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Genetic Stability and Disease Resistance Analysis of *Hrpzpsta* Gene in Transgenic Soybean Lines

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Abstract

This experiment was carried out to evaluate genetic stability and disease resistance in transformed soybean lines with *hrpZpsta* gene using PCR analysis, southern blotting, real-time quantitative PCR (qRT-PCR) and to analyze the resistance against *Phytophthora sojae* (*P. sojae*) and *Cercospora sojina* (*C. sojina*) after inoculation. The results obtained using PCR and southern blotting analytical methods showed that exogenous gene functional elements were stably inherited in transgenic soybean and *hrpZpsta* gene was successfully integrated into the soybean genome in a single copy. Results at high-generation (T_7, T_8) transgenic lines of *hrpZpsta* revealed that their relative expression of *hrpZpsta* gene was the highest in leaves followed by roots, and much lower in stems, flowers, and seeds. Activity change rates of peroxidase (POD), polyphenol oxidase (PPO) and phenylalanine ammonia lyase (PAL) showed that transgenic lines significantly enhanced receptor species. The resistance of transgenic strains T_7 and T_8 generations against *P. sojae* was significantly increased with artificial inoculation methods, and the resistance against *C. sojina* was increased from susceptibility to the level of resistance. Under natural conditions in the field, the response of T_8 transgenic lines to *C. sojina* reached disease resistance level. There were no significant differences in transgenic lines and recipient variety in maturing stage, leaf shape, flower color, plant height, 100-grain weight and quality content, and the two years average yield of plots increased to 11.59% and 8.19%, which significantly higher than recipient cultivar. The current results provide data support for the release of transgenic lines.

Keywords: disease resistance, *hrpZpsta* gene, genetic transformation, soybean

1. Introduction

Soybean (*Glycine max* L.) is a major food and oilseed crop worldwide and provides the largest source of vegetable oil and protein for humans and animals (Li, Zhang, Hao, Hua, Duan, Zhang, & Li, (2013). Its fungal disease is currently a major hurdle restricting the quality of soybean and causes damage to the crop's yield (Li, Luan, & Liu, 2015). However, the complex physiology of pathogenic bacteria makes it difficult to breed a resistant plant to break through the problem (Dorrance, Jia, & Abney, 2004; Kim, et al., 2013), and conventional breeding methods have limitations which make it difficult to fulfill the production needs (Molinar, 2012). Therefore, a combination of biotechnology and traditional breeding methods provides a new way to breed for disease resistant varieties and the renewal of germ plasm resources (Kamthan, Chaudhuri, Kamthan, & Datta, 2016).

Harpin protein is a class of nonspecific protein elicitors encoded by the *hrp* gene in gram-negative plant pathogenic bacteria which can: excite hypersensitive reaction (HR) in plants; make plants gain broad spectrum disease resistance (Alfano, & Collmer, 1997; Strobel, Ji, Goplan, Kuc, & He, 1996); can be induced by different signaling pathways to produce disease, insect and drought resistance and promote plant growth and other beneficial effects (He, Huang, Collmer, 1993; Pandey et al., 2005). Furthermore, it has important theoretical and practical values in the production of better application prospects. The gene encoding harpin protein was transferred into rapeseed (Ma et al., 2008), rice (Cheng & Xu, 2008), and wheat (Fu et al., 2014), all of which showed significant disease resistance. Studies have shown that harpin protein can induce resistance to 60 kinds of diseases in more than 40 kinds of crops. In addition, the physiological and biochemical mechanisms of resistance in harpin protein shows that it could induce the expression of POD, PPO, PAL and other protective enzymes in tomato, cucumber and eggplant, (Yuan & Meng, 2008), and as a consequence, induce plant defense

response and enhance plant resistance to diseases. In transgenic tobacco, hpa1Xoo can increase the POD, PPO, PAL and other defense enzymes, and enhance resistance to tobacco mosaic virus (Han & Chang, 2013).

The *hrpZpsta* gene derived from tobacco wildfire pathogen, is a broad-spectrum resistance gene which encodes the harpin protein and effectively stimulates the soybean plant to produce disease defense response so as to effectively improve its disease resistance and reduce yield loss caused by soybean diseases (Dorrance, Jia, & Abney, 2004; Wu, Zhang, Zhang, Gu, & Gao, 2017). Bioinformatics analysis showed that the gene and its encoded protein are not toxic for humans and animals. In this study, high-generation (T_7, T_8) transgenic lines of *hrpZpsta* were used to test the genetic stability of transformed *hrpZpsta* gene and the resistance of the transgenic lines was analyzed. In order to improve the ability of soybean to resist gray leaf spot and *phytophthora* root rot, it aims to reduce the disease to improve the yield of soybean.

