Study on the relationship between the *hrpZ$_{psta}$* resistance gene expression in transgenic soybean and resistance to gray leaf spot disease

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Abstract. *HrpZ$_{psta}$* gene is a broad spectrum disease resistance gene to plant disease. We transferred it into soybean with the promoter of 35S mediated by Agrobacterium and got T_5 and T_6 transgenic soybean-lines. To study the relationship between *hrpZ$_{Psta}$* gene expression in transgenic soybean and the resistance to gray leaf spot disease, we investigated the *hrpZ$_{psta}$* gene expression level in T_5 and T_6 transgenic soybean lines of JN29-705-21 and -22 in different tissues by real-time quantitative polymerase chain reaction (qRT-PCR) technique and resistance to gray leaf spot disease by spraying the spore of leaf spot disease. The results showed that the *hrpZ$_{Psta}$* gene was expressed in soybean roots, stems, leaves and seeds, the average relative expression levels of T_5 two lines were 2.6/2.8, 0.7/0.6, 4.0/4.2, 0.7/0.8, respectively for T_6 were 1.4/1.5, 0.3/0.3, 2.3/2.8, 0.5/0.4; And the resistance level of JN29-705-21 and JN29-705-22 in T_5 and T_6 transgenic lines to gray leaf spot disease were both medium-resistant, whereas the nontransgenic line was susceptible, the disease level of T_5 and T_6 transgenic lines all decreased, the range was from 1-9 of the nontransgenic line to 0-7 of transgenic lines. Correlation analysis showed that the expression levels of *hrpZ$_{Psta}$* gene in leaves of each transgenic line in the two generations were very significantly and negatively correlated with the disease level, the correlation coefficient was -0.92/-0.95 in T_5 transgenic lines and -0.91/-0.97 in T_6 transgenic lines respectively, but insignificantly correlated with other tissues. It showed that the higher the target gene expression in leaves was, the lower the disease level was, and the stronger the disease resistant ability becomes. The result indicated the expression of target gene *hrpZ$_{Psta}$* was positively correlated with the resistance ability to gray leaf spot, it did improve the disease resistance of soybean plants.

Introduction

Soybean is an important cash crop in China, rich in nutrients, but soybean diseases seriously affected the soybean yield and quality of soybean, gray leaf spot disease is a worldwide disease, which brought more serious losses of soybean production, breeding for resistance to gray leaf spot of soybean varieties to become one of the effective measures to solve the bottleneck problem. *Hrp* gene which widespread in Gram-negative pathogens in plant is a broad spectrum disease resistance gene, it decided to pathogenicity of pathogenic bacteria to host plant and to induce hypersensitive response of non-host resistance plant[1-3]. The *hrpZ$_{Psta}$* gene from tobacco wildfire pathogenic bacteria and pseudomonas syringae *hrpZ* gene homology is 100%, which is capable of encoding non-specific exciton hrpin protein, inducing non-host plants hypersensitive response, stimulating the plant to produce the immune mechanism of nature, causing the infected plants produce active defense reaction, and making plants produce broad-spectrum resistance for subsequent infection and not have a negative impact on the growth and development of plants[4-7].

QRT-PCR technology as a new technology was introduced in 1996 by the US Applied Biosystems[8], a method refers to add a fluorophore in PCR reaction system, use accumulation of fluorescence signal to monitor the PCR process in real time, and finally to have a quantitative

analysis to unknown template through the standard curve[9], it has the advantages of high sensitivity, specificity and reliability, high degree of automation, less pollution, real-time, accuracy[10-11]. The qRT-PCR technique is mainly nonspecific dye binding and fluorescence labeled probes two ways, SYBR Green I is the most commonly used DNA binding dye for fluorescence quantitative PCR , which can specifically combine with the minor groove of DNA to emit fluorescence signal, through monitoring intensity of the fluorescence signal in real time during the experiment, to achieve accurate quantitation of the target gene[12-13]. At present, the technology has been widely used in many fields of bacteria[14,15], virus[16], transgenic[17-19] and so on[20], for the qualitative detection of agricultural genetically modified crops, strains identification and detection of transgenic component content[21].

