BIOLOGICAL CONTROL

Effect of *Baculovirus spodoptera* Isolates in *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) Larvae and Their Characterization By RAPD

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Efeitos de Isolados do *Baculovirus spodoptera* em Lagartas de *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) e sua Caracterização por Meio de RAPD

RESUMO - Foram utilizados 22 isolados de vírus amostrados em diferentes regiões produtoras de milho do Brasil. Os vírus foram purificados e suas suspensões fornecidas a lagartas sadias do 3º e 4º ínstar de *Spodoptera frugiperda* (J.E. Smith). A mortalidade foi avaliada diariamente, e as lagartas infectadas foram congeladas logo após sua morte, o que em geral ocorreu do 5º ao 7º dia após ingestão do vírus. Os isolados foram usados em seis concentrações (10^3 a 10^8 poliedros/ml) e uma testemunha (água). Os percentuais de mortalidade, duração do período larval e período pupal, peso de pupa e a concentração letal (CL_{50}) foram determinados para todos os isolados. Foram observadas diferenças significativas entre todos os isolados e concentrações testadas para todos os parâmetros avaliados, e também foi constatada a presença da interação isolado x concentração, exceto para período pupal. Os padrões de amplificação de 54 marcadores RAPD, sendo 41 polimórficos, foram utilizados para avaliar a distância genética e a sua correlação com os índices de mortalidade das lagartas. A divergência genética calculada pelo coeficiente Jaccard utilizando os dados moleculares permitiu dividir os isolados em dois grupos, com um elevada confiabilidade. O agrupamento não apresentou associação com a taxa de mortalidade causada pelos isolados ou com sua distribuição geográfica. No entanto, um fragmento de RAPD OPW04.2280 apresentou-se altamente associado com a mortalidade das lagartas e com a CL_{50}, explicando 23% e 65% da variação fenotípica para essas características entre os isolados virais, respectivamente.

PALAVRAS-CHAVE: Patologia de insetos, lagarta do cartucho do milho, PCR

ABSTRACT - The total of 22 Baculovirus isolates surveyed in different corn producing regions in Brazil were used against fall armyworm, *Spodoptera frugiperda* (J.E. Smith). The viruses were purified and their suspensions were used to feed fall armyworm larvae from 4th and 5th instar. The mortality rate was checked daily and the infected larvae were frost after death, what generally occurred between the 5th and 7th day after virus ingestion. The 22 Baculovirus isolates were used in six concentrations (from 10^3 to 10^8 polyhedra/ml) and one check treatment with water. Mortality rate, larval period, pupal period, pupa weight and lethal concentration (LC_{50}) were determined for all isolates. Significant differences were found among all isolates and different concentrations, also interaction between isolate x virus concentration for all characteristics evaluated, except for pupal period. Amplification patterns of 54 RAPD markers, being 41 polymorphic among the isolates, were used to evaluate the genetic distance and its correlation with the fall armyworm larvae mortality rate. The genetic diversity calculated by the Jaccard's coefficient using the molecular data allowed a division of the isolates into two groups, with a high level of confidence. These groups did not present any association with the mortality rate caused by the isolates or with their geographical distribution. However, a RAPD fragment OPW04.2280 was highly associated with the larvae mortality rate and with LC_{50}, explaining 23 and 65% of the phenotypic variation for these traits among the isolates, respectively.

KEY WORDS: Insect pathology, fall armyworm larvae, PCR

The Baculoviridae is a large family of occluded viruses that is composed of two genera, the nucleopolyhedrovirus (NPV) and the granulovirus (GV), commonly called baculovirus. Baculovirus has long been recognized as an environmentally safe potential alternative to chemical pesticides. As the viruses are highly hostspecific, non-pathogenic to beneficial insects and other non-target organisms, including mammals (Monobrullah & Nagata 1999), they are attractive candidates for integrated pest management (IPM). Other advantages of baculovirus for pest control include a lack of toxic residues, allowing growers to treat their crops even shortly before harvest, with low probability to develop stable resistance (Monobrullah 2003). The NPV produce large polyhedron-shaped structures called polyhedra, which contain many virions, while the GV have smaller occlusion bodies called granules, which normally contain a single virion (Funk *et al*. 1997). The occlusion of the virus inside a protein coat is important to protect the infective particles in the transmission of the virus from insect to insect (Blissard & Rhormann 1990).