2. Materials and Methods

2.1 Materials and Experimental Design

2.1.1 Plant Materials and Strain

Transformed *hrpZpsta* gene stable lines JL30-187 and JL30-80 at T_7 to T_8, receptor cultivar named JL30 were provided by the Plant Biotechnology Center, Jilin Agricultural University, (Changchun, China). *P. sojae* PmC-1 and *C. sojae* CSJ-1 were provided by the Jilin Academy of Agricultural Sciences, (changchun, China).

2.1.2 Test Design

Plant material were planted the randomized block design (three replications) was used in the field trial, used for genetic stability analysis and field surveys. The experimental site is Jilin Agricultural University transgenic crop test base (Changchun, China). It is located in 125.13 degrees east longitude, Latitude 43 degrees 53 minutes north, 236.8 meters above sea level, The annual average temperature of 4.8℃, frost-free period of 148 days, accumulated temperature of more than 10℃ is 2921.8℃, Sunshine 2300-3000 hours per square centimeter annual radiation 120-130 kcal, Annual rainfall of 567 mm, The precipitation in the four seasons is the percentage of the year: the spring (3-5) is 11.4%; the summer (6-8) is 69.5%; the autumn (9-11) is 17.0%, the winter (12-2) is 2%. April average temperature of 7.2℃, July average temperature of 26.1℃, September 8.4 ℃, is a continental monsoon climate.

2.2 Genetic Stability Analysis of Target Genes

Transgenic plants at T_7 to T_8 were detected by PCR, Soybean Fresh leaves genome were collected from each of the transformed plants at 60 days after planting and DNA was extracted according to the manufacturer's instruction of NuClean Plant Genomic DNA kit (CWBIO, Beijing, China). Specific primers were designed to target these three genes *hrpZpsta*, *badh* and *35s-hrpZpsta-NOS was* designed with Primer 5.0 soft ware, and two pairs primers named QhrpZpsta, QActin were designed using the Primer 5.0 software to amplify *hrpZpsta* and *TUB4*(reference gene) for real-time quantitative PCR, respectively(Table 1).

PCR amplification system was 2.5uL loading buffer, 2.5 uL $MgCl_2$, 0.5uLdNTP, 1uL forward primer, 1uL reverse, 1uLDNA and 0.3uL Taq, add DDH_2O to 25uL. *hrpZpsta* PCR reaction conditions were pre-denaturation at 94℃for 3 min, followed by 36 cycles of 94℃for 40s, 54℃for 40s, 72℃for 40s, and 72℃for 8 min. PCR conditions for the *badh* were pre-denaturation at 94℃for 3 minutes, followed by 40 cycles of 94℃for 50s, 54℃ for 50s, 72℃for 50s, and 72℃for 8 min. and *35s-hrpZpsta-NOS* genes were similar to those of *hrpZpsta* except that 56℃annealing temperatures were used.

Table1. Primer sequence of PCR and qRT-PCR

Primer name	Forward Primer sequence（from 5′ to 3′）	Reverse Primer sequence（3′ to 5′）
hrpZpsta	ATGCAGAGTCTCAGCTTAAC	TCACCATTGGAATTGCTGTTG
35S-hrpZpsta-NOS	TTCAGAAAGAATGCTAACCCACAG	TGCGGGACTCTAATCATAAAAACC
BADH	TGTCGATCCCTATACCTTC	TTAAGGAGACTTGTACCAC
QhrpZpsta	GACTTGATGACACAGGTG	ACCATTGGAATTGCTGTT
QTUB4	GGCGTCCACATTCATTGGA	CCGGTGTACCAATGCAAGAA

According to the experimental PCR results, from the genome extracted from T_7 to T_8 soybean leaves, the target gene and the marker probe were labeled according to DIG High Prime DNA Labeling and Detection Starter Kit (Roche, America). The genomic DNA was digested with restriction endonuclease for 20 hours. The recombinant

plasmid digest was separated on 0.8% agarose gel electrophoresis. The digest was then denatured to enable transfer onto a membrane for further analysis.

