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Bioinformatics Analysis of Disease Resistance Gene *PR1* and Its Genetic Transformation in Soybeans and Cultivation of Multi-resistant Materials

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ABSTRACT

In agricultural production, a single insect-resistant and disease-resistant variety can no longer meet the demand. In this study, the expression vector pCAMBIA-3301-*PR1* containing the disease-resistant gene *PR1* was constructed by means of genetic engineering, and the *PR1* gene was genetically transformed to contain the *PR1* gene through the pollen tube method. In *CryAb-8Like* transgenic high-generation T_7 receptor soybean, a new material that is resistant to insects and diseases is obtained. For T_2 transformed plants, routine PCR detection, Southern Blot hybridization, fluorescence quantitative PCR detection, indoor and outdoor pest resistance identification and indoor disease resistance identification were performed. The results showed that there were 9 positive plants in the routine PCR test of T_2 generation. In Southern Blot hybridization, both *PR1* and *CryAb-8Like* genes are integrated in soybeans in the form of single copies. Fluorescence quantitative PCR showed that the expression levels of *PR1* and *CryAb-8Like* genes are different in different tissues. The average expression levels of *PR1* gene in plant roots, stems, and leaves are 2.88, 1.54, and 5.26, respectively. *CryAb-8Like* genes are found in roots, stems, and leaves. The average expression levels were 1.36, 1.39, and 4.25, respectively. The insectivorous rate of the *CryAb-8Like* gene in outdoor plants with positive insect resistance identification was 3.78%. The disc partition method was used indoors for pest resistance identification, and the bud length of transformed plants increased significantly. The average mortality rate of untransformed plants in indoor disease resistance identification was as high as 56.66%, and the average mortality rate of plants transformed with *PR1* gene was 10.00%, and disease resistance was significantly improved. Therefore, a new material with resistance to diseases and insects is obtained.

KEYWORDS

Soybean; *PR1* gene; *CryAb-8Like* gene; insect resistance; disease resistance

1 Introduction

Soybean (*Glycine max* L., Merr) is an important oil and food crop in the world [1]. It is one of the main sources of protein and fat for mankind. In China, the protein in soybeans accounts for about 40%, and the fat content accounts for about 20% [2,3]. In recent years, China's demand for soybeans has increased year by year. In 2017, the total domestic consumption of soybeans reached 1.2×10^{11} Kg. In 2019, the total domestic consumption of soybeans reached 1.964×10^{11} Kg, and soybean consumption accounted for 5.4% of the world [4]. This shows that soybeans play an important role in people's lives. Diseases and pests of

external environmental factors will greatly change the yield of soybeans. In severe cases, it will lead to no harvest of soybeans. Cultivating high-yield and high-quality soybeans is the current goal of crop breeders.

Soybean gray spot disease is also known as brown spot disease. The pathogen is (*Cercospora sojina Hara*), which is called soybean short fat cell. It belongs to the subphylum Deuteromycota. It is a worldwide disease [5]. The pathogen is *Cercosporidium sofinum*, which belongs to the subphylum Deuteromycota. The disease type is a fungal disease and spreads all over the world. This pathogen develops in soybean leaves, stems and seeds. The incidence is mainly concentrated in the high temperature and rainy period from July to August [6]. After the onset, the leaves of soybeans will fall off prematurely, which will affect the normal photosynthesis of soybeans, and reduce the yield and quality of soybeans [7]. In recent years, studies have found that the disease has a high incidence in the three northeastern provinces of China, causing a 25%–35% reduction in soybean production, and a serious reduction of more than 50%, which seriously affects soybean yield and quality, causing direct losses of up to 100 million RMB [8].

Pathogenesis related PR1 proteins (*PR1s*), when plants are invaded by certain pathogenic bacteria or stimulated by some specific compounds, the stress produces related proteins, which are closely related to system resistance and allergic necrosis [9]. In 1980, the *PR1* encoding protein gene was first discovered in tobacco [10], and subsequently found in both monocot and dicot plants [11,12]. The *PR1* gene encoding protein has been confirmed to be effective in resisting the spread of viruses and blocking fungi. Infringement and resisting the adverse external environment and other functions, the specific mechanism of action is not yet clear, and it has become one of the hot research topics in recent years [13].

Bacillus thuringiensis (Bt) has been discovered more than 100 years ago, and it has played an important role in the biological control of pests [14]. *Bacillus thuringiensis* can produce two types of endotoxin and exotoxin. Pests ingest the plants containing Bt gene. Pests will die due to starvation, blood necrosis, or neurotoxicity. It has an obvious control effect on Lepidoptera pests (Soybean Heartworm) [14].

This experiment uses biotechnology and genetic engineering principles to construct an overexpression vector for the disease resistance gene *PR1* gene, and uses the genetic transformation method to transfer the disease resistance gene *PR1* to the high-generation recipient soybean containing the insect resistance gene *CryAb-8Like*. In the process, through the identification of resistance to offspring, new insect-resistant, disease-resistant and multi-resistant materials are cultivated.

2 Materials and Methods

2.1 Experimental Materials

Soybean Jinong 28 and Jinong 38, *CryAB-8Like* transgenic insect-resistant soybean LND097, ZLHH21, pCAMBIA-3301-*PR1* gene expression vector, soybean gray spot and white net cover were used. All the experimental materials were provided by the Biotechnology Center of Jilin Agricultural University.

2.2 Experimental Reagenets

Plant DNA extraction kit (Kang Wei Reagent Company, Jiangsu, China), High purity plasmid extraction kit (Wegrath Biology Company, Beijing, China), Agarose gel extraction kit (Changchun Weijie Company, Changchun, China), Taq polymerase in PCR, dNTP, Buffering (Fermentas Company, Beijing, China), Southern Hybridization Kit (Roche Company, Shanghai, China), fluorescent quantification kits (Roche Company, Shanghai, China), and Synthetic primer (kumei Company, Changchun, China) were purchased while Other biological reagents were purchased from Beijing Dingguo Company (Beijing, China).

2.3 Identification of Plant Expression Vector pCAMBIA-3301-PR1

The *Escherichia coli* containing the target disease-resistant gene *PR1* was stored in the −80°C refrigerator. The bacterial liquid were activated in the LB solid medium containing kanamycin and

incubated overnight at 37°C. A single bacterial colony was taken and , placed it in LB liquid medium containing kanamycin. Then the liquid medium incubated overnight at 37°C. The plasmid containing the *PR1* gene sent to Changchun Kume Biotechnology for sequencing. The sequencing analysis identified the expression vector containing the target gene *PR1*, and the identification results are shown (Fig. 13). The homology reached 100%, indicating that the target gene *PR1* is on the overexpression vector.

2.4 Transformation of Recipient Soybean Plants

The method of genetic transformation was carried out by the pollen tube passage method, and the T$_2$ generation plants of transgenic plants are obtained by adding generation planting in the field. The specific operation of the pollen tube passage method is as follows: removed the keel flap, cut the stigma and tied with the rope, respectively. Then the plasmid was introduced in to the plant.

