Inheritance of resistance to *Helicoverpa armigera* of 3 kinds of transgenic *Bt* strains available in upland cotton in China

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Abstract There are 3 kinds of transgenic *Bt* strains, Shanxi 94-24, Zhongxin 94, and R19, in upland cotton in China. Their transgenic *Bt* insect-resistance cultivars or hybrids have been developed and grown by farmers. Genetic studies indicate that the resistance of the 3 transgenic *Bt* cotton strains to *Helicoverpa armigera* is controlled by one pair of non-allelic dominant genes. Linkage relationship between the resistant genes of R19 and Shanxi 94-24 transgenic *Bt* strains shows that they may be inserted in the same chromosome. F_1 hybrids crossed among the 3 strains show that high levels of protection from feeding damage are the same as that of their parents. Therefore, there is no co-suppression phenomenon in many transgenic plants. The results presented here afford a fundamental reliance in developing transgenic *Bt* insect-resistant cultivars and exploiting the heterosis of hybrids in upland cotton.

Keywords: upland cotton, transgenic *Bt* plant inheritance, insect-resistance, *Helicoverpa armigera*.

The insecticidal crystal protein produced by *Bacillus thuringiensis* (*Bt*) renders highly poisonous effects specifically toward lepidopteran pests. The development of transgenic *Bt* plants through transferring insecticide crystal protein gene generated in *Bacillus thuringinensis* into crops such as cotton is considered one of the breakthroughs of paramount importance in biotechnology in recent years[1]. Guo[2] in the Biotechnology Research Centre, CAAS synthesized GFM CryIA insecticide crystal protein gene for the first time in China in 1992. Using Agrobacterium mediated transformation and pollen tube transformation methods, the synthesized *Bt* gene has been successfully transferred into released cultivars in upland cotton cooperated with the Cotton Research Institute, Shanxi Academy of Agricultural Sciences (SAAS), and the Industrial Crop Institute, Jiangsu Academy of Agricultural Sciences (JAAS)[3,4]. Transgenic *Bt* cotton cultivars resistant to bollworm have been developed and grown by farmers in China. They showed a high level of resistance to bollworm indicated by the biological assay and field investigation. In this note, the inheritance pattern of resistance to bollworm of these transgenic *Bt* strains in *Gossypium hirsutum* L. is presented.

1 Materials and method

Transgenic *Bt* strains, Shanxi 94-24, Zhongxin 94 was and R19, were obtained from the Cotton Research Institute, SAAS, the Biotechnology Research Centre, CAAS, and the Industrial Crop Institute, JAAS, respectively. The strains have been bred true in agronomic traits and resistance to bollworm by pedigree selection consecutively for 4 generations and individual plant bioassay fed with bollworm before they were used in the present inheritance study. The transformation construct is the same for both Shanxi 94-24 and Zhongxin 94 strains, but with different transformation methods. Shanxi 94-24 was developed *via* the Agrobacterium mediated transformation method[4], whereas Zhongxin 94 developed *via* the pollen tube method[3]. From the 3 kinds of transgenic *Bt* strains, transgenic *Bt* insect-resistance cultivars or hybrids have been developed and applied to the production in China. Bioassay was conducted using laboratory culture (SuS_2) of *H.armigera*. The leaf damage degrees are divided into 4 classes: 1, 2, 3, and 4. Laboratory bioassay and the leaf damage degree demarcation were presented in our previous report[5].

The transformed strains were also confirmed and validated by PCR amplification. One specific

800 bp fragment could be amplified by synthesized primers of *Bt* gene from all plants of homozygous Shanxi 94-24 and Zhongxin 94 strains (results not listed). In our laboratory assay and PCR amplification, commercial cultivar Sumian 12 was always used as check.

2 Results

(i) Inheritance pattern of Shanxi 94-24 strain. The seedlings at 2 or 3 true leaf stages of F_1 plants, produced by crossing between Shanxi 94-24 and commercial cultivars such as Wuxi 272, Yan 8054, Chuan 109 and Zhongmiansuo 23, were picked for laboratory assay for their resistance. It was indicated that the mortality of neonate larvae accounted for 91.7%—100% after 5-d feeding (table 1). The survived larvae were generally first and second instar larvae and plant damage index was 1. On the other hand, the mortality of the bollworm larvae fed with true leaves of the check cultivar, Sumian 12, was only 6.2%. As a result, each F_1 hybrid showed high level of resistance to bollworm. The resistance of F_1 hybrids attained the same level with the parent strain, Shanxi 94-24. The result also showed that the insect resistance of this transgenic strain was inherited dominantly.

