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Genotypic Variation Amongst *Heliothis armigera* Baculovirus Isolates

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ABSTRACT

The restriction endonuclease patterns (REN) of three baculovirus isolates of *Heliothis armigera* collected from Anand (Gujarat), Faridabad (Haryana) and Pantnagar (U.P.) have been compared for genotypic variation. The digestion of dsDNA genomes with EcoRI and SalI restriction enzymes resulted in similar, but not identical fragmentation profiles showing minor differences in the size and number of several fragments. The genotypic heterogeneity of these baculovirus isolates is discussed.

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INTRODUCTION

Heliothis armigera is one of the most devastating insect pests among the pod borer (Reed and Pawar, 1982). Nuclear polyhedrosis virus (NPV) has been employed to control *H. armigera* as an alternate method of chemical control (Jayaraj *et al.*, 1987). Nuclear polyhedrosis viruses are members of the family *Baculoviridae*, subgroup A, which are characterized by enveloped rod shaped virions containing circular double-stranded DNA (Bilimoria, 1986). Genotypic heterogeneity within baculovirus population is a common phenomena (Mc Intosh *et al.*, 1987) which is indicated by the presence of submolar bands in restriction endonuclease profiles of virus DNA. REN analysis has been an efficient and precise method of distinguishing genotypic variants of NPVs (Miller *et al.*, 1980; Shapiro *et al.*, 1991).

Considerable differences in the infectivity of NPV isolated from different geographic locations of India in laboratory and pot trials suggest variation in the virulence of NPV isolates (Odak *et al.*, 1982). Similarly variation in the antigenic make up of virions has been shown in geographic isolates of HaNPV (Vyas and Lakhchaura, 1991).

The purpose of this study was to compare three representative HaNPV collected from Anand (Gujarat), Faridabad (Haryana) and Pantnagar (U.P.) for their genotypic variation by comparing EcoRI and SalI restriction profiles.

MATERIALS AND METHODS

Virus multiplication and purification of PIBs

The NPV isolates from Anand, Faridabad and Pantnagar were mass multiplied on

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Heliothis armigera larvae. The virus killed larvae were homogenised in 100 ml, 0.01 M Tris-HCl buffer, pH 7.6 and filtered through a fine polyester cloth. The filterate was centrifuged at 500 x g for 10 min and supernatant was again centrifuged at 2000 x g for 10 min to pellet the PIBs. Further purification of PIB was done by resuspending the pellet in 20 ml of 0.01 M Tris buffer, pH 7.6 containing 1.0% sodium dodecyl sulfate (SDS), 4 M Urea, 0.1% Mercaptoethanol and 0.5 M NaCl for 30 min at room temperature. After sonication for 30 sec at 20 W, the polyhedra were pelleted at 2000 x g for 10 min at 4°C. The partially purified PIB were subjected to sucrose density grodient centrifugation on a continuous linear gradient of 40-63% sucrose (w/w) at 100,000 g for 20 min. The PIB band was removed and washed thrice with tris-buffer followed by dialysis at 4°C overnight (Langridge *et al.* 1981).

Purification of virions and extraction of DNA

Purified occluded NPVs were disrupted with alkali according to the method of Miller and Dawes (1978) with some modification. An equal volume of alkaline solution of 0.1 M Na_2CO_3, 0.1 M NaCl, pH 11.0 was mixed with purified PIB and incubated at room temperature for 60 min and then at 4°C overnight. The mixture containing the liberated virions was centrifuged at 3000 rpm for 10 min at 4°C for removing the undigested polyhedra. The supernatant was collected and subjected to centrifugation at 100,000 x g for 90 min at 4° C. The pelleted virions were resuspended in 0.01 M Tris-HCl, 0.001 M EDTA, pH 7.6 and washed twice at 96,000 x g for 30 min. The purified pellet of virions was suspended in 0.01 M Tris, 0.001 M EDTA, pH. 7.6.

SDS was added to alkali liberated virus solution to a final concentration of 0.5% and the solution was heated to 65° C for 10 min. Th DNA was then separated from virions by pheno extraction method at room temperature (Mille and Dawes, 1978). The DNA was furthe purified by taking equal volume of chille chloroform : iso-amyl alcohol (24:1) and mixe with aqueous phase collected from pheno extraction. The contents were gently mixed and phases separated by centrifugation at 12000 x g for 5 Sec. The upper aqueous phase was collected and the entire procedure was repeated twice. The aqueous phase was collected in Eppendorf tube and equal volume of water saturated ether (chilled) was mixed gently and immediately centrifuged at 12000 x g for 5 sec. The upper layer of ether was carefully discarded. The DNA was precipitated by adding half volume of 7.5 M Ammonium acetate and double volume of 95% ethanol and kept at -20°C overnight. The precipitated DNA was pelleted at 12000 x g 15 min on Beckman microfuge T^{EM}. The traces of ethanol were evaporated under vacuum and purified DNA was resuspended in 0.01 M Tris, 0.001 M EDTA, pH 7.6. The ratio of optical density at 260/280 nm was 1.5. The DNA was quantified on the basis of one optical density at 260 nm equivalent to 50 micrograms of DNA.

