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Inheritance and expression of the *cry1Ab* gene in Bt (*Bacillus thuringiensis*) transgenic rice

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Abstract The inheritance and expression patterns of the *cry1Ab* gene were studied in the progenies derived from different Bt (*Bacillus thuringiensis*) transgenic *japonica* rice lines under field conditions. Both Mendelian and distorted segregation ratios were observed in some selfed and crossed F_2 populations. Crosses between *japonica* intra-subspecies had no significant effect on the segregation ratios of the *cry1Ab* gene, but crossing between *japonica* and *indica* inter-subspecies led to distorted segregation of the *cry1Ab* gene in the F_2 population. Field-release experiments indicated that the *cry1Ab* gene was stably transmitted in an intact manner via successive sexual generations, and the concentration of the Cry1Ab protein was kept quantitatively stable up to the R_6 generation. The *cry1Ab* gene, driven by the maize *ubiquitin* promoter, displayed certain kinds of spatial and temporal expression patterns under field conditions. The content of the Cry1Ab protein varied in different tissues of the main stems, the primary tillers and the secondary tillers. Higher levels of the Cry1Ab protein were found in the stems, leaves and leaf sheaths than in the roots, while the lowest level was detected in grains at the maturation stage. The content of the Cry1Ab protein in the leaves peaked at the booting stage and was lowest at the heading stage. Furthermore, the Cry1Ab content of *cry1Ab* expression in different tissues of transgenic rice varied individually with temperature.

Keywords Inheritance · Expression · *cry1Ab* gene · Bt (*Bacillus thuringiensis*) transgenic rice

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Introduction

Stable inheritance and expression of transgenes in transgenic plants is of paramount importance in the successful application of genetic engineering in crop improvement. Some research revealed that once foreign genes were integrated into host cells, they could be faithfully transmitted to progenies through sexual generations and retained high meiotic stability and expression stability (Duan et al. 1996; Fearing et al. 1997; Scott et al. 1998). However, there are also many instances where foreign genes were lost through meiosis or inactivated or silenced in the progenies of transgenic plants (Finnegan and McElroy 1994; Matzke and Matzke 1995; Srivastava et al. 1996; Zhang et al. 1996). Whether transgenes could be stably inherited and expressed in progenies of transgenic plants has remained a prime question during the course of the successful employment of transgenic plants into traditional breeding programs.

With the rapid advances in the genetic transformation technology of some cereals, like maize, greater difficulties have been encountered in rice manipulation. One successful example of transformation of rice has been with Bt (*Bacillus thuringiensis*) genes (Fujimoto et al. 1993; Wünn et al. 1996; Cheng et al. 1997, 1998; Ghareyazie et al. 1997; Nayak et al. 1997; Wu et al. 1997; Datta et al. 1998; Tu et al. 1998). The expression level of Bt transgenes and the insect resistance it confers in transgenic rice has been studied in some instances. However, to our knowledge, the inheritance and expression patterns of the Bt gene in successive advanced gen-

erations of transgenic rice, especially under field release conditions, has not yet been studied.

Transgenic rice displaying high insect-pest resistance was obtained through the *Agrobacterium*-mediated genetic transformation method in our laboratory, and the field release experiment of *cry1Ab* transgenic rice was approved from the Chinese Ministry of Agriculture in 1998 (Xiang et al. 1999). Since transgene stability data is necessary for future ecological risk assessment, insect resistance management and the sustainable utilization of *cry1Ab* transgenic rice, the inheritance and expression of the *cry1Ab* gene in transgenic rice under natural field conditions has now been analyzed.