2.5 Routine PCR Identification of Transgenic Plants

In the late three-leaf period of the plant, fresh soybean leaves were taken, and the DNA extraction kit of Kangwei Century Company (Beijing, China) was used to extract soybean plant DNA. Primer 5.0 was used to pair *PR1*, *CryAB-8Like*, screening gene *Bar*, promoter *35S*, and terminator *Nos*. Gene design specific primers (Table 1), plasmid as a positive control group, untransformed plants as a CK control group, and ddH$_2$O as a blank control group. The specific reaction conditions were as shown (Table 2).

Table 1: Primer sequence information

Name	Sequence (5'-3')
CryAB-8Like-A	CCTATGAACAGCAACAACCAAAAC
CryAB-8Like-AS	AAGTTCATCCTTCTCGGATCTAGTCC
PR1A	ATAATGGGATACTTGTGC
PR1AS	CTACAGTTCGTAGGGACTT
T-PR1A	ACTCTTGACCATGGTagatctATAATGGGATACTTGTGC
T-PR1-As	GGGGAAATTCGAGCTggtgaccCTACAGTTCGTAGGGACTT
35SA	TAGAGGACCTAACAGAAC
35sAS	CCGTGTTCTCTCCAAATG
Bars	TCAAATCTCGGTGACGGGC
BarAs	ATGAGCCCAGAACGACGC
NosA	GAATCCTGTTGCCGGTCTTG
NosAs	TTATCCTAGTTTGCGCGCTA
qCRYA	CTTCAGACCACGGAGATAACAG
qCRYAs	AATGCGTGTGGGTAAGAGATAG
qPR1s	GGTGAGTTCTCAATCACCAAGA
qPR1As	GTAAAGGTGACTGTGCACTGA
qActins	ATCTTGACTGAGCGTGGTTATTCC
qActinAs	GCTGGTCCTGGCTGTCTCC

Note: Underlines in sequences indicate the restriction enzyme sites.

Table 2: PCR specific amplification conditions

Gene name	Predenaturation	Denaturation	Annealing	Extend	Rearextension	Cycles
PR1	94°C, 5 min	94°C, 30 s	55°C, 60 s	72°C, 35 s	72°C, 8 min	40
CryAB-8Like	94°C, 5 min	94°C, 30 s	58°C, 50 s	72°C, 30 s	72°C, 8 min	35
bar	94°C, 5 min	94°C, 35 s	58°C, 30 s	72°C, 35 s	72°C, 8 min	30
35s	94°C, 5 min	94°C, 40 s	53°C, 35 s	72°C, 30 s	72°C, 8 min	30
Nos	94°C, 5 min	94°C, 40 s	52°C, 30 s	72°C, 40 s	72°C, 8 min	30

2.6 Southern Blot Hybridization Identification of T_2 Transgenic Lines

Use DNA genome extraction kit to extract the genome of the T_2 generation plant, and store it in the refrigerator at –20°C for subsequent enzyme digestion experiments. The *PR1* and *CryAB-8Like* plasmids were used as the positive control group, and the untransformed plants were used as the CK control group.

The soybean genome was digested with BgLII, and the specific experimental operation method was carried out according to the instructions of Roche's Southern blot hybridization kit.

2.7 Fluorescence Quantitative PCR Identification of T_2 Generation Transgenic Plants

RNA was extracted from the roots, stems, and leaves of T_2 transgenic plants, and then reverse-transcribed into cDNA using a fluorescent reverse transcription kit (TakaraBio, RR036A), and designed fluorescent quantitative primers using Primer Quest Tool software (Table 4), Mx3000P fluorescence Quantitative PCR was used to detect the relative expression levels of *PR1* gene and *CryAB-8Like* gene in roots, stems, and leaves, and use the formula $^{2-\Delta\Delta}CT$ for data analysis.

2.8 Outdoor Pest Resistance Identification of T_2 Transgenic Plants

In the outdoor transgenic field, the positive plants genetically transformed with the *PR1* gene were planted, and the untransformed soybean Jinong 28CK was used as the control group in the outdoor net shed. During the prevalence of soybean borer in late August, a large number of soybean borer was inserted into the net room. The insect-resistant, disease-resistant and multi-resistant plants and recipient plants containing *CryAB-8Like* gene and *PR1* gene were to be harvested. The plant was threshed and related statistical data analysis was carried out, the soybean insect-eating situation was investigated, and the soybean carbophagous insect-eating situation was photographed.

2.9 Indoor Pest Resistance Identification of T_2 Transgenic Plants

50 *PR1* gene pest-resistant and disease-resistant soybeans and 50 untransformed soybeans were taken, and conducted the pest resistance identification test. The disc separation method was used for soybean resistance identification [15]. The transformed and untransformed soybeans were soaked in sterilized water for 15 hours for subsequent tests.

Autoclave the glass disc and filter paper. Spread the sterilized filter paper on the surface of the glass dish. Place 10 soybean heartworms on the scoring position in the center of the petri dish. Spread them evenly on both sides of the scoring position on the dish. Soybeans, the transformed soybeans were placed on the left side, and the untransformed soybeans were placed on the right side, and three repeated control experiments were set. After 7 days, record the borer's condition, bud length and fresh weight (Fig. 1A). According to the actual growth status of soybean seeds, they were divided into insect resistance. In order to ensure the accuracy of the test, four boxes were prepared, and all four boxes were sterilized at high temperatures. The filter paper was spread on the surface of the box and was numbered 1, 2, 3, 4, place 40 transformed *CryAB-8Like* soybeans in boxes numbered 1, 2, 40 untransformed soybeans, Jinong 28 soybeans in boxes

3 and 4, and 12 soybean heartworms in boxes 1, 3, respectively. Insect resistance identification of transformed *CryAB-8Like* gene and Jinong 28 resistance identification. Boxes 2 and 4 were used for the identification of *CryAB-8Like* gene without insect resistance and Jinong 28 for the identification of insect resistance (Fig. 1B).

Figure 1: Disc segmentation method of insect resistance identification (A) Comparative experiment (B)
Note: Ab: Bt soybean seeds; CK: untransformed soybean seed; 1, 2: Soybean gene *CryAB-8Like* transfected; 3, 4: Untransformed soybean Jinong 28; 1, 3: Catch soybean heartworm

2.10 Indoor Disease Resistance Identification of T₂ Transgenic Plants

The *PR1* genetically modified soybeans were planted and artificially inoculated indoors. The plants containing the *PR1* gene and the CK plants of the control group were inoculated with the same bacteria in equal amounts, and the hypocotyls and leaf parts of soybean leaves were scratched. Soybean gray spot was inoculated on the scratched parts of soybean plants at room temperature 25°C At 91% under humid environment, three groups of repeated controls were set up, in which there were two CK plants in each group, and two plants with disease-resistant *PR1* gene were inoculated in the late three-leaf stage of soybeans, and soybean gray board disease was inoculated for 3 days and 7 days. Investigation The actual condition of the disease and record the data.

3 Results

3.1 GmPR1 Gene Source

The Gm*PR1* gene comes from high-throughput sequencing of the differential transcriptome to obtain differentially expressed genes. The over-expression vector pCAMBIA-3301-Gm*PR1* of this gene was provided by the Biotechnology Center of Jilin Agricultural University.

