check for updates

ARTICLE

Identification and Evaluation of Insect and Disease Resistance in Transgenic *Cry1Ab13-1* and *NPR1* Maize

Yongjing Xi, Zhou Yang, Yukun Jin, Jing Qu, Shuyan Guan, Siyan Liu and Piwu Wang[*]

Joint Laboratory of International Cooperation in Modern Agriculture Technology of Ministry of Education, Plant Biotechnology Center, Jilin Agricultural University, Changchun, China

[*]Corresponding Author: Piwu Wang. Email: peiwuw@163.com

ABSTRACT

PCR detection, quantitative real-time PCR (q-RTPCR), outdoor insect resistance, and disease resistance identification were carried out for the detection of genetic stability and disease resistance through generations (T_2, T_3, and T_4) in transgenic maize germplasms (S3002 and 349) containing the bivalent genes (insect resistance gene *Cry1Ab13-1* and disease resistance gene *NPR1*) and their corresponding wild type. Results indicated that the target genes *Cry1Ab13-1* and *NPR1* were successfully transferred into both germplasms through tested generations; q-PCR confirmed the expression of *Cry1Ab13-1* and *NPR1* genes in roots, stems, and leaves of tested maize plants. In addition, S3002 and 349 bivalent gene-transformed lines exhibited resistance to large leaf spots and corn borer in the field evaluation compared to the wild type. Our study confirmed that *Cry1Ab13-1* and *NPR1* bivalent genes enhanced the resistance against maize borer and large leaf spot disease and can stably inherit. These findings could be exploited for improving other cultivated maize varieties.

KEYWORDS

Maize; *NPR1* gene; *Cry1Ab13-1* gene; disease resistance; insect resistance

1 Introduction

As a food crop, maize (*Zea mays*) is crucial for the food security of the world, since it is a source of feed and energy [1,2]. Moreover, it has significantly increased the access of humankind to food. During the period from jointing to heading, if the rainfall is concentrated, the field humidity is high, and the temperature is suitable, it can cause an epidemic of diseases and insect pests, which will severely affect the yield and quality of corn. This will restrict the healthy development of the corn industry.

Corn borers belong to the order Lepidoptera, family *Moth*. There are mainly two kinds of corn border of Asian corn borer *Ostrinia furnacalis* (Guenée) and European corn borer *Ostrinia nubilalis* (Hubner) in the world. The Asian corn borer is widely distributed in the world, mainly in Southeast Asia, East Asia, India, Northeast Asia, Australia, and the Western Pacific Ocean; while the European corn borer is mainly distributed in Europe, North America and Northern Africa, and so on [3]. Spring maize in Northern China is mainly affected by the Asian corn borer. The surface of the earth has warmed by 0.74°C on average during the past 100 years as a result of the greenhouse effect [4]. High temperature prolongs the growth period of insects. With the continuous increase of temperature, the temperature is positively

correlated with the growth and development of insects. However, after the temperature exceeds the economic threshold, the temperature gets negatively correlated with the growth and development of insects [5,6]. The risk of harm to the Asian corn borer has significantly increased as a result of the rise in effective accumulated temperature brought on by global warming. The Asian corn borer found in the central region of Jilin Province has changed from having an incomplete second generation to having a full second generation every year. As a result, the problem of insect pests will become more and more serious, which will have a greater impact on the production of corn. Corn borer is a destructive pest of crops such as corn, sorghum, millet, and cotton. It is found throughout China, ranging from the southern tropical region to the northern freezing zone, and it may reproduce year-round at a rate of seven or more generations per year. Since the corn borer damages the plant by burrowing it as it begins to erode the corn plant, it will display a straight line of insect feeding holes and so on, posing a serious threat to the growth and development of the corn. Every year, the crop will experience significant harvest losses, with at least 10% yield loss in spring corn and 20%–30% loss in summer corn. In some instances, it may even exceed 30% [7]. The breeding of insect-resistant corn is a significant means of resolving the issue, because the corn borer poses a serious threat to the growth state of corn while affecting food security in China [8]. This results in an annual reduction of millions of tons of corn production, causing enormous economic losses to the corn production and farming industry of China, and severely restricting the economic development of the country [9,10].

Grey leaf spot (GLS) disease is one of the most prevalent ailments that affect maize during its growth and development, and it may be found everywhere where maize is cultivated. The GLS produces a productivity loss of approximately 30%, which poses a serious threat to the corn harvest [11]. Numerous variables such as disease resistance, crop rotation scheme, meteorological circumstances, and management practices of the inbred line have an impact on the prevalence of maize big patches. The large spot is most affected by them when it comes to temperature and humidity in the weather. Due to the cold temperatures and high air humidity in northern China, grey leaf Spot disease is more common. Generally, the relative humidity is above 80%, the daily temperature is 14°C~25°C, sunlight is available for less than 6 h, and continuous cloudy days are 5D~7D. These conditions will greatly increase the probability of grey leaf Spot disease [12]. After the maize tasseling, lesions initially appeared on the lower leaves. Later, the virus slowly spread to the upper leaves of the maize plant. When a large leaf spot occurs in maize, the symptoms are blue-gray water-soaked spots on the leaves. Later, the spots are longitudinally split, and several small spots are connected into larger and irregular mold spots.

Traditional chemical pesticide spraying and control not only pollutes the environment but is also time-consuming and labor-intensive. Moreover, they cannot solve the problem from the root cause. Discovering resistant resources and improving the understanding of corresponding defense mechanisms are particularly important for breeding resistant varieties, but traditional breeding techniques are time-consuming, time-consuming, and labor-intensive [13]. To open up a new and effective way to prevent and control these pests and diseases, and improve yield and quality, my country is also actively carrying out the development and application of genetic engineering in crops [14,15].

The utilization of genetically modified disease-resistant and insect-resistant varieties has become one of the most economical, effective, and environmentally friendly control methods [16]. The use of transgenic breeding technology to breed corn varieties with strong and durable resistance to corn borer and great leaf spot disease provides a powerful reference for molecular breeding of corn [17].