The fall armyworm *Spodoptera frugiperda* NPV, denominated *Baculovirus spodoptera* (SfMNPV) shows a great protential to be used as a biocontrol agent. The Embrapa Milho e Sorgo owns 22 baculovirus isolates (Table 1) that were collected in different corn producing regions in Brazil (Valicente 1989, Valicente & Barreto 1999). Larvae normally are infected by ingestion of occlusion bodies (OB), although vertical transmission and injection by parasitoids may occur (Moscardi 1999).

The efficiency of *B. spodoptera* on controlling the fall armyworm via irrigation system was shown by Valicente & Costa (1995). Baculoviruses are also considered very safe to humans (Burges *et al.* 1980), and their great potential as a biocontrol agent of insect pests results from their characteristic ability to produce virions sequestered within the proteinaceous matrix (polyhedrin or granulin) of a nuclear occlusion body (Rohrmann 1992). This occlusion body facilitates virus survival and dispersal in the environment and is a convenient, safe, and simply manipulated product (Bulach *et al*. 1999). Agudelo *et al.* (1983) reported that fall armyworm larvae were infected by a strain of SfNPV in a corn crop in Venezuela and that 6×10^6 pol/ml caused 100% mortality on 7- and 10-day old larvae, with a LT_{50} of 4.7 and 7.3 days, for each group, respectively. Also, results reported by Valicente & Cruz (1991) evidenced baculovirus efficiency against the fall armyworm under laboratory conditions.

The ivestigation of phylogenetic relationships between baculoviruses and their hosts may provide understanding of biological adaptations such as virus host specificity, but only broad aspects of virus-host associations have so far been identified (Zanotto *et al.* 1993). However, some other basic studies are needed in order to better understand the relationship between baculovirus, fall armyworm and also studies regarding baculoviruses themselves.

The main objectives of this research were to check the susceptibility of fall armyworm to different geographical baculovirus isolates and to evaluate the genetic diversity of these isolates using RAPD markers.

Table 1. *Baculovirus spodoptera* isolates sampled from different regions in Brazil.

Isolate number (stock)	Place sampled – State[1]
01	Penha, Local I - PR
02	Penha, Local II- PR
03	Melissa, Local I - PR
04	Melissa, Local II - PR
05	Cascavel - PR
06	Cascavel - PR
07	Marechal Cândido Rondon, Local I - PR
08	Marechal Cândido Rondon, Local II - PR
09	Penha, Local I - PR
10	Penha, Local II - PR
11	Melissa, Local I - PR
12	Penha, Local II - PR
13	Melissa, Local I - PR
14	Melissa, Local III - PR
15	Melissa, Local I - PR
16	Cascavel - PR
17	Cascavel - PR
18	Sete Lagoas - MG
19	Sertaneja - PR
20	Patos de Minas - MG
21	Patos de Minas - MG
22	Marechal Cândido Rondon, Local III - PR

[1]PR, Paraná State and MG, Minas Gerais State
All isolates were collected by Fernando Hercos Valicente, except isolate 19 that was kindly provided by Dr. Flávio Moscardi.

Materials and Methods

In order to obtain uniform material, larvae infected with 22 baculovirus isolates were macerated individually with autoclaved bi-distilled water and the polyhedra are purified using sucrose gradient. Virus suspensions were offered to healthy larvae from the artificial rearing. Corn leaves washed with sodium hypochlorite and distilled water were inoculated with virus suspensions and offered to 3rd- and 4th-instar fall armyworm that were kept individually in plastic containers (50 ml). These leaves were replaced by new ones two days later; after 48h the leaves were replaced by artificial diet. Infected larvae were maintained at 25ºC, 70% of humidity and photo phase of 14hL/10hD. Mortality evaluation was checked daily, and dead larvae were frozen at -20ºC.