3.2 Sequence Analysis of Soybean PR1 Gene

Using the NCBI website to perform Blast analysis, the nucleotide sequence of soybean is as much as 100% homologous to Gm*PR1* (Fig. 2), confirming that this gene is the target gene. The full length of the cDNA is 525 bp, and the ORF sequence is 525 bp in length, which encodes 174 amino acids. The molecular weight of the protein is 18.83 kilodaltons, and the theoretical isoelectric point PI is 4.54.

3.3 The Signal Peptide Prediction and Subcellular Localization Analysis of Soybean PR1 Encoded Protein

Signalp online analysis was used to predict protein signal peptides (Fig. 3). The protein encoding the *PR1* gene is a secreted protein. The maximum signal peptide (Sec/SPI) is 0.9695, and a cleavage site appears at the 27–28 amino acid position, with a probability of 0.8692. The results of PSORT II software on the protein subcellular location analysis of the Gm*PR1* gene are shown (Fig. 4). The percentage of K-NN prediction 44.4% on the endoplasmic reticulum, 22.2% on the mitochondria and Golgi apparatus were obtained. This result proving that the gene mainly functions on the endoplasmic reticulum.

Secondly, it functions on mitochondria and Golgi apparatus, and it can be seen from the side that this gene is a secreted protein.

Figure 2: NCBI prediction results of *PR1* target gene

Figure 3: Prediction results of *PR1* gene signal peptide

Figure 4: Protein subcellular localization of soybean *PR1* gene

3.4 Research on Hydrophilicity and Hydrophobicity of Soybean PR1 Encoded Protein and Analysis of Transmembrane Region

ProtScale online analysis of the hydrophilicity and hydrophobicity of the protein encoded by soybean PR1, Hphob/Kyte & Doolittle is the prediction standard, and the results are as follows (Fig. 5). There are many relatively low peaks, and the amino acids at positions 111–119 have obvious hydrophilic regions. Where the amino acid at position 115 has the smallest value, which is –2.756, where the hydrophilicity is the strongest. However, the amino acid at position 16 has the largest value, which is 3.189, where the amino acid has the strongest hydrophobicity. The hydrophilic amino acids in the entire polypeptide chain account for 72.45%, and the hydrophobic amino acids account for 27.55%. The overall performance is hydrophilic, so the protein is a soluble protein. Using TMHMM Server 2.0 to analyze the transmembrane region of the protein online, the results are shown (Fig. 6). The GmPR1 protein contains a transmembrane structure. It is a transmembrane protein that migrates in the cell.
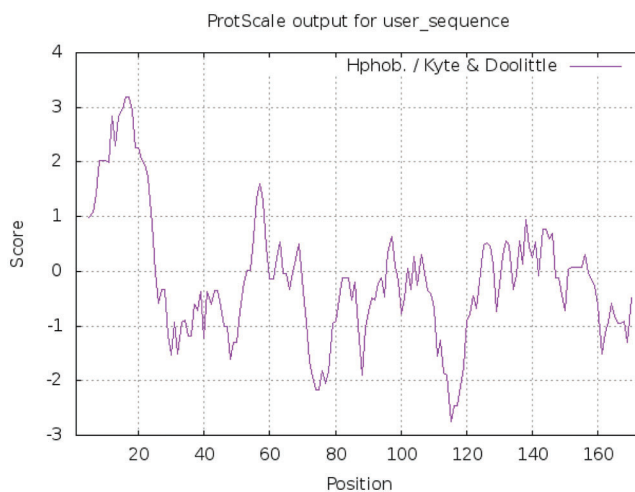

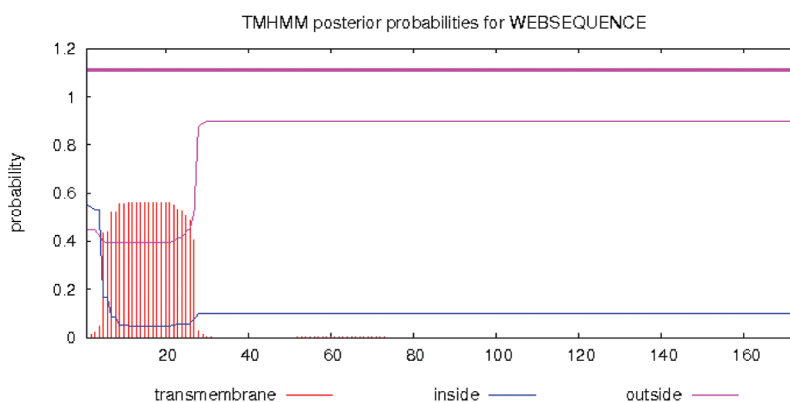**Figure 5:** Study on the hydrophilicity and hydrophobicity of *PR1* protein

Figure 6: Prediction of transmembrane region of *PR1* gene encoding protein

3.5 Prediction of the Phosphorylation Site of Soybean PR1 Protein

NetPhos online analysis for *PR1* encoded protein phosphorylation site are shown (Fig. 7). The GmPR1 protein has 19 potential phosphorylation sites, including 10 serine (Ser) phosphorylation sites, 3 threonine (Thr)) Phosphorylation sites, 6 tyrosine (Tyr) phosphorylation sites.
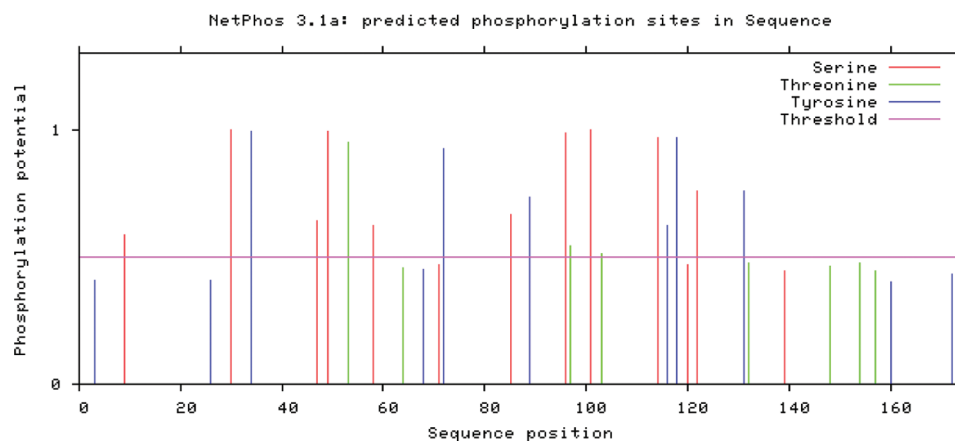
Figure 7: Prediction analysis of phosphorylation of soybean *PR1* gene encoding protein

