A transgenic wheat with a stilbene synthase gene resistant to powdery mildew obtained by biolistic method

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Abstract Stilbene, a kind of phytoalexin, plays an important role in resistance to fungal and

bacterial infection in plants. It strongly inhibits the growth of fungi and sprout of spore. Stilbene synthase gene (*Vst1*) obtained from grapevine has been transferred into common spring wheat Jinghong 5 by using the biolistic transformation method. Five transgenic plants (T_0) were obtained from the bombarded 2014 immature embryos. One immune plantlet and 3 plantlets with mid-resistance to powdery mildew were identified from the transgenic plants of T_3 generation which came from 2 T_0 transgenic plants.

Keywords: wheat, transformation by biolistic method, stilbene synthase, phytoalexin, powdery mildew.

Phytoalexin, a chemical resistant to fungi and bacteria synthesized by plant itself, possesses the resistance to fungal and bacterial infection[1]. Some plants like grapevine (*Vitis vinifera* L.), peanut (*Arachis hypogaea* L.), pine (*Pinus sylvestris* L.), etc. can synthesize and accumulate phytoalexin such as stilbene at their wounded sites when they are infected by pathogens so as to prevent the further invasion of pathogenic organism[2]. The biosynthesis of stilbene-like phytoalexin is catalyzed by the key enzyme——stilbene synthase (STS). Although the action substrate of STS generally exists in plants, but most of the plants are short of stilbene synthase gene, so they can not synthesize phytoalexin[3]. Two linked STS genes (*Vst1* and *Vst2*) were isolated from the genomic library of grapevine (*Vitis vinifera* L.)[4,5]. STS genes had been transferred into tobacco[4,5], oilseed rape[6], rice[7], barley[2], etc. by other researchers, and transgenic plants with enhanced disease resistance were obtained. Although the STS gene has been successfully transferred into wheat[2], reports on obvious enhanced disease resistance of wheat have not been found. In this study, STS gene *Vst1* has been transferred into common spring wheat variety and plants with good resistance to powdery mildew of wheat were obtained from the T_3 segregating transgenic plants. The results are as follows.

1 Materials and methods

(i) Experimental materials and plant culture. The common spring wheat (*Triticum aestivum* L.) variety Jinghong 5 that is highly susceptible to powdery mildew was used as material. Immature seeds at 12—14 d after pollination were taken and the young embryos in 1.0 mm diameter were selected as the receptor of gene *Vst1*. The embryonic donor plants and transgenic plants were cultured by using the method as described by Weeks et al.[8].

(ii) Particle bombardment parameter. During the period from 6 h before bombardment to 18 h after bombardment, the young embryos were put onto the MS solid medium supplemented with 0.5 mol/L of mannitol[9]. A PDS-1000/He gun produced by the BioRad company was used in this experiment. The diameter of gold particle was 0.4—1.0 μm. The helium gas pressure of 7 584 kPa was used for bombardment. About 50 immature embryonic scutella in each dish were bombarded with 80 μg gold particles and 0.4 μg plasmid DNA. The conditions of other bombardment were as described by Becker et al.[10].

(iii) Callus culture of immature embryo after bombardment. Recovery culture of wheat embryos was carried out by using MS solid medium supplemented with 2 mg/L of 2,4-D and the cultural period was about 9—11 d. MS solid medium supplemented with 5 mg/L of bialaphos and 2 mg/L of 2,4-D was used for induction of embryonic calli and the induction period was about 15—17 d. The 1/2 MS solid medium supplemented with 3 mg/L of bialaphos, 1 mg/L of zeatin, and 1 mg/L of IAA was used as callus differentiation medium and the concentration of sucrose was 3%. The 1/2 MS solid medium supplemented with 3 mg/L of bialaphos and without growth-promoting hormone was used for root strengthening of plantlets and the concentration of sucrose was 8%.