These 22 baculovirus isolates were purified again and tested against fall armyworm, using six different concentrations (10^3, 10^4, 10^5, 10^6, 10^7 e 10^8 POB/ml) and a check treatment (water). Mortality rate, larval period, pupal

period and pupa weight were evaluated in a randomized complete block with four replicates, each replicate containing 12 larvae and a total of 48 larvae per baculovirus concentration. Lethal concentration (LC_{50}) was determined for all isolates. Due to a high mortality observed when concentrations of 10^7 e 10^8 POB/ml were used, larval and pupal period, and weight of pupa were analyzed only at the concentrations of 10^3 a 10^6 POB/ml.

Purification of the virus followed the method described by Maruniak (1986). Sample homogenization was performed using Tris 0.01M, pH 7.8. SDS was added to the suspension that was filtered and centrifuged at 10,000 g for 10 min. Pellet was cleaned in a linear sucrose gradient (40% to 63%) in Tris 0.01M, pH 7.8 and EDTA 0.001M, and spinned at a 100,000 g for 30 min at 4ºC.

Viral DNA was isolated from the purified baculovirus polyhedra adding 100 µL of Na_2CO_3 1M to a suspension of 1 ml of polyhedra in a concentration of 5 x 10^7 POBs/ml (Protein Inclusion Body). After incubation at 37ºC in order to solubilize the polyhedrin, SDS 20% and proteinase K 5 mg/ml were added maintaining the solution at 37ºC for 6h to 12h. The extraction was performed three times with phenol: chloroform: isoamyl alcohol (25:24:1) and two times with chloroform: isoamyl alcohol (24:1). Samples were subjected to dialysis overnight using Tris/EDTA buffer, which was changed three times and DNA was frozen at -20ºC. Viral DNA was amplified using 12 RAPD primers of random sequence (Operon Technologies, Inc.), whose sequences are listed in the Table 2. PCR reactions consisted of 10 ng of viral DNA, 0.4 mM of primer, 100 mM of each dNTP, 10 mM Tris-HCl (pH 8.6), 50 mM KCl, 2 mM $MgCl_2$ and 1 U of Taq polimerase in a total volume of 25 ml. Amplification steps were: 95ºC/1min, followed by 35 cicles at 94ºC/1s, 36ºC/1 min, 72ºC/2 min and a final extension at 72ºC/7 min. PCR products were separated in 1% agarose gel, stained with ethidium bromide and photographed under UV light. Each reaction was repeated at least twice.

Amplification patterns were evaluated as presence or absence of the band, and scored as 1:0 binary table. The genetic distance was calculated using the complement of Jaccard coefficient and a dendrogram representing the distance matrix was generated using the algorithm UPGMA. A bootstrap analysis of the dendrogram was done by the software BOOD (Coelho 2000) and the genetic diversity

Table 2. Average mortality rate (%) of fall armyworm larvae, *S. frugiperda* when fed with different concentrations of *B. spodoptera*.

