Rice Science, 2005, 12(3): 202－206
http://www.ricescience.org

Effects of Transgenic Bt Rice on the Food Consumption, Growth and Survival of *Cnaphalocrocis medinalis* (Guenée) Larvae

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Abstract: The transgenic rice KMD1, expressing a synthetic *Cry1Ab* gene from *Bacillus thuringiensis*, showed effective resistance to the older (third- to fifth-instar) larvae of the rice leaf-folder (RLF), *Cnaphalocrocis medinalis* (Guenée) in laboratory bioassay. Significant declines were revealed in food consumption and growth of the older RLF nymphs fed on the cut-leaves of transgenic KMD1 plants. The increase rate of food consumption by larvae fed on KMD1 was drastically lower than those on Xiushui 11. Food consumption was varied with different instars when the larvae fed on the Bt rice. Those of fourth- and fifth-instar larvae were different compared to the third-instar, lower than those on the non-transgenic rice but still increased a little when the feeding time prolonged. It is indicated that younger RLF larvae are more sensitive to Bt rice than older ones. Also, about 81%, 78% and 68% of the third-, fourth- and fifth-instar RLF larvae died within 72 hours bioassay period on KMD1 leaves, respectively. These results demonstrated that Bt-transgene in KMD1 rice confers substantial protection against infestations with older RLF larvae.
Key words: *Caphalocrocis medinalis; cry1Ab* gene; transgenic rice; resistance to insects

Bacillus thuringiensis is a bacterium that occurs naturally in the soil. It was first discovered in Japan in 1902 and has been used for more than 50 years as a biopesticide [1–3]. The most distinctive feature of this bacterium is protein crystals formed during sporulation. The crystal proteins are dissolved by the alkaline gut juices in the insect's midgut lumen, and converted into toxic core fragments by gut proteases. However, the biopesticide could not be popularized due to some of the defects such as lack of stability, narrow specificity and penetration in the specific plant tissues.

Rice is one of the most important staple food crops and is grown extensively in different parts of the world. The rice leaf-folder (RLF), *Cnaphalocrocis medinalis* (Guenée) (Lepidoptera: Pyralidae), is one of the most serious destructive pests of rice. Among the alternative tactics for its control, transgenic plants expressing toxic proteins of *Bacillus thuringiensis* not only offer an effective method to prevent the crop losses caused by lepidopteran insect pests, but also break the bottlenecks of biopesticides. In short, the cloning and expression of insecticidal Bt-*cry* genes in crop plants serves as an effective strategy for protection of crops against insect damage. The crystal proteins are specifically toxic to lepidopteran insect pests [4–9].

The transgenic Bt rice line, KMD1, produced by *Agrobacterium*-mediated transformation, is an elite line resistant to lepidopteran rice pest species [10]. Shu et al [10] had assessed the resistance of this line to eight lepidopteran pest species in paddy field, including the rice leaf folder, and observed that 100% mortality of this insect when its newly hatched or third-instar larvae were fed KMD1 leaf tissues in laboratory bioassays whereas only 9.65% of the neonates and none of the third-instar larvae died when fed the leaf tissues of the non-transgenic control.

The current experiments were undertaken to evaluate the resistance of the transgenic line KMD1 against the older (from the third- to fifth-instar) RLF larvae, including the effect on the food consumption and larvae weight, and the larvae mortality.

MATERIALS AND METHODS

Experimental materials

The transgenic rice KMD1 and the non-transgenic

Received: 4 March 2005; **Accepted:** 5 July 2005
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parental commercial cultivar Xiushui 11 were employed in the experiment. The transgenic line harbored the *cry1Ab* gene under the control of the maize ubiquitin-1 promoter, linked in tandam with *gus* (encoding the β-glucuronidase), *hpt* (encoding the hygromycin phosphotransferase), and *npt* (encoding the neomycin phosphotransferase) genes [11, 12].

Culturing of the leaf-folder larvae

The initial colony of the RLF was obtained from the experimental farm of Zhejiang University. Adults were maintained in a nylon net cage (50 cm × 50 cm × 50 cm) for mating and oviposition. Eggs were then inoculated on the rice leaf, and then kept in the nylon net cage (50 cm × 50 cm ×50 cm) at 26±1℃, 70–80% relative humidity, and lighting cycle of 16 h light / 8 h dark until hatching.

Laboratory tests

The tests were conducted with the transgenic and non-transgenic rice leaves inside a tube (1.5 cm diameter, 7.5 cm long).

For each test, the newly emerged young rice leaves were freshly excised from the plants and brought to the laboratory in the labeled containers. Some wet cotton was used inside the tube to keep leaves fresh. Each leaf was 4 cm long and was placed separately inside a tube. It was artificially infested with one leaf folder larva, which has been starved for two hours. Every 30 larvae at the same age was treated as a group. Each group was repeated three times. The Bt rice KMD1 and the control Xiushui 11 were artificially infested with the third- to fifth-instar larvae. Food consumption and larvae weight were measured individually after 24, 72 and 240 h. Larva mortality was also recorded. The rice leaves without larvae were also weighed to correct weight change due to evaporation.