Table 1 Resistance expression of F_1 hybrid crossed between transgenic *Bt* strains, Shanxi 94-24 and Zhongxin 94 and commercial cultivars[a)]

Combinations or strains	No. of plants	Mortality of larvae (%)	Instar distribution of survived larvae			Damage degree
			1st	2nd	3rd	
(Wuxi272×Shanxi 94-24)F_1	4	91.7±4.5	33	66.7	0	1±0
(Yan8054×Shanxi 94-24)F_1	4	98.3±4.5	100	0	0	1±0
(Chuan 109×Shanxi 94-24)F_1	6	99.0±2.5	100	0	0	1±0
(ZMS23×Shanxi 94-24)F_1	4	100	0	0	0	1±0
Shanxi 94-24	4	100	0	0	0	1±0
Sumian 12[b)]	4	6.2±5.5	0	96.8	3.2	4±0
(Zhongxin 94×T582)F_1	14	67.1±20.2	0	100	0	—
(Zhongxin 94×Zhong 1098)F_1	13	69.2±17.5	15.8	84.2	0	—
(Zhongxin 94×Jiwu 2913)F_1	16	61.3±27.8	3.2	96.8	0	—
(Zhongxin 94×Sumian 12)F_1	14	60.0±23.5	13.0	87.0	0	—
(ZMS 23×Zhongxin 94)F_1	15	84.0±13.5	33.3	66.7	0	—
(Zhongxin 94×Taicang 520)F_1	18	81.1±26.0	17.6	82.3	0	—
Average		72.3±10.0	13.8±11.8	86.2±11.8	0	—
Sumian 12	19	30.5	1.5	7.7	90.8	4

a) Fed with 5 larvae per leaf; b) insufficient diet due to small seedlings affected the development of larvae.

The insect-resistant and susceptible plants were segregated out in F_2 and BC_1 populations produced between Shanxi 94—24 and 5 commercial cultivars (table 2). The leaf damage index of susceptible plants was generally 3 and 4, and survived larvae at 3rd instar were the same as the check cultivar, Sumian 12, whereas that of resistant plants of transgenic *Bt* strain was 1 and 2, and no 3rd instar larvae survived. So the plants with 3 and 4 in leaf damage index and 3rd instar larvae survival were classified as the susceptible, and those of 1 and 2 in leaf damage index and no 3rd instar larvae survival as the resistant after 5-d feeding in laboratory assay. Following this standard, segregation of resistant and susceptible plants fits 3 ∶ 1 ratio in 6 F_2 populations and fits 1 ∶ 1 ratio in 5 backcrossing ones. The results indicated that the resistance of Shanxi 94-24 strain to *Helicoverpa armigera* was controlled by one pair of dominant genes (table 2). Additionally, individual plant of (Shanxi 94-24×T586)F_2 was PCR-amplified using synthesized *Bt* primers in our inheritance test. Among 233 amplified plants, there were 166 plants from which around 800 bp DNA fragment of *Bt* gene were amplified. The result is also in consistence with 3 ∶ 1 ratio (χ_c^2=1.558; P>0.10).

(ii) Inheritance of resistance of Zhongxin 94 strain. The larvae mortality rate amounted to 100% after 5-d feeding with the seedling leaves of (Zhongxin 94×Zhong 1098)F_1, which produced the same resistant level as its parent, Zhongxin 94 strain. The resistance level of F_1 hybrids crossed between Zhongxin 94 and other commercial cultivars gradually decreased at the later developmental stages. The average mortality rate of larvae was 72.3%, the 1st and 2nd instar larvae amounted to 13.8% 5 d after being fed with mainstem leaves of 6 F_1 hybrids at flowering stage. Compared with that

of the check, Sumian 12, there is still a great significant resistance in transgenic *Bt* cotton. Furthermore, (Zhongxin 94×Sumian 12) Zhongxin 94 population produced all resistant plants. From these results, the resistance to *Helicoverpa armigera* for this transgenic strain was dominantly inherited.