Restriction endonuclease digestion

The viral DNAs were specifically cleaved by digesting 2 ug of DNA with 5 to 10 units of EcoRI and SalI restriction enzymes in 40 µl of total system containing 0.01 M Tris (pH 7.6) 0.01 M $MgCl_2$, 0.1 M NaCl and 1 mM dithiothreitol. After 2 hr of incubation, the digestion was stopped by heating at 65° C for 15 min in a water bath, then cooled on ice prior to gel electrophoresis. Lambda DNA (CSIR center for Biochemicals, New Delhi) digested with Hind III (New England Biolabs, USA) was our standard molecular weight marker. The buffer for Hind III

was 0.01 M tris (pH 7.6), 0.05 M NaCl, 0.01 M $MgCl_2$ and 1 mM dithiothreitol.

Electrophoresis of DNA fragments

The DNA fragments produced by REN digestion of viral DNAs were fractionated by electrophoresis through 0.6% agarose. The buffer for the agarose solution and for the electrophoresis contained 10.8 g Tris base, 0.93 g EDTA, sodium salt and 5.5 g boric acid in 1 liter of distilled water (pH 8.3). The DNA fragments were loaded into the wells under TBE buffer plus tracking dye (0.25% bromo phenol blue in 40% sucrose (w/w). A constant current of approximately 20 mA was applied across the gel for approximately 15 hr until the bromo phenol blue marker reached the acryl amide plug at the bottom of the gel. The gels were stained with ethidium bromide (0.5 µg/ml) for 20 min, then destained twice with distilled water for 15 min each. The restriction fragments were visualised with an UV transilluminator (Fotodyne) and photographed with an MP-4 land camera. The sizes of DNA fragments were estimated by comparison to the standard molecular weight marker lambda Hind III.

RESULTS AND DISCUSSION

The fragmentation profiles resulting from the digestion of *Heliothis* NPV DNAs of AND (Anand, Gujarat), FRD (Faridabad, Haryana) and PNT (Pantnagar, Uttar Pradesh) with EcoRI and SalI retriction enzymes are presented in Figures 1 and 2, respectively.

The restriction fragmentation patterns produced by EcoRI digestion of the three isolates were similar with minor variation by