Material and methods

Plant materials and experiment design

All the transgenic rice lines were the progenies of the commercial *japonica* rice variety Xiushui 11 genetically transformed with a *cry1Ab* gene through the *Agrobacterium*-mediated method (Xiang et al. 1999). The T-DNA region of binary vector pKUB used for transformation is shown in Fig. 1. Hemizygous transgenic lines PRIV23, TR60, TR78 and homozygous TR69, TR77, PRIV16 lines were selected for field experiment. PRIV16 and PRIV23 lines were named as KMD1, at an advanced generation, and so were TR78, TR69 and TR77 as KMD2 (Shu et al. 1998, 2000). Lines KMD1, KMD2 and TR60 are derived from different primary transformants. Seeds of TR69 line at the R_4, R_5 and R_6 generation were individually selected for *cry1Ab* expression study under field conditions. For studying temperature effects, three replications with 50 seeds of each transgenic TR 69 line at the R_4 generation were incubated in phytotrons at 20°C, 25°C, 30°C, 35°C and 40°C under a light intensity of 6,000 lux and a 12-h photoperiod. In the autumn of 1998, transgenic rice lines (PRIV16, TR69 and TR77) were crossed with the non-transgenic *indica* variety Zhefu 504, and *japonicas* 9663, 9402, 9331. All the transgenic rice seeds, together with the F_2 seeds derived from single transgenic rice plants in F_1 population from different crosses, were sown on the farm of Zhejiang University on May 21, 1999, and transplanted on June 18, 1999. A completely randomized block design of three replications, with 60 transgenic plants per replication, was employed. Field management was traditional except that no pesticide was applied throughout the rice developmental stage.

Fig. 1 T-DNA region of the pKUB binary vector in *Agrobacterium* used for rice transformation. The vector was constructed by inserting the *Pubi-cry1Ab* gene into the unique *Hin*dIII site in binary vector pKHG4. BR, right border; BL, left border; *hpt*, hygromycin phosphotransferase gene; *nptII*, neomycin phosphotransferase gene; *cry1Ab*, synthetic insecticidal protein gene from *B. thuringiensis*; *Pubi*, maize *ubiquitin* promoter; *Pnos*, nopaline synthase promoter; *P35S*, CaMV 35S promoter; *NT*, 3´ termination signal of nopaline synthase

GUS histochemical assay

The *gus* coding sequence was used as a reporter for the *cry1Ab* gene. Previous study has proved that the *gus* sequence and the *cry1Ab* gene were closely linked in the selfed and crossed transgenic rice populations (Wu et al. 2000). GUS histochemical assay was performed as described by Rueb and Hensgens (1989).

Southern blot

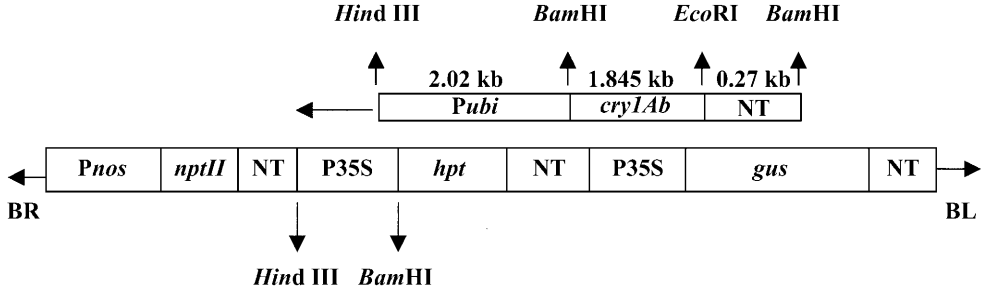
Leaves of ten rice plants from the R_4, R_5 and R_6 of TR69, together with the Xiushui 11 control, were randomly chosen at the tillering stage, respectively, and were ground to a fine powder using liquid nitrogen. DNA isolation was performed as described by Lu and Zheng (1992). About 15 µg of rice DNA was digested with *Hin*d III; the DNA fragments were subsequently separated overnight on a 0.8% agarose gel and transferred to a Hybond N^+ nylon membrane following the method described by Sambrook et al. (1989). The 559-bp DNA fragment amplified with one pair of primers specific for the *cry1Ab* gene was used as a hybridization probe. Probe labelling, membrane pre-hybridization and hybridization was carried out according to the hybridization-kit instructions supplied by Amersham Pharmacia Biotech Company. After hybridization, the membrane was sealed with a plastic sheet and exposed to X-ray film.

Western blotting

The Western analysis protocol was essentially adopted from that described by Sardana et al. (1996). About 0.4 g of above powder was transferred to 1.5-ml Eppendorf tubes with 0.5 ml of extraction buffer (Na_2CO_3 50 mM, DTT 10 mM) for protein extraction. After homogenizing, the tubes were kept at 4°C for 3 h then centrifuged at 12,000 rpm for 10 min, and the supernatant was collected for subsequent protein analysis. After electrophoresis on a 10% SDS-PAGE polyacrylamide gel, the protein was electrophoretically transferred to a nitrocellulose filter. The filter was developed using immunoaffinity purified polyclonal goat antibodies specific for the Cry1Ab protein in TSW blocking solution (NaCl 0.9%, gelatin 0.25%, Triton X-100 0.1%, SDS 0.02%). Rabbit anti-goat antibody conjugated to alkaline phosphatase was used to bind to the primary antibody and was detected by development solution (pH 9.4 AP buffer, 100 mM Tris-HCl, $MgCl_2$ 5 mM, NaCl 100 mM 10 ml, 50 mg/ml of Nitro Blue Tetrazolium 66 µl, 50 mg/ml of 5-bromo-1-chloro-3-indolyl phosphate 33 µl).