HrpZ$_{Psta}$ resistance gene was transfered into soybean receptor species Jinong 29 in previous work of our laboratory , and has obtained T_5 and T_6 transgenic lines which can be inherited stably with *hrpZ*$_{Psta}$ gene. In this experiment, the amount of expression of *hrpZ*$_{Psta}$ gene in 2 lines different tissues of T_5 and T_6 transgenic soybean were measured by SYBR Green qRT-PCR technology, relative expression difference of *hrpZ*$_{Psta}$ gene were analyzed in different tissues of transgenic soybeans, and transformed lines were identified by artificial inoculation through the foliage spraying method to observe the performance of their resistance to gray leaf spot disease, and study the relationship between the expression of *hrpZ*$_{Psta}$ gene and identification results of resistance to gray leaf spot disease.

1 Materials and methods

1.1 Materials

1.1.1 Plant material

Soybean variety JN29 as the receptor for breeding obtained *hrpZ*$_{Psta}$ gene T_5, T_6 transgenic lines JN29-705-21 and JN29-705-22; Each line separately for 60 plants, were inoculated for identification, 15 lines selected randomly from which verified stably inherited were determined by qRT-PCR, materials provided by the Biotechnology Center of Jilin Agricultural University.

1.1.2 Pathogenic bacteria

Cercosporidium sojinum CsJ-1, provided by the Department of Pathology, Jilin Agricultural University College of Agriculture.

1.2 Method

1.2.1 PCR identification of *hrpZ*$_{Psta}$ gene

Genomic DNA was extracted from T_5, T_6 JN29-705-21, JN29-705-22 transformed plants and control plants. Corresponding specific primers (*hrpZ*$_{Psta}$-S: 5'- ATGCAG AGTCTCAGTCTTAAC -3'; *hrpZ*$_{Psta}$-AS:5'- TCA CCA TTG GAA TTG CTG TTG -3 ') was designed according to *hrpZ*$_{Psta}$ gene sequence , the reaction conditions used were as follows: predegeneration at 94 °C for 5 min, degeneration at 94 °C for 40 s, annealing at 55 °C for 40 s, extension at 72 °C for 40 s, with 35 cycles; and another extension at 72 °C for 8 min for PCR amplification of *hrpZ*$_{Psta}$ gene.

1.2.2 Southern blotting detection of genetically modified soybean

Genomic DNA was extracted from T_5, T_6 transgenic plant leaves with positive PCR detection, with endonuclease Hind III restriction and DIG DNA Labeling and Detection Kit I (Roche products), southern blotting was performed according to the instruction labeled probe, template DNA by enzyme digestion, electrophoresis separation, transfer film, hybridization,washing the membrane, color and othersteps, which using the principle of complementary base pairing[22], and using DNA as a probe to detect DNA chains to verify integration of *hrpZ*$_{Psta}$ gene in the plant genome.

1.2.3 Determination of *hrpZ*$_{Psta}$ gene expression

1.2.3.1 Extraction of RNA and Synthesis of cDNA .

Total RNA was extracted from roots, stems, leaves,seeds of positive plants and control plants; RNA as the template, TaKaRa company's PrimeScript^{TM}RT Master Mix reverse transcription kit was used to make total RNA reverse transcript into the corresponding cDNA, and concentrations were determined by protein nucleic acid detection instrument.

1.2.3.2 Design of quantitative PCR primer

*TUB*4 (Genbank no: EV263740) gene was selected as a reference gene in test , *TUB*4 gene and *hrpZ*$_{Psta}$ gene specific primers (Table 1) were designed by the software of Primer Premier 5.0, which synthesized by Beijing Sunbiotech Co. Ltd.

