.6 best cocoons had the highest production, while in contrast, hybrid 111×116 with only 30.6 best cocoons showed significant differences with the others. These results with respect to the number of live larvae and pupae of these groups of hybrids and the survival rate of pupae in the cocoons can be explained. Also while hybrid 113k×108k had 2.8 bad cocoons, it was in the *a* statistical group because it produced the smallest number of poor cocoons. Hybrids 111×116 and 111×114 respectively had 11.2 and 20 poor cocoons (Table 2).

The mean weight of each cocoon in hybrid 101433×116 was 2.180 g which was the highest individual cocoon weight, and hybrid 113k×120k with a mean of 1.602 g had the lowest cocoon weight. The cocoon shell ratio in hybrid 111×112 with a mean of 23.72% was the highest, while hybrid 113k×120k at 19.18% was the lowest (Table 2).

One of the most important factors for selecting superior groups under viral infection is the resistance index or the total weight of cocoons produced by each hybrid. Since, the resistance index involves the cocoon weight and the number of cocoons, as an index for selection, it is a reliable factor. Hybrid 1014133×114 is in an appropriate position, although the four hybrids 107k×108k, 31×32, 107k×124k, and 101433×112 are also in a good position compared to the other groups (Fig. 1).

Hierarchal cluster analysis of various hybrids categorized the hybrids into two groups on the basis of the structure of characteristics related to survival and resistance of the larvae under viral stress. Three hybrids, 103×104, 111×114, and 111×116, were classified in the susceptible group. In the other group which included most of the hybrids the abundance

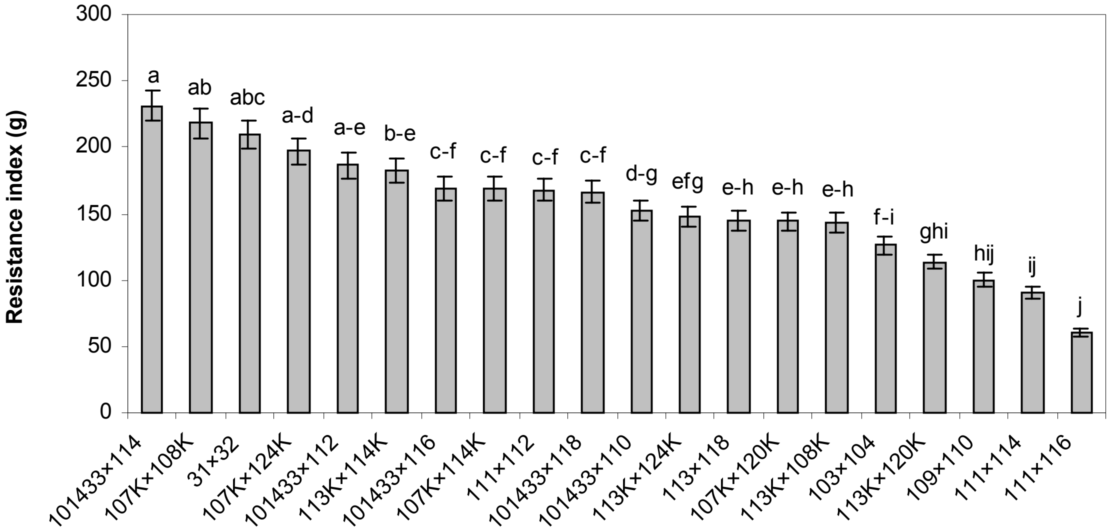

Fig 1. Resistance index for different hybrids of commercial silkworms. Column with the same letters do not significantly differ at the $P < 0.05$ level; Resistance index (g) = no. of best cocoons × best cocoons weight.

diversity was also observed whereas three hybrids of 113×118, 109×110 and 101433×116 were more susceptible compared to the other groups and had less resistance and were closer to the other three hybrids (Fig. 2).

On the basis of production characteristics including cocoon weight and cocoon shell weight, the cluster analysis of data divided the hybrids into two groups with high yield and low yield. In this way, the three hybrids of 101433×116, 31×32, and 111×112, had the highest yields with great similarity to each other, but many hybrids were separated as a subgroup from hybrids with the potential of high production (Fig. 3).

It is obvious that the groups with potential for high production are occasionally more resistant to a virus. But some groups with low resistance have high potential for cocoon production.

Silkworm breeds with complete susceptibility to BmNPV may be more productive but will be very difficult to rear under field conditions (Sivaprasad *et al.*, 2003). Therefore, selection on the basis of a combination of survival and yield, which are included in the resistance index, are suggested as the best index for selection.

The silkworm's resistance to NPV disease is controlled by both a dominant major gene on the autosome and a modificator gene on sex chromosome Z, belonging to qualitative and quantitative categories (Chen *et al.*, 2003). Its heredity is quite complicated. Traditional breeding can only rely on screening with NPV infection, whose progress is quite slow. It is also influenced by a number of environmental factors. Selection based on molecular markers can accurately trace fragments linked to resistance (Chen *et al.*, 2003).

This tool is very important for the efficient hybridization of silkworm lines. Although the evaluation of resistance in productive lines is very time consuming, it is necessary for characterization of productive hybrids. So, identifying the best selection index is very important.