3.6 Analysis of the Results of Soybean PR1 Gene Encoding Protein

SOPMA online was used to analyze the secondary structure of the amino acid sequence of Gm*PR1*, and the results are shown (Fig. 8). Gm*PR1* protein consists of α-helix (32.76%), extended chain (17.82%), β-turn (5.17%), and random coils (44.25%). The four parts of Gm*PR1* protein composed of random coils accounted for the highest proportion, followed by α-helical structure, β-turn, and the lowest was β-turn. SWISS-MODEL was used to analyze the tertiary structure of the amino acid sequence of Gm*PR1* online for predictive analysis. The results show that the tertiary structure and the secondary structure are consistent, and they are composed of four parts (Fig. 9). The Gm*PR1* encoded protein is in the three-dimensional structure database. The homology to *Arabidopsis thaliana* is as high as 82.52%.
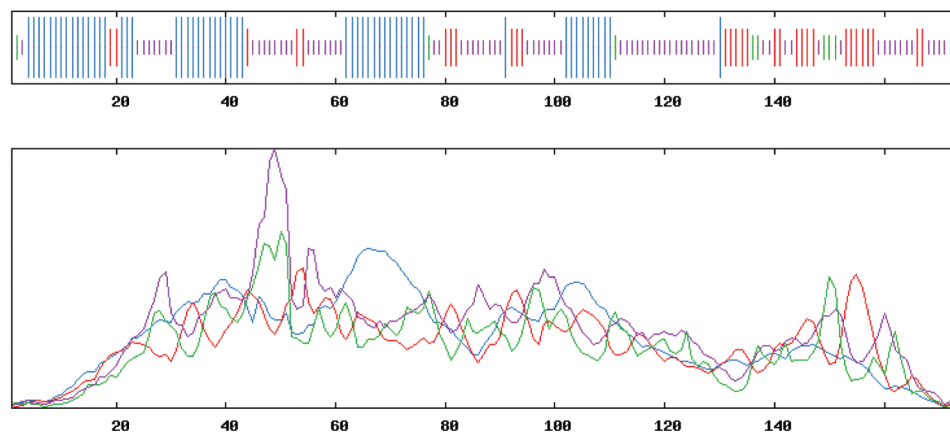

Figure 8: *PR1* protein secondary structure prediction

3.7 Protein Interaction Analysis of Soybean PR1 Gene

STRING online analysis of Gm*PR1* gene protein interaction analysis are shown (Fig. 10). *PR1* protein is mainly related to defensin-like protein, oxidized allene synthase, chloroplast allene oxide synthase, chitinase class I, peptidase M10A family, NPR1-2 protein, tubulin, etc., interact with each other. Protein interaction analysis showed that GmPR1 protein played an important role and function in response to disease stress.
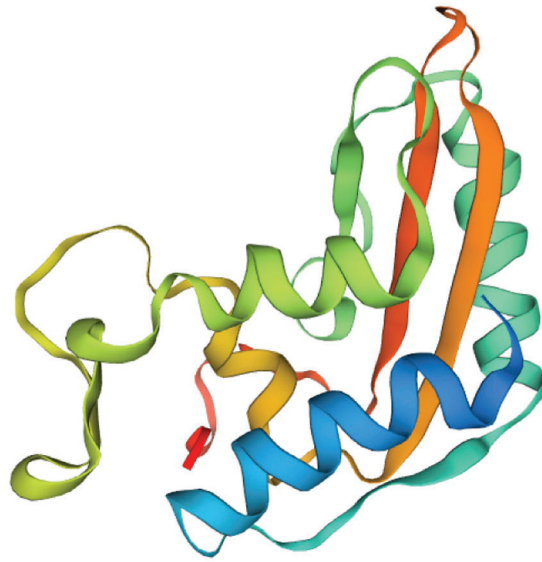
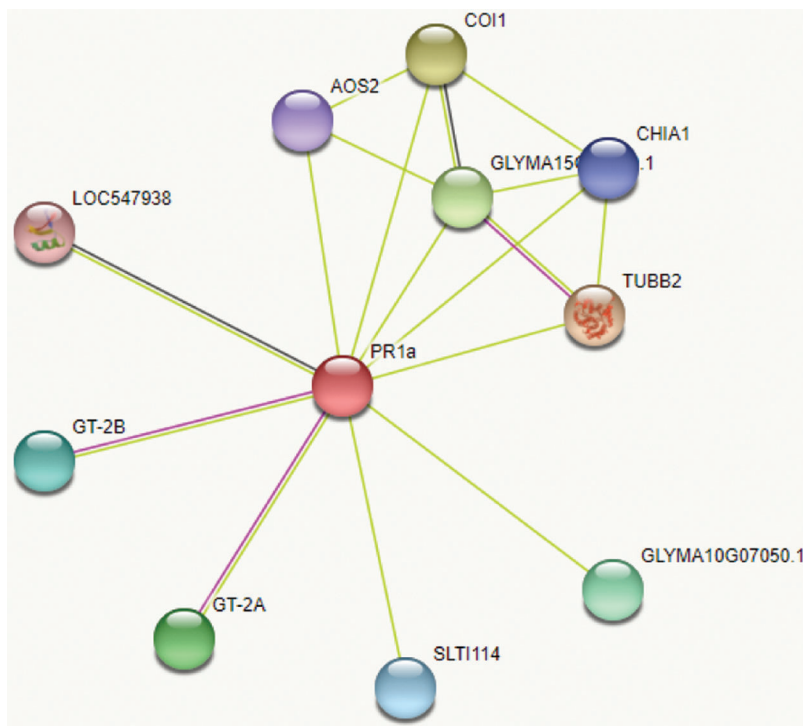**Figure 9:** Prediction of the tertiary structure of *PR1* protein

Figure 10: *PR1* protein interaction analysis

Note: LOC547938 defensin-like protein; AOS2 oxidized allene synthase, chloroplast allene oxide synthase; SLTI114 peptidase M10A family; CHIA1 chitinase class I; GLYMA15G13320.1 NPR1-2 protein; TUBB2 tubulin.

3.8 Construction of Plant Expression Vector pCAMBIA-3301-PR1

Primer 5.0 software was used to design primer to specifically amplify the nucleotide sequence of the target gene GmPR1 by PCR. Using 1.5% concentration agarose gel electrophoresis at 220 V, 110 mA, the

target band is 525bp (Fig. 11) and the size of the expected fragment is consistent. It is preliminarily considered that this fragment is the target gene fragment. In this experiment, the pCAMBIA-3301-*PR1* gene expression vector was provided by the Plant Biotechnology Center of Jilin Agricultural University. The vector structure diagram of the disease resistance gene is (Fig. 12) DNA man software and CE design2.0 software were used to design a double digestion. The restriction sites are BglII and BsteII. Seamless cloning technology was used to seamlessly clone the cloned target gene and pCAMBIA-3301 large fragment. Then transformed into *Escherichia coli* DH5α, LB+Kan (kanamycin) medium plate coating. Sent to the Kumei Biotechnology Company in Changchun City for sequencing. The sequencing results were compared using DNAman homology results as shown (Fig. 13).
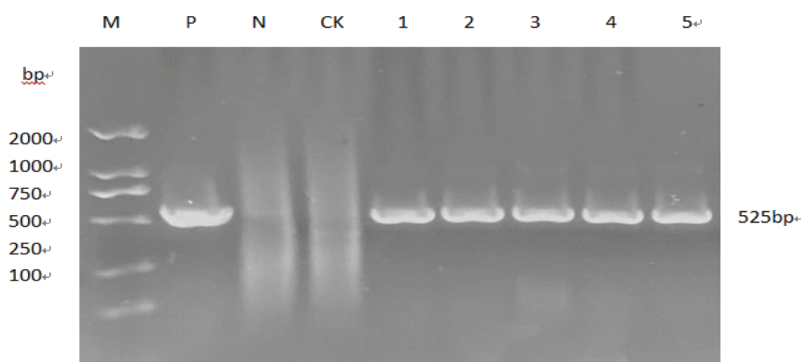