Statistical analysis

The data obtained were subjected to analysis of variance (ANOVA) using DPS [13]. Duncan's Multiple Range Test was conducted for comparison of means at $P \leq 0.05$ to evaluate the level of significance. The food consumption by larvae was calculated as losses of the leaves weight before and after being exposed the leaves to the larvae, also the water losses of the leaves would take into consideration by using leaves only without larvae as a control. The relative growth of the larvae was measured before and after the exposure of the larvae to the Bt rice and the control Xiushui 11. Also the corrected mortality of the larvae was calculated as the difference between the tested mortality (TM) (the rate death of the larvae fed on Bt rice) and control mortality (CM) (the rate death of the larvae fed on Xiushui 11) divided by 100 minus the control mortality, i.e. [(TM－CM)/(100－CM)].

RESULTS

Effects of transgenic Bt rice on food consumption of RLF larvae

The bioassay results revealed that the food consumption of the RLF larvae on Bt rice at different ages was significantly lower than that on the non-transgenic rice considered as a control (Table 1).

RLF larvae fed normally on control plants, Xiushui 11, and the accumulated consumption increased as the feeding period prolonged. However, the increase rate of food consumption by larvae fed on KMD1 was drastically lower than those on Xiushui 11. Food consumption was varied with different instars when the larvae fed on the Bt rice. Those of the fourth- and fifth-instar larvae were different compared to the third-instar: lower than those on the non-transgenic rice but still increased a little when the feeding time prolonged.

Effects of transgenic Bt rice on growth of RLF larvae

It is evident that the growth and development of the RLF larvae feeding on KMD1 were distinctly retarded as compared to those on Xiushui 11 (Table 2). The relative increase of the body weights of the RLF larvae feeding on KMD1 was negative while those on Xiushui 11 were positive.

Effects of transgenic Bt rice on corrected mortality of RLF larvae

The high mortality of RLF larvae was observed when they fed on the leaf tissue of transgenic Bt rice

Table 1. Effects of KMD1 (Bt rice) and Xiushui 11(check) on food consumption of RLF at different ages.

Instar	Rice cultivar	Time after inoculation (h)	Sample size of the insects	Food consumption ($\overline{X}$ ± SE)(mg/larva)
3rd	KMD1	24	90	219.5 ± 17.5 cC
		72	90	40.1 ± 18.5 eE
		240	90	76.6 ± 1.2 dDE
	Xiushui 11	24	90	106.8 ± 8.0 dD
		72	90	395.3 ± 8.7 bB
		240	90	933.7 ± 5.6 aA
4th	KMD1	24	90	53.0 ± 25.5 eD
		72	90	69.9 ± 40.4 eD
		240	90	3.9 ± 2.3 cC
	Xiushui 11	24	90	226.2 ± 33.3 dC
		72	90	524.1 ± 11.2 bB
		240	90	956.7 ± 0.7 aA
5th	KMD1	24	90	6.2 ± 0.5 eD
		72	90	138.2 ± 18.5 eE
		240	90	82.8 ± 5.1 deCD
	Xiushui 11	24	90	200.4 ± 39.4 cC
		72	90	467.3 ± 69.0 bB
		240	90	991.3 ± 1.0 aA

Means within column followed by the same letters are not significantly different based on ANOVA (Duncan's Multiple Range Test, $P \leq 0.05$). Mean food consumption (± SE) was calculated from data of only three groups (each group consists of 30 individuals, the tested larvae didn't include the dead because of age).

Table 2. Effects of KMD1 (Bt rice) and Xiushui 11(check) on the relative growth of RLF at different ages.

Instar	Rice cultivar	Time after inoculation (h)	Sample size of the insects	Relative growth ($\overline{X}$ ± SE) (mg/h)
3rd	KMD1	24	90	-34.4 ± 7.1 dD
		72	90	-9.5 ± 2.0 cC
		240	90	-5.3 ± 0.3 cC
	Xiushui 11	24	90	9.0 ± 0.1 bB
		72	90	12.8 ± 0.4 bB
		240	90	66.7 ± 0.5 aA
4th	KMD1	24	90	-100.3 ± 1.1 fE
		72	90	-89.2 ± 5.8 dE
		240	90	-73.8 ± 3.2 dD
	Xiushui 11	24	90	183.9 ± 3.1 cC
		72	90	301.3 ± 0.3 bB
		240	90	998.9 ± 0.7 aA
5th	KMD1	24	90	-38.5 ± 31.9 bC
		72	90	-47.8 ± 30.5 cC
		240	90	-6.8 ± 0.5 bC
	Xiushui 11	24	90	45.5 ± 6.21 cB
		72	90	200.1 ± 0.8 bA
		240	90	253.9 ± 4.3 aA

Means within column followed by the same letter are not significantly different based on ANOVA (Duncan's Multiple Range Test, $P \leq 0.05$). Mean body weight (± SE) was calculated from data of only three groups (each group consists of 30 individuals, the tested larvae didn't include the dead because of age).

as compared to those on the non-transgenic control (Table 3). About 81, 77 and 68% of RLF larvae of the third-, fourth and fifth-instar died by 72 h after they fed on the Bt rice as compared to the non-transgenic control, respectively. It is also indicated that younger RLF larvae are more sensitive to Bt rice than elder ones.

