No Effect of Bt-transgenic Rice on the Tritrophic Interaction of the Stored Rice, the Maize Weevil Sitophilus Zeamais and the Parasitoid Wasp Theocolax elegans

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During Bt transgenic rice storage, Bt Cry1Ab/Cry1Ac fused protein is exposed to the maize weevil Sitophilus zeamais and the parasitoid wasp Theocolax elegans. We have carried out a long-term risk assessment for Bt rice to these non-target organisms in the storehouse. Effects of Bt rice on S. zeamais and T. elegans have been carefully detected in a laboratory experiment of over 5 years. The survival, development, fecundity, and longevity of the maize weevil were compared between Bt rice and non-Bt rice treatments for every 5 generations from generation 1 to 25. Moreover, the development, adult body size and sex ratio of T. elegans were compared between them parasitizing S. zeamais feeding on Bt rice or non-Bt rice. We found that although Bt Cry1Ab/Cry1Ac fused protein exists in the Bt rice grains and S. zeamais digestive tracts, Bt rice is not harmful to the maize weevil S. zeamais and its parasitoid T. elegans.

Rice (Oryza sativa L) is one of the most important grain crops. It serves as a staple diet for more than half of the world's population1. The major insect pests of rice production are hemipteran sap-sucking planthoppers and lepidopteran stem borers and leaf folders. The latter includes yellow stem borer (Scirpophaga incertulas Walker), striped stem borer (Chilo suppressalis Walker), pink stem borer (Sesamia inferens Walker), and leaf folder (Cnaphalocrosis medinalis Guenee), which cause severe yield losses annually2. Every year, insecticides have been extensively applied to control these lepidopteran pests during the commercial rice production, which is expensive and environmentally unfriendly.

With wide use of modern biotechnology in rice breeding programs, many foreign genes have been introduced into rice to generate genetically modified (GM) rice varieties that resist these lepidopteran pests3,4. Among them, the Bacillus thuringiensis crystal (Bt Cry) insecticidal protein (δ-endotoxin) genes have shown good performance in public and private sector rice breeding programs5. These Bt-crystal genes are highly selective and represent a class of numerous proteins with insecticidal action on larvae from various orders. For example, Cry1 and Cry2 are toxic to lepidopteran pests, specially6. So far, rice has been genetically transformed with genes of Bt cry1Ab, cry1Ac, cry1Ca, cry2A and cry1Ab/cry1Ac fusion to control these stem borers and leaf folders7. Field study with a Bt rice line in China revealed an increase in yield by 6–9% and a reduction in pesticides usage by 80%8.

However, before a Bt rice line can be cultivated, the risks to the environment and human health must be assessed. This includes the evaluation of potential adverse effects on non-target invertebrate and vertebrate animals, as well as the ecosystem services they provide8–12. For this purpose, extensive investigations have been conducted in the rice field. First, several studies focused on the effects of Bt-transgenic rice lines on the non-target arthropods in the rice field were performed. These studies revealed that Bt-transgenic rice does not directly affect the growth, development, abundance, and diversity of brown planthopper13–15, leafhopper13, ground-dwelling collembolans16, pond wolf spider and other rice field predator natural enemies15,17–20. Second, biosafety of

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Bt-transgenic rice to agroecosystems, including soil and aquatic ecosystem, was assessed. It was reported that Cry1Ab and Cry1Ac toxins from Bt rice plant tissues could persist in paddy fields for a long period of time. These Cry proteins could accumulate further in the soil by binding to soil particles and remain biologically active. However, transgenic Bt rice is safer to aquatic ecosystems than its non-transgenic counterpart. The abundance and diversity of zooplankton were significantly higher in Bt rice fields than non-Bt rice fields, because Bt rice required reduced pesticide application therefore produced lower pesticide residues in the field water body. Finally, safety of Bt-transgenic rice to the vertebrates were investigated. Impact of Bt transgenic rice on frogs, rats, rats, pigs, and macaques was intensively studied. Through a 90-days feeding assay, no differences were observed between the rats feeding on genetically modified rice and non-genetically modified rice in term of a range of parameters, including microflora composition, intestinal permeability, epithelial structure, fecal enzymes, bacterial activity, and intestinal immunity.