Cry1Ab protein quantification

At the stage of seedling, tillering, booting and heading stage, about a 1.5-cm leaf segment from the upper-most leaves of the main stems of 20 transgenic rice in each replication of TR 69 line was taken for Cry1Ab protein quantification. At the heading and maturing stages, 15 main stems, primary tillers and secondary tillers were randomly chosen from 15 rice plants of the TR69 line at the R_4 generation, and each was dissected into root, stem, leaf, leaf sheath, grain husk and grain. The content of Cry1Ab in roots was calculated as the average value of the roots of different tillers because it is hard to distinguish between different roots of differ-



ent tillers. For whole transgenic rice plant sampling, 15 transgenic rice plants from the TR-69 line at the R_4 generation were randomly chosen and dissected into root, leaf, leaf sheath, stem, grain husk five parts, and the identical tissues were well-mixed for protein extraction. For the samples of the temperature experiment, 30 plants from each replication were sampled into leaf, stem and root parts when the seedling grew to a 3-leaf stage. All the tissues were ground to a fine powder using liquid nitrogen. Protein extraction was performed as described above.

Total protein determination

The Coomassie Brilliant Blue G-250 method (Bradford 1976) was employed to quantify the total protein content in the extractions.

Western dot-blotting and calculation of the Cry1Ab content

Two microliters of the leaf protein extract, as well as the pure Cry1Ab protein with standard concentration, was spotted onto a methanol-wetted nitrocellulose membrane, and hybridization was performed as described above. Then the membrane was analyzed with SXI98 software provided by the Chinese SXI Company. The Cry1Ab protein-content was expressed as a percent of the total soluble protein.

Results

Inheritance of the *cry1Ab* gene in transgenic rice

Table 1 and 2 show the GUS histochemical-assay results of rice plants both from selfed and crossed populations. Because the *gus* reporter gene and the *cry1Ab* gene are closely linked in progenies of transgenic rice (Wu et al. 2000), the segregation of the *cry1Ab* gene could be deduced from the segregation of GUS staining. In the selfed populations, only the TR60 line displayed a one-locus Mendelian segregation ratio, while the other two lines displayed an abnormal segregation ratio (Table 1).

Table 1 Segregation of GUS staining in the selfed progenies of different hemizygous transgenic rice lines

Line	No. of plants tested	No. of GUS^+ plants	No. of GUS^- plants	GUS^+/GUS^-	$(\chi^2)^a$
TR60	180	136	44	3.09:1	0.007(3:1)
PRIV-23	515	350	165	2.12:1	13.423(3:1)
TR-78	469	332	137	2.42:1	4.214(3:1)

[a] $\chi^2_{(0.05)}=3.841$

In the F_2 population, the *cry1Ab* gene in all crosses between *japonica* and *japonica* rice displayed a 3:1 one-locus Mendelian segregation ratio, while the cross between *indica* and *japonica* displayed a distorted segregation ratio. No difference in the *cry1Ab* inheritance pattern was found between the reciprocal crosses with TR69 alone. This indicated that a different genetic background does not affect the inheritance pattern of the *cry1Ab* gene in the crosses between *japonica* and *japonica* rice. But distorted segregation of the *cry1Ab* gene in the cross between *indica* and *japonica* was found, as could be seen in Table 2. The *cry1Ab* gene in the crosses of 9663/PRIV16 and TR77/9402 displayed a one-locus Mendelian segregation ratio (Table 2), which meant one insert of the *cry1Ab* gene in transgenic PRIV16 and TR77 lines. Because lines PRIV16 and PRIV23, and TR77 and TR78, are derived from the same primary transformants, respectively, the *cry1Ab* gene in PRIV23 and TR78 should also display a one-locus Mendelian segregation ratio, whereas in fact it displayed an abnormal segregation ratio (Table 1). Meanwhile, PCR analyses for all the GUS-negative plants in both the selfed and F_2 population found no amplification of the expected 559-bp fragment (data not shown), which indicated that distorted segregation was not due to inactivation of the *gus* gene in the transgenic rice. Meanwhile, the presence of plants homozygous for the *gus* gene excluded the possibility that the transgene itself was functioning as a recessive lethal. Combined with the segregation ratio in the F_2 population, the most probable reason for the distorted segregation in the selfed population could be attributed to the low viability of the transgenic pollen instead of a complicated *cry1Ab* integration pattern in the rice genome. And the distorted segregation in the F_2 population from the cross between *indica* and *japonica* rice may be due to the low fertility of progenies and the low viability of transgenic pollen.