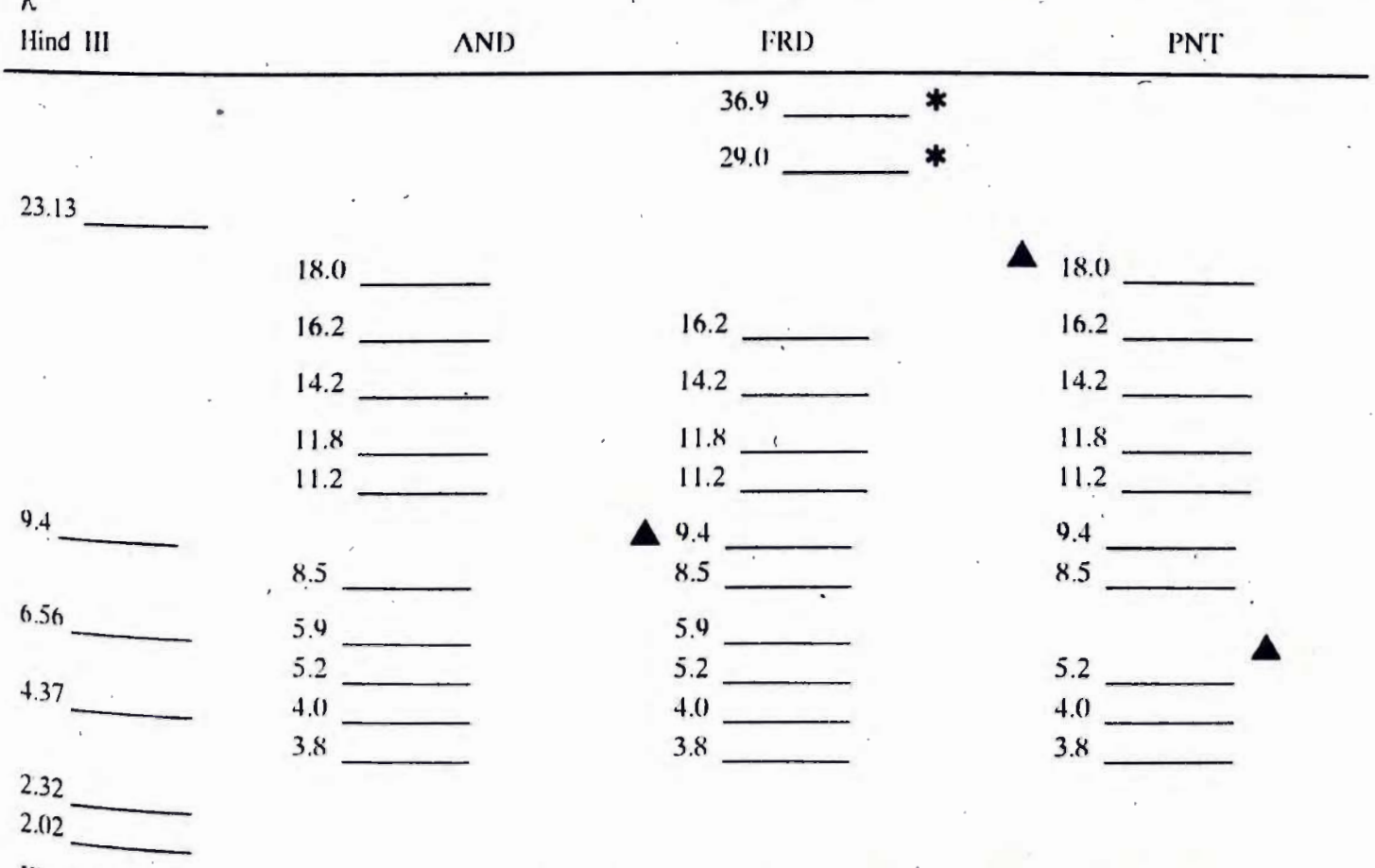


Fig. 1 : A schematic representation of EcoRI fragmentation profiles of the DNA from AND, FRD and PNT *Heliothis baculovirus* isolates.

way of one or two additional or missing fragments (Fig.-1). The FRD isolate produced two additional fragments of 36 and 29 kbp which were absent in AND and PNT isolates. Similarly, a 9.4 kbp fragment was observed in FRD and PNT isolates but absent from AND DNA profile. It can be seen from Fig.-1 that a 5.9 kbp band was present in FRD and AND isolates but could not be detected in PNT isolate. Conversely, a 18 kbp fragment was absent in FRD isolate. However, over all similarities were observed in the profiles of these three isolates.

The REN profiles produced by Sal digestion has been presented schematically in Fig-2. The REN patterns of these isolates were characteristically similar, but not identical

