转 Bt-cry1Ab 玉米花粉对异色瓢虫生长发育及体内三种代谢酶活性的影响

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摘要：转 Bt-cry1Ab 玉米花粉对异色瓢虫生长发育及体内三种代谢酶活性的影响。结果显示，异色瓢虫取食混有适量蚜虫的转基因玉米花粉时与取食混有适量蚜虫的非转基因亲本玉米花粉时相比，各虫态发育历期没有显著差异；取食转基因玉米花粉对异色瓢虫的体重增加无明显影响。多数龄期内取食转基因玉米花粉的异色瓢虫体内的乙酸萘酯酶活性、乙酰胆碱酯酶活性以及谷光甘肽转移酶活性与对照组相比没有显著差异。用酶联免疫（ELISA）方法在取食转基因玉米花粉的瓢虫体内未检测到 Bt 杀虫蛋白。转基因玉米花粉对异色瓢虫生长发育没有显著负面影响，初步证明转基因玉米花粉对异色瓢虫是安全的。

关键词：转 Bt-cry1Ab 玉米；花粉；异色瓢虫；生长发育；代谢解毒酶；酶活性

Effects of transgenic Bt-cry1Ab corn pollen on the growth and development and the activity of three metabolic enzymes in Harmonia axyridis Pallas Coleoptera Coccinellidae

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Abstract In order to explore the effects of transgenic Bt-cry1Ab corn pollen on the multicolored Asian lady beetle MALB Harmonia axyridis Pallas a series of experiments were conducted with a non-Bt isolate as the control in the laboratory. The H. axyridis beetles were fed with transgenic Bt-cry1Ab corn pollen mixed with a certain proportion of pea aphids Acyrthosiphon pisum Harris in the laboratory and changes in their developmental duration and body weight at different stages were observed. The activity changes of three metabolic enzymes a-naphthylacetate esterase acetylcholinesterase and glutathione-S-transferase in H. axyridis reared on transgenic Bt-cry1Ab corn pollen were also determined. Moreover, we monitored the sediment of Bt-Cry1Ab toxin protein in the bodies of H. axyridis by ELISA method. The results indicated that there was no marked difference in the developmental duration at each stage between these beetles fed on transgenic Bt-cry1Ab corn pollen and those on non-Bt isolate corn pollen. The transgenic Bt-cry1Ab corn pollen also had no notable side effects on the body weight of H. axyridis at different stages. The activities of three metabolic enzyme activities in H. axyridis fed on transgenic Bt-cry1Ab corn pollen changed slightly compared with the control which had no visible effect on the growth of H. axyridis. The sediment of Bt-Cry1Ab toxin protein in the bodies of H. axyridis at different developmental stages was so scarce that it could not be detected by ELISA method. It was concluded that there was no significantly harmful effect of transgenic Bt-cry1Ab corn on the growth and development of the beetle.

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Key words Transgenic Bt-cry1Ab corn pollen Harmonia axyridis growth and development metabolic enzymes enzyme activity

1 材料与方法

1.1 供试玉米花粉

- Bt-cry1Ab MON810 Bt DK647 Bt Monsanto
- 12 % 6 2 : 1 10 % 1 mL pH 8.0
- 0.1 % Triton X-100
- 4℃ 10 000 × g
- 30 min

1.2 供试昆虫及饲养方法

- H. axyridis Acrhythosiphon pisum
- 26℃ RH 60% – 70%
- D = 13 : 11
- 100 cm × 100 cm × 100 cm
- 10 cm
- 100 mg
- 1 %

1.3 转基因玉米花粉对异色瓢虫生长发育影响的室内测定

- 30 8 4
- 2 : 1
- 45 1

1.4 瓢虫体内代谢解毒酶和 Bt-Cry1Ab 毒蛋白含量的测定

1.4.1 30 % 12 % 6 % 2 : 1
- 10 %
- 4℃

1.4.2 Van Aspe 1962
- Ellman 1961
- -S-Booth 1961

1.4.3 Bt-Cry1Ab ELISA AGDIA Bt-Cry1Ab 2
- 50 1
- 0.8 mL
离心。按照试剂盒操作说明，取匀浆上清液加入已用单克隆一抗包被的酶标板孔内（重复，次），在同一酶标板上其他两行孔中加不同梯度的-杀虫蛋白作标准曲线，然后每孔加入过氧化氢酶偶联的二抗溶液，完成后置冰箱过夜。用磷酸缓冲液洗板次。拍除余液后每孔加底物，于37恒温箱黑暗孵育后显色。在酶标仪处测定各个点样孔吸光值。另参照的方法测定样品提取液中总可溶性蛋白含量。整理测定结果，依据标准曲线，确定瓢虫体内-杀虫蛋白占总可溶性蛋白的比例。本试验数据均采用中文版软件进行统计分析。