RNA was extracted from different tissues (roots, stems, leaves, flowers and seeds) of the plant at anthesis using RNAiso Plus kit (Takara), with three replicates. Using the above RNA as a template, reverse transcription into cDNA was made, and the specific primers were designed with primer 5.0 for the *hrpZpsta* gene sequence and soybean TUB4 (GeneBank No.EV263740) as shown in Table 1. The relative expression was analyzed using Mx3000P instrument (America), according to manufacturer's instructions for which each sample mRNA were detected three times. The relative quantitative calculation was performed by $2^{-\Delta CT}$ method. CDNA reverse transcription and real-time PCR analysis were performed according to the instructions of All-in-One TM First-strand cDNA Synthesis Kit (GeneCopoeia, beijing China) and All-in-One TM qPCR Mix (GeneCopoeia, beijing China).

2.3 Enzyme Activity Determination for Resistance against P. Sojae

In order to eliminate the effects of temperature and humidity on enzyme activity, two sets of parallel experiments were conducted, transgenic lines at T_7 generation and non-transgenic receptors were planted at pots, three strains were planted per barrel, each treated three timesa total. After the opposite leaf of soybean plant was completely grown, the hypocotyls were used to induce the test material. one group was inoculated and the other group was not. Leaves were taken at 0h, 24h, 48h, 72h, 96h and 120h after inoculation. Samples were frozen using liquid nitrogen and placed in -80 ℃ refrigerator for preservation.

Leaves picked at different times to weigh 0.2g, and 5 ml of sample extract (boric acid buffer pH 8.8, 5 mmol/L mercaptoethanol, 1 mmol/L EDTA and 5% glycerol) was added to make the homogenate at 6×104r/min, 4 ℃ and centrifuged for 20 min. The supernatant was extracted for enzyme solution and placed in 4 ℃refrigerator and then centrifuged again for 20 min. POD, PPO and PAL activities were determined as previously described (Cheng & Xu 2013; Xu & Chang, 2014; Zhang, Xu, Wu, & Chen, 2008). In order to eliminate the background enzyme activity of each strain, the change rate of enzyme activity was taken as the standard.

Change rate of enzyme activity % = (A1-A0 / A0) * 100

where A_1: vaccinated leaves activity.

A_0: unvaccinated leaves activity.

2.4 Disease Resistance Analysis against P. Sojae and C. Sojae

T_7 and T_8 soybeans were tested for *P. sojae* PmC-1, the pathogenic strain of *P. sojae*, by hypocotyl staining method after the soybean leaves were completely grown. After inoculation, keep the wound humidity, shed temperature control in 20-25 ℃, humidity maintained at 90% or more. Investigation and statistics of transgenic lines and control receptor varieties Vaccination rate of *Phytophthora* infestans at five days after inoculation. Resistance evaluation criteria were: resistant (R)- plant mortality less than 30 %; moderately resistant (MR)-mortality rate from 31 % to 69 %; susceptible (S)- plant mortality rate of 70 % or more.

In the flowering stage of soybean to the early flowering stage of inoculation, the foliar leaching method was used to test the inoculation test of T_7 transgenic lines and acceptor varieties。After the inoculation, the humidity was 80% -100%, the control temperature was about 25-27℃, and the disease was investigated after 15 days. The incidence and incidence of the whole soybean were counted. To evaluate the resistance to gray leaf spot at field natural environment, soybean plants at the T8generation at flowering stages were Investigation, using the diagonal 5 points sampling, each investigation 100 cases. Soybean resistance to gray spot disease identification of disease levels, the use of 1-9 ratio assessment, 1 represents no disease spots, 9 on behalf of the leaves there are many lesion area of more than 50%. Specific resistance evaluation criteria are: immune (IM), the disease index was 0; high resistance (HR), disease index of 2 and below; disease resistance (R), disease index was 2.1~15.0; moderately resistant (MR), disease index was 15.1~40.0; moderately susceptible (MS), disease index was 40.1~60; susceptible (S), disease index was 60.1~80; highly susceptible (HS), disease index of 80 or more.

2.5 Analysis of Agronomic Traits

During anthesis, the phenotypic characters of the plant material at T_7 to T_8 were investigated. After maturation, 10 plants were randomly selected from each plot to investigate the agronomic traits of yield, including growth period, leaf shape, color, coat color, plant height，node，100-see weight，branches, Pods，seed weight，plot yield. NIRS DS2500 (Denmark) was used to analyze the quality of plant material at T_8 generation. The yield of the plot was measured and the data were statistically analyzed by MS Exceland DPS data processing system.