NPR1 gene was discovered from Arabidopsis mutants. *NPR*1 gene is a key gene in plant systemic acquired disease resistance (SAR) and plays an important role in regulating the broad-spectrum disease resistance of plants [18–20]. *NPR1* homologous gene exists widely in plants and has been studied as a disease resistance-related gene. It plays an important role in regulating salicylic acid signaling in plant

disease resistance and pathogenesis in Arabidopsis [21], and in wheat, (*Triticum aestivum*) [22], Rice (*Oryza Sativa*) [23], Barley (*Hordeum vulgare*) [24] have been reported in other species.

The *Cry1Ab13-1* gene is a member of the *Bacillus thuringiensis (Bt)* gene family. After spore formation, *Cry1Ab13-1* can produce proteins that are toxic to pests. These include Lepidoptera, Hymenoptera, Homoptera, Diptera, Coleoptera, etc. Foreign research on Bt gene insect-resistant maize started early [25], and GM Insect-Resistant Corn Begins Commercially in 1996 [26]. Many recent studies have found that Bt corn can reduce 99% of borer tunnels and 84% of leaf damage.

In the initial stage of this experiment, *Cry1Ab13-1* and *NPR1* pCAMBIA3301-Cry1Ab13-1-NPR1 genes were transformed into maize varieties S3002 and 349, which improved the disease resistance and insect resistance of maize. Therefore, this experiment was used to identify and evaluate the disease resistance and insect resistance of T_2, T_3, and T_4 of the transgenic *Cry1Ab13-1* and *NPR1* bivalent maize varieties S3002 and 349.

2 Materials and Methods

2.1 Test Materials

The tested varieties are S3002 (*Cry1Ab13-1+NPR1*), 349 (*Cry1Ab13-1+NPR1*), three generations of maize lines T_2, T_3, and T_4, and their corresponding wild types provided by the Plant Biotechnology Center of Jilin Agricultural University (China). The insect taken into consideration was the Asian corn borer, provided by the Institute of Plant Protection, Jilin Academy of Agricultural Sciences (China). The test agent was 10% Glufosinate Ammonium (10% Solution).

2.2 Test Methods

A split-plot experimental design was adopted, with inbred varieties as the main plot, generations as the subplots, and three-row plots with a row length of 5.5 m, repeated three times. The transgenic T_2, T_3, and T_4 generation seeds and wild-type recipient seeds of lines S3002 and 349 were planted in the transgenic experimental field of Jilin Agricultural University, with three rows per generation, and three rows of wild type were planted beside the corresponding lines. 20 rows of corn were sown. The transgenic plants were examined at the seedling stage, and 10 plants that tested positive were identified by rubbing herbicide (glufosinate-ammonium) on their leaves.

2.3 PCR Detection of Transformed Plants

The DNA of the positive plant leaves was extracted using a plant genomic DNA extraction kit (CWBio, Jiangsu, China). The *Cry1Ab13-1* and *NPR1* plasmids were used as positive controls, wild-type genome was used as a negative control, and water was used as blank control. Perform PCR testing. PCR amplification conditions and primer sequences are demonstrated in the table given below (Table S1).

2.4 Fluorescence Quantitative Real-Time PCR Detection of Transgenic Positive Plants

In reference to the method suggested by Xie et al. [10], to extract the total RNA of the roots, stems, and leaves of the plants that tested positive in 1.2.1, a fluorescent quantitative reverse transcription kit (TakaraBio, RR036A) was used to reverse transcribe into cDNA and use the online design primer website (Primer Quest Tool) (https://sg.idtdna.com/pages/tools). The Mx3000P fluorescence quantitative PCR instrument (Agilent Technologies, United States) was used to detect the relative expression levels of the target genes *NPR1* and *Cry1AB13-1* in the roots, stems, and leaves of the three-leaf stage of transformed plants, and the expression was calculated as $2^{-\Delta\Delta CT}$. Later, the relative expression level was calculated [27,28].

2.5 Outdoor Insect Resistance Test Method of Transgenic Positive Plants

When corn grew to the six to eight leaf stage, 20 positive plants were randomly selected from each line, and 20 to 30 2-instar corn borer larvae were placed in the corn core leaves. Later, they were inoculated again after 3D to resist insects. The sex identification standard adopted the nine-level grading standard formulated by the International Corn Borer Collaborative Group [29] (Table S2).

2.6 Outdoor Disease Resistance Test Method of Transgenic Positive Plants

The large leaf blight bacteria (*Exserohilum turcicum* (Pass.) Leonay et Suggs) was cultivated on the Potato Dextrose Agar (PDA) medium and then inoculated on the sorghum grain medium with the cultured strains. Later, the sorghum grains were covered with bacteria spare with silk. The Grey spot inoculation test on the genetically modified land was conducted on June 25th. In reference to the method suggested by Xia et al. [30], at the 9–11 leaf stage of the corn plant, sorghum grains covered with mycelium were sprinkled into the corn core leaves, and 10–15 grains were sprinkled into each corn plant. 10 corn plants were randomly investigated in each row, and the survey objects were three leaves above the ear and three leaves below the ear. According to the nine-level evaluation system (Table S3), each maize plant was investigated for GLS at the maize milk maturity stage [30].

2.7 Statistical Analysis

All the experiments were carried out with replicates of three and the same was repeated three times. The statistical evaluation was performed to determine the significant difference by one-way ANOVA Duncan test and two-way ANOVA Duncan test. Values are means ± SE of independent biological replicates of three. The mean values under each treatment were compared at $0.01 < p < 0.05$. All the analyses were performed by GraphPad Prism 6.0 software and SPSS 23.0 software.

3 Results

3.1 PCR Detection of Transgenic Plants

3.1.1 PCR Detection of T_0, T_2, T_3, and T_4 Generation Transgenic Plants

By employing seamless cloning technology and double enzyme digestion, the original GUS region of 3301 was replaced with *NPR1*, and *Cry1Ab13-1* was inserted by exploiting the various cloning sites on the vector, as demonstrated in Fig. S1.