(iv) Plasmid DNA used for transformation. Plasmid DNA used for transformation in the experiment was pJL392A (fig. 1(a)) and pJL392B (fig. 1(b)). Both of pJL392A and pJL392B carry *Vst1* gene (supplied by BAYER Company, Germany) and *bar* gene. The length of the plasmid is 11.1 kb. The *Vst1* gene has its own promoter and there is a 4-fold enhancer of the 35s promoter coming from the upstream of the promoter. The promoter of *bar* gene is *ubiquitin-1*. The difference between the two genes is that the direction of the promoters of *Vst1* gene and *bar* gene is different, and pJL392A is promoted in the same direction while the pJL392B is promoted in the opposite direction. The extraction

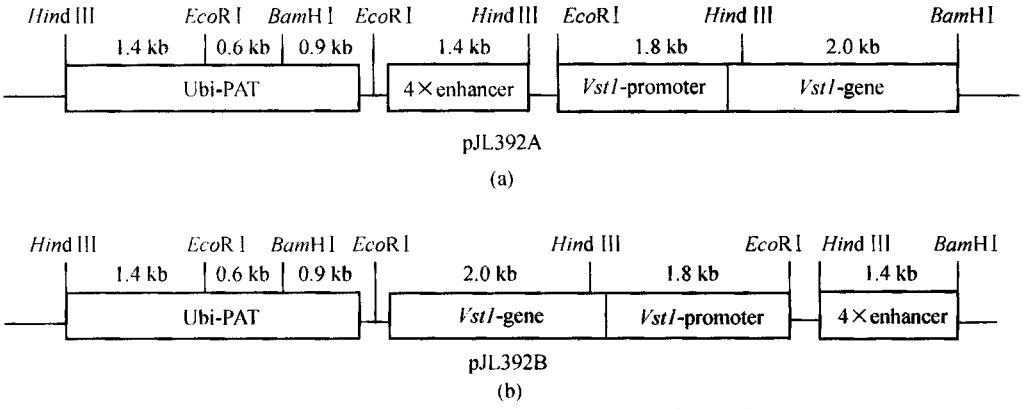

Fig. 1. Plasmid constructs used for transformation in experiments.

of plasmid DNA was done by using the method as described by Weeks et al.[8].

(v) Tests on transgenic plants for resistance to herbicide. All differentiated plants of current generation and transgenic plant progenies were tested for resistance to herbicide Basta according to the method reported by Becker et al.[10].

(vi) PCR analysis of the transgenic plantlets. PCR analysis of the transgenic plantlets of T_0 and their progeny were carried out by using the method described by Futterer et al.[11]. The primers used for *bar* probe: primer I 5′ TGCACCATCGTCAACCACTA 3′; primer II 5′ ACAGCCACCACGCTCTTGAA 3′. The primers used for *Vst1* probe: primer I 5′ AATCCGTCACCTGTGGTAGC 3′; primer II 5′ ACCGAAGAAATGCTTGAGGA 3′. The reaction system was for 1 min at 94℃, 30 s at 60℃, and 1 min at 72℃ for total of 30 cycles.

(vii) Southern blot analysis of the transgenic planlets. The extraction of total genomic DNA from the leaves of wheat and Southern hybridization were carried out by using the method described by Weeks et al.[8]. Using *bar* fragment (550 bp) digested from pDM302 with *Sma* I and *Vst1* fragment (870 bp) amplified from pJL392A or pJL392B by PCR were used as probe DNA and tagged by employing the random primer method. The fragments of plasmid pJL392A digested by *Eco*R I (*bar*) or *Bam*H I (*Vst1*) were used as a positive control.

(viii) Assay of disease resistance of transgenic plants. The resistance to powdery mildew of T_3 plants of transgenic wheat was tested. The mixed races of the dominant powdery mildew (races No. 11 and No. 15) in current production were inoculated by employing traditional method at the growth stage of seedlings with one leaf and one head. After 8—10 d of inoculation, the reaction types of plants to invasion of powdery mildew were investigated and recorded according to the 5-grades method: grade 0 is immune, grade 1 is highly resistance, grade 2 is mid-resistant, grade 3 is mid-susceptible, grade 4 is highly susceptible. Assay of disease resistance was carried out in the Institute of Plant Protection, the Chinese Academy of Agricultural Sciences.