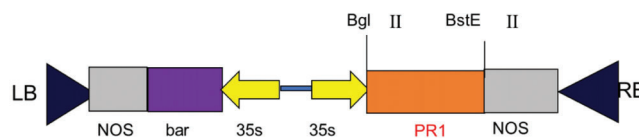**Figure 11:** Specific amplified fragment of *PR1* gene

Figure 12: Schematic diagram of pCAMBIA-3301-*PR1* vector structure
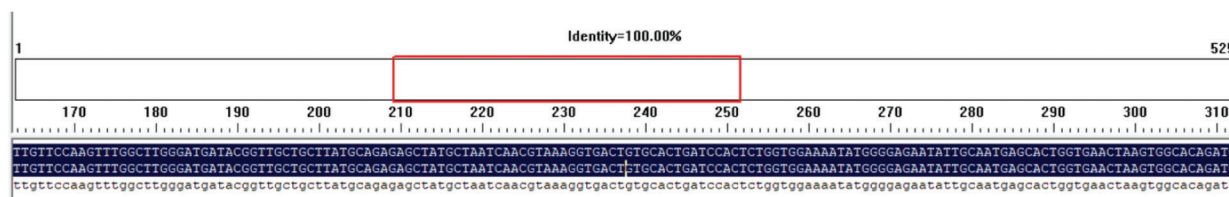

Figure 13: DNAman homology comparison result of *PR1* gene

3.9 Transforming Recipient Soybeans

The pollen tube pathway was used to genetically transform 10 T_1 positive plants of pCAMBIA-3301-*PR1* and 18 T_2 positive plants.

3.10 Routine PCR Identification of Transgenic Positive Plants

3.10.1 Identification of T_1 Generation Positive Plants

The soybean leaf DNA genome of T_1 plant was extracted, and the *PR1* gene, *Cry-8Like* gene, *Bar* gene, *35S* gene, and *NOS* gene were specifically amplified, and the corresponding fragment sizes were 525, 1987,

552, 500 and 198 bp, respectively (Fig. 14), consistent with the expected results, it can be preliminarily considered that the gene is integrated into the genome of the T_1 plant.
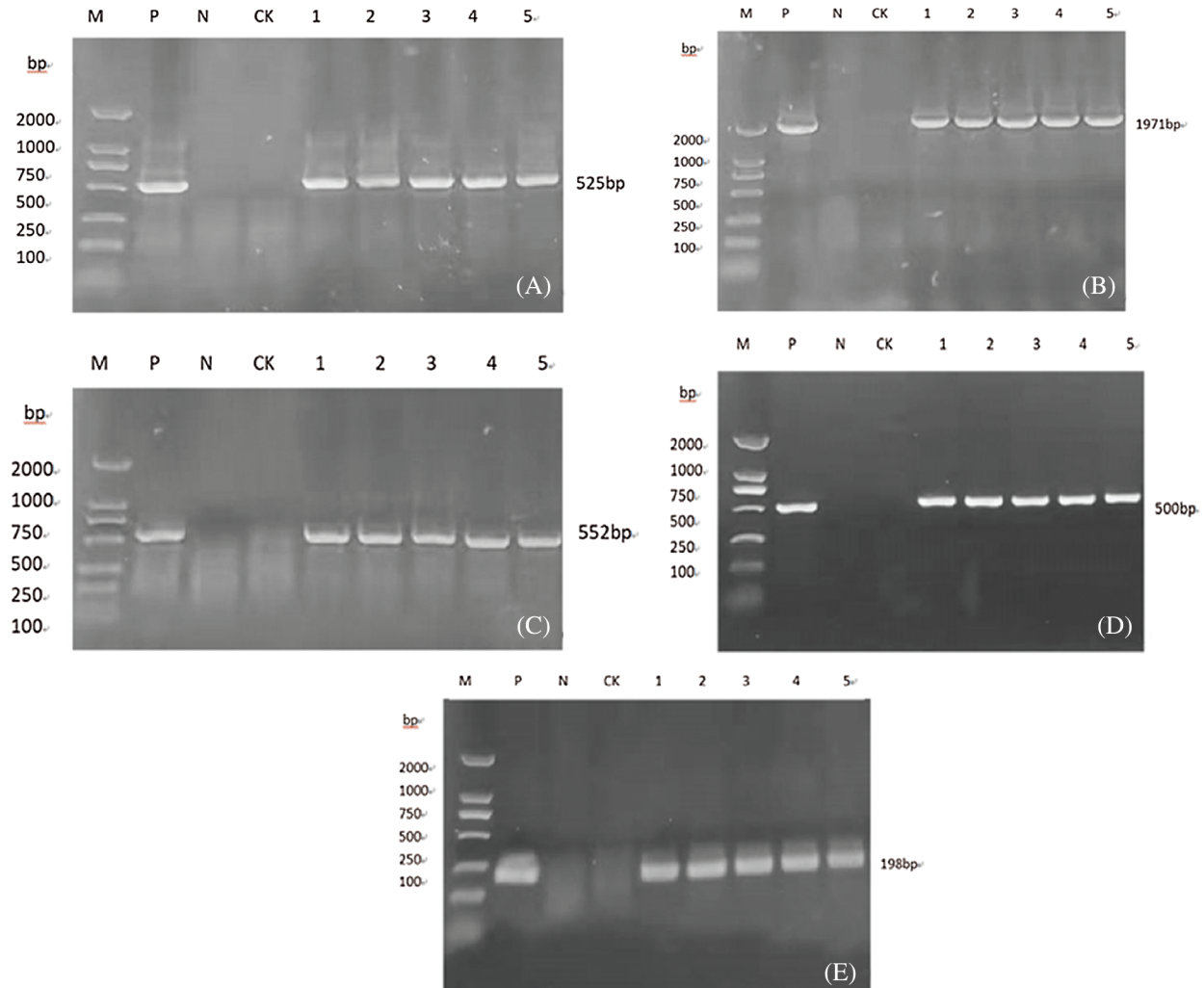
Figure 14: Molecular identification of T_1 generation plants
Note: A, B, C, D, E are respectively *PR1*, *Cry8like*, *bar*, *35s*, *Nos* gene detection; M: DL2000 DNA Marker; P: Plasmid (positive control); N: H$_2$O; CK: untransformed plant 1- 5:Transformed plants.

3.10.2 Identification of T_2 Generation Positive Plants

The soybean leaf DNA genome of T_2 plant was extracted, and the *PR1* gene, *Cry-8Like* gene, *Bar* gene, *35S* gene, and *NOS* gene were specifically amplified, and the corresponding fragment sizes were 525, 1987, 552, 500 and 198 bp, respectively (Fig. 15), consistent with the expected results, it can be preliminarily considered that the gene is integrated into the genome of the T_2 plant.