The size of the amplified target bands was 500, 552, 205, 525, and 1657 bp, which was consistent with the size of the target gene *35S*, *Bar*, *Nos*, *NPR1*, and *Cry1Ab13-1* fragments, indicating that the functional genes and marker gene fragments have been integrated into T_0, T_2, T_3, and T_4 generation lines (Figs. S2–S5). However, due to the small number of seeds obtained from T_0 generation plants, subsequent analysis of these plants was not carried out.

3.2 Fluorescence Quantitative Real-Time PCR Detection of Transgenic Maize

3.2.1 Fluorescence Quantitative Real-Time PCR Detection of Cry1Ab13-1 Gene in Transgenic Maize

By detecting the relative expression levels of *Cry1Ab13-1* gene in roots, stems and leaves of S3002 and 349 T_2, T_3 and T_4 generations at V3 stage, the contents of T_2 generation stems of S3002 and 349 were used as controls, respectively, the quantitative Real-time PCR (qRT-PCR) was performed, and the results were shown in Figs. 1A and 1B. By calculating the mean value of *Cry1Ab13-1* gene in roots, stems and leaves of T_2, T_3 and T_4 generations of S3002 and 349, it could be seen that there was no significant difference in the expression of *Cry1Ab13-1* gene in the same tissue of T_2, T_3 and T_4 generations of S3002 and 349. There were significant differences in the expression levels of *Cry1Ab13-1* gene in different tissues of T_2, T_3 and T_4 generations of S3002 and 349. The results showed that the highest relative expression of *Cry1Ab13-1* gene in S3002 was in leaves, followed by roots, with the lowest content in stems, the highest relative expression of *Cry1Ab13-1* gene in 349 was in leaves, followed by stems, with the lowest content in roots.
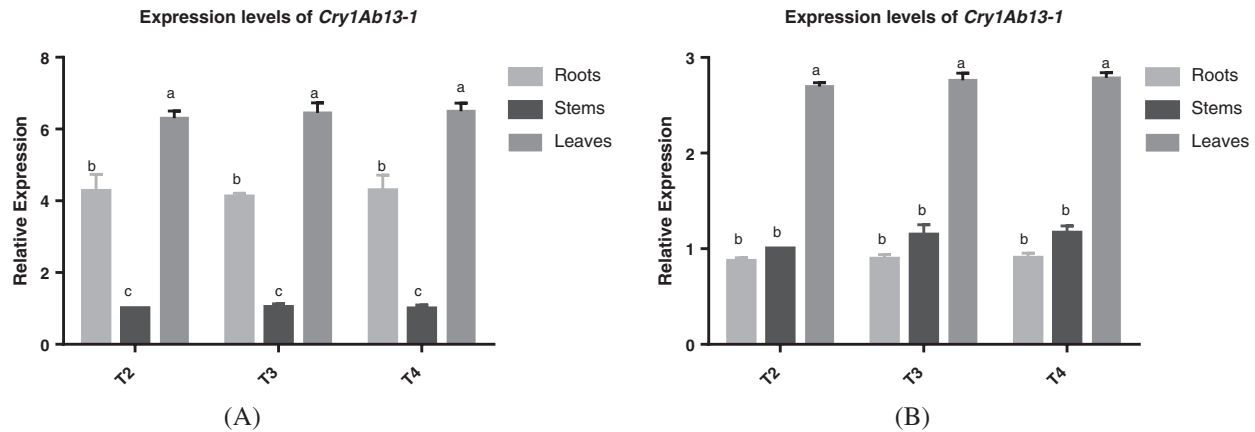
Figure 1: (A) Relative expression of *Cry1Ab13-1* gene in roots stems and leaves of V3 transgenic maize of S3002 generations T_2, T_3, and T_4; (B) Relative expression of *Cry1Ab13-1* gene in roots stems and leaves of V3 transgenic maize of 349 generations T_2, T_3, and T_4. All values were the mean of three replicates (mean ± SE). Different letters represent significant differences between each treatment at $0.01 < p < 0.05$, calculated using two-way ANOVA Duncan test. The small letter (i.e., a and b)indicated significant differences between different organizations

3.2.2 Fluorescence Quantitative Real-Time PCR Detection of NPR1 Gene in Transgenic Maize

The relative expression levels of *NPR1* gene in roots, stems and leaves of S3002 and 349 T_2, T_3 and T_4 generations at V3 stage were detected. The content of T2 stem of S3002 and 349 was used as the control for qRT-PCR detection, and the results were shown in Figs. 2A and 2B. By calculating the mean value of *NPR1* gene in roots, stems and leaves of T_2, T_3 and T_4 generations of S3002 and 349, it could be seen that there was no significant difference in the expression of *NPR1* gene in the same tissue of T_2, T_3 and T_4 generations of S3002 and 349. The expression levels of *NPR1* gene in different tissues of T_2, T_3 and T_4 generations of S3002 were significantly different. The expression of *NPR1* gene in roots and leaves of 349 T_2, T_3 and T_4 generations was significantly different from that in stems. The results showed that the highest relative expression of *NPR1* gene in S3002 and 349 was in leaves, followed by roots, with the lowest in stems.
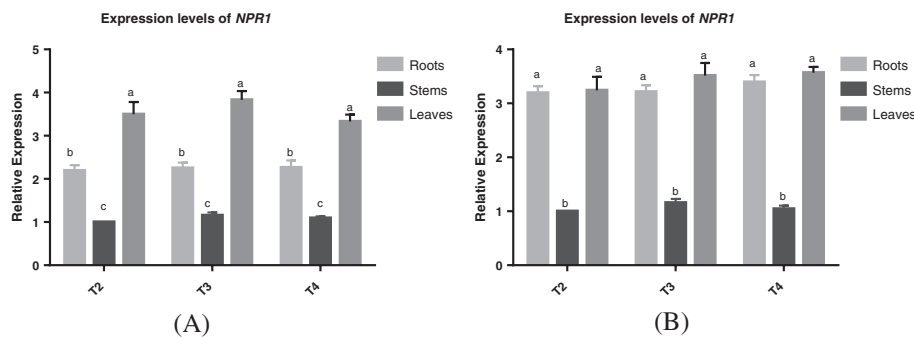