DISCUSSION

Many Asian countries have reported the serious RLF infestations on rice, including China, India, Japan, Korea, Malaysia, Sri Lanka and Vietnam. The RLF larvae damage plants by folding the leaves and scraping the green leaf tissues within the fold, causing yield loss by reducing leaf photosynthetic activity.

Transgenics are an additional tool to supplement conventional pest resistance programs. It is important to integrate transgenic technology into the on-going insect pest management (IPM) programs. Advances in molecular biology have opened new opportunities to introduce transgenes into crop plants from diverse systems.

Fujimoto et al [14] bred rice variety Nipponbare with *cry1Ab* gene under the control of 35S promoter, revealing the increase of resistance to the striped stem borer and RLF [14]. Many laboratories have produced transgenic rice carrying Bt genes for tolerance to the stem borers [15–18]. Wünn et al [18] introduced *cry1Ab* gene into rice variety IR58 through particle bombardment, and the transgenic plants showed significant insecticidal effect on several lepidopteran insect pests. Feeding studies showed up to 100% mortality for the yellow stem borer and the striped stem borer. Nayak et al transformed IR64 through particle bombardment using *cry1Ac* gene placed under the control of the maize ubiquitin-1 promoter [16]. The transferred synthetic *cry1Ac* gene was stably expressed in the T_2 of these lines and the transgenic IR64 were highly toxic to larvae of the yellow stem borer, which caused little feeding damage. Ghareyazie et al [15] transformed aromatic rice variety "Tarom Molali" carrying a synthetic truncated *cry1Ab* toxin gene. The transgenic lines showed high levels of tolerance to the yellow stem borer. Alam et al [19] introduced *cry1Ab* gene driven by 35S promoter through biolistic method. The integration of the *cry1A*b gene in the transgenic rice (IR68899B) showed resistant to the yellow stem borer.

Table 3. Effects of Bt rice KMD1 on the corrected mortality of RLF at different ages.

Instar	Time after inoculation (h)	Sample size of the insects	Corrected mortality ($\overline{X}$ ± SE) (%)
3rd	24	90	17.78 ± 1.11 cC
	72	90	81.11 ± 1.11 bB
	240	90	96.58 ± 0.85 aA
4th	24	90	11.90 ± 1.19 cC
	72	90	77.78 ± 1.11 bB
	240	90	93.33 ± 1.67 aA
5th	24	90	17.78 ± 1.11 cC
	72	90	68.06 ± 0.14 bB
	240	90	72.22 ± 1.11 aA

Means within column followed by the same letters are not significantly different based on ANOVA (Duncan's Multiple Range Test, $P \leqslant 0.05$).

Mean corrected mortality (± SE) was calculated from data of only three groups (each group consists of 30 individuals).

KMD1, which was the transgenic line harbored the *cry1Ab* gene under the control of the maize ubiquitin-1 promoter, linked in tandam with *gus* (encoding the β-glucuronidase), *hpt* (encoding the hygromycin phosphotransferase), and *npt* (encoding the neomycin phosphotransferase) genes. Shu et al [10] had already showed that KMD1 caused high mortality of the newly hatched or third-instar larvae of eight lepidopteran pest species, including the RLF, the yellow stem borer and the striped stem borer, when they were fed on this transgenic rice line. Our results revealed that KMD1 has also highly toxic to the older RLF larvae.

When the older RLF larvae fed on the Bt rice, the rate of increases in food consumption of larvae fed on KMD1 was drastically lower than those on XS11. Food consumption was varied with different instars. Those of the fourth- and fifth-instar larvae were different compared to the third-instar: lower than those on the non-transgenic rice but still increased a little when the feeding time prolonged. It is also evident that the growth and development of the RLF larvae feeding on KMD1 were distinctly retarded as compared to those on the non-transgenic rice. The high mortality of older RLF larvae was observed when they were fed on the leaf tissues of the transgenic Bt rice, about 81, 78 and 68% of RLF larvae of the third-, fourth and fifth-instar died by 72 h after they fed on Bt rice, respectively, indicating that younger RLF larvae are more sensitive to Bt rice than older ones. This situation is in accordance with the results as were observed with the rice stem borers and European corn borer [14–20].

From the results above, it can be concluded that the development of KMD1 can serve as an effective strategy for protection of rice. But long-term impact of transgenics on development of resistance to insects should be considered from cross-resistance point of view. We should carefully examine the environmental and public concerns and biosafety issues before the release of transgenics for commercial planting.

ACKNOWLEDGEMENT

This work is funded by the National Basic Research Program (001CB109004) from the Ministry of Science and Technology of China.

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