Isolate	Concentration (POB/ml)					
	10^3	10^4	10^5	10^6	10^7 ns*	10^8 ns*
01	0.287 hi	0.472 efg	0.670 def	0.937 a	0.930	1.000
02	0.190 ij	0.440 fgh	0.602 fg	0.875 a	1.000	0.977
03	0.847 a	0.817 ab	0.932 ab	0.977 a	1.000	1.000
04	0.292 hi	0.677 bcd	0.640 ef	0.897 a	0.977	1.000
05	0.545 bcdef	0.610 cde	0.960 a	0.977 a	1.000	1.000
06	0.605 bcd	0.745 bc	0.980 a	0.937 a	1.000	1.000
07	0.487 defg	0.577 def	0.787 cd	0.725 b	0.980	1.000
08	0.337 h	0.712 bcd	0.792 bcd	0.865 ab	1.000	1.000
09	0.632 bc	0.762 b	0.872 abc	1.000 a	1.000	1.000
10	0.165 ij	0.260 ijk	0.705 def	0.980 a	1.000	1.000
11	0.670 b	0.732 bc	0.872 abc	0.935 a	1.000	1.000
12	0.560 bcde	0.360 ghi	0.260 I	0.892 a	1.000	1.000
13	0.397 gh	0.375 ghi	0.750 cde	0.940 a	1.000	1.000
14	0.090 j	0.115 l	0.607 efg	0.895 a	1.000	1.000
15	0.662 b	0.750 bc	0.887 abc	0.980 a	1.000	1.000
16	0.407 fgh	0.325 hi	0.692 def	0.955 a	1.000	1.000
17	0.337 h	0.305 hij	0.802 bcd	1.000 a	0.980	1.000
18	0.495 cdefg	0.157 kl	0.395 hi	0.870 a	1.000	1.000
19	0.907 a	0.940 a	0.880 abc	0.980 a	1.000	1.000
20	0.105 j	0.170 jkl	0.452 h	0.937 a	0.980	1.000
21	0.180 ij	0.267 ijk	0.472 gh	0.872 a	0.977	1.000
22	0.425 efgh	0.685 bcd	0.787 cd	0.960 a	1.000	1.000

Means followed by the same letter within the same column are not significantly different using Tukey's test at α = 5 %.
*ns = não significativo

analysis was performed using GQMol (www.ufv.br/dbg/gqmol/gqmol.htm) and Statistica v 4.5 (StatSoft 1993) softwares. Associations between RAPD markers and mortality rate of fall armyworm larvae were calculated using linear regression models, considering the markers as independent variables. The determination coefficient (r^2) was interpreted as the estimative of the proportion of the phenotypic variance explained by the marker and the analysis were done using the JUMP v 3.1.6.2 (SAS Institute 1989).

Results and Discussion

Significant differences were observed among all isolates and dosage concentrations used for all characteristics observed. Also, an interaction baculovirus isolate x concentration was observed for all characteristics evaluated, except for the pupal period. The isolates 03 and 19 showed the highest mortality rate (> 80%) when used in the concentrations of 10^3 e 10^4 POB/ml, and the isolates 14 and 20 showed the lowest rates of mortality in a concentrations of 10^3 and 10^4. All isolates showed high mortality (>72%) when using concentrations equal and/or superior to 10^6 POB/ml (Table 2). However, seven isolates showed mortality above 87% when concentration of 10^5 POB/ml was used. The isolates 11 and 19 increased the average duration of the larval period and the isolate 02 increased the average larval period at 10^3 and 10^6 POB/ml (Table 3). Additionally, the isolates 02, 06, 09, 11 and 19 increased the average duration of the pupal period in most of the concentrations used (Table 4). The isolate 20 showed the lowest average larval period in the concentration of 10^3 (Table 3). Many isolates showed a slight increase in the average of pupae weight when concentrations of 10^3, 10^4, 10^5 and 10^6 were used (Table 5). Only isolates 16 and 17 spent a short period to kill fall armyworm larvae in all concentrations used; the isolate 16 took the shortest period (4.9 days) at the concentration of 10^8 POB/ml (Table 6), still showing a good results in killing fall armyworm larvae (Table 2). Overall, all insects that survived to virus infection were able to keep pupae weight and no abnormal adults were observed.

Fall armyworm larvae showed the same pattern of infection for all isolates. Internal organs were totally destroyed, integument showed a light pink color and total liquefaction at the death time, as previously reported by Weinzierl & Henn (1989). According to Valicente (1988), discoloration, paleness, integument liquefaction and lack of apetite are typical symptoms of an infected insect with baculovirus. In some

Table 3. Average larval period (days) of *S. frugiperda* in different concentrations of *B. spodoptera*.