Stability of *cry1Ab* inheritance and expression via sexual generation

Southern-blot analysis of *Hin*dIII-digested DNA randomly chosen from plants at the R_4, R_5 and R_6 generations of the TR69 line showed the expected 4.1-kb DNA fragment consisting of the *ubiquitin* promoter, the *cry1Ab* gene and the *nos* terminator. No rearrangement

Table 2 Segregation of GUS staining in F_2 progenies of different crosses

Cross	Type of cross[a]	No. of plants tested	No. of GUS^+ plants	No. of GUS^- plants	GUS^+/GUS^-	$(\chi^2)^b$
Zhefu504/PRIV16	I/J	293	171	122	1.40:1	42.377 (3:1)
9663/PRIV16	J/J	100	80	20	4:1	1.08 (3:1)
TR77/9402	J/J	60	43	17	2.53:1	0.200 (3:1)
TR69/9331	J/J	60	42	18	2.33:1	0.556 (3:1)
TR69/9663	J/J	60	48	12	4:1	0.556 (3:1)
9663/TR69	J/J	60	45	15	3:1	0.022 (3:1)
Xiushui11/TR69	J/J	60	48	12	4:1	0.556 (3:1)
9402/TR69	J/J	60	45	15	3:1	0.022 (3:1)

[a] I: *indica*; J: *japonica*
[b] $\chi^2_{(0.05)}=3.841$

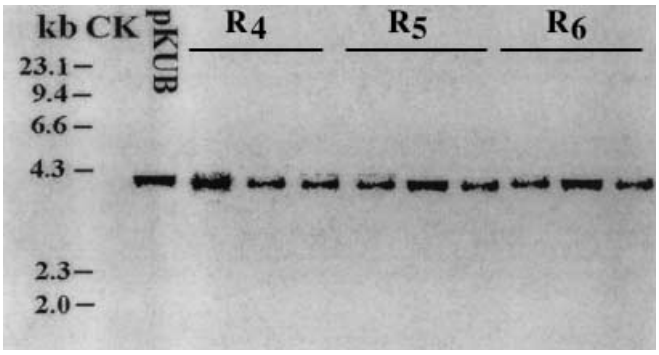


Fig. 2 Southern blot of *Hin*dIII-digested DNA from transgenic rice TR69 containing a *cry1Ab* gene. CK: Xiushui 11; R_4, R_5, R_6: transgenic rice at different generations

of the *cry1Ab* gene was found in the progenies, which indicated that the *cry1Ab* gene was faithfully transmitted to progenies via the sexual generation and the expression unit was kept intact in successive generations (Fig. 2). Western-blot analysis of protein extracts from above samples indicated the presence of the expected 68-kDa bands as shown in Fig. 3, which demonstrated that expression of the *cry1Ab* gene remained stable through successive sexual generations.

Fig. 3 Western blot of protein extract of transgenic rice TR69 containing a *cry1Ab* gene. CK: Xiushui 11; R_4, R_5, R_6: transgenic rice at different generations

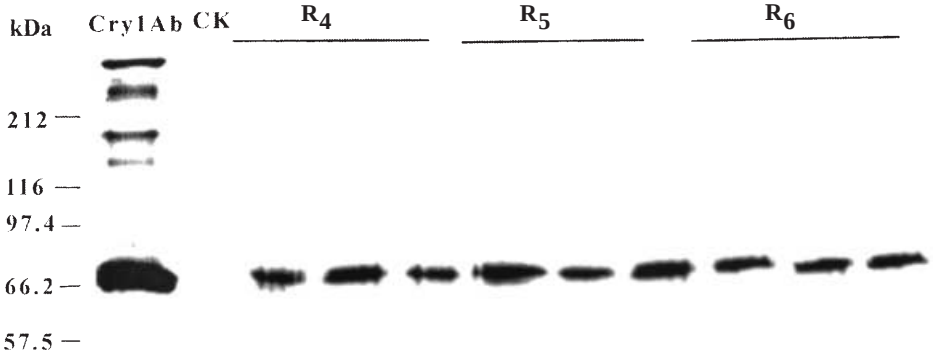


Fig. 4 Content of Cry1Ab in different tissues of transgenic rice at the heading stage