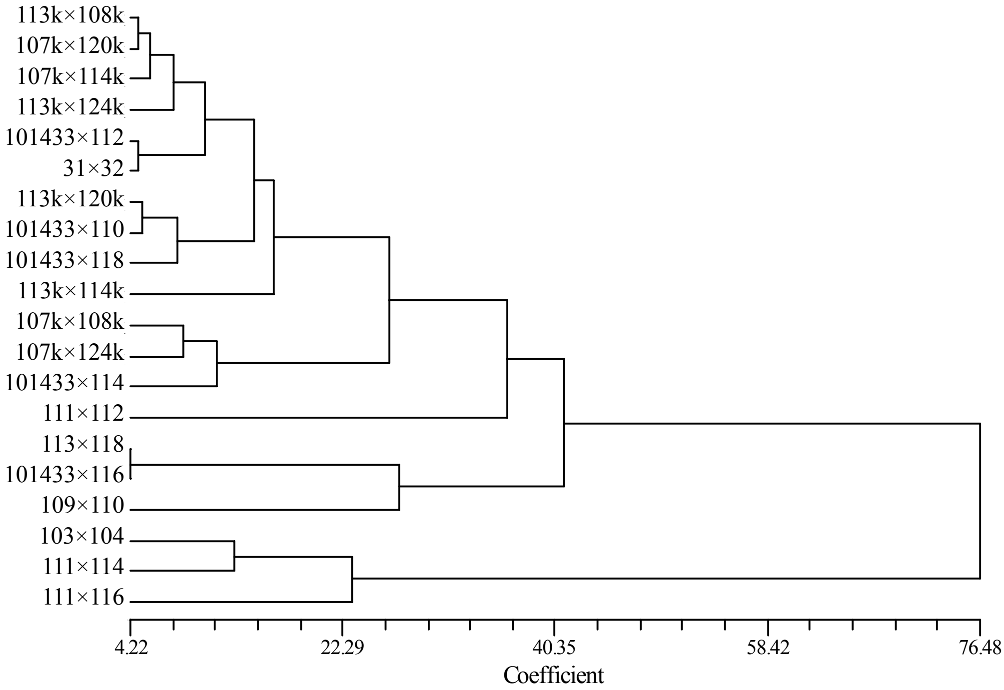

Fig. 2. Agglomerative cluster analysis of different hybrids of silkworm based on survival and resistance parameters.

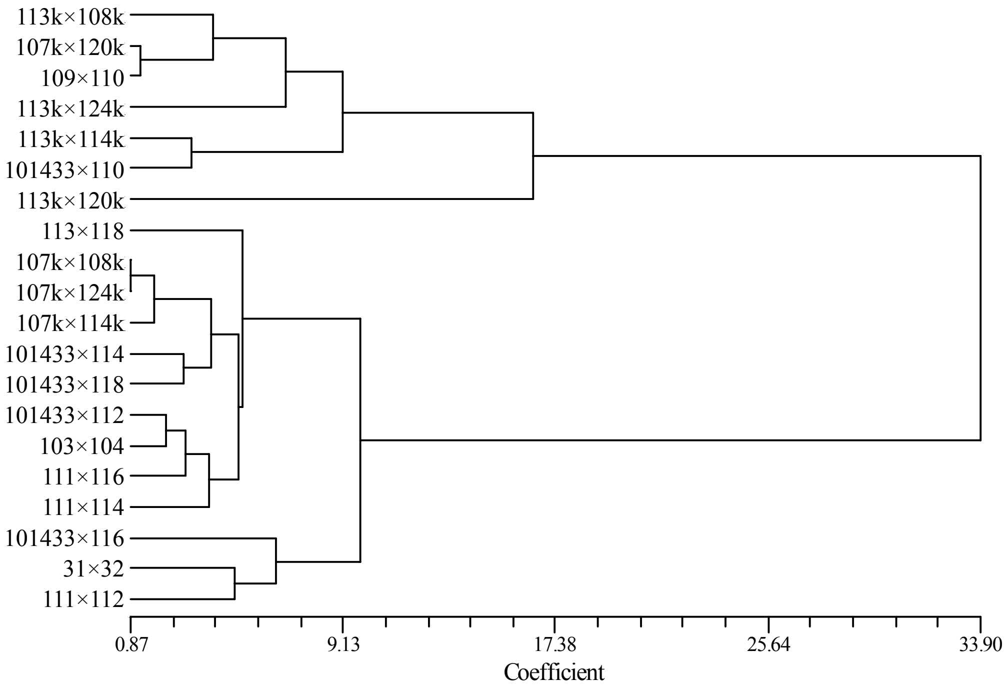

Fig. 3. Agglomerative cluster analysis of different hybrids of silkworm based on cocoon yield parameters.

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評估20種商用家蠶雜交品系對核多角體病毒的抵抗能力

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摘　要

核多角體病毒是家蠶 (*Bombyx mori*) 最普遍的病毒疾病，本研究評估 20 種新的伊朗雜交家蠶對核多角體病毒 (NPV) 的抵抗能力。第 3 次蛻皮後的家蠶幼蟲，經口餵飼接種家蠶 NPV (550 ppm)，一直飼養到夏季吐絲期，並調查各處理組的一些生物學和經濟特性。根據試驗結果顯示，107k×124k、107k×108k 和 101433×114 雜交品系較其他品系對此疾病具有顯著性 ($P < 0.05$) 的最高抵抗能力。依抗性程度，使用 Agglomerative 叢聚分析，可將不同的雜交品系分成兩大類，其中以 111×114、103×104 和 111×116 雜交品系對 BmNPV 最爲敏感。

關鍵詞：核多角體病毒、家蠶、抵抗能力、雜交。