Figure 2: (A) Relative expression of *NPR1* gene in roots, stems, and leaves of V3 stage of transgenic maize of S3002 T_2, T_3, and T_4 generations; (B) Relative expression of *NPR1* gene in roots, stems, and leaves of V3 stage of transgenic maize of 349 T_2, T_3, and T_4 generations. All values were the mean of three replicates (mean ± SE). Different letters represent significant differences between each treatment at $0.01 < p < 0.05$, calculated using two-way ANOVA Duncan test. The small letter (i.e., a and b) indicated significant differences between different organizations

The results indicated that *Cry1Ab13-1* and *NPR1* genes were expressed in the roots, stems, and leaves of the T$_2$, T$_3$, and T$_4$ progeny of transgenic S3002 and 349. The expression levels of the *Cry1Ab13-1* gene and *NPR1* gene in the same tissues of the same cultivar and different generations have no significant difference indicating that *Cry1Ab13-1* and *NPR1* genes can be expressed in various parts of the transgenic maize plant and can be stably inherited.

3.3 Identification of Outdoor Insect Resistance of Transgenic Maize

After the 7D, 14D, and 21D field investigations (Fig. 3), the plants were processed for data processing (Figs. 4 and 5). At the 7D after inoculation, the T$_2$, T$_3$, and T$_4$ generations of S3002 all reached the resistance (R) level, while the wild insect resistance of the type was the susceptible (S) level. T$_2$, T$_3$, and T$_4$ generations of the 349 line had no significant differences in leaf-eating grades, and their insect resistance reached the resistance (R) level, while the insect resistance of the 349 wild-types was at the insect-susceptible (S) level.
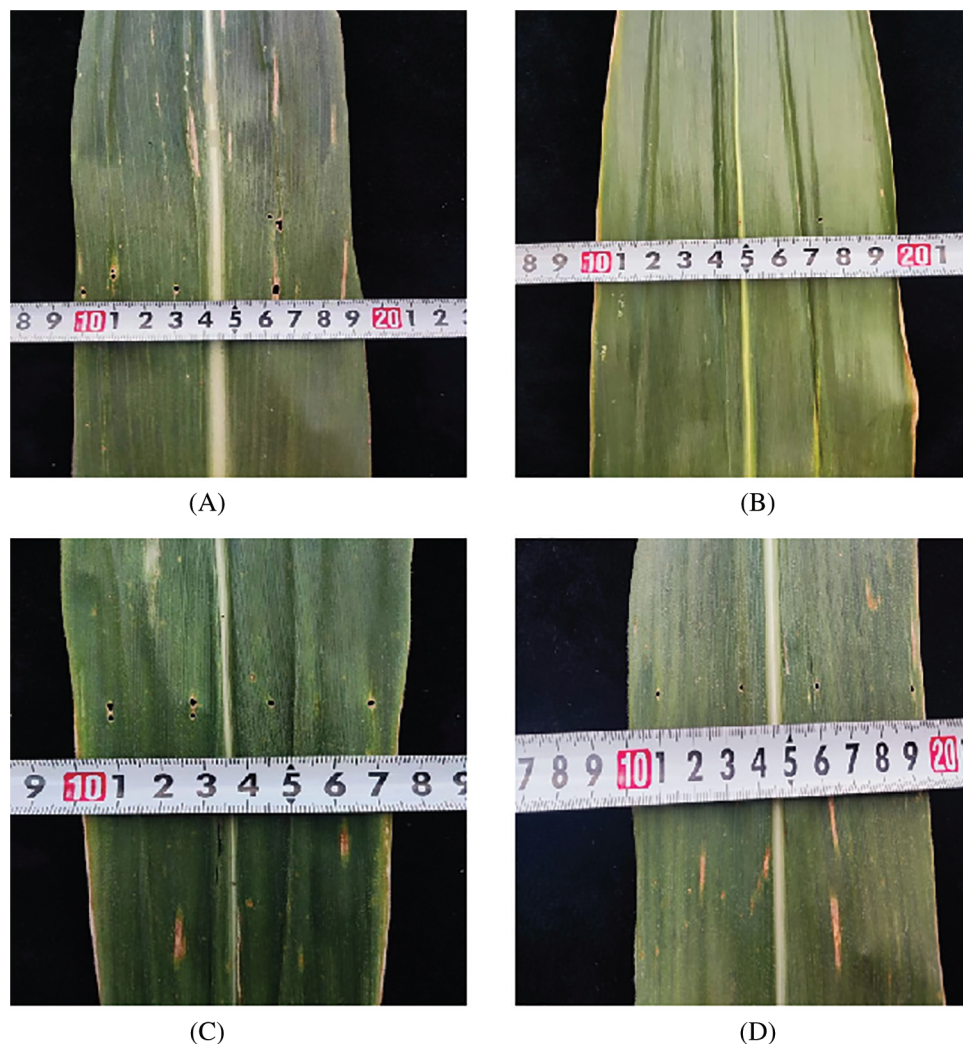

(A)   (B)

(C)   (D)