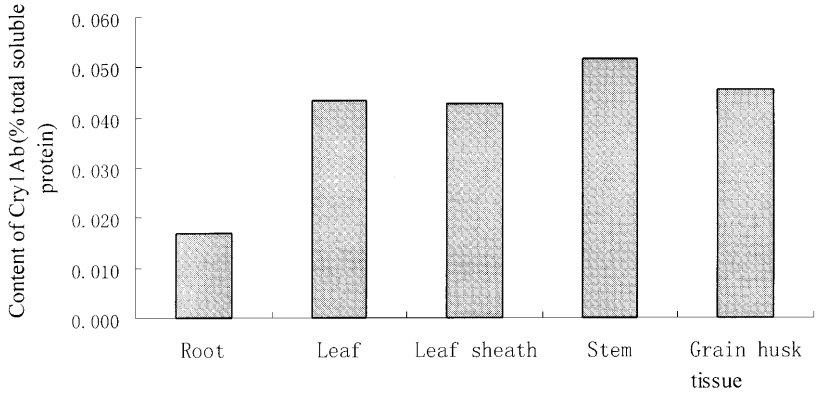


Fig. 5 Content of Cry1Ab in different tissues of various tillers of transgenic rice at the heading stage

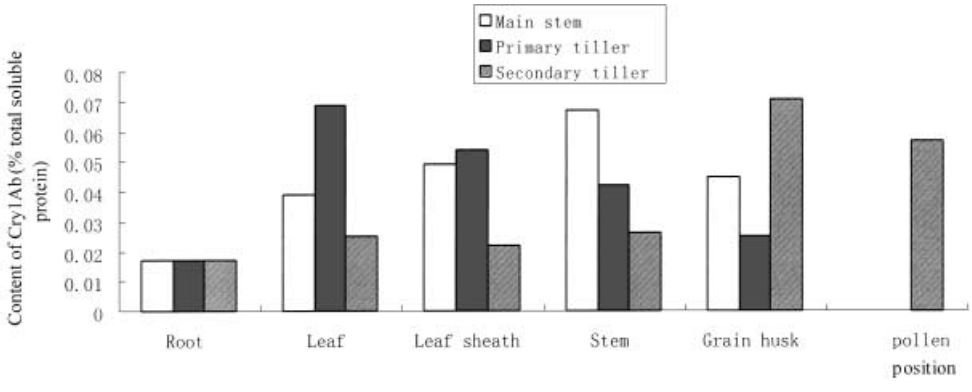


Table 3 Content of Cry1Ab protein (% total soluble protein) in leaf of transgenic rice at different generations[a]

Generation	Seedling	Tillering	Booting	Heading	Maturing
R_4	0.018±0.011 a	0.090±0.022 a	0.136±0.042 a	0.028±0.018 a	0.079±0.018 a
R_5	0.038±0.025 ab	0.077±0.031 a	0.094±0.041 a	0.020±0.010 a	0.079±0.022 a
R_6	0.029±0.009 b	0.080±0.033 a	0.135±0.098 a	0.014±0.009 a	0.067±0.011 a

[a] The mean values followed by a same letter in the same column are not significantly different at the P=0.05 level

Table 4 Effect of different temperatures on the expression of the *cry1Ab* gene in transgenic rice (% total soluble protein)[a]

Tissue	20°C	25°C	30°C	35°C	40°C
Root	0.059±0.020 b	0.090±0.025 ab	0.056±0.022 b	0.135±0.057 a	0.041±0.042 b
Sheath	0.042±0.010 a	0.021±0.008 bc	0.023±0.008 b	0.015±0.011 cd	0.006±0.003 d
Leaf	0.026±0.010 b	0.032±0.014 a	0.007±0.005 c	0.016±0.024 c	0.026±0.019 b

[a] The mean values followed by a same letter on the same line are not significantly different at the P=0.05 level