Rice production does not end in the field. Newly harvested rice is not sold immediately but stored for months before it is consumed. However, biosafety of Bt-transgenic rice to animals in the storehouse has not been well studied. Various stored pests can cause severe damage to the storage rice. Among them, the maize weevil, Sitophilus zeamais is a common pest of grain storage world widely. It attacks stored cereal products, including maize, wheat, rice, sorghum, oats, barley, as well as other types of stored, processed cereal products such as pasta, cassava. Because of its high reproductive capacity and strong infectivity, it is considered to be the most harmful pest in grain storage. Thecolax elegans is a parasitoid wasp that parasitizes larvae and pupae of economically important stored-product insect pests. In this paper, we studied the effects of transgenic Bt stored rice (Bt cry1Ab/cry1Ac) on the non-target coleopteran storage pest S. zeamais and its natural enemy T. elegans.

Results
Effects of Bt rice on the maize weevil S. zeamais. We first assessed the effects of Bt rice on the survival and development of S. zeamais immature stages. As shown in Table 1, when S. zeamais were reared on non-GM rice under the conditions of 28 ± 1 °C, 80% relative humidity, and total darkness, the final survival rate during the developmental stages from egg to nonage was about 82% and the developmental time for this period was about 73 days. These numbers were stable from the 1st to 25th generation and no significant difference was detected when comparing the beetles feeding on non-GM rice or Bt rice (Table 1, p > 0.05), indicating that Bt rice does not affect S. zeamais development.

We then examined the effects of Bt rice on the longevity and fecundity of S. zeamais adults. When reared on non-GM rice under the experimental conditions, the S. zeamais adults lived 107.18 ± 8.74 days, oviposition period was 85.64 ± 5.26 days, and each female laid 276.34 ± 12.92 eggs. The life span, oviposition period, and total number of eggs laid by each female were all comparable among beetles of different generations and between beetles feeding on non-GM rice and Bt rice (Table 2, p > 0.05), suggesting that no obvious effect of Bt rice on the longevity and fecundity of S. zeamais adults was observed.

Effects of Bt rice on the parasitoid wasp T. elegans. Bt rice does not affect the development, longevity and fecundity of S. zeamais. Whether does it affect the parasitoid wasp of the maize weevil, T. elegans? We compared the developmental time, body size, and sex ratio of T. elegans feeding on non-GM rice or Bt rice. When T. elegans parasitized on the S. zeamais feeding on non-GM rice, the wasps took about 44 days to develop from eggs to nonage; the body length of female and male was 1.46 ± 0.13 mm and 1.29 ± 0.12 mm, respectively; and female/male ratio was 4.02 ± 0.37. In all of these biological characteristics, no obvious difference was detected between T. elegans that parasitized the S. zeamais feeding on non-GM rice and T. elegans that parasitized the S. zeamais feeding on Bt rice (Table 3, p > 0.05).

Determination of Bt Cry protein in the stored rice, the maize weevil and the parasitoid wasp. As we did not detect any significant influence of Bt rice on S. zeamais and T. elegans. We were wondering if Bt Cry protein exists in the Bt rice, S. zeamais and T. elegans. To address this question, we examined Bt Cry protein in all three players in the tritrophic relationship, the stored Bt rice, the stored-product pest S. zeamais, and the parasitoid wasp T. elegans. As shown in Table 4, we found that Bt Cry1Ab/Cry1Ac protein did exist in the Bt rice, the maize weevil larvae, and the digestive tract of maize weevil adults. But no Bt Cry1Ab/Cry1Ac protein was detected in the maize weevil adult tissues other than digestive tract. 10 days after the maize weevils were transferred from Bt rice to non-GM rice, Bt Cry1Ab/Cry1Ac protein disappeared from the maize weevil adult digestive tract. Moreover, Bt Cry1Ab/Cry1Ac protein was not detectable in the parasitoid wasp T. elegans.

Discussion
Risk assessment should be carried out when the genetically modified plants (GM plants) are cultured, stored, transported, disposed of or used. However, most non-target risk assessments of GM rice are mainly focused on the field ecosystems, whereas coarse rice, the final product of rice production, is usually stored for a long period of time before consumption. Therefore, we focused our study on the risk assessment of Bt rice to the non-target organisms during storage.

