COMPARATIVE CYTOLOGICAL STUDIES ON A SUSCEPTIBLE AND RESISTANT STRAINS OF PINK BOLLWORM, PECTINOPHORA GOSSypiELLA (SAUND.)

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Abstract

The parent strain of Pectinophora gossypiella (Saund.) was collected from cotton fields and reared in the laboratory for several generations free from insecticidal pressure. Selection of newly hatched larvae with cyanophos for 24 generations resulted in a 32.51-fold increase in resistance.

Cytological behaviour of chromosomes in meiotically dividing cells was studied at the 24th selected generation. Also, the cytological aberration in relaxed strain for one generation was studied.

Results show that the percentage of abnormal cells was 26.08% at the 24th selected generation. Relaxed strain showed a slight decrease of percentage of abnormalities (22%) as compared with the susceptible strain (28%). The most frequent chromosomal aberrations were stickiness of chromosomes and lagging chromosomes. Irregular distribution of chromosomes was also observed in a few cells.

INTRODUCTION

Pink bollworm, Pectinophora gossypiella (Saunders) causes considerable damage to cotton in Egypt. The widespread and prolonged use of insecticides led to rapid development of resistance in field strains of P. gossypiella. Therefore, predictions or interpretations of rates of development or reversion of resistance are attainable without an understanding of the mode of inheritance of the resistance character. In order to understand how this pest develops resistance to pesticides, certain genetic studies have to be performed, especially on its cytogenetics.

This study aims to verify the effect of continuous application of cyanophos against 1st instar larvae of P. gossypiella on the rate of development of resistance,
and to explore the relation between resistance and the cytological effects occurring
in the gonads of the full grown larvae.

MATERIALS AND METHODS

1. Strains of pink bollworm

The susceptible strain (S) was obtained from the Bollworm Research Division,
plant Protection Research Institute, Dokki, Giza (Rashad & Ammar, 1984).

The resistant strain (R) was obtained from the parent strain after being
reared in the laboratory for several generations free from insecticidal pressure. It
was selected by cyanophos for 24 generations using the newly hatched larvae at the
LC30 level. 25th generation was left without insecticidal pressure to obtain relaxa-
tion strain (RS) for one generation (Rofai et al., 1995).

2. Selection procedure

LC30 concentration was calculated for every generation and topically applied
on artificial diet in glass tubes (2 x 7.5 cm). Every tube was infested with neonatal
larvae and capped with a cotton plug. Concentration was adjusted and increased with
the increase of resistance.

3. Cytological studies

Cyanophos selected strain larvae at generation 24 (G24), Relaxed line at gen-
eration 24 and untreated control larvae were collected for the cytological studies.

The techniques used were those adopted by North et al. (1981), Shalabi et al.
(1983) and El-Sorady et al. (1992) with slight modifications. The gonads were ob-
tained from larvae dissected in Belar’s hypotonic saline solution, then placed into
hypotonic colchicine solution at 37°C for 45 minutes. Each lobe of the gonads was di-
vided into three or four pieces, placed on a slide and fixed by acetic acid (45%) for
3-10 minutes. The tissues were stained with aceto-orcein solution (2%) and the
cover slips were positioned. The slides were slightly heated over a low flame and
squashed by using thumb present with stripes of filter papers over the cover slips.
The aceto-orcein stain was filtered each time through microfilter just before use to
prevent any precipitation in the stain which may lead to the formation of artifacts.
Prepared slides were stored in sealed slide staining dishes over bibulous paper satu-
rated with acetic acid (45 %) at 20°C for 5 days.
RESULTS AND DISCUSSION

1. Toxicological studies

Data presented in Table 1 show the resistance level to cyanophos during selection in generations 17, 20, 24 and the relaxed G24. Resistance ratio was 37.35, 29.13, 32.51, and 27.38, respectively. The corresponding slopes of the regression line were 2.86, 2.37, 2.44 and 2.24 indicating a considerable degree of homogeneity toward the development of cyanophos resistance. Results show a slight decrease in the resistance ratio in the relaxed G24 as compared with the selected one.

<table>
<thead>
<tr>
<th>Generation</th>
<th>LC50</th>
<th>Slope</th>
<th>R.R.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Susceptible</td>
<td>3.577</td>
<td>1.79</td>
<td></td>
</tr>
<tr>
<td>G12</td>
<td>133.62</td>
<td>2.86</td>
<td>37.35</td>
</tr>
<tr>
<td>G20</td>
<td>103.025</td>
<td>2.37</td>
<td>29.13</td>
</tr>
<tr>
<td>G24</td>
<td>116.30</td>
<td>2.44</td>
<td>32.51</td>
</tr>
<tr>
<td>Relaxation</td>
<td>57.97</td>
<td>2.24</td>
<td>27.38</td>
</tr>
</tbody>
</table>

2. Cytological studies

Observations indicated that the metaphase stage, Fig. 1, A & B was the highest frequent stage. The chromosomes were highly compact, and appeared as short rods with no details of structure or points of bending indicating the position of centromeres. Such observations might be due to the high construction of the chromosomes or to the presence of diffused centromeres (Davidson, 1974; Hassan, 1985).