Table 1 Primer pairs for real-time quantitative PCR

Gene	Name	Sequence(5′ →3′)
*TUB*4	Q*TUB*4	GGCGTCCACATTCATTGGA
*TUB*4	QA*TUB*4	CCGGTGTACCAATGCAAGAA
hrpZ$_{Psta}$	Q *HrpZ*$_{Psta}$	GACTTGATGACACAGGTG
hrpZ$_{Psta}$	QA *HrpZ*$_{Psta}$	ACCATTGGAATTGCTGTT

1.2.3.3 Reaction system and condition

The PCR reaction system (20μL) comprised the following: 10 μL of SYBR® Green Real time PCR Master Mix; 2 μL of template cDNA (2mg/μL); 0.4 μL of each primer (20umol/L); 7.2 μL of ddH2O; mRNA detected in each template repeated 3 times by qRT-PCR, reference gene repeated 1 times. Amplification conditions were as follows: predegeneration at 95 °C for 30 s, degeneration at 95 °C for 5 s, annealing at 55 °C for 30 s, extension at 72 °C for 30 s, with 40 cycles.

1.2.3.4 Calculation of quantitative results

The relative quantitative method is to compare differences in expression of target gene transcription between the experimental group and control group samples[23]. $2^{-\triangle\triangle Ct}$ method was selected in this experiment .

Formula: ① foldchange＝$2^{-\Delta\Delta Ct}$; ②Δ Δ Ct＝（Ct target gene -Ct reference gene) $_{\text{test group}}$ - (Ct target gene -Ct reference gene) $_{\text{control group}}$ [24-25].

1.2.4 Identification of resistance to gray leaf spot disease in transgenic soybean

Foliar spraying method was selected to infect soybean by Gray Leaf Spot Pathogen , investigation after inoculation 15 days, identification according to the method of "NY/T 495-2002 soybean frogeye leaf spot identification technical specifications", specific resistance evaluation(Table 2, 3).

Table 2 Disease-level classification of soybean resistance identification to gray leaf spot disease

Disease levels	Symptom description
0	Plants without spot.
1	Only a few leaf disease, lesion 2 mm below, lesion area below 1% .
3	The most plant minority leaf disease, lesion diameter 2 mm, lesion area 1% ~ 5%..
5	Most plant disease, lesion diameter 2mm or more medium-sized spots, lesion area 6% ~ 20%.
7	The leaves of the plants common disease, lesion more, lesion diameter ranged from 3 to 6 mm, lesion area 21% ~ 50%.
9	Plants were generally incidence, leaves covered with lesions, sometimes contiguous lesion, lesion accounted for more than 50% of the leaf area.

$$\text{The disease index} = \frac{\Sigma(\text{Incidence level representative value} \times \text{number of the level disease})}{\text{Total number of investigation} \times \text{highest incidence level representative value}} \times 100$$

Type: Σ is the sum of all levels of product value.

Table 3 Standard of soybean resistant evaluation for Frogeye leaf spot

Disease Index	Evaluation of resistance
0	IM
≤2.0	HR
2.1～15.0	R
15.1～40.0	MR
40.1～60.0	MS
60.1～80.0	S
≥80.1	HS

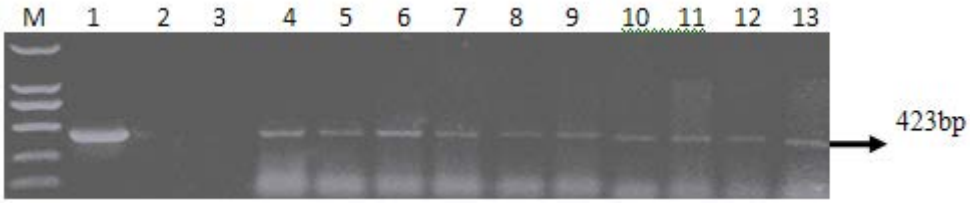
1.2.5 Correlation analysis between expression and identification results of resistance to gray leaf spot disease

Correlation was analyzed between expression in different tissues of the transgenic lines and gray leaf spot disease level to study the relationship between the expression of *hrpZ*$_{Psta}$ gene in different tissues and the resistance ability to gray leaf spot.