Table 2 Segregation of F_2 and BC populations crossed between Shanxi 94-24, Zhongxin 94 and commercial cultivars

Combinations	No. of resistant plants	No. of susceptible plants	Ratio	Chi-square value χ_c^2	Year	Tested stage
(ZMS 23×Shanxi94-24)F_2	103	35	3 : 1	0	1998	full blossom
(Xiangmian16×Shanxi94-24)F_2	51	17	3 : 1	0	1998	full blossom
(Tong 6580×Shanxi94-24)F_2	67	17	3 : 1	0.778	1998	full blossom
(Sumian12×Shanxi94-24)F_2	75	24	3 : 1	0.003	1997	seedling
(Shanxi94-24×Tong 6580)F_2	65	17	3 : 1	0.585	1997	seedling
(Sumian 9×Shanxi94-24)F_2	25	4	3 : 1	1.390	1997	seedling
Total	386	114	3 : 1	1.176		
Heterogeneity	—	—	—	1.58	—	—
(ZMS23×Shanxi94-24)BC_1[a)]	79	79	1 : 1	0.006	1998	full blossom
(Zhong1098×Shanxi94-24)BC_1[a)]	34	28	1 : 1	0.403	1998	full blossom
(Jiwu2031×Shanxi94-24)BC_1[a)]	13	15	1 : 1	0.036	1998	full blossom
(Ekangmian1×Shanxi94-24)BC_1[a)]	36	24	1 : 1	2.016	1998	full blossom
(Sumian12×Shanxi94-24)BC_1[a)]	44	38	1 : 1	0.305	1998	Seedling
Total	206	184	1 : 1	1.131	—	—
Heterogeneity	—	—	—	1.635	—	—
(Zhongxin94×Jiwu2913)F_2	68	24	3 : 1	0.014	1998	seedling
(Zhongxin94×Sumian12)F_2	86	19	3 : 1	2.314	1998	seedling
(Zhongxin94×Zhong1098)F_2	85	20	3 : 1	1.679	1998	seedling
(Zhongxin94×Taicang 520)F_2	77	27	3 : 1	0.013	1998	seedling
Total	316	90	3 : 1	1.589	—	—
Heterogeneity	—	—	—	2.431	—	—
(Simian3×Zhongxin94)BC	24	17	1 : 1	0.878	1999	seedling
(Zhongxin94×Sumian 12)Sumian 12	22	0	—	—	1999	seedling

a) Recurrent parents were commercial cultivars.

Segregation results of 4 F_2 hybrids crossed between Zhongxin 94 and Jiwu 2913, Sumian 12, Zhong 1098, and Taicang 520 for resistant and susceptible plants are given in table 2. The identification criteria for plants which were classified as the resistant or susceptible were the same as that of Shanxi 94-24. It showed that the segregation of the resistant and susceptible plants was also in conformity with 3 : 1 and 1 : 1 for F_2 and BC populations, i.e. the insect resistance trait of Zhongxin 94 was controlled by one pair of dominant genes, too.

(iii) Allelic relationship among the 3 kinds of transgenic strain. Transgenic *Bt* strain, R19, was proven to have a high level resistance to bollworm just as Shanxi 94-24 and Zhongxin 94 do. Genetic analysis indicated that the resistance was controlled by one pair of dominant genes too (see our previous paper)[5]. So the resistance of the 3 kinds of transgenic *Bt* cotton available in China at present is all controlled by one pair of dominant genes, i.e. one copy of *Bt* gene was inserted in them. Their resistant level and differential expression tendencies at whole developmental stage were fundamentally alike *(published in another paper)*. So to reveal the allelic relationship among these 3 kinds of transgenic *Bt* insect-resistant strains might be of paramount importance to the exploitation of these germplasm resources.