In any ecosystem, there is usually a high number of non-target organism species exposing to the GM plants. There are several criteria suggested for species selection to conduct risk assessment for GM plants, for example, selecting species that are representative of their genera and/or of particular functional groups, including herbivores, pollinators, natural enemies (predators and parasitoids) of pest organisms, and decomposers of plant material. Based on these criteria, extensive investigations were performed in the field ecosystems to assess the potential risk of Bt-rice to non-target herbivore pests–planthoppers, their predators–spiders, and their parasitoids–wasps. As the maize weevil is one of the most important stored pests of rice, we evaluated the effects of Bt rice on the tritrophic interaction of the stored rice-S. zeamais-T. elegans. We found that Bt rice
| Generation | stages | Parameter | Rice cultivars | | | Non-GM rice | Bt rice | p-value |
|------------|--------|-----------|---------------|---|---|-----|-----|--------|
| 1          | Egg    | Survival rate (%) | 94.56 ± 2.86* | 95.18 ± 2.48 | 0.56 |
|            |        | Duration (d)     | 8.68 ± 1.24 | 8.46 ± 1.58 | 0.41 |
|            | Larva  | Survival rate (%) | 92.46 ± 3.64 | 91.66 ± 3.85 | 0.95 |
|            |        | Duration (d)     | 18.84 ± 1.97 | 18.27 ± 2.02 | 0.96 |
|            | Pupa   | Survival rate (%) | 88.34 ± 3.91 | 89.26 ± 3.59 | 0.61 |
|            |        | Duration (d)     | 9.27 ± 1.08 | 9.58 ± 1.26 | 0.40 |
|            | Nonage | Survival rate (%) | 82.25 ± 4.86 | 83.54 ± 5.23 | 0.98 |
|            |        | Duration (d)     | 35.87 ± 2.86 | 36.05 ± 2.13 | 0.68 |
| 5          | Egg    | Survival rate (%) | 95.76 ± 3.05 | 95.44 ± 2.62 | 0.55 |
|            |        | Duration (d)     | 8.04 ± 1.82 | 8.52 ± 1.36 | 0.31 |
|            | Larva  | Survival rate (%) | 92.94 ± 2.89 | 92.13 ± 3.57 | 0.51 |
|            |        | Duration (d)     | 19.26 ± 1.96 | 18.53 ± 2.19 | 0.24 |
|            | Pupa   | Survival rate (%) | 87.92 ± 3.64 | 88.06 ± 2.93 | 0.47 |
|            |        | Duration (d)     | 9.46 ± 1.17 | 8.94 ± 1.44 | 0.49 |
|            | Nonage | Survival rate (%) | 82.42 ± 4.67 | 82.76 ± 4.56 | 0.17 |
|            |        | Duration (d)     | 36.23 ± 2.55 | 35.75 ± 3.02 | 0.58 |
| 10         | Egg    | Survival rate (%) | 96.38 ± 2.14 | 96.12 ± 2.62 | 0.66 |
|            |        | Duration (d)     | 7.98 ± 1.86 | 8.26 ± 1.64 | 0.76 |
|            | Larva  | Survival rate (%) | 90.95 ± 4.21 | 91.86 ± 3.86 | 0.50 |
|            |        | Duration (d)     | 18.71 ± 2.08 | 19.02 ± 2.43 | 0.80 |
|            | Pupa   | Survival rate (%) | 88.81 ± 3.76 | 87.72 ± 3.53 | 0.96 |
|            |        | Duration (d)     | 9.12 ± 1.33 | 9.28 ± 1.51 | 0.67 |
|            | Nonage | Survival rate (%) | 83.18 ± 4.73 | 82.48 ± 4.28 | 0.81 |
|            |        | Duration (d)     | 36.19 ± 2.08 | 36.47 ± 2.64 | 0.64 |
| 15         | Egg    | Survival rate (%) | 95.64 ± 2.76 | 96.44 ± 1.96 | 0.27 |
|            |        | Duration (d)     | 8.06 ± 1.66 | 8.42 ± 1.72 | 0.91 |
|            | Larva  | Survival rate (%) | 93.47 ± 3.03 | 92.49 ± 2.67 | 0.88 |
|            |        | Duration (d)     | 18.79 ± 2.75 | 19.04 ± 2.37 | 0.90 |
|            | Pupa   | Survival rate (%) | 90.22 ± 3.87 | 88.49 ± 3.12 | 0.59 |
|            |        | Duration (d)     | 8.86 ± 1.59 | 9.07 ± 1.37 | 0.69 |
|            | Nonage | Survival rate (%) | 82.87 ± 4.62 | 82.16 ± 4.32 | 0.91 |
|            |        | Duration (d)     | 35.86 ± 2.96 | 35.28 ± 3.27 | 0.86 |
| 20         | Egg    | Survival rate (%) | 94.98 ± 2.94 | 95.40 ± 2.24 | 0.81 |
|            |        | Duration (d)     | 8.56 ± 1.48 | 8.36 ± 1.54 | 0.91 |
|            | Larva  | Survival rate (%) | 93.09 ± 3.51 | 92.43 ± 2.69 | 0.67 |
|            |        | Duration (d)     | 18.41 ± 2.63 | 18.59 ± 2.14 | 0.74 |
|            | Pupa   | Survival rate (%) | 87.91 ± 4.03 | 89.59 ± 3.92 | 0.89 |
|            |        | Duration (d)     | 9.34 ± 1.45 | 9.23 ± 1.06 | 0.43 |
|            | Nonage | Survival rate (%) | 83.02 ± 4.81 | 82.86 ± 4.53 | 0.92 |
|            |        | Duration (d)     | 36.12 ± 2.81 | 35.74 ± 2.29 | 0.78 |
| 25         | Egg    | Survival rate (%) | 95.46 ± 2.68 | 95.56 ± 2.36 | 0.48 |
|            |        | Duration (d)     | 8.44 ± 1.32 | 8.18 ± 1.52 | 0.54 |
|            | Larva  | Survival rate (%) | 92.58 ± 2.87 | 91.86 ± 2.98 | 0.86 |
|            |        | Duration (d)     | 19.07 ± 2.73 | 18.83 ± 2.16 | 0.76 |
|            | Pupa   | Survival rate (%) | 88.64 ± 3.65 | 88.51 ± 3.26 | 0.71 |
|            |        | Duration (d)     | 9.48 ± 1.53 | 9.19 ± 1.48 | 0.92 |
|            | Nonage | Survival rate (%) | 82.81 ± 4.29 | 82.53 ± 4.74 | 0.92 |
|            |        | Duration (d)     | 35.82 ± 2.77 | 35.49 ± 2.23 | 0.59 |