2. Results and Analysis

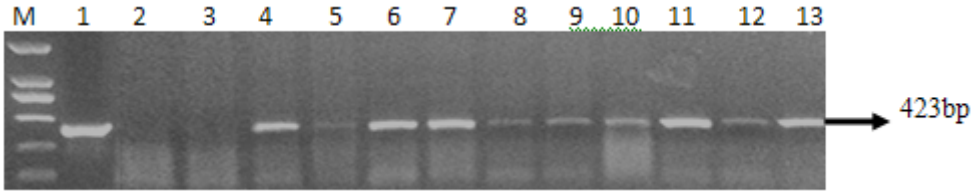
2.1 PCR identification of *hrpZ*$_{Psta}$ gene

T_5, T_6 transgenic lines JN29-705-21 and JN29-705-22 were detected by PCR, the results in Figure 1, figure 2, showed the same specific band as the location 423bp of positive control, initially proved stable inheritance of *hrpZ*$_{Psta}$ gene in T_5, T_6 transgenic lines.



Note: M: DNA Marker DL-2000; 1: Positive control; 2: Negative control; 3: H_2O; 4-8: T_5 JN29-705-21; 9-13: T_5 JN29-705-22

Fig. 1 PCR detection of T_5 transgenic soybean



Note: M: DNA Marker DL-2000; 1: Positive control; 2: Negative control; 3: H_2O; 4-8: T_6 JN29-705-21; 9-13: T_6 JN29-705-22

Fig. 2 PCR detection of T_6 transgenic soybean

2.2 Southern blot detection of transgenic soybean

Genomic DNA was extracted from PCR positive plants, 1301-*hrpZ*$_{psta}$ recombinant plasmid was selected as a positive control, untransformed receptor plants as a negative control, the DNA were digested by restriction endonuclease Hind III, and *hrpZ*$_{psta}$ integration condition in the plant genome was detected through the Southern blot method, results shown in Figure 3,4, no hybridization signals appeared on non-transformed plants, but obvious hybridization signals appeared on the 4 transgenic plants, and as a single copy form integrated in the soybean genome, but integration sites were not the same, which proved *hrpZ*$_{psta}$ gene can be stably inherited in T_5, T_6 transgenic plants.

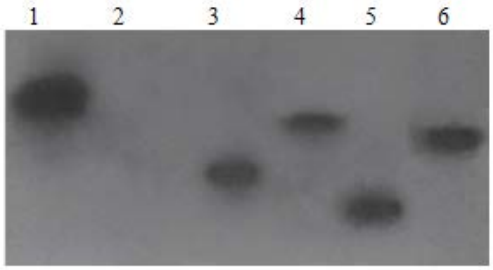


Figure 3 Southern blot detection for T_5 strains 1: Positive control; 2: Negative control; 3-6: T_5 transgenic plants

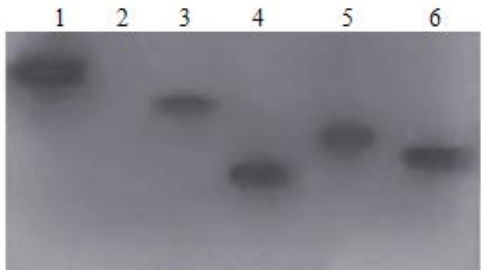


Figure 4 Southern blot detection for T6 strains 1: Positive control; 2: Negative control; 3-6: T6 transgenic plants

2.3 Expression analysis of *hrpZ*$_{Psta}$ gene

2.3.1 Melting curve analysis

Target gene and reference gene melting curve (Figure 5) which got from SYBR Green Ⅰ real-time quantitative PCR of experimental group and the control group showed a single peak and no other peak, non-specific double stranded DNA products or primer dimers did not appear in PCR process, it indicated showed a good specificity of amplification product[26]. The amplification curve (Figure 6) showed the trend was normal, inflection point clear, overall parallel also good, and baseline level without rising phenomenon, which suggested that the amplification efficiency of target gene was consistent with that of reference gene, and accorded with the relative quantitative conditions under the situation of no making standard curve[27].