3.11 Southern Blot Hybridization Identification of Transgenic T_2 Plants

Southern Blot hybridization was performed on T_2 plants to identify transformed plants and untransformed plants. Hybridization signals appeared in the lanes of transformed plants, and hybridization signal bands appeared in untransformed plants (Fig. 16A) and *Cry-8Like* gene and (Fig. 16B) genes are

integrated in the recipient soybean plant as a single copy, which is consistent with the results of conventional PCR detection. The hybridization bands in the lanes are not used. The position appeared indicates that the integration site of the foreign gene in the soybean genome is inconsistent. The denaturation solution (Nacl, NaOH), neutralization solution (Nacl,Tris.HCl), 20 × SSC (Nacl, sodium citrate, pH = 7.0), mother liquor, washing solution 1, washing solution 2, washing buffer, the molecular probes, and 0.8% agarose gel without nucleic acid dye were prepared to perform the experiment. Finally, the nitrocellulose membrane was hybridized in a hybridization oven and color was developed.
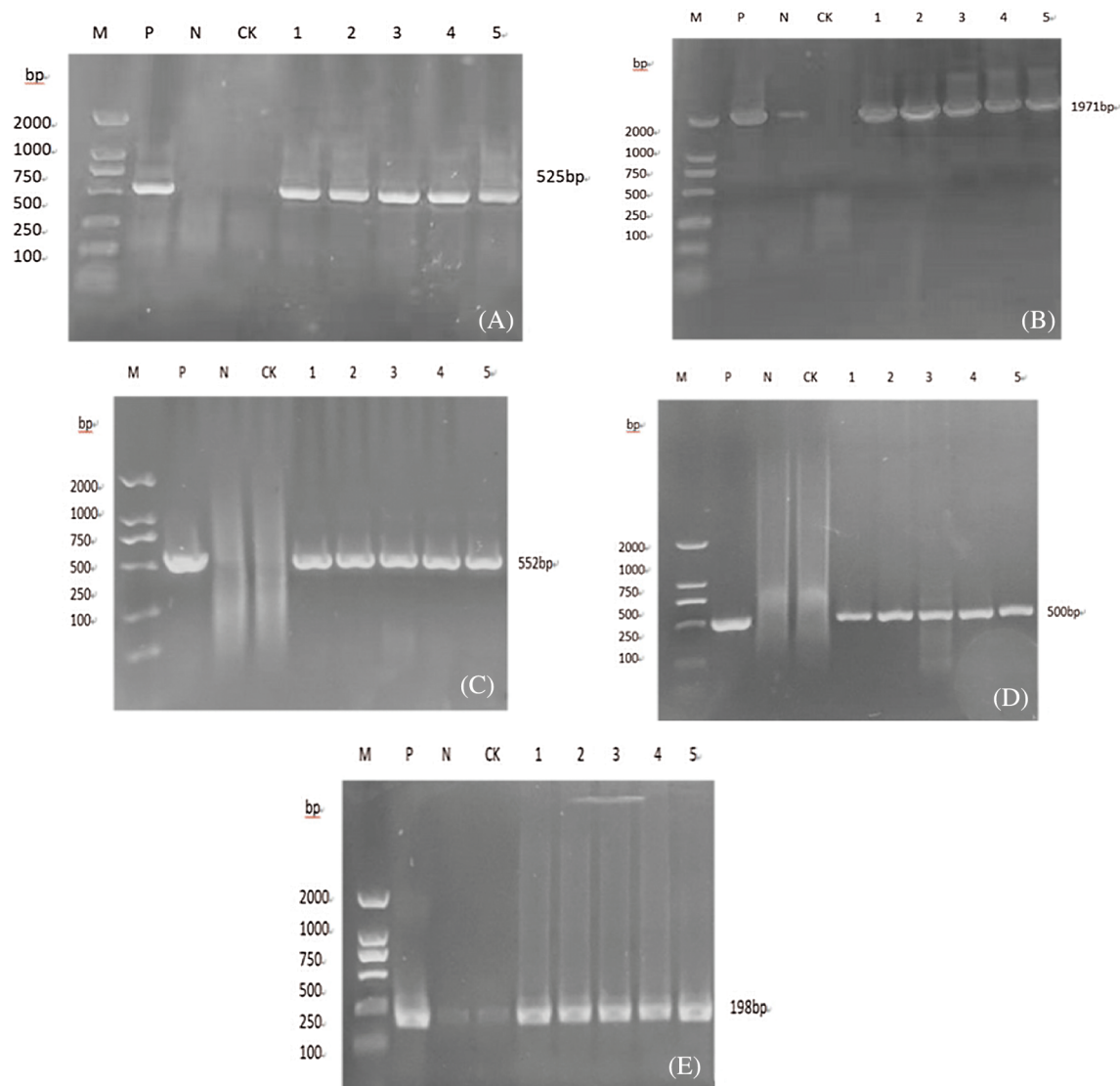

Figure 15: Molecular identification of T$_2$ generation plants

Note: A, B, C, D, E are respectively *PR1*, *Cry8like*, *bar*, *35 s*, *Nos* gene detection; M: DL2000 DNA Marker; P: Plasmid (positive control); N: H$_2$O; CK: untransformed plant; 1-5: Transformed plants.
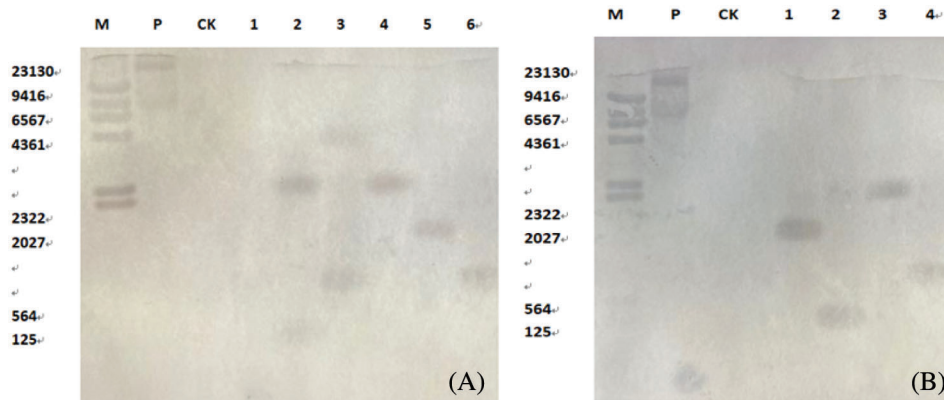
Figure 16: Southern Blot hybridization of T$_2$ transgenic plants

Note: A: *Cry8Like*; B: *PR1*; M: Southern dedicated Marker; P: Positive control; CK: untransformed plants; A, B: 1-4/6: transformed plants.