Table 1. Effect of Bt rice on survival and development of S. zeamais immature stages. Note: *All values are means ± standard deviations.

expressing Bt Cry1Ab/Cry1Ac fusion protein has no adverse effect on the growth and development of the maize weevil S. zeamais, as well as that of the parasitoid wasp T. elegans. When comparing the beetles reared on Bt rice and non-GM rice, there is no significant difference in all biological traits observed, including developmental speed, survival rate, and adult longevity and fecundity (Tables 1 and 2). Similarly, when T. elegans parasitized S. zeamais feeding on Bt rice and non-GM rice, no significant difference was found in the development, adult body sizes and female/male ratio (Table 3). These results are not due to lack of Bt Cry1Ab/Cry1Ac protein in the stored
Bt rice and the maize weevils. On the contrary, we detected Bt Cry1Ab/Cry1Ac protein existing in the stored Bt rice and in the S. zeamais larvae or adults that feed on Bt rice (Table 4).

These findings are consistent with the results of several investigations in the rice field. For example, when feeding on Cry1 or Cry2-expressing rice pollen, non-target coleopteran species Micraspis discolor and Propylea japonica are not affected. The adult survival, longevity, and fecundity of these beetles are not different when they feed on Bt rice or non-Bt rice pollen. Similarly, Bt rice is not harmful to the parasitoid wasps of non-target insects in the rice field. Anagrus nilaparvatae and Pseudogonatopus flavifemur are two parasitoid wasp species of rice plant hoppers. Previous researches indicate that no significant difference on the development, survival, longevity, fecundity, prey consumption, and population dynamics of these parasitoid wasps is detected between the Bt and non-Bt rice treatments. Furthermore, when A. nilaparvatae is exposed to Bt proteins by feeding on plant hopper N. lugens honeydew produced from Bt rice, the development, longevity, emergence rate and fecundity of the wasp is not affected, too.

When considering the biosafety of Bt crops, one big concern is the long-term effects of Bt toxin. Most of the risk assessment of Bt rice to the non-target organisms have been conducted within a relative short period of time and limited generations or production cycles. In this study, we evaluated the biosafety of Bt rice grain to the non-target organisms in the storehouse, including a storage pest and its natural enemy. Our results show that, under the laboratory trials of five years and 25 generations, Bt rice expressing Bt Cry1Ab/Cry1Ac fusion protein does not pose a risk to the maize weevil S. zeamais and its parasitoid T. elegans.