3. Results and Analysis

3.1 Genetic Stability Analysis of Target Genes

The results of PCR detection of the target gene are shown in Fig. 1a. Target gene *hrpZpsta, 35S-hrpZpsta-NOS* expression full-length sequence screening marker, and gene *BADH* were detected in the T_7 and T_8 generation in transgenic soybean lines. The effect of exogenous inserts of different generations in transgenic soybeans was genetically stable.

The results of Southern blot analysis of *hrpZpsta* gene are shown in Fig. 1b. Hybridization bands were detected in T_7 and T_8 transgenic soybean lines, while the receptor was not detected in the control sample. The target gene *hrpZpsta* was integrated at different sites into the recipient genome in a single copy, andwas stably inherited in the transgenic lines.

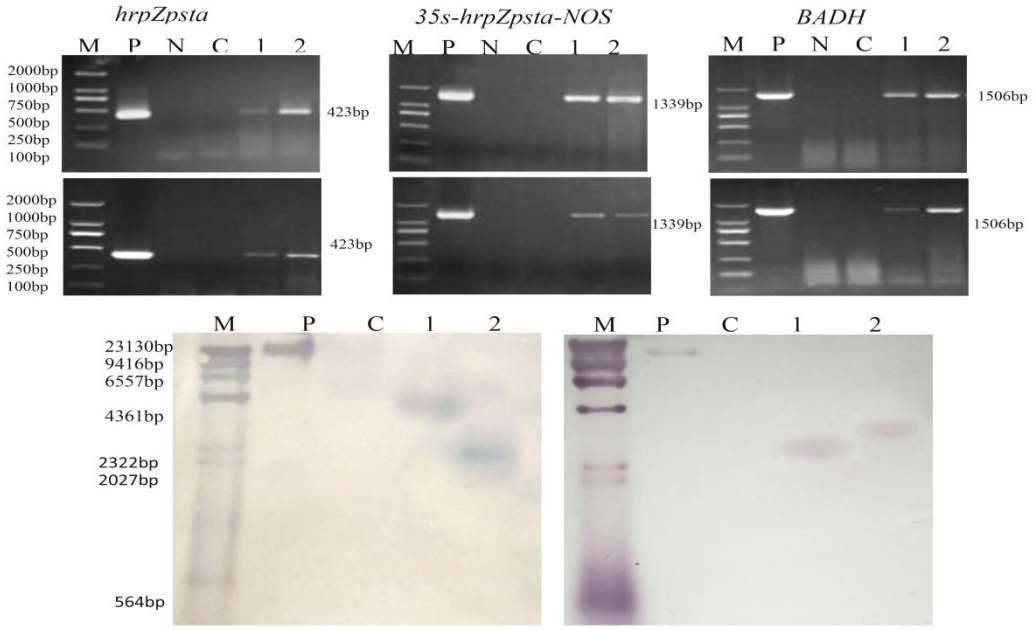

Figure 1. Genetic stability analysis of target genes

Note: (a): PCR analysis of T7 and T8 positive transgenic lines using *hrpZpsta*, 35s-*hrpZpsta*-NOS, and badh primer, respectively. (Note: M= DL2000 DNA marker, P= Positive control, N= Negative control, C= CK Jilin30, 1= Transformed Line JL30-187, 2= Transformed Line JL30-80). (b): Southern blot analysis of T7 and T8 transgenic plants (M=Southern DNA marker, P=Positive control, C= CKJilin30, 1-2= transgenic soybean plants).

The results showed that the target gene *hrpZpsta* was expressed in the roots, stems, leaves, flowers and grains of soybean (Fig. 1). The relative expression level of *hrpZpsta* gene in T7 generation transgenic soybean lines was the highest while the relative expression average of JL30-187 and JL30-80 were 8.477 and 6.971, respectively. In the roots, the average relative expression of JL30-187 and JL30-80 were 4.903 and 5.816, respectively. The expression levels of JL30-187 in stems, flowers and grains were lower at 1.423, 1.467 and 0.756, respectively, while the average expression levels were 0.686, 0.757 and 0.683, respectively for JL30-80. The relative expression level of *hrpZpsta* gene in T8 generation transgenic soybean lines was the highest with a relative expression average of 9.237 in JL30-187 and of 6.67 for JL30-80. In the roots, the average relative expression of JL30-187 and JL30-80 were 5.495 and 4.754, respectively. The expression levels in stems, flowers and grains were low. The results showed that the expression level of the target gene in different tissues was different. The gene of *hrpZpsta* and the transgenic materials could be stably expressed in different generations.