Figure 3: Picture of external transplanting of transformed plants. Note: (A) Untransformed inbred line 349; (B) Transformed inbred line 349; (C) Untransformed inbred line S3002; (D) Transformed inbred line S3002
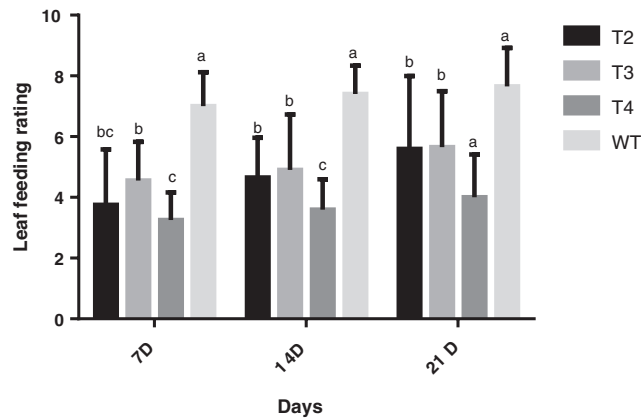
Figure 4: Leaf feeding levels of T_2, T_3, and T_4 of transgenic positive plants S3002. All values were the mean of three replicates (mean ± SE). Different letters represent significant differences between each treatment at $0.01 < p < 0.05$, calculated using two-way ANOVA Duncan test. The small letter (i.e., a and b) indicate significant differences among different generations
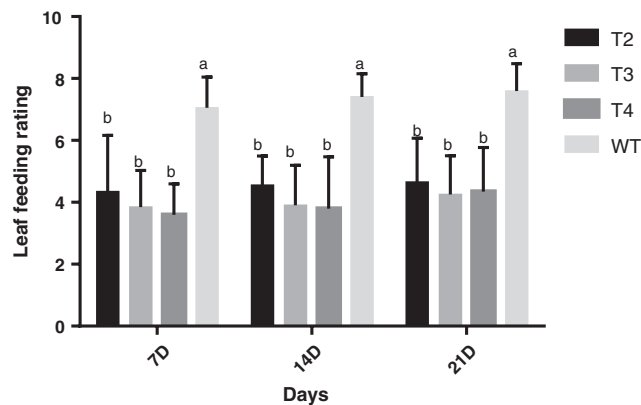

Figure 5: Leaf feeding levels of T_2, T_3, and T_4 of transgenic positive plants 349. All values were the mean of three replicates (mean ± SE). Different letters represent significant differences between each treatment at 0.01 $< p < 0.05$, calculated using two-way ANOVA Duncan test. The small letter (i.e., a and b) indicated significant differences among different generations

On the 14D after inoculation, the insect resistance of the three generations T_2, T_3, T_4, and the wild type of S3002 was the insect resistance (R) level, while the wild type was all susceptible (S) level, respectively. Among them, the differences in the leaf-eating grades of the T_2 and T_3 generations were not significant, the differences in the leaf-eating grades between the T_2 and T_4, with the T_3 and T_4 generations were significant, and the three generations of the S3002 line and the wild type had significant leaf-eating grades. The insect resistance of the three generations of 349 continued at the level of insect resistance (R), while the insect resistance of the wild type was at the susceptible (S) level, and there were significant differences in the leaf-eating levels of the three generations of T_2, T_3, and T_4 from wild type.

On the 21D after inoculation, the insect resistance of each line of S3002 and 349 and the wild type was consistent with the insect resistance after inoculation 14D. The transgenic lines were all at the resistance (R) level, while the wild type was all at the susceptible (S) level, and there is no change in leaf-feeding level with the wild type, all of which are significantly different.

3.4 Identification of Outdoor Disease Resistance of Transgenic Positive Plants

As demonstrated in Fig. 6, it is the survey pictures of S3002, 349 transgenic maize and wild-type maize large leaf spot, and Figs. 7 and 8 demonstrated the diseased resistance grade of outdoor transformed plant grey leaf Spot disease. In the late stage of maize milk maturity, 10 transgenic maize plants were selected for S3002 and 349 for investigation, and there were wild-type plants. The disease resistance grades are depicted in Fig. 7. The average disease resistance grades of S3002 and 349 transgenic lines were 4.00 and 3.20, respectively, and the disease resistances were both resistant (R). By variance analysis, S3002, 349 transgenic maize significantly improved disease resistance compared with wild-type maize.
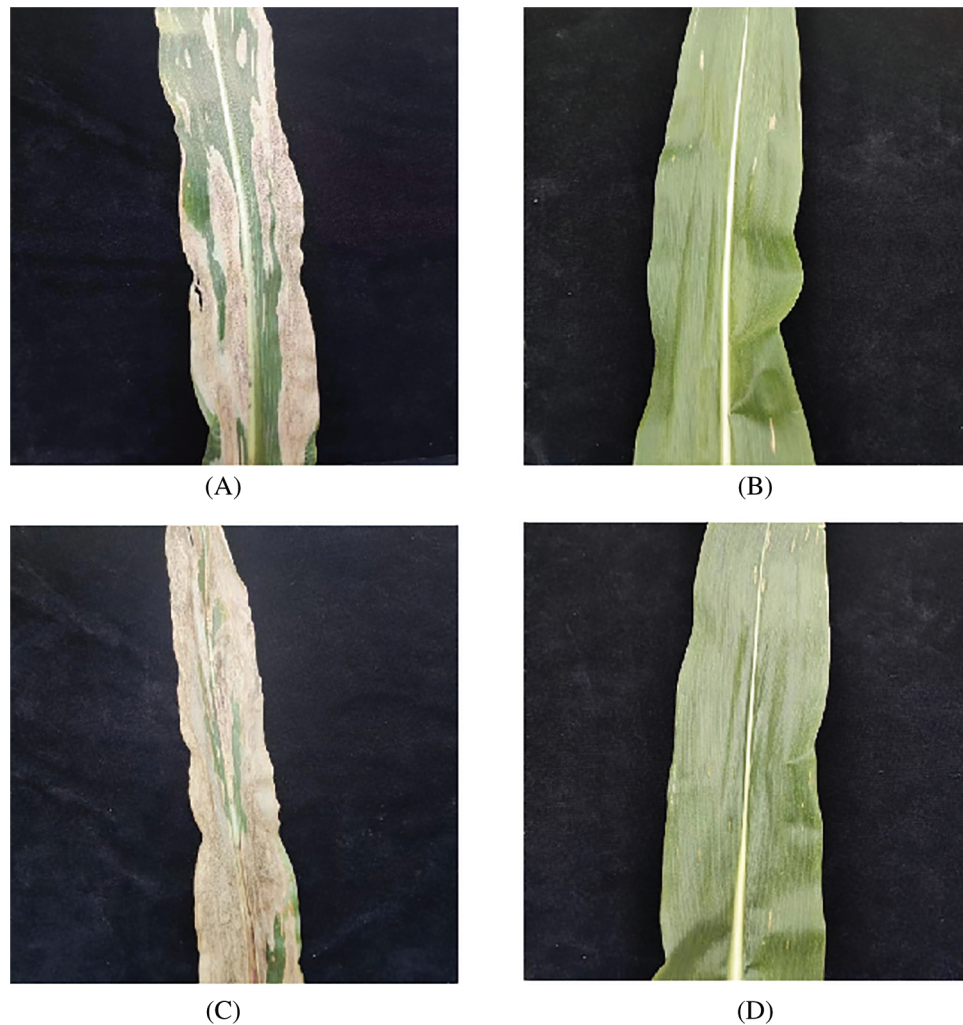