Table 2. Effect of Bt rice on longevity, oviposition period and fecundity of S. zeamais adults. Note: *All values are means ± standard deviations. **Fecundity is presented by number of eggs laid by each female in 10 days starting from the first egg-laying.

Table 3. Effects of S. zeamais feeding on Bt rice on development, adult body size and sex ratio of T. elegans. Note: *All values are means ± standard deviations.

| Generation | Parameter | Rice cultivars                | p-value |
|------------|-----------|-------------------------------|---------|
|            |           | Non-GM rice | Bt rice |       |
| 1          | Longevity (d) | 107.18 ± 8.74* | 111.26 ± 9.67 | 0.96   |
|            | Oviposition period (d) | 85.64 ± 5.26 | 86.72 ± 4.98 | 0.87   |
|            | Fecundity** | 276.34 ± 12.92 | 281.17 ± 12.36 | 0.79   |
| 5          | Longevity (d) | 109.53 ± 9.51 | 105.45 ± 8.92 | 0.83   |
|            | Oviposition period (d) | 86.22 ± 6.82 | 87.19 ± 5.25 | 0.60   |
|            | Fecundity | 275.49 ± 11.33 | 273.51 ± 10.94 | 0.88   |
| 10         | Longevity (d) | 108.76 ± 8.36 | 107.64 ± 9.27 | 0.34   |
|            | Oviposition period (d) | 84.96 ± 6.34 | 85.17 ± 5.67 | 0.37   |
|            | Fecundity | 279.26 ± 12.45 | 275.38 ± 11.41 | 0.88   |
| 15         | Longevity (d) | 112.71 ± 9.94 | 109.34 ± 7.69 | 0.50   |
|            | Oviposition period (d) | 83.93 ± 6.84 | 86.03 ± 5.43 | 0.52   |
|            | Fecundity | 272.58 ± 13.58 | 278.43 ± 10.27 | 0.41   |
| 20         | Longevity (d) | 106.83 ± 8.19 | 108.39 ± 9.64 | 0.81   |
|            | Oviposition period (d) | 85.11 ± 5.69 | 86.25 ± 5.31 | 0.88   |
|            | Fecundity | 280.11 ± 12.37 | 274.19 ± 10.59 | 0.85   |
| 25         | Longevity (d) | 108.47 ± 8.35 | 110.51 ± 7.97 | 0.85   |
|            | Oviposition period (d) | 87.04 ± 5.48 | 85.49 ± 6.07 | 0.80   |
|            | Fecundity | 272.97 ± 11.67 | 279.65 ± 12.62 | 0.71   |

Table 4. Effects of S. zeamais feeding on Bt rice on development, adult body size and sex ratio of T. elegans. Note: *All values are means ± standard deviations.
Male and female ratio was calculated. Means to analyze the data on rice and the conventional control (SAS, version 8.1).

Bt rice. Parasitoid wasps were expelled 24 hours later and this time was counted as the starting point of the parasitoid wasps to select their hosts freely. Six repeats were set up for both non-GM brown rice and brown Bt gans. T pupae until the emergence of all the brown rice, respectively, and removed 24 hours later to propagate the 2nd generation of beetles on Bt brown rice or adult beetles reared on non-GM brown rice or Bt brown rice were transferred into new jars containing non-GM 25th generation of S collected from the wheat-round sealed box (20 cm in diameter and 10 cm in height) with vent holes at the top. 24 pairs of S. 28 insects. Plant material. Materials and Methods

Insect species. The maize weevil S. zeamais beetles were from a laboratory colony rearing on wheat at 28 ± 1 °C, 80% relative humidity, and under total darkness. T. elegans colony was collected from the parasitized S. zeamais in a cereal storehouse and maintained at 28 ± 1 °C, 60% relative humidity, and 12 L:12D photoperiod.

S. zeamais development. S. zeamais adults reared on wheat were transferred to a jar containing non-GM brown rice or Bt brown rice for 24 hours. Then, the beetles were removed and the brown rice grains with the egg granules were put into a new jar, which was counted as the 1st generation of beetles on rice. The 1st generation adult beetles reared on non-GM brown rice or Bt brown rice were transferred into new jars containing non-GM brown rice or Bt brown rice, respectively, and removed 24 hours later to propagate the 2nd generation of beetles on rice. This procedure was repeated thereafter until 25th generation.