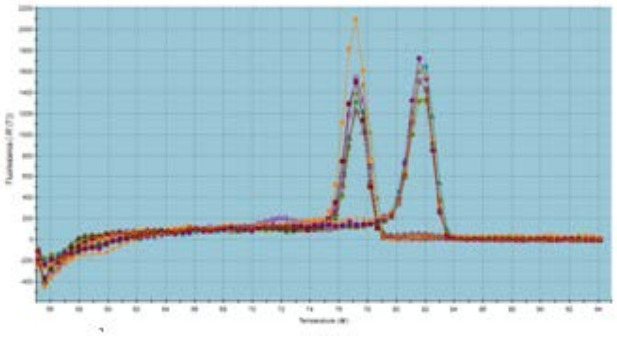


Fig.5 Melting curve

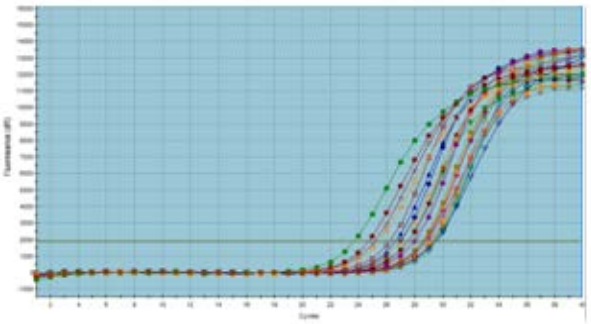


Fig.6 Amplification curve

2.3.2 Expression analysis of *hrpZ*$_{Psta}$ gene in transgenic plant roots, stems, leaves, seeds

Took the qRT-PCR results Ct values into the formula 2-△△Ct, which showed that: hrpZPsta gene expression in nontransgenic lines was close to 0, but hrpZPsta gene was expressed in the roots, stems, leaves and seeds of transgenic lines JN29-705-21 and JN29-705-22, and it got the highest expression in leaf, the average expression of JN29-705-21 on T5 was 4.0, while the average expression of JN29-705-22 was 4.2; The average expression of JN29-705-21 on T6 was 2.3, while the average expression of JN29-705-22 was 2.8; secondly, higher expression were in roots, where the average expression of JN29-705-21 on T5 was 2.6, and the average expression of JN29-705-22 was 2.8; The average expression of JN29-705-21 on T6 was 1.4, while the average expression of JN29-705-22 was 1.5; Lower expression were in the stems and grains, the average expression of JN29-705-21 on T5 in the stems was 0.7, JN29-705-22 was 0.6; JN29-705-21 on T6 was 0.3, JN29-705-22 was 0.3; And average expression in grains of JN29 -705-21 on T5 was 0.7, JN29-705-22 was 0.8, JN29-705-21 on T6 was 0.5, JN29-705-22 was 0.4. HrpZPsta gene expression existed obvious difference in different tissues, but also not the same expression in the same tissues in different generations and different lines, shown in Figure 7,8.

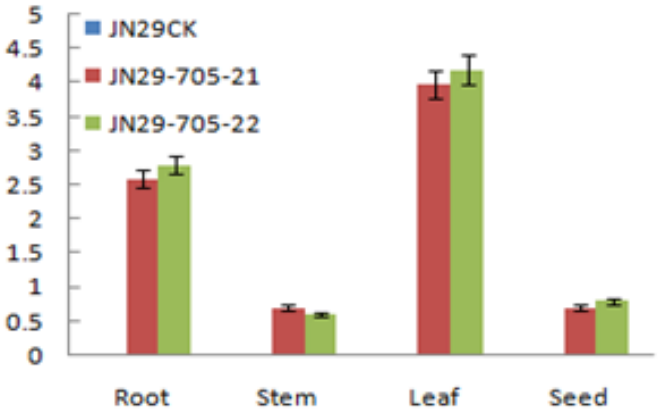


Fig. 7 *HrpZ*$_{Psta}$ gene relative expression results in different tissues of T_5 JN29

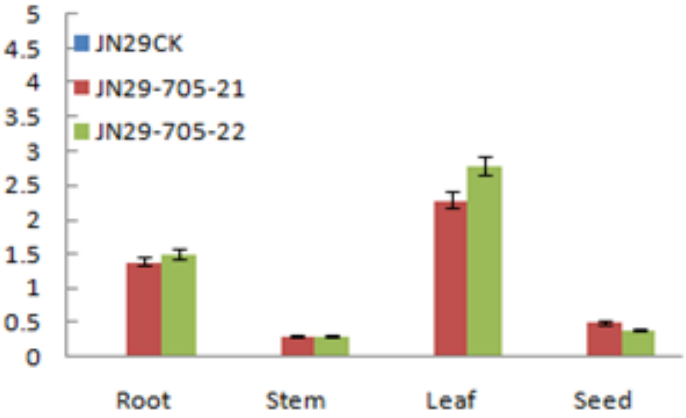


Fig. 8 *HrpZ*$_{Psta}$ gene relative expression results in different tissues of T_6 JN29