1.5 数据处理

SAS 9.0

2 结果与分析

2.1 瓢虫取食 Bt 玉米花粉和非 Bt 玉米花粉的生长发育情况

表 1 瓢虫取食 Bt 玉米花粉(处理组)和非 Bt 玉米花粉(对照组)后的发育历期

<table>
<thead>
<tr>
<th>Agl</th>
<th>Treatment groups</th>
<th>Control groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.33 ± 0.02 a</td>
<td>0.27 ± 0.01 a</td>
</tr>
<tr>
<td>3</td>
<td>1.25 ± 0.00 a</td>
<td>0.98 ± 0.10 b</td>
</tr>
<tr>
<td>5</td>
<td>5.80 ± 0.50 a</td>
<td>4.51 ± 0.40 a</td>
</tr>
<tr>
<td>7</td>
<td>13.38 ± 0.80 a</td>
<td>11.45 ± 0.90 a</td>
</tr>
<tr>
<td>9</td>
<td>28.00 ± 0.80 a</td>
<td>26.48 ± 1.50 a</td>
</tr>
<tr>
<td>11</td>
<td>29.22 ± 0.50 a</td>
<td>26.49 ± 0.70 b</td>
</tr>
<tr>
<td>13</td>
<td>28.17 ± 0.40 a</td>
<td>26.66 ± 0.70 a</td>
</tr>
<tr>
<td>15</td>
<td>28.40 ± 0.80 a</td>
<td>28.00 ± 0.90 a</td>
</tr>
<tr>
<td>17</td>
<td>27.98 ± 0.70 a</td>
<td>26.81 ± 0.90 a</td>
</tr>
<tr>
<td>19</td>
<td>29.40 ± 0.60 a</td>
<td>28.23 ± 0.80 a</td>
</tr>
<tr>
<td>21</td>
<td>30.09 ± 0.50 a</td>
<td>28.48 ± 2.80 a</td>
</tr>
<tr>
<td>23</td>
<td>32.49 ± 1.00 a</td>
<td>31.33 ± 1.10 a</td>
</tr>
<tr>
<td>25</td>
<td>31.91 ± 0.60 a</td>
<td>31.22 ± 1.00 a</td>
</tr>
<tr>
<td>27</td>
<td>31.63 ± 0.90 a</td>
<td>31.94 ± 1.00 a</td>
</tr>
<tr>
<td>29</td>
<td>31.88 ± 0.60 a</td>
<td>31.01 ± 1.80 a</td>
</tr>
<tr>
<td>45</td>
<td>32.65 ± 1.00 a</td>
<td>31.09 ± 1.40 a</td>
</tr>
</tbody>
</table>

乙酸奈酯酶活性在0龄期较取食非玉米花粉的异色瓢虫显著降低，在，龄期显著升高，在,龄期、蛹期、成虫期在对照均无显著变化。取食玉米花粉的异色瓢虫乙酰胆碱酯酶活性在0龄期较取食非玉米花粉的显著降低，而在， 龄期显著升高，其他发育时期无明显变化。取食玉米花粉的异色瓢虫体内谷胱甘肽转移酶活性在各个发育时期与对照均没有显著差异。在所测定的样品中，转玉米花粉处理的0龄、，龄、，龄、蛹、成虫(，天)体内均检测不到-杀虫蛋白(数据未列出)。

取食玉米花粉的异色瓢虫与取食非玉米花粉的异色瓢虫相比，各龄期历期均没有显著差异，初步说明转玉米花粉对异色瓢虫的发育历期无明显负面影响。
表3 异色瓢虫幼虫期和成虫期取食转基因玉米花粉与非转基因玉米花粉对代谢酶活性的影响

Table 3 The activity of metabolic enzymes in *H. axyridis* reared on pollen of transgenic

<table>
<thead>
<tr>
<th>Stage</th>
<th>Enzyme activity (μmol L⁻¹·mg⁻¹·min⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Treatment groups</td>
</tr>
<tr>
<td>2nd instar</td>
<td>0.06 ± 0.01 a</td>
</tr>
<tr>
<td>3rd instar</td>
<td>0.07 ± 0.01 a</td>
</tr>
<tr>
<td>4th instar</td>
<td>0.06 ± 0.01 a</td>
</tr>
<tr>
<td>Pupa</td>
<td>0.06 ± 0.00 a</td>
</tr>
<tr>
<td>Adult</td>
<td>40 d</td>
</tr>
<tr>
<td>Adult</td>
<td>50 d</td>
</tr>
</tbody>
</table>

The difference between the treatment and control groups is tested only within an enzyme.

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