Isolate	Concentration (POB/ml)			
	10^3	10^4	10^5	10^6
01	21.76 cde	21.42 defg	23.04 bcd	21.00 c
02	23.71 abc	20.46 defgh	21.86 bcdef	29.00 a
03	17.13 g	16.50 i	19.00 efg	16.00 d
04	22.39 bcd	20.33 defgh	22.49 bcde	20.25 c
05	20.96 cdef	21.65 def	20.50 cdefg	20.00 c
06	21.77 cde	22.17 cde	22.00 bcdef	21.00 c
07	18.35 efg	16.88 hi	17.79 g	18.96 cd
08	16.10 defg	18.54 fghi	18.85 fg	18.25 cd
09	22.00 bcd	22.83 bcd	23.63 abc	20.57 c
10	19.51 defg	18.94 efghi	19.33 efg	20.18 c
11	25.54 ab	25.88 ab	25.38 ab	24.67 b
12	18.02 fg	18.64 efghi	18.17 g	19.00 cd
13	17.32 g	16.30 i	17.50 g	16.00 d
14	17.45 fg	18.67 efghi	19.79 defg	19.50 cd
15	21.94 cde	27.08 a	24.13 ab	20.68 c
16	19.14 defg	18.64 efghi	17.75 g	16.00 d
17	18.99 defg	18.00 ghi	18.42 fg	20.57 c
18	18.13 fg	18.62 efghi	19.23 efg	19.63 d
19	27.13 a	25.67 abc	26.75 a	26.00 b
20	12.76 h	18.13 fghi	18.24 g	18.00 cd
21	19.48 defg	19.16 efghi	19.59 defg	20.00 c
22	19.70 defg	19.13 efghi	20.00 defg	18.00 cd

Means followed by the same letter within the same column are not significantly different using Tukey's test at α = 5 %.

Table 4. Average pupal period (days) of *S. frugiperda* in different concentrations *of B. spodoptera*.

Isolate	Concentration (POB/ml)			
	10^3	10^4	10^5	10^6
01	11.52 def	11.17 cd	11.56 de	9.50 defg
02	15.07 ab	10.59 def	9.94 efg	15.50 a
03	8.00 k	9.25 defghi	7.66 i	9.00 efgh
04	10.33 efghij	10.81 cde	11.24 de	10.75 de
05	10.71 efg	11.16 cd	14.00 bc	9.00 efgh
06	14.71 ab	15.29 a	16.00 ab	15.00 ab
07	8.16 k	8.56 ghi	7.87 hi	9.81 defg
08	8.19 k	7.93 i	8.66 ghi	8.50 fgh
09	15.69 a	15.33 a	16.38 a	10.47 def
10	8.78 ghijk	8.57 fghi	8.10 ghi	10.47 def
11	13.27 bcd	13.84 ab	14.13 bc	13.33 bc
12	8.65 hijk	8.90 efghi	8.97 fghi	7.00 h
13	8.28 k	8.55 ghi	8.12 ghi	8.66 fgh
14	9.54 fghijk	10.41 defg	10.75 def	11.50 cd
15	13.88 abc	13.33 ab	12.75 cd	10.47 def
16	10.61 efgh	10.60 def	8.60 ghi	11.00 de
17	10.57 efghi	10.17 defgh	9.87 efgh	10.47 def
18	8.51 jk	8.35 hi	8.44 ghi	8.37 gh
19	12.00 cde	13.67 bc	12.13 cd	15.00 ab
20	8.50 jk	8.712 fghi	8.91 fghi	9.33 efg
21	8.57 ijk	8.53 ghi	8.15 ghi	8.00 gh
22	8.12 k	8.12 i	7.20 i	9.00 efgh

Means followed by the same letter within the same column are not significantly different using Tukey's test at $\alpha = 5$ %.

cases, as observed in infected larvae with granulosis virus, larvae may increase weight and longevity (Whitlock 1974, Valicente 1989).

The lethal time (LT_{50}) was 5.5 days using the concentration of 10^6 to the isolate 18 (Valicente 1988). Regarding the mortality average, results were similar to those described by Valicente *et al*. (1989), with fall armyworm larvae at the concentration of 10^6 POB/ml. Mortality caused by baculovirus was significant and increased with high doses when sprayed using irrigation water (Valicente & Costa (1995). Valicente (1988) reported that fall armyworm larvae treated with NPV reached pupal period within 18 days (isolate 18). Similarly in our research, the isolate 18 took 19 days to become a pupae. However, some isolates took 29 days to kill fall armyworm larvae, delaying the larvae time to reach the pupae stage (Table 4).