2.4 Results analysis of resistance test

2.4.1 Identification of gray leaf spot disease

After inoculation test on T5, T6 transgenic lines JN29-705-21 and JN29-705-22, receptor control and transgenic lines showed varying degrees of susceptibility phenomenon, effect shown in figure 9.

Note: A:JN29-705-21 of T_5 Gray leaf spot disease; B: JN29-705-22 of T_5 Gray leaf spot disease; C:JN29-705-21 of T_6 Gray leaf spot disease; D: JN29-705-22 of T_6 Gray leaf spot disease;

Fig. 9 T_5、T_6 transgenic plants with gray leaf spot inoculation experimental identification

The survey data on T_5, T_6 plants of gray leaf spot pathogen was counted according to the statistical method from “soybean frogeye leaf spot identification technical specifications”, the disease levels of transgenic plants and receptor control plant were shown in Table 4,5, and the identification results were shown in Table 6, 7:

Table 4 Disease-level and expression situation of T_5 transgenic plants

Line	Number	Disease level		Relative expression		Line	Number	Disease level		Relative expression	
		Test group	Control group	Test group	Control group			Test group	Control group	Test group	Control group
JN29-705-21	1	5	7	2.69	0.019	JN29-705-22	1	7	9	2.36	0.019
	2	1	3	5.04	0.019		2	3	5	4.87	0.019
	3	5	7	3.37	0.019		3	3	7	4.33	0.019
	4	3	5	4.36	0.019		4	3	7	4.06	0.019
	5	7	9	2.27	0.019		5	1	3	5.34	0.019
	6	1	3	4.85	0.019		6	1	5	5.26	0.019
	7	5	7	2.51	0.019		7	0	1	5.87	0.019
	8	3	7	3.41	0.019		8	5	9	2.97	0.019
	9	1	3	4.71	0.019		9	5	7	3.58	0.019
	10	0	1	5.92	0.019		10	3	5	3.95	0.019
	11	0	3	5.67	0.019		11	0	3	5.73	0.019
	12	3	7	4.01	0.019		12	5	7	2.91	0.019
	13	3	5	3.3	0.019		13	3	5	3.72	0.019
	14	3	7	3.05	0.019		14	3	7	3.55	0.019
	15	1	3	4.46	0.019		15	1	5	5.15	0.019
	Average	3	5	3.97	0.019		Average	3	6	4.24	0.019

Table 5 Disease-level and expression situation of T_6 transgenic plants

Line	Number	Disease level		Relative expression		Line	Number	Disease level		Relative expression	
		Test group	Control group	Test group	Control group			Test group	Control group	Test group	Control group
JN29-705-21	1	5	7	2.39	0.013	JN29-705-22	1	7	9	1.66	0.013
	2	5	7	2.12	0.013		2	3	5	3.22	0.013
	3	5	7	2	0.013		3	1	5	3.37	0.013
	4	3	5	2.52	0.013		4	3	5	3.18	0.013
	5	1	3	2.91	0.013		5	5	7	1.97	0.013
	6	1	3	3.16	0.013		6	3	5	2.7	0.013
	7	5	7	1.87	0.013		7	0	3	4.03	0.013
	8	3	5	2.11	0.013		8	5	9	2.09	0.013
	9	0	1	3.83	0.013		9	3	5	2.94	0.013
	10	0	3	3.61	0.013		10	3	7	2.76	0.013
	11	7	9	1.77	0.013		11	3	5	2.81	0.013
	12	3	5	2.33	0.013		12	0	1	3.75	0.013
	13	5	7	2.04	0.013		13	5	7	2.26	0.013
	14	5	7	1.8	0.013		14	5	9	2.34	0.013
	15	3	5	2.18	0.013		15	1	7	3.49	0.013
	Average	3	5	2.44	0.013		Average	3	6	2.83	0.013