2 Results and discussion

(i) Effects of the plasmid structure on the frequency of gene transformation. The data in table 1 show that using plasmid pJL392B as the transformation vector, the frequency of the Basta-resistant plants was 2.3%, which was 2 times as much as that by using pJL392A as the transformation vector and the difference between them reached significant level in statistics. Although the frequency of transgenic plants did not reach the significant level in difference due to the less number of transgenic plants, but the frequency of transgenic plants obtained by using plasmid pJL392B as a transformation vector was 4 times as much as that by using pJL392A as the transformation vector. The only difference between the two plasmids was the different directions of the promoters of *bar* gene and *Vst1* gene. The direction of the two promoters of plasmid pJL392A is identical while that of the two promoters of plasmid pJL392B is opposite. This indicates that the direction of the two promoters in plasmid resulted in effect on the

frequency of gene transformation.

Table 1 Effect of the plasmid structure on expression of gene

Plasmid	No. of bombarded embryo	No. of Basta-resistant plants (frequency)	No. of transgenic plants (frequency)	Origin of transgenic plants
pJL392A	1 045	11 (1.1%)[a)]	1 (0.1%)[a)]	$T_0$8
pJL392B	969	22 (2.3%)[a)]	4 (0.4%)[a)]	$T_0$4, 6, 9, 10

Plant material: Spring wheat Jinghong 5. a) Entries with the same letter are not significantly different, $P>0.05$ (Chi-square test).

(ii) Stable transformation of wheat with *Vst1* gene. PCR analysis on *Vst1* gene and *bar* gene of the above-mentioned 33 differentiated resistant plants (R_0) was made and the results are shown in fig. 2(a), (b). The expected specific bands of 870 bp (*Vst1*) and 310 bp (*bar*) were amplified in 5 of these differentiated resistant plants and they showed a pattern identical to that of the amplified plasmid in size. The corresponding bands have not been amplified from the non-transformed plants of control.

Four plants produced seeds except one plant ($T_0$4) among the 5 transgenic plants (T_0). The inserted gene was lost in T_2 generation of one plant ($T_0$6) and the results of Southern blot analysis on the progeny plant of the remaining 3 plants are shown in fig. 3(T_1) and fig. 4(T_2). The results showed that the expected 6.1 kb (*Vst1*) band appeared from Southern hybridization when DNA from the leaves of transgenic progeny plants was digested via *Bam*H I (*Vst1*) and the band is identical in size to the band of hybridization of digested plasmid. No signal of hybridization appeared in non-transgenic plants

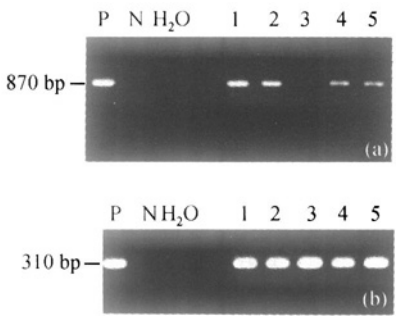

Fig. 2. PCR analysis of the transgenic T_0 plants. P, Positive control (plasmid DNA); H_2O, water as control; N, negative control (DNA from nontransformed plant). 1−5, DNAs from transformed plants. Two primers were used to amplify a 870 bp coding region of *Vst1* gene (a) and a 310 bp coding region of *bar* gene (b).

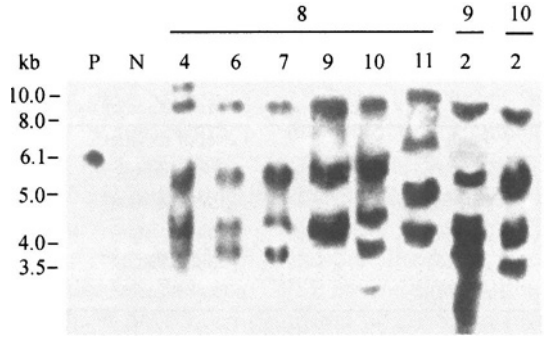

Fig. 3. Southern blot analysis of T_1 plants. From left to right: P, Positive control (pJL392A) digested with *Bam*H I; N, nontransgenic plantlet genomic DNA digested with *Bam*H I. $T_1$8-4, 8-6, 8-7, 8-9, 8-10, 8-11, 9-2, 10-2 plantlet genomic DNAs digested with *Bam*H I, respectively.