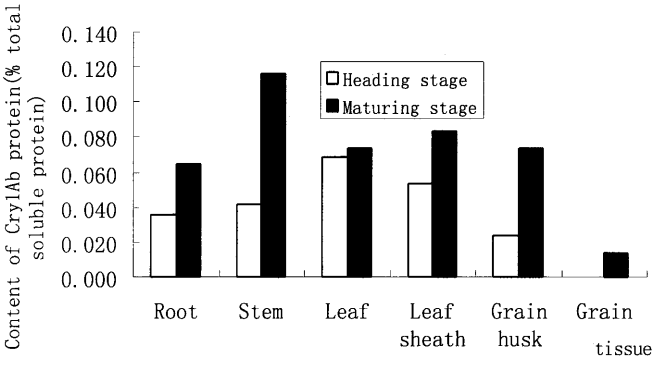


Fig. 6 Content of Cry1Ab protein in different tissues in the primary tiller at different stages

Spatial and temporal expression patterns of the *cry1Ab* gene in transgenic rice under field conditions

Because the *cry1Ab* gene was put under control of the constitutive *ubiquitin* promoter, just as expected, the *cry1Ab* gene was expressed in all parts of the transgenic rice plants. At the heading stage, the content of the Cry1Ab protein in different tissues was ranked as stem>grain husk>leaf>leaf sheath>root (Fig. 4). From the seedling to maturing stage, the content of the Cry1Ab protein in the upper-most leaves gradually increased and peaked at the booting stage, then decreased to the lowest at the heading stage, and again increased slightly afterwards (Table 3). This changing tendency of Cry1Ab content was similar from the R_4 to the R_6 generation. Except for the seedling stage, no significant difference in the Cry1Ab content in leaves of different generations of transgenic rice was found, which indicated that expression of the *cry1Ab* gene remained quantitatively stable through successive sexual generations (Table 3). At the heading stage, the Cry1Ab protein in different tissues of different tillers varied, but the highest contents of the Cry1Ab protein were detected in stems, leaf sheaths and leaves. In particular, the Cry1Ab protein was also detected in the pollen of transgenic rice plants (Fig. 5). The content of Cry1Ab protein in different tissues of primary tillers increased from the heading to the maturing stage. A significant increase was found in the stem while the lowest change was in the leaf (Fig. 6). At the maturing stage, the highest content of Cry1Ab in the primary tiller was found in the stem and the lowest was in the rice grains (Fig. 6).

Effect of temperature on *cry1Ab* expression in transgenic rice

To deepen our understanding of the expression patterns of the *cry1Ab* gene in transgenic rice, the Cry1Ab content was studied with transgenic rice plants grown under different temperatures. Table 4 summarizes the expression profiles of the *cry1Ab* gene in transgenic rice plants of the temperature experiment. The content of the Cry1Ab protein in different tissues was greatly influenced by temperature; the degree of influence by temperature varied in different tissues, resulting in a different change of the Cry1Ab protein in different tissues (Table 4). The highest content of Cry1Ab protein was found in roots grown at 35°C and the lowest was at 40°C, while in the leaf the highest was at 25°C and the lowest was at 30°C, and the content of Cry1Ab protein in the sheath tended to decrease with the increase in temperature.

Discussion

The inheritance and expression stability of foreign genes has been widely studied in different transgenic plants. Duan et al. (1996) demonstrated that the introduced potato proteinase inhibitor-II transgene in rice was stably inherited and expressed for more than four generations. Müller et al. (1987) also reported a high fidelity of meiotic transmission of the foreign transgene to progeny. Meanwhile, stable expression of foreign genes in transgenic plants has also been observed in alfalfa (Micallef et al. 1995) and in maize (Fearing et al. 1997). However,

instability of transgene inheritance and expression, including transgene loss, rearrangement and transgene inactivation or transgene silencing, was also reported (Finnegan and McElroy 1994; Matzke and Matzke 1995; Srivastava et al. 1996; Zhang et al. 1996). The mechanisms for the instability of transgene inheritance and expression have not been well understood. In this paper, PCR, Southern-blot and Western-blot analyses with DNA and protein extracts from the different progenies from transgenic rice lines confirmed the stable inheritance and expression of the *cry1Ab* transgene up to the R_6 generation under field conditions.