3.12 *Fluorescence Quantitative PCR Identification of T$_2$ Generation Plants*

For transgenic lines identified by conventional PCR and Southern blot hybridization, the roots, stems, and leaves of the plants were extracted after seed planting, and the cDNA was reverse-transcribed using a fluorescent quantitative reverse transcription kit. Soybean cDNA was used as a specific amplification template. β-actin was used as an internal reference gene in soybean. The relative expression levels of *CryAB-8Like* gene on roots (Fig. 17A) were 1.47, 0.88, 1.75, respectively. The relative expression levels on stems were 1.34, 1.16, 1.67 and the relative expression levels on leaves were 5.19, 4.12, 3.44, respectively (Fig. 17A). The results indicated that the relative expression of *CryAB-8Like* gene is the highest in leaves, followed by stems and roots. The relative expression levels of the *PR1* gene on the roots (Fig. 17B) were 2.74, 2.65, 3.25, respectively and the relative expression levels on the stems were 2.35, 1.32, and 0.95. The relative expression levels on the leaves were 5.10, 5.65, and 5.05, respectively. The relative expression level of *PR1* gene in leaves is 5.65. The relative expression level in the stem is the lowest 0.95. Both *PR1* and *CryAB-8Like* genes are foreign genes. From the above data, it can be shown that the two genes are expressed in different roots, stems, and leaves, and their relative expression levels are not the same.
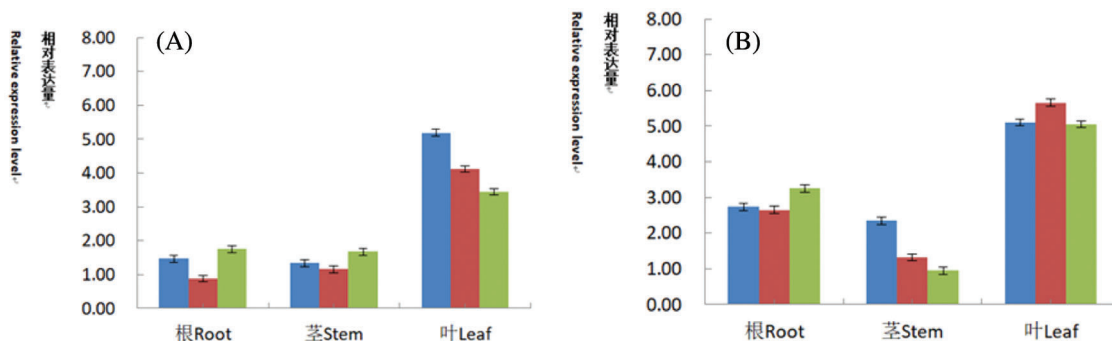

Figure 17: Fluorescence quantitative PCR identification of T$_2$ generation plants

Note: A: Relative expression level of *CryAB-8Like* gene in roots, stems and leaves; B: Relative expression level of *PR1* gene in roots, stems and leaves.

3.13 Outdoor Pest Resistance Identification of T₂ Positive Plants

The transgenic plants containing the *CryAB-8Like* gene and the recipient plants (JN28) were harvested in the transgenic experimental field net laboratory for individual plant test analysis, and single plant threshing for statistical analysis of data (Fig. 18). Among them, 15 transgenic plants were randomly selected for statistical data analysis (Table 3).

Figure 18: Outdoor net room planting situation

Table 3: Average statistics of 15 transgenic plants

Transgenic plants	Plant height (cm)	Pod number (uni)	Branch (uni)	Section (uni)	Number of insects (uni)	100 grain weight	Total weight	Total grains (uni)	Moth-eaten ratio	Insect resistance level
1	94	88	4	19	7	19.65	34.03	187	3.74	MR
2	83	125	7	18	7	18.26	29.47	168	4.16	MR
3	77	185	8	17	12	21.70	56.05	375	3.20	R
4	97	57	4	16	4	13.28	16.22	96	4.16	MR
5	80	49	3	16	5	15.20	17.23	87	5.74	MR
6	92	62	5	18	3	21.39	24.34	127	2.36	R
7	96	75	3	17	4	21.94	25.40	117	3.41	R
8	94	60	3	15	6	19.98	19.02	138	4.34	MR
9	97	44	2	16	2	17.10	15.70	97	2.06	R
10	92	73	3	20	7	16.92	20.67	147	4.76	MR
11	90	52	4	17	3	20.05	17.05	162	1.85**	HR
12	96	36	2	19	4	19.20	16.70	76	5.26	MR
13	95	78	3	16	2	2219	26.77	112	1.78**	HR
14	81	55	2	15	2	22.25	26.39	141	1.41**	HR
15	97	39	2	18	2	18.73	15.21	88	2.27	R

Note: HR: high resistance (0–2%); R: resistance (2–4%); MR: medium resistance (4–6%); S: sensitivity (6–10%); H: high sensitivity (10–100%) **Extremely significant at the 0.01 level; different letters indicate different levels.

The method of identification of resistance to soybean heartworm and its identification criteria refer to the research of Zhang et al. [16]. The comparison of transgenic plants with JN28CK does not occur in agricultural traits such as plant height, branching, number of nodes, and number of pods, except for insect

resistance. Comparing JN28 receptors and genetically modified materials, JN28 insectivorous rate was 7.5%, and the average insectivorous rate of plants belonging to the S-sense (6%~10%) *CryAB-8Like* gene was 3.78% in the grade while R-anti (2%~4%). The insectivory rate of transgenic plants has changed significantly, and the insectivory rate of lines 11, 13 and 14 is extremely significant compared with other transformed lines. The average total weight of soybeans after transformation reached 24.01 g, and the total weight of the acceptor material was 17.55 g. The weight of the transformed material was significantly higher than that of the untransformed acceptor material. Receptor transgenic *CryAB-8Like* soybeans have obvious insect resistance effects and play an important role in improving soybean yield and quality.

3.14 Indoor Pest Identification

The method used for indoor insect resistance was the disc segmentation method (Table 4). The transformed bud length was 13.4–20.2 cm, with an average of about 17.4 cm. The highest number of borers was 2 heads, the least was 1 head, and the average was 2 heads. The bud length of untransformed plants was 6–12 cm, with an average of about 10.5 cm. The number of borers was 7 at most, 5 at least, and 6 on average. It shows that the transformed grains will release some pheromone during the germination process, which has a certain deworming effect. Under the same circumstances, the growth of transformed plants was better than that of untransformed plants. Some of the untransformed plants even stopped growing, and soybean borer had a certain inhibitory effect on the growth and development of untransformed plants (Fig. 19).

Table 4: Disc separation method in terms of shoot length, fresh weight, and number of borers

Gene name	Average bud length (cm)	Average fresh weight (g)	Average moth (uin)
CRYAB-8Like	19.3	4.8	2
JN28CK	11.4	3.2	6

Figure 19: Kernel growth of transformed plants. Note: AB: Transformed seedlings; CK: Untransformed seedlings

3.15 Indoor Disease Resistance Identification

The transformed plants and untransformed plants under the same conditions. After the three-leaf period, a knife was used to lightly scratch the soybean leaves, scratch the hypocotyl part of the neck, and connect the soybean to the leaf wound and the soybean hypocotyl. The result after 3 days (Fig. 20), 3 days after the

soybean gray spot disease, the leaves of the recipient plant showed a tendency to turn yellow and gradually wilt, but the positive plants hardly changed. One week after the soybean gray spot disease was inoculated, the recipient plants wilt severely, were broken, and tended to die. However, the transgenic plants are significantly stronger than the recipient plants, and their disease resistance has been improved.

Figure 20: Transformed plants inoculated with soybean gray spot disease
Note: A: transformed plants; B: untransformed plants; C, D: diseased leaves.

In the survey of the mortality of large gray spot disease in the transformed T_2 generation plants, the average mortality rate of untransformed plants was as high as 56.66%, and the average mortality rate of transformed *PR1* gene plants was 10.00%, and the mortality rate has changed significantly (Table 5). The disease resistance has been improved, indicating that the insect-resistant materials transformed into *PR1* gene plants have obvious disease resistance and improved the disease resistance of soybeans.

Table 5: Soybean inoculation mortality

Strain	Number of shares	Number of surviving plants	Number of dead plants	mortality rate (%)	Resistance evaluation
Untransformed plants	30	13	17	56.66	S
Transformed plant	30	27	3	10.00	MR

Note: S: Susceptible; MR: Medium resistance.