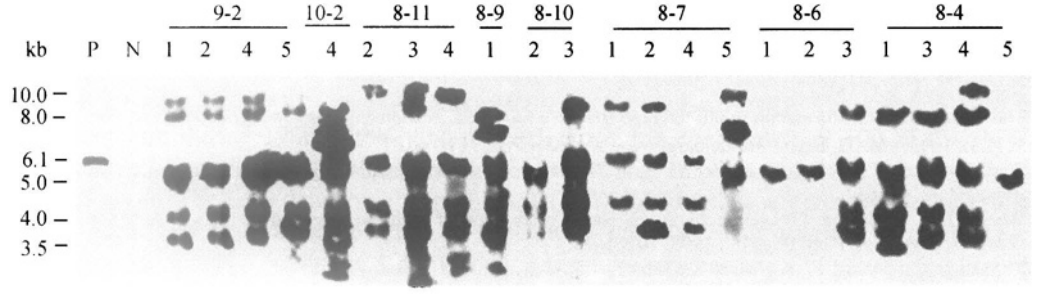

Fig. 4. Southern blot analysis of T_2 plants. From left to right: P, Positive control (pJL392A) digested with *Bam*H I; N, nontransgenic plantlet genomic DNA digested with *Bam*H I. T_2 9-2-1, 9-2-2, 9-2-4, 9-2-5, 10-2-4, 8-11-2, 8-11-3, 8-11-4, 8-9-1, 8-10-2, 8-10-3, 8-7-1, 8-7-2, 8-7-4, 8-7-5, 8-6-1, 8-6-2, 8-6-3, 8-4-1, 8-4-3, 8-4-4, 8-4-5 plantlet genomic DNAs digested with *Bam*H I, respectively.

of negative control. This shows that the structural gene of pJL392A or pJL392B has been stably and completely integrated into the plant cell genome. Southern blot analysis of *bar* gene of the corresponding plants also showed the same results as mentioned above (not shown in figs.). One or several strongly hybridized bands of about 3.5 kb were observed in part of the transgenic progeny plants, which may result from the incomplete integration of *Vst1* gene. In addition, there are some other fragments in different sizes, which shows that the size of fragments are changed due to the rearrangement of transforming plasmid or the loss of *Bam*H I site.

(iii) Obtaining of transgenic wheat plants resistant to powdery mildew. The resistance to powdery mildew of progeny plants of $T_0$8, 9, 10 which came from T_0 transgenic plants was tested in this experiment. It was found that only $T_1$8-4 progeny plants expressed good resistance to powdery mildew in 6 T_1 transgenic plants which came from $T_0$8 plants. Four individual plants in T_2 generation, $T_2$8-4(1), $T_2$8-4(3), $T_2$8-4(4) and $T_2$8-4(5), were obtained and 21 T_3 transgenic plants were obtained from them. Among these plants, one is immune to powdery mildew, 2 are mid-resistant, 9 are mid-suceptible, and 9 are highly susceptible. The proportion of plants which are immune to and mid-resistant to powdery mildew is 14.3% of the total (table 2). Only one transgenic T_1 plants ($T_1$9-2) was obtained, from which 3 T_2 progeny plants, $T_2$9-2(2), $T_2$9-2(4), and $T_2$9-2(5), were obtained. Among the 10 T_3 transgenic plants which came from $T_2$9-2(5), one is mid-resistant to powdery mildew, 7 are mid-susceptible, and 2 are highly susceptible to powdery mildew. The regenerated R_3 progeny plants (regenerated plants on medium without addition of bialaphos) of the control wheat cv. Jinghong 5 expressed highly susceptible to powdery mildew. The results indicated that the resistance of these plants to powdery mildew might come from *Vst1* gene. We will further study the relationship between the disease-resistance of the transgenic plants and the quantity of *Vst1* gene expression as well as the factors influencing the quantity of *Vst1* gene expression in plants.

Table 2 Assay of resistance of the progeny transgenic plants to powdery mildew

Plant No.	Level of resistance	Origin of T_1 plants	Origin of T_0 plants
T_3 8-4(3)-11	immune	T_1 8-4	$T_0$8
T_3 8-4(3)-12	mid-resistant	T_1 8-4	$T_0$8
T_3 8-4(4)-8	mid-resistant	T_1 8-4	$T_0$8
T_3 9-2(5)-1	mid-resistant	T_1 9-2	$T_0$9
Jinghong 5	highly susceptible	non-transgenic plants	

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