3.2 Enzyme Activity Determination against P. Sojae

The changes in enzyme activities of the transgenic line JL30-187 and receptor cultivar JL30 after inoculation with *P. sojae* are shown in Fig. 2. The change rates of POD, PPO and PAL activities in the leaves of the transgenic line showed no significant changes ($P < 0.01$) after 0 h and 120 h inoculation. However, 24 h inoculation resulted in a change rate in POD activity in the transgenic line, being181.39 % higher than in the recipient cultivar. After 48h inoculation, the rate of change in POD and PAL activities in the transgenic lines for the receptor species increased by 273.95 % and 464.99 %, respectively. After 72h inoculation, POD, PPO and

PAL activities in the leaves (transgenic lines) of receptor species increased to 156.52 %, 127.91 % and 106.99 %, respectively. The activity change rate in POD, PPO and PAL in transgenic lines increased to 163.76 %, 60.58 % and 27.51 %, respectively compared with the recipient cultivars after 96 h inoculation. The results showed that the activity of disease-resistant enzymes increased in the transgenic lines, which could be one of the causes of the resistance of the transgenic plants to infection by pathogenic fungi.

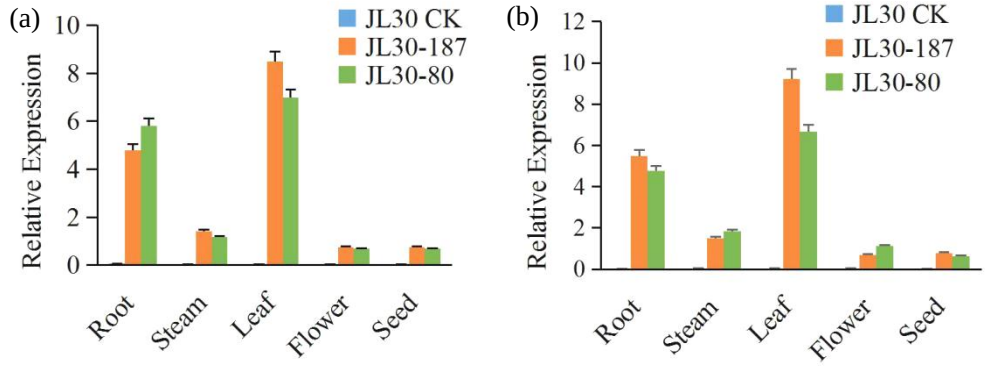

Figure 2. Analysis of target gene expression stability

Note: (a): T7 generation expression of *hrpZpsta* gene in different parts of plants. (b): T8 generation expression of *hrpZpsta* gene in different parts of plants.