The 12 RAPD primers tested in the 22 baculovirus isolates amplified 54 bands, being 41 (76%) polymorphic ones. Analysis of genetic divergence using molecular data allowed to divide the baculovirus isolates into two main groups, one including the isolates 03, 07 and 13, and a second larger group composed by all other 19 isolates (Fig. 1). Both groups were highly supported using 10,000 bootstrap sampling, with a probability of 73.3% and 58.5% for the smaller and larger groups, respectively (Fig. 1). Indeed, the sub-groups of isolates formed within the larger group were weakly supported by the bootstrap analysis, suggesting that they should not be considered in the diversity analysis. Grouping of baculovirus isolates based on these molecular data showed no relationship with mortality rate or with their geographical distribution. These results may be explained by the reduced number of RAPD markers used to evaluate genetic variability among the isolates. However, significant differences in biological activity of baculovirus were found regarding different geographical regions (Shapiro & Robertson 1991).

Despite the diversity analysis had no relation with the biological characteristics of the baculovirus isolates, a RAPD fragment OPW04.2280 showed a significant association with larvae mortality in two concentrations of the isolates (10^3 and 10^4 pol/ml) and with the LC_{50} (Table 7). This marker was negatively associated with the mortality rate, explaining 23% of the phenotypic variation and positively associated with the LC_{50} of the isolates, explaining 65% of the phenotypic variation . In the Table 8, the different directions of the linear regression with the RAPD marker can be explained, once the isolates 19 and 03 showed the lowest LC_{50} values and the highest mortality rate in both concentrations. Additionally, the OPW04.2280 was present only in the isolates with highest

Table 5. Average pupa weight (grams) of *S. frugiperda* in different concentrations of *B. spodoptera*.

Isolate	Concentration (POB/ml)			
	10^3	10^4	10^5	10^6
01	0.2375 cd	0.2391 def	0.2426 bcde	0.2374 cdef
02	0.2670 abcd	0.2679 abcde	0.2494 abcde	0.2848 ab
03	0.2347 cd	0.2385 ef	0.2218 e	0.2333 cdef
04	0.2398 cd	0.2535 bcdef	0.2464 abcde	0.2781 ab
05	0.2689 abc	0.2510 bcdef	0.2780 ab	0.2158 ef
06	0.2882 a	0.2990 a	0.2827 a	0.2798 ab
07	0.2297 d	0.2548 bcdef	0.2655 abcd	0.2669 abc
08	0.2563 abcd	0.2382 ef	0.2485 abcde	0.2400 cdef
09	0.2882 a	0.2761 abcd	0.2782 ab	0.2527 bcde
10	0.2446 bcd	0.2364 ef	0.2331 de	0.2578 bcd
11	0.2795 ab	0.2777 abc	0.2778 abc	0.2635 abcd
12	0.2487 bcd	0.2503 bcdef	0.2203 e	0.2361 cdef
13	0.2453 bcd	0.2463 bcdef	0.2371 de	0.2115 f
14	0.2593 abcd	0.2650 abcdef	0.2568 abcde	0.2402 cdef
15	0.2666 abcd	0.2281 f	0.2661 abcd	0.2612 abcd
16	0.2319 cd	0.2506 bcdef	0.2500 abcde	0.2329 cdef
17	0.2587 abcd	0.2646 abcdef	0.2797 ab	0.2527 bcde
18	0.2451 bcd	0.2507 bcdef	0.2402 de	0.2487 bcdef
19	0.2537 abcd	0.2805 ab	0.2565 abcde	0.2953 a
20	0.2471 bcd	0.2444 bcdef	0.2195 e	0.2475 bcdef
21	0.2482 bcd	0.2416 cdef	0.2367 de	0.2577 bcd
22	0.2442 bcd	0.2503 bcdef	0.2404 cde	0.2281 def

Means followed by the same letter within the same column are not significantly different using Tukey's test at $\alpha = 5$ %.