Table 6 Identification results on resistance to gray leaf spot disease of T_5 plants

Strain	total number	Disease level						disease index	resistance evaluation
		0	1	3	5	7	9		
JN29 receptor	60	0	2	7	9	37	5	68.89%	S
JN29-705-21	60	6	30	17	7	0	0	38.67%	MR
JN29 receptor	60	0	3	7	7	36	7	69.26%	S
JN29-705-22	60	7	29	16	8	0	0	39.00%	MR

Obvious lesion appeared on the receptor plant leaves(Figure 9), and the lesion appeared on transgenic plants leaf was relatively less, which proved that the resistance ability to gray leaf spot disease of transgenic plant offspring improved more than that of receptor plants. Meanwhile shown in Table 4,5, compared with the receptor, the disease levels of T_5, T_6 transgenic lines JN29-705-21 and JN29-705-22 significantly reduced, and the disease index also decreased significantly. JN29-705-21 of T_5 decreased from 68.89% to 38.67%, JN29-705-22 decreased from 69.26% to 39%; While JN29-705-21 of T_6 decreased from 69.26% to 39.33%, JN29-705-22 decreased from 69.62% to 39.67%; The resistant evaluation of transgenic lines all improved, JN29-705-21 and JN29-705-22 both increased from susceptible to anti, which showed that to transfer $hrpZ_{Psta}$ resistance gene into plants effectively improve the ability of resistance to gray leaf spot disease.

Table 7 Identification results on resistance to gray leaf spot disease of T_6 plants

Strain	total number	Disease level						disease index	resistance evaluation
		0	1	3	5	7	9		
JN29 receptor	60	0	3	6	9	35	7	69.26%	S
JN29-705-21	60	6	30	16	8	0	0	39.33%	MR
JN29 receptor	60	0	2	7	9	35	7	69.62%	S
JN29-705-22	60	5	31	16	8	0	0	39.67%	MR

2.5 Correlation analysis between expression of $hrpZ_{Psta}$ gene and disease levels of gray leaf spot disease

Correlation was analyzed between expression of $hrpZ_{Psta}$ gene in different tissues of T_5,T_6 transgenic lines and disease levels of gray leaf spot disease, the expression of $hrpZ_{Psta}$ gene in leaves were very significantly and negatively correlated with the disease level of gray leaf spot disease（$P < 0.01$）, the correlation coefficient was -0.92/-0.95 in T_5 transgenic lines and

-0.91/-0.97 in T_6 transgenic lines respectively, but insignificantly correlated with other tissues，shown in Table 8, 9. It showed that the higher the target gene expression in the leaves was, the lower the disease level was, and the stronger the disease resistant ability becomes. The result indicated the expression of *hrpZ*$_{Psta}$ gene in leaves of T_5,T_6 transgenic lines was positively correlated with the resistance ability to gray leaf spot disease, the *hrpZ*$_{Psta}$ gene which transfered into plants did improve the resistance ability to gray leaf spot disease of soybean plants.

Table 8 Correlation analysis between *hrpZ*$_{Psta}$ gene expression in different organization of T_5 each strain and disease levels

Strain		Leaves	Stems	Roots	Seeds
JN29-750-21	Stems	0.06			
	Roots	-0.12	0.18		
	Seeds	-0.44	-0.36	-0.28	
	Disease Level	-0.92**	-0.06	0.01	0.4
JN29-750-22	Stems	-0.28			
	Roots	0.08	-0.1		
	Seeds	-0.3	-0.33	-0.01	
	Disease Level	-0.95**	0.27	-0.18	0.37

Table 9 Correlation analysis between *hrpZ*$_{Psta}$ gene expression in different organization of T_6 each strain and disease levels

Strain		Leaves	Stems	Roots	Seeds
JN29-750-21	Stems	-0.13			
	Roots	0.32	-0.17		
	Seeds	-0.67	0.43	-0.39	
	Disease Level	-0.91**	-0.09	0.04	0.3
JN29-750-22	Stems	-0.36			
	Roots	0.11	0.31		
	Seeds	-0.23	-0.46	-0.07	
	Disease Level	-0.97**	0.19	0.25	0.41

*and**means significantly related at 0.05 and 0.01 probability level respectively.