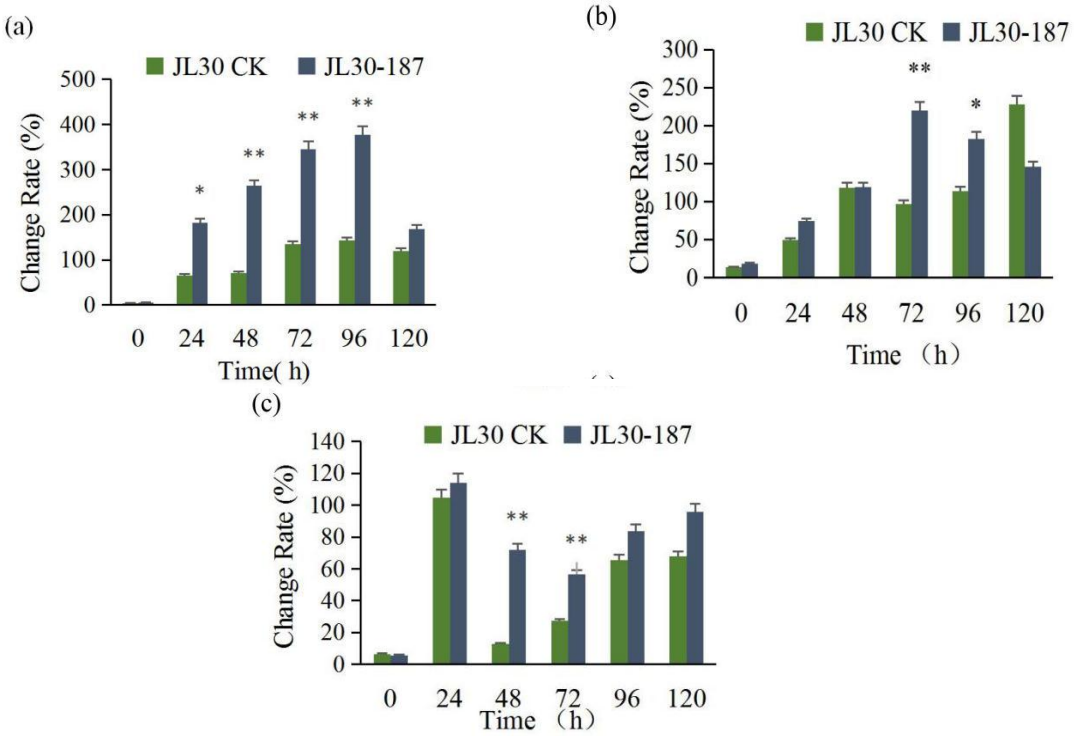

Figure 3. Changes in enzyme activity of leaves after inoculation with *P. sojae*

(a): POD activity change rate. (b): PPO activity change rate. (c): PAL activity change rate. * Indicates that the difference in treatment level was significant at 0.05 level ($p < 0.05$). ** Indicates that the difference in treatment level was significant at 0.01 level ($p < 0.01$).

3.3 Disease Resistance Analysis of P. Sojae and C. Sojina

Identification vaccination of *P. sojae* results are shown in Table 2 and Fig. 3. During the continuous 2-year comprehensive evaluation, JL30-187 and JL30-80 inoculation mortality rates were 6.94 % and 19.44 %, respectively, as all showed R. In the recipient variety, JL 30 inoculation mortality rate was 50.00 %, the performance of the MR. The resistance of the transgenic lines to *Phytophthora* root rot was evaluated from the MR to the R level.

Table 2. Identification vaccination *P. sojae*

Generation	Cultivar and lines	Detected No.	Alive No.	Death No.	Death rate (%)	Resistance reaction
	JL30 CK	18	9	9	50	MR
T_7	JL30-187	18	17	1	5.55	R
	JL30-80	18	15	3	16.67	R
	JL30 CK	36	15	15	50	MR
T_8	JL30-187	36	33	3	8.33	R
	JL30-80	36	28	8	22.22	R

Identification results of *C. sojina* are shown in Table 3. From Fig. 4, it can be seen that the lesion is obvious on the leaves of the recipient plant and the spots on the leaves of transgenic progeny are relatively few. Artificial inoculation results showed that T_7 transgenic lines JL30-187 and JL30-80 disease index was 16.94 % of which 16.42 % showed MR; receptor species JL30 disease index was 61.59 %, expressed as S. The resistance of the transgenic lines to gray leaf spot was improved from S to MR.

The results of field investigation showed that the disease index of JL30-187 was 13.93 % and JL30-80 was 14.60%, which indicated R. The disease index of JL30 was 60.29 % thus indicating S. Resistance evaluation of transgenic lines to gray leaf spot was improved from S to R level.

Table 3. Identification of Resistance to *C.sojina*

Method	Cultivar and line	Death rate (%)	Disease index	Resistance evaluation
Artificial vaccination (T7)	JL30-CK	96.67	61.59±2.35 A	S
	JL30-187	78.33	16.42±1.35 AB	MR
	JL30-80	76.67	17.33±2.15 AB	MR
Field investigation (T8)	JL30-CK	90.67	60.29±2.45 A	S
	JL30-187	49.67	13.93±1.65 AB	R
	JL30-80	50.33	14.60±3.25 AB	R

Note:The data in the table are mean ± standard deviation, Different lowercase letters indicate that the difference in treatment level is significant ($p < 0.01$).

3.4 Investigation of Agronomic Traits

The results of agronomic traits at T_7 and T_8 are shown in Table 4. The growth period of the transgenic lines were 132 days with the sharp leaves, gray hairs, white flowers and podding habits sub-limited and yellow-hued indicative of no significant differences from the recipient cultivar. Pod numbers and total grain weight were significantly different from those of recipients ($p < 0.5$). The yield of JL30-187 and JL30-80 increased 9.47% and 4.54%, respectively, compared to the control plot in 2015, and the yield of the plot increased by 13.46% and 11.83% in 2016.