Three F_1 hybrids crossed among the 3 kinds of transgenic *Bt* strains have the same resistant level to bollworm as their parents (table 3). It means that in heterozygous status, there is no co-suppression phenomenon that will cause gene silence and resistance decrease. Additionally, the insect-susceptible plants were segregated out for 3 F_1 hybrids, which demonstrated that *Bt* gene might be inserted into different chromosomes for these 3 transgenic *Bt* strains. There were 341 vs 26, and 251 vs 22 resistant and susceptible plants segregated out respectively for (Zhongxin 94×R19)F_2 and (Shanxi 94-24×Zhongxin 94)F_2. These values closely fit 15 : 1 segregation ratio. Independent inheritance or different

chromosome insertion of *Bt* genes for Shanxi 94-24 and Zhongxin 94 strains was concluded. However, there were 608 resistant plants and 23 susceptible ones in (Shangxi 94-24×R19) F_2. This segregation value does not fit 15 : 1 segregation ratio of two pairs of independent dominant genes. The same chromosome insertion and linkage relationship of the *Bt* genes for the 2 transgenic *Bt* strains was supposed.

Table 3 Segregation of F_2 hybrids crossed among 3 kinds of transgenic *Bt* strains

Combinations	No. of resistant plants	No. of susceptible plants	χ_c^2 (15 : 1)	Probability	Assayed organ	Year
(Zhongxin 94×R19)F_1	12	0	—	—	mainstem leaf	1997
(Shanxi 94-24×Zhongxin 94) F_1	14	0	—	—	mainstem leaf	1997
(Shanxi 94-24×R19)F_1	19	0	—	—	mainstem leaf	1997
(Zhongxin 94×R19) F_2	16	1	0.192	0.75—0.50	young plant	1997
(Zhongxin 94×R19)F_2	125	8	0.005	>0.90	young plant	1998
(Zhongxin 94×R19)F_2	200	17	0.679	0.50—0.25	mainstem leaf	1998
Total	325	25	0.336	0.75—0.50	—	—
(Shanxi 94-24×Zhongxin 94)F_2	40	4	0.218	0.75—0.50	young plant	1997
(Shanxi 94-24×Zhongxin 94)F_2	51	1	1.005	0.50—0.25	young plant	1998
(Shanxi 94-24×Zhongxin 94)F_2	160	17	2.850	0.10—0.05	mainstem leaf	1998
Total	251	22	1.230	0.50—0.25	—	—
(Shanxi 94-24×R19)F_2	3	0	—	—	young plant	1997
(Shanxi 94-24×R19)F_2	105	3	1.669	0.25—0.10	young plant	1998
(Shanxi 94-24×R19)F_2	500	20	4.726	<0.05	mainstem leaf	1998
Total	608	23	6.870	<0.01	—	—

3 Discussion

Inheritance research in this note shows that the insect-resistance of the 3 kinds of transgenic *Bt* strains in upland cotton is all controlled by one pair of dominant genes. The simple inheritance pattern afforded a relatively convenient approach for exploiting the 3 insect-resistant germplasm resources in cotton breeding. The *Bt* gene is relatively readily transferred to other commercial cultivars to develop the insect-resistance cultivars with high yield, good fiber quality, and disease-resistance.The development of transgenic *Bt* plant makes *Bt* toxins highly desirable for use as one component of integrated pest management (IPM). Breeding and production practice in China and USA completely confirmed such point.

It is known that in our molecular breeding, the target gene to be integrated should be preferably one copy gene, otherwise the gene silencing will be induced[6]. However, present studies proved that there is no co-suppression phenomenon in F_1 hybrid crossed among the 3 kinds of transgenic *Bt* strains, which was not consistent with the former reports[6,7], but was in conformity with Hobbs et al.[8] in tobacco. In their research, they found that F_1 population from a cross between two transformants with a high level of *vidA* RNA and GUS activity inserted at different loci had GUS activity levels equal to the parents, and individuals in the F_2 populations had levels up to twice the parental levels. So, if there are accumulative effects of transgenic *Bt* genes, it may be possible to transfer multiple different *Bt* genes into one commercial cultivar to increase the resistant level and to exploit the heterosis of F_2 generation of intervarietal hybrids of transgenic *Bt* strains. Dose effect expression of transgenic *Bt* genes in cotton will be published later.

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