LC50 and low mortality rates, mainly in the concentration of 10^3 (Table 7). Correlation values between LC_{50} and mortality rate at the concentration of 10^3 and 10^4 were also high and negative (-0.71 and -0.78, respectively).

Even with a low number of RAPD primers evaluated, it was possible to identify one fragment OPW04.2280 that was closely associated with the non-virulence ability of these baculovirus against fall armyworm larvae. Thus, it might be interesting to improve the characterization of this RAPD marker and to increase the molecular analysis of these baculovirus to better understand these isolates and their potential as biological control agents.

Baculovirus isolates showed different intensities of efficiency against *S. frugiperda*. The isolates 03, 09, 11 and 19 were shown more efficient regarding fall armyworm mortality, while the isolate 19 showed the lowest LC_{50} and the highest mortality rate in lower concentrations. However, all other isolates were also efficient on killing fall armyworm larvae when concentrations of 10^6 POB/ml and above were used. The gross pathology showed the same symptoms regarding larvae mortality (discoloration, paleness, integument liquefaction and lack of appetite).

Although the genetic analysis based on RAPD markers provided no differentiation among the isolates related to geographical distribution and mortality rates, one RAPD marker (OPW04.2280) was associated with mortality rate of fall armyworm larvae in two polyhedra concentrations and with the LC_{50} of the baculovirus isolates.

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Table 6. Average time (days) needed to kill *S. frugiperda* larvae in different concentrations of *B. spodoptera*.

Isolate	Concentration (POB/ml)					
	10^3	10^4	10^5	10^6	10^7	10^8
01	10.83 cde	9.35 efg	8.17 efgh	7.86 efg	7.13 de	7.28 bcd
02	9.37 defghi	7.71 ghij	7.84 fgh	8.47 cde	6.70 e	6.67 bcd
03	9.28 efghi	9.95 bcde	8.87 cdef	8.47 cde	7.71 bcde	7.07 bcd
04	8.75 fghi	8.06 fghij	8.42 defg	7.97 ef	7.79 bcde	6.41 cde
05	8.35 ghij	8.96 efg	7.99 efgh	7.51 efg	7.23 de	7.40 bcd
06	11.69 bc	11.43 ab	11.28 a	10.38 ab	9.15 abc	8.30 ab
07	10.96 cd	9.65 cdef	8.79 cdef	8.90 bcde	9.26 ab	7.23 bcd
08	13.17 b	11.03 abcd	10.60 ab	10.77 a	8.62 abcd	7.91 abc
09	10.93 cde	12.40 a	10.82 ab	10.09 abc	8.68 abcd	8.29 ab
10	10.00 defg	8.17 fghij	7.87 fgh	7.63 efg	7.06 de	7.00 bcd
11	10.35 cdef	11.13 abcd	10.20 abc	10.21 ab	9.18 abc	9.10 a
12	8.32 hij	8.62 efgh	11.46 a	8.86 bcde	7.45 de	7.20 bcd
13	9.67 defgh	9.00 efg	8.29 efgh	7.75 efg	7.08 de	7.00 bcd
14	8.00 ij	6.62 j	6.74 h	5.66 h	7.08 de	7.00 bcd
15	10.38 cdef	11.27 abc	10.74 ab	9.68 abcd	9.62 a	8.15 ab
16	6.91 j	6.92 ij	6.76 h	6.78 fgh	6.90 e	4.92 e
17	8.06 hij	7.06 hij	6.85 gh	6.31 gh	6.55 e	5.84 de
18	10.00 defg	9.00 efg	9.58 bcde	8.21 def	7.04 de	5.94 de
19	10.85 cde	9.59 def	10.72 ab	10.98 a	9.87 a	8.10 ab
20	9.67 defgh	8.50 efghi	10.02 abcd	8.11 def	7.313 de	6.67 bcd
21	16.88 a	11.85 a	8.88 cdef	9.93 abc	7.60 cde	7.80 abc
22	10.22 cdef	8.47 efghi	8.25 efgh	7.75 efg	7.08 de	5.87 de

Means followed by the same letter within the same column are not significantly different using Tukey's test at $\alpha = 5\ \%$.