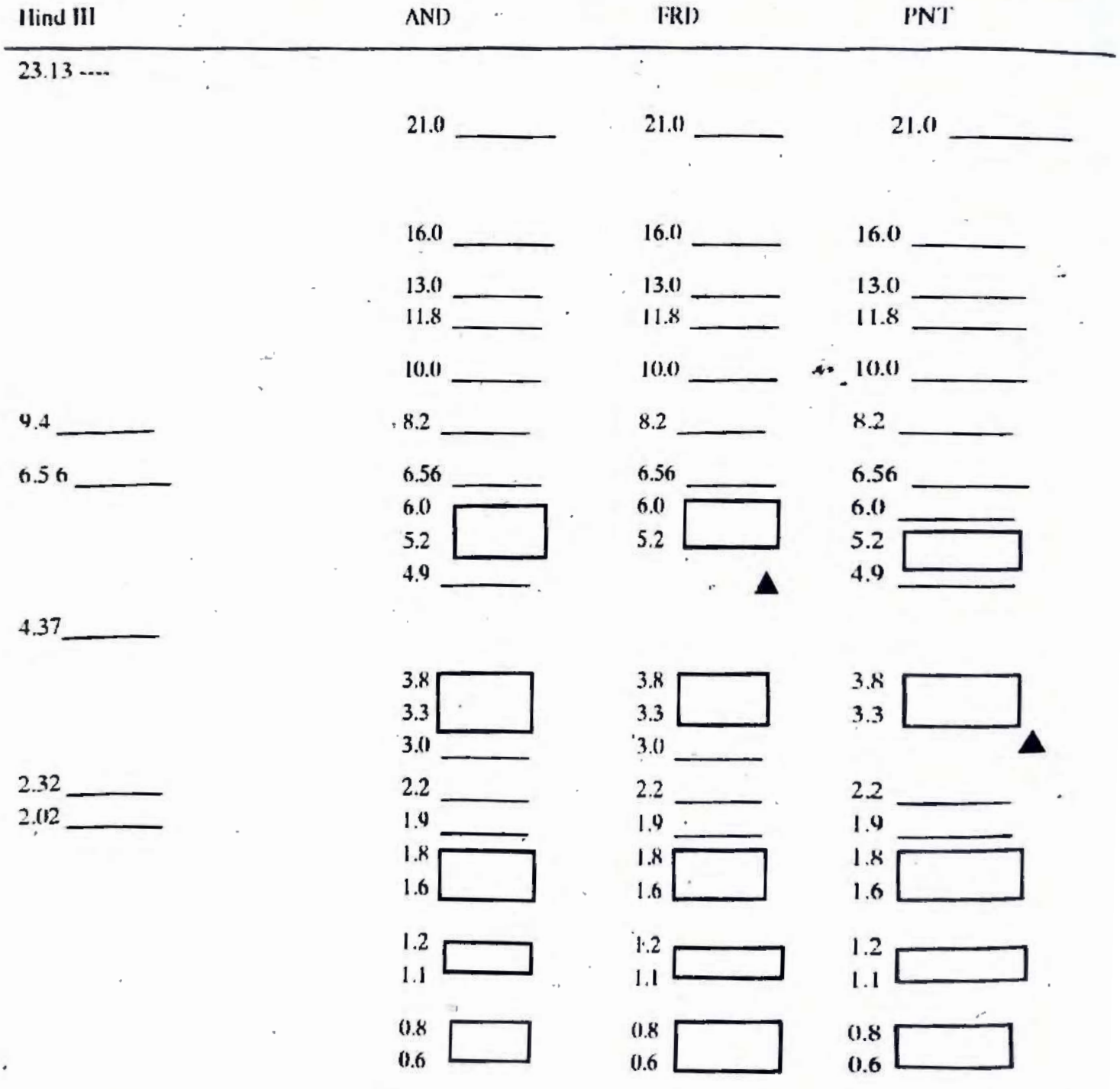


Fig. 2 : A schematic representation of Sall fragmentation profiles of the DNA from AND, FRD and PNT *Heliothis baculovirus* isolates.

There was less heterogeneity in the DNAs. A 4.9 kbp fragment was absent in FRD isolate of HaNPV but present in AND and PNT profiles. Similarly, a 3.0 kbp fragment could not be detected in PNT isolate, but present in AND and FRD profiles.

Diffused multiple bands in the REN profile between 0.6-0.8, 1.1- 1.2, 1.6-1.8, 3.3-3.8 and 5.2-6.0 kbp in the Sall digestion suggests more number of molar equivalents.

A comparison of EcoRI digestion profiles obtained in this study and the reported by Gettig and McCarthy (1982) of Indian strain of HaSNPV revealed that 8.5, 5.9, 5.2, 4.0 and 3.0 kbp fragments were comparable. However, geographic location of HaSNPV of Indian strain is not evident in their study except the fact that it was obtained from southern India. The HaSNPV had the unique EcoRI and Hind III fragmentation profiles which showed additional fragments of 2.43, 1.62, 1.21, 0.92 and 0.51 kbp in EcoRI digestion when the profiles of USSR, China, South Africa, Botswana, Australia and India were compared (Gettig and McCarthy, 1982). In our study, fragments lower than 3.8 kbp could not be resolved properly, hence it is difficult to have an accurate comparison of EcoRI digestion products.

Heliothis MNPV and SNPV genomes show little simalarility in their REN profiles and thus probably represent two distinct genotypes of *Heliothis* NPVs. Similar differences have been reported in MNPV and SNPV of *Trichoplusia ni* (Smith and Summers, 1978). The occurence of genotypic variants in wild isolates of baculovirus populations is a well documented phenomena (Kislev and Edelman, 1982; Shapiro *et al.*, 1991). These variants could be recognised by presence of submolar fragments in REN digestion products. The exact cause of variation in the REN profiles is unknown. However, variation in restriction patterns of NPV may result from (a) duplication of viral sequences (b) insertions of host DNA into the viral genome and/or point mutations (Brown *et al.*, 1985).

The precise history of all the isolates used in this study is not known. The Anand Isolate of Gujarat was indentified by Patel *et al.*, 1968 and is being used since then. However, genomic analysis has not been done. It is difficult to know whether any mutations has taken place or not. The history of Faridabad isolate is also not available. The Pantnagar isolate was isolated from the fields of chickpea (*Cicer arietinum* L.) during 1988 from Crop Research Station, Pantnagar.

The results of our study is a representative case of only three isolates. These isolates may be genotypic variants of a particular NPV strain. No attempt was made to plaque purify these viral isolates. It is thus, likely that the patterns obtained may reflect the genomic character of only the major variant in the population. The restriction analysis of large number of isolates from many geographic locations of India, plaque purification and bioassay studies will be fundamental to their ecology, development of commercial microbial pesticide and perhaps to an understanding of their evolution.

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