Figure 6: Pictures of transformed plants exposed to disease outside. Note: (A) Untransformed inbred line 349; (B) Transformed inbred line 349; (C) Untransformed inbred line S3002; (D) Transformed inbred line S3002
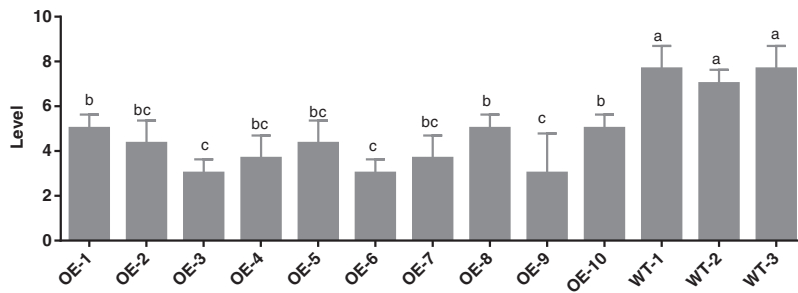
Figure 7: Identification of resistance grade of untransformed inbred line S3002 and transformed inbred line S3002. All values were the mean of three replicates (mean ± SE). Different letters represent significant differences between each treatment at $0.01 < p < 0.05$, calculated using one-way ANOVA Duncan test. The small letter (i.e., a and b) indicated significant differences among different lines
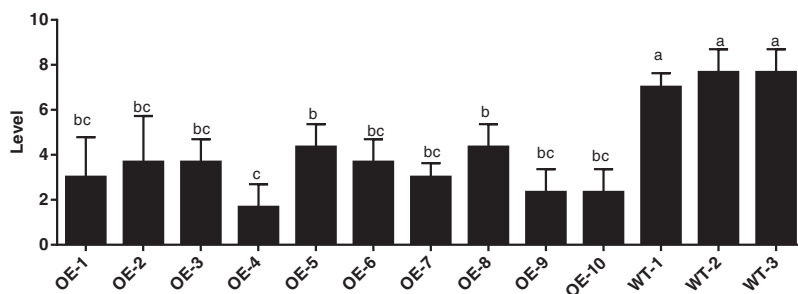

Figure 8: Identification of resistance grade of untransformed inbred line 349 and transformed inbred line 349. All values were the mean of three replicates (mean ± SE). Different letters represent significant differences between each treatment at $0.01 < p < 0.05$, calculated using one-way ANOVA Duncan test. The small letter (i.e., a and b) indicated significant differences among different lines

This study confirmed that the T_2, T_3, and T_4 generation of maize varieties S3002 and 349 containing *NPR1* and *Cry1Ab13-1* bivalent gene maize materials can better enhance insect resistance and disease resistance. The progeny of T_2, T_3, and T_4 transgenic lines of maize varieties S3002 and 349 were tested by conventional PCR, qRT-PCR, outdoor insect resistance, and disease resistance. Through the identification of outdoor insect resistance and disease resistance of the two receptors and wild-type plants and the fact that transgenic maize has insect resistance and disease resistance, it is confirmed that the bivalent gene polymerized maize line is more resistant to insect and disease than the wild type. The disease resistance ability has been improved, and it can be stably inherited for multiple generations. The results obtained from this research provided a good foundation for breeding new maize germplasm with insect resistance and disease resistance.

4 Discussion

There are few research reports on bivalent plant expression vectors in maize. In this study, we examined the genetic stability and resistance in the T_2, T_3, and T_4 generation maize materials containing bivalent genes and their corresponding wild types transformed with the insect-resistance gene *Cry1Ab13-1* and the disease-resistance gene *NPR1* double-resistance gene sick ability. The results showed that the bivalent gene polymerized maize line had improved insect and disease resistance compared with the wild type.

Sprays with the insecticidal protein *Bt* have been used for decades to control pests in agriculture, forestry, and public health. Due to its precise and very effective target features, transgenic Bt maize is

well-liked by growers [31,32]. Since 1996, Bt crops have been recognized for their ability to control target pests and reduce impacts on non-target organisms [33]. Many researchers have transferred the Bt gene into the maize backbone line and obtained insect-resistant transformants and Bt-transgenic insect-resistant maize inbred lines.

In China, transgenic *Cry1Ab* maize has good field insect resistance to Asian corn borer [34]. In 1995, the study by Armstrong et al. proved that the structure of *Cry1Ab* toxin protein endowed it with strong insecticidal activity against the diamondback moth *Plutella xylostella* and the tobacco hawkmoth *Manduca sexta*. Tang et al. [35] demonstrated that the structure of *Cry1Ab* toxin protein endowed it with strong insecticidal activity against rice. Subsequently, many researchers from China and other countries introduced the *Cry1Ab* gene into maize and proved that the transgenic maize expressing the *Cry1Ab* protein was effective against the Asian corn borer *Ostrinia* furnacalis and European corn borer *Ostrinia* nubilalis has good field resistance and can effectively control the damage of corn borer at the heart leaf stage and ear stage. The results of this experiment are consistent with the previous results, the transgenic plants obtained after the *Cry1Ab13-1* gene was transformed into the recipient improved the insect resistance from the susceptible to the resistant.