Table 7. Association of the RAPD marker with mortality rates of fall armyworm larvae in different concentrations of *B. spodoptera* isolates (10^3 and 10^4 POB/ml) and with LC_{50}.

Marker	Larvae mortality rate				LC_{50}	
	10^3 POB/ml		10^4 POB/ml			
	R^2 (%)	P	R^2 (%)	P	R^2 (%)	P
OPW04.2280	23.1	0.0185	22.5	0.0198	66.6	< 0.0001

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Table 8. LC_{50}, larvae mortality rate using the concentration of 10^3 and 10^4 POB/ml and presence (1) and absence (0) of the RAPD fragment OPW04.2300 for *B. spodoptera* isolates.

Isolate	LC_{50}	Mortality rate (10^3)	Mortality rate (10^4)	OPW04.2300
19	0.029	0.907	0.940	0
3	8.760	0.847	0.817	0
11	215.399	0.670	0.732	0
15	286.047	0.662	0.750	0
6	383.497	0.605	0.745	0
9	417.215	0.632	0.762	0
5	1,252.211	0.545	0.610	0
22	2,265.639	0.425	0.685	0
7	2,360.130	0.487	0.577	0
8	3,223.913	0.337	0.712	0
4	6,189.799	0.292	0.677	0
13	7,594.436	0.397	0.375	0
16	9,347.877	0.407	0.325	-
17	9,507.090	0.337	0.305	0
1	11,068.527	0.287	0.472	0
12	13,872.272	0.560	0.360	0
2	21,679.767	0.190	0.440	-
10	22,350.416	0.165	0.260	0
18	25,450.242	0.495	0.157	0
21	46,877.225	0.180	0.267	0
14	58,442.899	0.090	0.115	1
20	65,635.444	0.105	0.170	1

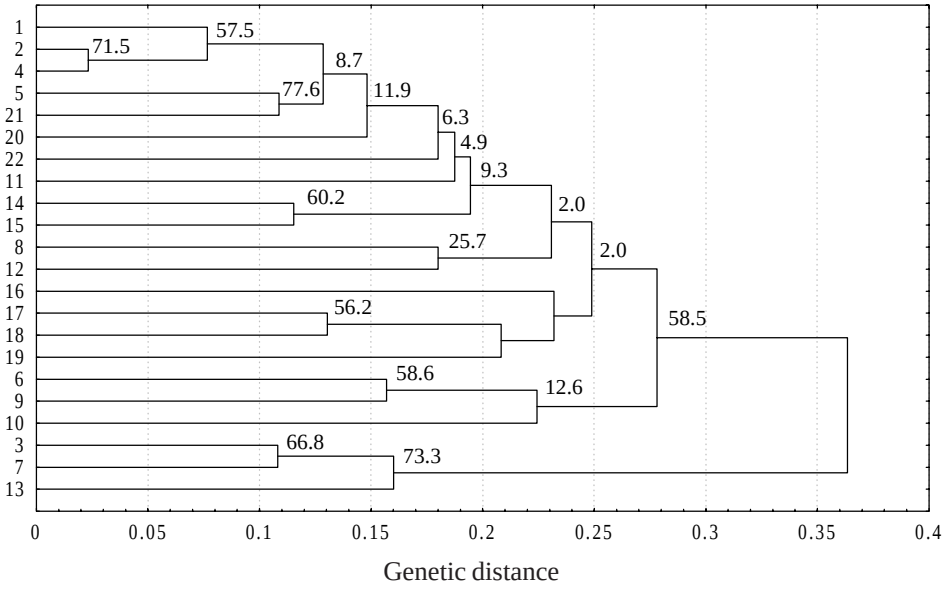

Figure 1. Dendogram representing the genetic distance among the 22 Baculovirus isolates from two geographical regions in Brazil. Isolates 18, 20, 21 and 22 are from Minas Gerais State, and all other isolates are from Paraná State. The numbers in each group indicate the frequency of the grouping calculated with 10,000 bootstraps.

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