4 Discussion

Soybean gray spot is a worldwide disease. It occurs most severely in northeastern China. It mainly occurs in the cotyledons, stems, node pods and seeds of soybeans, resulting in decreased photosynthesis and yield of soybean plants. The severity of soybean gray spot disease mainly depends on the number of bacterial sources. Due to the high temperature, long-term rainy weather, little sunshine, and relative air humidity, serious gray spot disease can be caused. The fungal source of cotyledons and node pods are different. The fungal source of leaf parts is the source of overwintering fungi, but the fungal source of node pods comes from the re-infection of diseased leaf spores. It can be seen that the characteristics of soybean gray spot infection are continuous, and the infection process is from work to pod to seed [17]. At present, the prevention of soybean gray spot disease usually adopts conventional cross breeding and molecular assisted breeding. New soybean varieties resistant to gray spot disease are obtained through

hybrid breeding, and molecular-assisted breeding which often uses transgenic technology. The disease-related protein gene *PR1*, which encodes a protein, has been proven to resist the spread of viruses and block fungal damage to resist bad external environments and other functions. Some studies have been reported on soybean [18], rice [19], potato [20] and corn [21]. *Bacillus thuringiensis* (Bt) *Bacillus thuringiensis* can produce both endotoxins and exotoxins. When pests eat plants containing Bt genes, the pests will die due to starvation, blood necrosis, or neurotoxicity. It has an obvious control effect on Lepidoptera pests (Soybean Heartworm) [22]. The protein encoded by the *CryAb-8Like* gene is one of the Bt toxic proteins, and Lin [23] and others have functionally verified that it has a significant anti-insect effect in soybeans.

The pollen tube channel method, when the plant is pollinated, it will form a line that runs through the entire style, and the pollen will enter the ovary along the channel and combine with the ovule to complete fertilization. Pollination within a certain period of time, the pollen tube channel will not be occluded, at this time the foreign DNA fragments along the pollen tube channel deep into the embryo sac [24]. Deng et al. [25] used laser confocal microscopy to clarify that the pathway for foreign DNA to enter the embryo sac is the outer channel of the pollen tube, that is, the foreign DNA enters through the gap between the tissue bundle and the pollen tube, not from the inner cavity of the pollen tube. Factors affecting the efficiency of genetic transformation include the size of inserted DNA fragments, DNA purity, DNA concentration, pH value of DNA vector buffer and different introduction periods. The transformed progeny has a wide variety of traits and rich types, and the various quantitative and quality traits of crops are obvious. Including plant morphology, growth and development, plant physiological and biochemical characteristics, resistance characteristics, yield, quality and many other aspects. At present, the pollen tube passage method is widely used in cotton [26], soybean [27], corn [28], sorghum [29] and many other crops.

There are very few reports about the transfer of foreign genes into resistant transgenic plants through pollen tube passage method to obtain insect-resistant and disease-resistant materials. In this experiment, the plant expression vector pCAMBIA-3301-*PR1* containing the Bar marker gene was successfully constructed, and the expression vector was introduced into the recipient soybean containing the insect-resistant gene *CryAB-8Like* through the pollen tube channel method, and routine PCR identification was performed. Southern blot hybridization of the *PR1* gene and *CryAB-8Like* gene are single copies integrated into the genome of the recipient plant at different sites. In the fluorescence quantitative PCR, the *PR1* gene and *CryAB-8Like* gene are located on the roots, stems, and leaves. Certain expression levels, among which *CryAB-8Like* gene has the highest expression level on leaves and the lowest expression level on roots. In the outdoor insect resistance test, the insectivory rate of plants containing *CryAB-8Like* gene was 3.78%, which was classified as R-resistant (2%~4%). The insectivory rate of transgenic plants has changed significantly. Indoor insect resistance test, disc separation method, the transformed plants have obvious insect resistance to soybean coreworm, and their growth is better than untransformed plants, and there is a big difference in bud growth, indicating that the transformed plants have a certain resistance to soybean coreworm. It can be preliminarily judged that the transformed plants have certain insect repellency. In the indoor disease resistance test, 30 *PR1* disease-resistant gene plants and 30 untransformed plants were planted respectively. In the survey of the mortality of soybean gray spot disease in the transformed T_2 generation plants, the average mortality rate of untransformed plants was as high as 56.66%, and the average mortality rate of transformed *PR1* gene plants was 10.00%. The mortality rate has changed significantly, and the transformed plants have certain resistance. Disease effect.

There are many breeding methods to produce insect-resistant and disease-resistant plants using backcross breeding, introduction of germpla sm, and distant hybridization. However, these methods are considered to be time-consuming and labor-intensive. Now a days, the application of biotechnology in crop breeding has solved the challenges related to time and labor. Previous studies only improved a

single trait, but few improved two or more traits at the same time. Comparing JN28 receptors and genetically modified materials, JN28 insectivorous rate was 7.5%, and the average insectivorous rate of plants belonging to the S-sense (6%~10%) *CryAB-8Like* gene was 3.78% in the grade while R-anti (2%~4%). The weight of the transformed material was significantly higher than that of the untransformed acceptor material. The average mortality rate of untransformed plants was as high as 56.66%, and the average mortality rate of *PR1* gene transformed plants was 10.00%, and the mortality rate has changed significantly (Table 5).

5 Conclusion

The total length of the *PR1* gene cDNA is 525 bp, and the ORF sequence is 525 bp in length, which encodes 174 amino acids. The molecular weight of the protein is 18.83 kilodaltons, and the theoretical isoelectric point PI is 4.54. Among the protein subcellular locations, 44.4% are located on the endoplasmic reticulum, and 22.2% are located on the endoplasmic reticulum and 22.2% of the mitochondria and the Golgi apparatus. This gene mainly functions on the endoplasmic reticulum, followed by functions on the mitochondria and the Golgi apparatus. It is a secreted protein. The Gm*PR1* protein contains a transmembrane structure. It is a transmembrane protein that migrates in the cell. Analysis of phosphorylation sites of *PR1* encoded protein: Gm*PR1* protein has 19 potential phosphorylation sites, including 10 serine (Ser) phosphorylation sites, 3 threonine (Thr) phosphorylation sites, and 6 tyrosines (Tyr) phosphorylation site. Gm*PR1* protein is composed of four parts: α-helix (32.76%), extended chain (17.82%), β-turn (5.17%), and random coils (44.25%). Gm*PR1* protein has the highest proportion of random coils. Gm*PR1* encodes a protein. The homology with *Arabidopsis thaliana* in the three-dimensional structure database is as high as 82.52%. Protein interaction analysis showed that Gm*PR1* protein played an important role and function in response to disease stress.

Authors Contribution: Huimin Cui is the executive of the experimental design and experimental research of this study; Huimin Cui and Shuo Qu completed the data analysis and the writing of the first draft of the paper; Shuo Qu, Abraham Lamboro and Yaolei Jiao participated in the experimental design, analyzed data, edit the final manuscript; Piwu Wang managed and guided the whole project. All authors have read and agreed to the final text.

Conflicts of Interest: The authors declare that they have no conflicts of interest to report regarding the present study.

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