3 Discussion and Conclusions

HrpZ$_{Psta}$ which from tobacco wildfire pathogenic bacteria is a functional gene with broad-spectrum disease resistance, it is capable of encoding non-specific exciton hrpin protein, inducing non-host plants hypersensitive response, causing the infected plants produceactive defense reaction, and making plants produce broad-spectrum resistance for subsequent infection[28-29]. In this study , *hrpZ*$_{Psta}$ resistance gene T_5 and T_6 transgenic lines were experiment materials, the PCR and Southern blot detection results showed that the target gene *hrpZ*$_{Psta}$ has been successfully integrated into the soybean genome, with a single copy integration and different integration sites; Determination of *hrpZ*$_{Psta}$ gene relative expression in soybean organizations by qRT-PCR technology, found the relatively expression content of *hrpZ*$_{Psta}$ gene in leaves and roots was higher, lower in stems and seeds，which indicated that the exogenous gene *hrpZ*$_{Psta}$ was expressed at the transcriptional level; Meanwhile the results of resistance identification showed that the expression of disease resistance gene *hrpZ*$_{Psta}$ improved the resistance to gray leaf spot disease of soybean plants, the quantitative relationship between the target gene expression and disease resistance changes was initially established, and it provide a strong reference basis for next analysis of the expression of exogenous gene in other transformed soybean.

QRT-PCR technique was not only quickly accepted by the people for the study of conventional quantitative analysis of target gene expression at the level of RNA [30], also has been widely used for the detection of genetically modified organisms copy number. In 2001, Ingham et al.[31] found certain differences in transgenic plants copy number by

using Southern blot and qRT-PCR technique; In 2002, P. Song et al.[32] detected the copy number of exogenous gene in transgenic corn by Southern blot and qRT-PCR technique, found the less the copy number in transgenic corn was, the stronger the correlation between two methods became, but it has not yet been reported to determine exogenous gene expression in different tissues of transgenic soybean by southern blot technology combined with qRT-PCR technology for studying the relationship between the target gene expression and disease resistance changes. Although Liu et al.[33] analyzed the relative expression level on the exogenous gene Vitreoscilla hemoglobin gene (*vgb*) in transgenic cotton leaves by qRT-PCR technology, it was only limited to determine the target gene expression level in the leaves, not to verify the function performance of target gene . Du et al.[34] studied on the heredity, expression and disease resistance of the RDV movement protein deficient gene (RDV MP) in transgenic resistance to dwarf mosaic virus maize, which was only limited to determine the basic content of soluble protein by Coomassie brilliant blue method[35], a significant relationship was showed between RDV MP gene expression and disease resistance of transgenic plants according to estimation of difference analysis, but the target gene expression in different tissues of transgenic plants cannot be accurately measured.

HrpZ$_{Psta}$ gene has been expressed in the roots, stems, leaves and seeds of T_5,T_6 soybean transgenic lines JN29-705-21 and JN29-705-22 through the qRT-PCR technology, the average relative expression levels of T_5 two lines were 2.6/2.8, 0.7/0.6, 4.0/4.2, 0.7/0.8; Respectively for T_6 were 1.4/1.5, 0.3/0.3, 2.3/2.8, 0.5/0.4; Results of resistance identification showed the resistant ability to gray leaf spot disease improved more than that of receptor control plants, correlation analysis showed the expression of *hrpZ*$_{Psta}$ gene in leaves was very significantly and negatively correlated with the disease level of gray leaf spot disease, which indicated the higher the target gene expression in leaves was, the lower the disease level was, and the stronger the disease resistant ability becomes. Results showed expression of *hrpZ*$_{Psta}$ gene in leaves was positively correlated with resistance ability to gray leaf spot, to transfer *hrpZ*$_{Psta}$ resistance gene into plants effectively improved the resistance ability to gray leaf spot disease of soybean plants, it laid the foundation for cultivating the new soybean germplasm resources with good agronomic traits, significant broad-spectrum disease resistance and stable genetic resistance gene by using molecular biology method.

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