Numerous crops exhibit the broad-spectrum of disease resistance of *the NPR1* gene. In grapes (*Vitis vinifera L.*), rice (*Oryza sativa*) [23], apples (*Malus domestica*) [36], bananas (*Musa nana Lour.*) [37], and other plants, several *NPR1* homologous genes have been cloned consecutively. The results from the research conducted by Quilis et al. [38] demonstrated that *NPR1*-mediated rice had a positive regulatory effect on the defense response of biological and abiotic stress in rice growth and development, and the plants with transformed *NPR1* gene developed resistance to fungal pathogens that caused rice blast and rice seedling disease, respectively, and resistance to bacterial pathogens that caused foot rot, filling the gap in maize research. There are few research reports on bivalent plant expression vectors in maize. This study verifies that *NPR1* and *Cry1Ab13-1* bivalent gene maize materials can better enhance insect resistance and disease resistance than non-transgenic positive plants and can be more stable in inheritance from generation to generation.

The experimental findings of this work support the use of bivalent gene transformation vectors in maize. Currently, bivalent gene transformation vectors are employed in soybeans, sweet potatoes, alfalfa, and kale rape. However, there remain few research reports on corn. New germplasm resources are provided for disease-resistant and insect-resistant varieties, and the bivalent expression vector has a good breeding prospect in disease-resistant and insect-resistant breeding.

References

1. Shiferaw, B., Prasanna, B. M., Hellin, J., Bänziger, M. (2011). Crops that feed the world 6 past successes and future challenges to the role played by maize in global foodsecurity. *Food Security, 3(3),* 307–327.

2. Amon, T., Amon, B., Kryvoruchko, V., Machmüller, A., Hopfner-Sixt, K. et al. (2007). Methane production through anaerobic digestion of various energy crops grown in sustainable crop rotations. *Bioresource Technology, 98(17),* 3204–3212.

3. Mutuura, A., Munroe, E. (1970). Taxonomy and distribution of the European corn borer and allied species: Genus ostrinia (lepidoptera: *pyralidae*). *The Memoirs of the Entomological Society of Canada, 71(S71),* 1–112.

4. St Louis, M. E., Hess, J. J. (2008). Climate change: Impacts on and implications for global health. *American Journal of Preventive Medicine, 35(5),* 527–538.

5. Vucic-Pestic, O., Ehnes, R. B., Rall, B. C., Brose, U. (2011). Warming up the system: Higher predator feeding rates but lower energetic efficiencies. *Global Change Biology, 17(3),* 1301–1310.

6. Clusella-Trullas, S., Blackburn, T. M., Chown, S. L. (2011). Climatic predictors of temperatu-re performance curve parameters in ectotherms imply complex responses to climate change. *The American Naturalist, 177(6),* 738–751.

7. Du, D., Geng, C., Zhang, X., Zhang, Z., Zheng, Y. et al. (2014). Transgenic maize linesexp-ressing acry1c*gene are resistant to insect pests. *Plant Molecular Biology Reporter, 32(2),* 549–557.

8. Dowd, P. F., Lagrimini, L. M., Herms, D. A. (1998). Differential leaf resistance to insects of transgenic sweetgum (*Liquidambar styraciflua*) expressing tobacco anionic peroxidase. *Cellular and Molecular Life Sciences, 54(7),* 712–720.

9. Archer, T. L., Schuster, G., Patrick, C., Cronholm, G., Bynum, E. D. et al. (2000). Whorland stalk damage by European and Southwestern corn borers to four events of bacillus thuringiensis transgenic maize. *Crop Protection, 19(3),* 181–190.

10. Xie, W., Ali, T., Cui, Q., Huang, J. K. (2017). Economic impacts of commercializing insect-resistant gm maize in China. *China Agricultural Economic Review, 9(3),* 340–354.

11. Weems, J. D., Bradley, C. A. (2018). Exserohilum turcicum race population distribution in the North Central United States. *Plant Disease, 102(2),* 292–299. DOI 10.1094/PDIS-01-17-0128-RE.

12. Levy, Y., Pataky, J. K. (1992). Epidemiology of northern leaf blight on sweet corn. *Phytoparasitica, 20(1),* 53–66. DOI 10.1007/BF02995636.

13. Zhao, W., Wang, T., Qi, R. D. (2015). Ypt1 gene-based detection of phytophthora sojae in a loop-mediated isothermal amplification assay. *Journal of Plant Diseases and Protection, 122(2),* 66–73. DOI 10.1007/BF03356533.

14. Liu, Q., Hallerman, E., Peng, Y., Li, Y. (2016). Development of *Bt* rice and *Bt* maize in China and their efficacy in target pest control. *International Journal of Molecular Sciences, 17(10),* 1561. DOI 10.3390/ijms17101561.

15. Dale, P. J., Clarke, B., Fontes, E. M. G. (2002). Erratum: Potential for the environmental impact of transgenic crops. *Nature Biotechnology, 20(8),* 843. DOI 10.1038/nbt0802-843b.

16. Munkvold, G. P., Martinson, C. A., Shriver, J. M., Dixon, P. M. (2001). Probabilities for profitable fungicide use against gray leaf spot in hybrid maize. *Phytopathology, 91(5),* 477–484. DOI 10.1094/PHYTO.2001.91.5.477.

17. Haudenshield, J. S., Song, J. Y., Hartman, G. L. (2017). A novel, multiplexed, probe-based quantitative PCR assay for the soybean root- and stem-rot pathogen, Phytophthora sojae, utilizes its transposable element. *PLoS One, 12(4),* e0176567. DOI 10.1371/journal.pone.0176567.

18. Cao, H., Glazebrook, J., Clarke, J. D., Volko, S., Dong, X. (1997). The Arabidopsis *NPR1* gene that controls systemic acquired resistance encodes a novel protein containing ankyrin repeats. *Cell, 88(1),* 57–63. DOI 10.1016/S0092-8674(00)81858-9.

19. Ding, Y., Sun, T., Ao, K., Peng, Y., Zhang, Y. et al. (2018). Opposite roles of salicylic acid receptors NPR1 and NPR3/NPR4 in transcriptional regulation of plant immunity. *Cell, 173(6),* 1454–1467.e15. DOI 10.1016/j.cell.2018.03.044.

20. Kinkema, M., Fan, W., Dong, X. (2000). Nuclear localization of NPR1 is required for activation of *PR* gene expression. *The Plant Cell, 12(12),* 2339–2350. DOI 10.1105/tpc.12.12.2339.