2016 seed quality test results are shown in Table 5. Seed protein content of JL30-187 and JL30-80 were reduced by 3.59 % and 0.52 %, respectively, compared with JL30 and fat content decreased by 2.08 % and 3.45 %. There were no significant differences in the contents of amino acids and fatty acids between transgenic lines and recipient cultivar.

Figure 4. Identification of resistance against *P. Sojae*

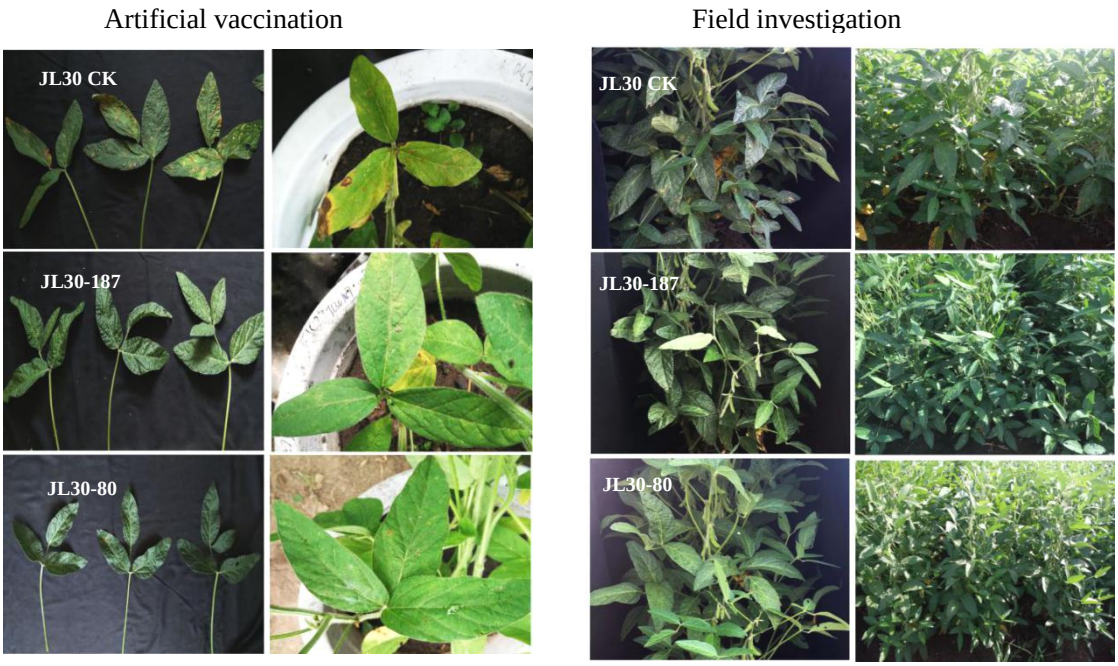

Figure 5. Identification of Resistance to *C.sojina*

Table 4. Investigation on Field Phenotypic Traits of transgenic lines

generation	Material	Plant height	Node	100-seed weight	Branches	Pods	Seed weight	Yield (㎡)	Increasing rate（%）
	JL30 CK	98.37±0.85a	17.6±0.08a	17.21±0.15a	1.90±0.21a	62.37±5.37a	29.35±2.61a	0.404±0.24a	
T_7	JL30-187	101.67±0.12a	17.67±0.12a	17.31±0.35a	2.23±0.40a	69.10±2.84a	32.71±0.98a	0.475±0.37a	9.71
	JL30-80	101.30±0.57a	17.87±0.05a	17.57±0.17a	2.5±0.08a	64.77±1.61a	31.13±0.98a	0.408±0.19a	4.54
	JL30 CK	89.57±0.26a	16.37±0.32a	19.36±0.05a	0.37±0.05a	29.7±1.65a	11.77±0.13a	0.362±0.63a	
T_8	JL30-187	92.03±0.37a	16.63±0.05a	19.31±0.06a	0.50±0.08a	40.60±1.13b	16.84±0.17b	0.418±0.56b	13.46
	JL30-80	93.10±0.45a	16.67±0.12a	19.84±0.03a	0.53±0.05a	37.90±2.11b	17.31±1.40b	0.412±0.49b	11.83

Note: The data in the table are mean ± standard deviation, Different lowercase letters indicate that the difference in treatment level is significant ($p < 0.05$).