Dividing meiotic cells of the male gonads of the pink bollworm displayed chromosomal aberrations, Table 2. Treatment increased such abnormalities. The most frequent aberrations induced by treatment were stickiness, Fig. 7 and lagging bivalents, Fig. 3. Irregular distribution of bivalent, Fig. 4 was also observed in few cells in all treatments and untreated control line. No abnormal cells with bridge formation were observed. Similar results were obtained by El-Wakil et al. (1988), El-Sorady et al. (1992) and Massoud et al. (1992).

The above-mentioned results showed that the percentage of abnormal cells was almost equal (26%) for cyanophos selected line in generation 24 (G24). On the other hand, the relaxation for one generation in Cyanophos selected strain in G24 showed a slight decrease in the percentage of abnormalities (22%). This observation
may be attributed to the stability of Cyanophos resistant strain with a considerable degree of homogeneity towards the development of Cyanophos resistance (Rofail et al., 1995).

Table 2. The percentage of the different types of abnormalities in the abnormal meiotic cells of treated larvae of Pectinophora gossypiella.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of divided cells</th>
<th>No. of abnormal cells</th>
<th>% Abnormality</th>
<th>Type of abnormality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyanophos selected line G24</td>
<td>345</td>
<td>90</td>
<td>26.087</td>
<td>3.768</td>
</tr>
<tr>
<td>Relaxed line G24</td>
<td>255</td>
<td>55</td>
<td>21.569</td>
<td>4.314</td>
</tr>
<tr>
<td>Untreated line (Control)</td>
<td>325</td>
<td>9</td>
<td>2.768</td>
<td>0.615</td>
</tr>
</tbody>
</table>

Generally speaking, the chromosomal aberrations observed in treated cells were similar to treated cells from different organisms when subjected to different pesticides. These findings go in line with the results obtained by Devadas et al. (1987), Jain and Sarbhoj (1987) in plants and Mishra and Benerjee (1987) and El-Wakil et al. (1988) in insects. Chromosomal stickiness is generally regarded as a physiological unspecific disturbance and has been attributed to the action of basic chromosomal proteins, e.g., histones (Devadas et al., 1987).

Saleem and Al-Najjar (1984) suggested that stickiness of chromosomes in wheat cells treated with common fungicides may be due to the presence of attached methyl group in all the fungicides used in their experiment. Lagging chromosomes were due to delayed terminal, of perhaps, as a result of the stickiness of chromosomes. The same conclusion was reached by Kaur and Grover (1985). Another explanation was given by Jain and Sarbhoj (1987) on the phenomenon of lagging chromosomes. They stated that pesticide treatment hindered the chromosomes from reaching the poles and remained scattered in the cytoplasm.

It was obvious, however, that there were irregularities in chromosomal distribution in some treated cells. This may be attributed to the disturbance of spindle apparatus (El-Feel et al., 1990).

In conclusion, chromosomal anomalies resulting from treatment were most frequent chromosomal aberrations causing stickiness of chromosomes and lagging chromosomes.
Fig. 1. Normal metasphase showing a complement of chromosomes in P.gossypiella.

(A) Side view   (B) Polar view.

Fig. 2. Metaphase I showing sticky bivalents of P.gossypiella larvae.
Fig. 3. Metaphase I showing lagging bivalents of selected P.gossypiella larvae.

Fig. 4. Metaphase I showing irregular distribution of bivalents in selected larvae of P.gossypiella.
REFERENCES


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دراسات سينتولوجية مقارنة على السلالات الحساسة والمقاومة للدورة اللوز

ф Pectinophora gossypiella

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جمعت سلالة من دورة اللوز الفرنسية Pectinophora gossypiella من حقول القطن وربت
معقلها لعدة أجيال دون إستخدام أي مبيدات، وتم الانتخاب بسبب سينتولوجيا قلة 14 جيل على
البرغيات حديثة الفقس والتي تأتي إلى زيادة القدرات على ال 41 هبنا. ودراسة سينتولوجي
كروموسومات الإنقسام الميوزي في الجيل الانتخابي الرابع والثامن ونقبل السلاله المستخر
عة جيل واحد بعد أن نسبة الخلايا الشائعة في الجيل الرابع والعشرين (27/1) أما السلالة
المستخرجاء لدة جيل واحد فقد أظهرت انخفاضاً طفيفاً في نسب الخلايا الشائعة (28/4) مقارنة
بالسلالة الحساسة (26/8). وكانت أكثر الاختلافات الكروموسومية تركاراً في ظهور
الكروموسومات القصيرة والكروموسومات المثلاة أو المنقرضة، كما ظهر التنوع غير المنتظم
الكروموسومات في عدد قليل من الخلايا.