For the 1st, 5th, 10th, 15th, 20th, and 25th generation of S. zeamais reared on non-GM brown rice or Bt brown rice, 100 brown rice grains that each carried one maize weevil egg were picked up and reared in a separate jar. During the experiment, new brown rice grains of non-GM or Bt rice were added into the jars when it is necessary. Development of the maize weevils were closely monitored for the stadium and survival rate of each developmental stages, such as egg, larva, pupa, and nonage. Nonage is referred to the developmental stage from eclosion to the first egg-laying of the female adults. All experiments were conducted in six replicates, so that each of them started from 600 maize weevil eggs. Means ± standard deviations are presented in the tables. T-tests were used to analyze the data on Bt rice and the conventional control (SAS, version 8.1).

S. zeamais adult longevity, oviposition period and fecundity. For the 1st, 5th, 10th, 15th, 20th, and 25th generation of S. zeamais reared on non-GM brown rice or Bt brown rice, paired male and female adults were put into separate bottles immediately after the eclosion. Their longevity and oviposition period were recorded. Number of eggs laid by individual female in the first 10 days of its oviposition period was used as an indicator for its fecundity. 100 pairs of male and female adults with six replicates were examined in each experiment. Means ± standard deviations are presented in the tables. T-tests were used to analyze the data on Bt rice and the conventional control (SAS, version 8.1).

T. elegans development, adult body size and sex ratio. 100 non-GM brown rice or Bt brown rice grains that bore 4th instar larvae of 25th generation S. zeamais were placed in a petri dish, which was put in a round sealed box (20 cm in diameter and 10 cm in height) with vent holes at the top. 24 pairs of T. elegans wasps collected from the wheat-S. zeamais-T. elegans tritrophic system were released into the sealed box, allowing the parasitoid wasps to select their hosts freely. Six repeats were set up for both non-GM brown rice and Bt brown rice. Parasitoid wasps were expelled 24 hours later and this time was counted as the starting point of the T. elegans development. Development of T. elegans was closely monitored by randomly dissecting S. zeamais larvae or pupae until the emergence of all the T. elegans. Body size of T. elegans adults was measured under microscope. Male and female ratio was calculated. Means ± standard deviations are presented in the tables. T-tests were used to analyze the data on Bt rice and the conventional control (SAS, version 8.1).

Bt Cry1Ab/Cry1Ac protein detection. Bt Cry protein was examined using a rapid Bt Cry1Ab/Cry1Ac protein test kit produced by Chongqing Jinbiao Biotech Company Ltd (Chongqing, P. R. China). 20 gm of Bt rice grains or pooled S. zeamais and T. elegans tissues were homogenized in the Eppendorf tubes with 200 µl lysis buffer provided by kit. Paper strips for Bt Cry protein test were put into the sample tubes to detect Bt Cry1Ab/Cry1Ac protein by immune-affinity chromatography assay according to the instruction manual of the kit. Each assay was repeated for three times.

| Material                                      | Bt Cry1Ab/Cry1Ac protein |
|-----------------------------------------------|-------------------------|
| Bt rice                                       | +                       |
| Non-GM rice                                   | –                       |
| S. zeamais–Eggs (25th generation)             | –                       |
| S. zeamais–Larvae (25th generation)           | +                       |
| S. zeamais–Pupa (25th generation)             | –                       |
| S. zeamais–Adult digestive tract (25th generation) | –                       |
| S. zeamais–Adult without digestive tract (25th generation) | –                       |
| S. zeamais–Adult digestive tract (10 days after transferring to non-GMO rice) | –                       |
| S. zeamais–Adult without digestive tract (10 days after transferring to non-GMO rice) | –                       |
| T. elegans–Adult                              | –                       |

Table 4. Determination of (Bt Cry1Ab/Cry1Ac) protein. Note: +, Bt protein exists; –, Bt protein does not detectable.
Compliance with Ethical Standards. Ethical approval: This article does not contain any studies with human participants performed by any of the authors.

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Author Contributions

Q.T., Z.Y. and J.W. conceived the experiment, analyzed the results, and prepared the manuscript. Q.T., Z.Y., R.H., Y.Z. and C.S. conducted the experiment. All authors reviewed the manuscript.

Additional Information

Competing Interests: The authors declare no competing interests.

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