Table 5. Seed quality analysis of T_8 transgenic lines

Quality / Cultivar	Protein	Fat	CYS	ARG	MET	PHE	C160	C180	C181	C182	C183
JL30 CK	40.08±0.31	21.13±0.33	0.53±0.00	3.01±0.05	0.45±0.00	2.08±0.02	9.34±0.11	3.25±0.13	12.63±0.33	62.21±0.27	4.33±0.21
JL30-187	38.64±0.72	20.69±0.11	0.52±0.00	2.88±0.08	0.42±0.03	2.05±0.03	9.18±0.13	3.35±0.21	12.86±0.79	61.60±0.53	4.36±0.27
JL30-80	39.87±0.40	20.4±0.16	0.53±0.00	2.97±0.02	0.44±0.01	2.09±0.00	9.04±0.11	3.28±0.11	12.32±0.51	62.39±0.21	4.10±0.16

Note: The data in the table are mean ± standard deviation.

4. Results and Discussion

The preliminary work of this experiment proved that T_1-T_6 generations of transgenic lines of the target gene can be stable (Zhang & Qu2011; Yin, Wang, Zhang, & Ma, 2013; Gu, Liu, & Wang, 2015). This study analyzes the genetic stability of target genes in T7, T8 transgenic lines; whether the presence of the primary PCR, the number of copies verified by Southern blotting or the expression of the transcriptional level detected by qRT-PCR confirmed that the transformed *hrpZpsta* gene was stably inherited and expressed in the recipient cultivar.

In recent years, a number of genetically modified soybean germ plasm have been obtained by genetic engineering. Zhou et al (2014) transferred *GmAKT2* gene into Williams82 to obtain transgenic material resistant to soybean mosaic virus disease. Fan et al (2012; 2015) cloned resistance genes SDR1 and Glym4l from anti-*Phytophthora sojae* variety Suinong 10, and transferred them into the susceptible cultivar Dongnong 50 to significantly increased its *Phytophthora* root rot capacity. Zhang, (2011) transferred *BnERF104* gene into Dongnong 50 and obtained significantly reduced lesion area of genetically modified soybean material. In this study, *hrpZpsta* gene was a broad-spectrum disease resistance gene, and its progenies were significantly improved in resistance to *Phytophthora* root rot and soybean gray leaf spot under indoor artificial inoculation conditions. The natural disease incidence of gray leaf spot disease in the field showed that the resistant ability of the transgenic lines reached the R level, which further proved the general resistance of the *hrpZpsta* gene.

Plant disease severity and disease resistance and plant defense enzyme activity changes are closely related (Qin, Sun, Zhao, Zhao, & Zhao,.2014; Liu et al., 2011). The results showed that the activities of POD, PPO and PAL in the transgenic lines increased rapidly after inoculation with *P. sojae*. The activity level was significantly higher than non-transgenic varieties, but the activity change rates of PAL, PPO and POD in the transgenic line was not significantly higher than in the untransformed variety which indicated that transformed *hrpZpsta* gene soybean had the ability to respond quickly to pathogen infection. This is consistent with reports of other plants on enzyme activity (Shi et al., 2014; Lei et al., 2010).

The results showed that there were no significant changes in the growth period, leaf shape, coat color, flower color, podding habit, hilum color, protein content and fat content of the transgenic lines.However, the number of pods per plant and plot yield were higher than that of the receptor control which may be associated with the insertion of the disease-resistance *hrpZpsta* gene as it reduces the risk of pathogens. Similar results have also been reported on other transgenic crops (Zhao, Zhu, Cai, Bai, & Ji, 2012; Guo, Zhu, Li, Bai, & Cai, 2008).

5. Conclusion

Molecular biology test, disease resistance analysis and agronomic traits of transgenic high-generation lines showed that transformed *hrpZpsta* gene has stable inheritance, and this significantly enhanced soybean root rot and gray leaf spot resistance. The main agronomic traits of the transgenic lines were not significantly different from recipient variety, but the number of pods per plant and plot yield were significantly higher than in the recipient variety. High-generation transgenic soybean lines showing resistance to *Phytophthora* root rot and *C. sojina* were obtained, which provides data support and theoretical basis for environmental release of transgenic lines.

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