Due to the complicated and random integration of foreign genes in the host genome, the inheritance of foreign genes in transgenic plants often displayed complex patterns. Typically, a single foreign-gene insert in the host genome often leads to the expected 3:1 segregation ratio in the selfed population (Shimamoto et al. 1989; Datta et al. 1990; Christou et al. 1991; Peng et al. 1992; Rathore et al. 1993) and 1:1 in the backcross population (Peng et al. 1992; Hiei et al. 1994; Casas et al. 1995; Dillen et al. 1997). Under particular circumstance, the segregation of foreign genes with a single insert in transgenic plants will deviate from the classic one-locus Mendelian segregation ratio (Peng et al. 1995; Sachs et al. 1998). Some mechanisms responsible for this phenomenon, including the lower viability of transgenic pollen, lower fertilization ability (Zhang et al. 1996) or transgene inactivation (Spencer. et al. 1992; Walters et al. 1992), the recessive lethal (Scott et al. 1998), were proposed in recent years. When multiple inserts of the foreign gene occurs, it will be inherited as different unlinked dominant genes (Tomes et al. 1990); but, in most cases, the foreign gene displayed complicated segregation profiles instead of Mendelian segregation (Spencer et al. 1992; Wan and Lemaux 1994; Somers et al. 1994). In this experiment, one line out of the three derived from different primary transformants displayed a one-locus Mendelian segregation pattern in the selfed population, whereas the other two deviated from the 3:1 segregation ratio. Combining the segregation results in the F_2 population, it could be concluded that the *cry1Ab* gene was integrated into the rice genome as one insert. The presence of plants homozygous for the *gus* gene excluded the possibility that the transgene itself was functioning as a recessive lethal, and PCR analyses also excluded inactivation of the *gus* gene. It could be concluded that the deviated segregation ratios in the selfed progenies of PRIV23, the TR78 lines may be due to the low viability and fertilization ability of the transgenic pollen. While the distorted segregation ratio in the F_2 population derived from the cross between PRIV16 and Zhefu504 may be due to the gamete selection (Mulcahy 1979; Lyttle 1991), the low fertility of the cross between *japonica* and *indica* subspecies, may result from the low viability and fertilization ability of the transgenic pollen. No significant segregation difference in GUS segregation was observed in the F_2 population from the crosses between transgenic plants and *japonica* rice when the transgenic plant was either used as female parent or as male parent, which indicated that the intra-species *japonica* cross had no direct effect on the segregation pattern. While the cross between inter-species of *japonica* and *indica* led to the distorted segregation pattern, the genetic background had a great effect on the segregation pattern, consistent with the conclusion drawn by Scott et al. (1998).

No further research concerning the spatial and temporal expression of the Bt gene in transgenic plants under environmental release conditions was reported except for transgenic tobacco (Carozzi et al. 1992), transgenic maize (Kozeil et al. 1993; Fearing et al. 1997) and transgenic cotton (Sachs et al. 1998).The content of the Cry1Ab protein in the transgenic tobacco plant with a CaMV35S+*cry1Ab* gene under field conditions increased with the growth stage, and reached the highest content accounting for the 0.01% of the total soluble protein in the 3rd week after flowering; the *cry1Ab* gene was expressed constitutively in all parts of the tobacco plants, except seeds, with the highest content in the root (Carozzi et al. 1992). Kozeil et al. (1993) found that a higher content of Cry1Ab protein was detected in leaf, pith and the root of transgenic maize with a CaMV35S+*cry1Ab* transgene under field conditions; expression was also found in seeds but not in pollen. Cry1Ab protein was detected in significant quantities only in leaves and pollen with non-quantifiable trace levels in kernels, roots and pith of transgenic maize with a *cry1Ab* gene under the control of PEPC or a Maize pollen specific promoter, and whole-plant Cry1Ab levels per g fresh weight were highest at the seedling stage, with levels decreasing during the growing season (Fearing et al. 1997). Sachs et al. (1998) also reported that *cry1Ab* gene expression in terminal leaves is variable and influenced by genetic and environmental factors. However, only a very limited number of researches on expression of the Bt gene in transgenic rice plants have been reported to-date (Fujimoto et al. 1993; Wünn et al. 1996; Ghareyazie et al. 1997; Nayak et al. 1997; Cheng et al. 1998). Furthermore, all these researches were carried out under laboratory conditions, and some transgenic rice had a defect of a low expression level of the *cry1Ab* gene and a low insect resistance. To our knowledge, no results on the inheritance and expression of the *cry1Ab* gene in transgenic rice under natural field conditions have been reported so far, whereas this step is indispensable for the future utilization of transgenic rice plants in breeding programs. In this experiment, the content of Cry1Ab protein in leaves of transgenic rice reached 0.9% and 0.14% of the total soluble protein in 1998 and 1999, respectively, which was higher than most of the reported content. Meanwhile, all the transgenic rice lines conferred a very high degree of resistance to eight kinds of rice insect pests under field conditions (Shu et al. 2000). In this experiment, the *cry1Ab* gene was put under the control of the maize-derived *ubiquitin* promoter. The strong plant-derived promoter not only enabled the high expression of *cry1Ab* in transgenic rice and high insect resistance, but also conferred specific

spatial and temporal expression characteristics of the *cry1Ab* gene in transgenic rice grown under field conditions. As for the temporal characteristics, the content of Cry1Ab protein was highest at the booting stage and lowest at the heading stage; and, as for the spatial characteristics, the content of Cry1Ab protein differed in the different tissues of the main stem, primary tiller and secondary tiller, with the highest in the stem, leaf and leaf sheath. This kind of expression characteristic is of great importance in controlling rice insect pests, such as the striped stem borer, the yellow stem borer and the leaf-folder, because these insect pests often infest rice plants at the booting stage, heading stage, and always destroy rice plants by feeding on the rice leaf, stem and leaf sheath. Even at the lowest expression of the *cry1Ab* gene stage (heading stage), no infestation of transgenic rice plants by insect pests was observed under natural field conditions. This meant that the transgenic rice plants themselves conferred enough insect resistance to rice insect pests, and no further auxiliary measures were needed to control rice insect pests.

The expression stability of foreign genes in transgenic plants has caused great concern so far. Transgenic *Arabidopsis* demonstrated a progressive loss of kanamycin resistance over four generations of inbreeding (Kilby et al. 1992), but stable expression of a transgene in transgenic maize (Fearing et al. 1997) and wheat (Stoger et al. 1999) was also reported. In our experiment, the content of Cry1Ab protein in TR69 remained quantitatively stable through sexual generations under field conditions, and no significant difference in Cry1Ab protein content was detected among different generations throughout the developmental stages, except for the seedling stage. While displaying the stable expression of *cry1Ab* in different transgenic rice generations, all the transgenic rice plants conferred a considerably high degree of resistance to insect pests, and this was indispensable for the future employment of these transgenic plants into traditional insect-resistant breeding programs. Meanwhile, a high content of Cry1Ab protein was also found in the pollen of transgenic rice, which implies that some protective measures must be taken to avoid the transmission of transgenic pollen onto non-target organisms so as to prevent unpredictable ecological risks. It was also found that expression of foreign genes in transgenic plants was greatly affected by environmental conditions, especially when the foreign genes were put under the control of inducible promoters. Sachs et al. (1998) found that *cry1A* gene expression in terminal leaves of transgenic cotton was variable and strongly influenced by environmental factors; thus a significant difference in Cry1Ab protein was found in leaves of transgenic cotton planted in different years and different places. A nearly 10-fold difference in *cry1Ab* gene expression was also found in the same transgenic rice plants planted in different years in our experiment (data not shown), which demonstrated the paramount importance to investigate the expression profiles of the *cry1Ab* gene, together with the infestation status caused by rice insect pests, under different field conditions before commercialization of the transgenic rice. Takimoto et al. (1994) found that the Ubi-1 gene was not regulated systemically but rather that the individual cell responded independently to heat stress and changed its tissue-specific expression in response to stress treatment. In our experiment, we also found that the expression of the *cry1Ab* gene under the control of the *ubiquitin* promoter was greatly influenced by temperature, and different tissues of transgenic rice plants also individually responded to temperature and led to the specific change tendency of Cry1Ab protein content in transgenic rice plants. As the *cry1Ab* gene expression remained quantitatively stable in progenies through successful sexual generations and conferred a high degree of resistance to rice insect pests, the transgenic rice could be used as a precious insect-resistant germplasm to be employed in traditional breeding programs. Therefore, the breeding utilization of transgenic rice by means of crossing and backcrossing with elite commercial *indica* and j*aponica* is being undertaken, and rapid progress has been made in the development of insect-resistant transgenic rice with elite agronomically important traits. Meanwhile the results presented here provide primary data for further research on Bt transgenic rice, including the potential risk assessment, sustainable utilization and implementation of integrated pest management in Bt transgenic rice.

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