21. Castelló, M. J., Medina-Puche, L., Lamilla, J., Tornero, P. (2018). NPR1 paralogs of Arabid-opsis and their role in salicylic acid perception. *PLoS One, 13(12),* e0209835. DOI 10.1371/journal.pone.0209835.

22. Wang, X. D., Bi, W. S., Gao, J., Yu, X. M., Wang, H. Y. et al. (2018). Systemic acqui-red resistance, *NPR1*, and pathogenesis-related genes in wheat and barley. *Journal of Integrative Agriculture, 17(11),* 2468–2477. DOI 10.1016/S2095-3119(17)61852-5.

23. Yuan, Y., Zhong, S., Li, Q., Zhu, Z., Lou, Y. et al. (2007). Functional analysis of rice NPR1 like genes reveals that OsNPR1/NH1 is the rice orthologue conferring disease resistance with enhanced herbivore susceptibility. *Plant Biotechnology Journal, 5(2),* 313–324. DOI 10.1111/j.1467-7652.2007.00243.x.

24. Gao, J., Bi, W., Li, H., Wu, J., Yu, X. et al. (2018). *WRKY* transcription factors associated with *NPR1*-mediated acquired resistance in barley are potential resources to improve wheat resistance to *Puccinia triticina*. *Frontiers in Plant Science, 9,* 1486. DOI 10.3389/fpls.2018.01486.

25. Gordon-Kamm, W. J., Spencer, T. M., Mangano, M. L., Adams, T. R., Daines, R. J. et al. (1990). Transformation of maize cells and regeneration of fertile transgenic plants. *The Plant Cell, 2(7),* 603–618. DOI 10.2307/3869124.

26. Denolf, P., Hendrickx, K., van Damme, J., Jansens, S., Peferoen, M. et al. (1997). Cloning and characterization of Manduca sexta and Plutella xylostella midgut aminopeptidase Nenzymes related to Bacillus thuringiensis toxin-binding proteins. *European Journal of Biochemistry, 248(3),* 748–761.

27. Javdi, M., Haghnazari, A., Tohidfar, M., Negari, S., Ghareyazi, B. (2010). Zygosity identifica-tion in transgenic cotton (*Gossypium hirsutum*) by real-time quantitative PCR. *Euphytica, 173(2),* 185–191.

28. Lu, S., Zhang, M., Zhang, Z., Wang, Z., Wu, N. et al. (2018). Screening and verification of genes associated with leaf angle and leaf orientation value in inbred maize lines. *PLoS One, 13(12),* e0208386.

29. Abel, C. A., Berhow, M. A., Wilson, R. L., Binder, B. F., Hibbard, B. E. (2000). Evaluation of conventional resistance to European corn borer (Lepidoptera: *Crambidae*) and western corn rootworm (*Coleoptera: Chrysomelidae*) in experimental maize lines developed from abackcross breeding program. *Journal of Economic Entomology, 93(6),* 1814–1821.

30. Xia, H., Gao, W., Qu, J., Dai, L., Gao, Y. et al. (2020). Genetic mapping of northern corn leaf blight-resistant quantitative trait loci in maize. *Medicine, 99(31),* e21326.

31. Milne, A. E., Bell, J. R., Hutchison, W. D., van den Bosch, F., Mitchell, P. D. et al. (2015). The effect of farmers' decisions on pest control with Bt crops: A billion dollar game of strategy. *PLoS Computational Biology, 11(12),* e1004483.

32. Hutchison, W. D., Burkness, E. C., Mitchell, P. D., Moon, R. D., Leslie, T. W. et al. (2010). Areawide suppression of European corn borer with Bt maize reaps savings to non-Bt maize growers. *Science, 330(6001),* 222–225.

33. Klümper, W., Qaim, M. (2014). A meta-analysis of the impacts of genetically modified crops. *PLoS One, 9(11),* e111629.

34. He, K., Wang, Z., Zhou, D., Wen, L., Song, Y. et al. (2003). Evaluation of transgenic Bt corn for resistance to the Asian corn borer (Lepidoptera: *Pyralidae*). *Journal of Economic Entomology, 96(3),* 935–940.

35. Tang, W., Lin, Y. J. (2007). Field experiment of transgenic cry1Ab insect resistant rice. *Yi Chuan = Hereditas, 29(8),* 1008–1012.

36. Zhao, J. T., Huang, X., Chen, Y. P., Chen, Y. F., Huang, X. L. (2009). Molecular cloningand characterization of an ortholog of *NPR1* gene from dongguan dajiao (*Musa* spp. ABB). *Plant Molecular Biology Reporter, 27(3),* 243–249. DOI 10.1007/s11105-008-0074-z.

37. Chupeau, M. C., Pautot, V., Chupeau, Y. (1994). Recovery of transgenic trees after electr-oporation of poplar protoplasts. *Transgenic Research, 3(1),* 13–19. DOI 10.1007/BF01976022.

38. Quilis, J., Peñas, G., Messeguer, J., Brugidou, C., San Segundo, B. (2008). The *Arabidopsis AtNPR1* inversely modulates defense responses against fungal, bacterial, or viralpathoge-ns while conferring hypersensitivity to abiotic stresses in transgenic rice. *Molecular Plant-Microbe Interactions, 21(9),* 1215–1231. DOI 10.1094/MPMI-21-9-1215.

Appendix

Table S1: PCR amplification conditions and primer sequence

| Name of the sequence	Primer sequence	Annealing temperature
*Cry1Ab13-1*S	AGGAACCAGGCTATCTCA	58.5°C
*Cry1Ab13-1*AS	TCATCCTTCTCGGAGTTA	
NPR1-S	ATGGGATACTTGTGCATTAAGG	54.5°C
NPR1-AS	CTACAGTTCGTAGGGACTTTCACCA	
35S-S	TAGAGGACCTAACAGAAC	54.5°C
35S-AS	CCGTGTTCTCTCCAAATG	
Nos-S	AGATTGAATCCTGTTGCC	55.0°C
Nos-AS	CCCGATCTAGTAACATAG	
Bar-S	TCAAATCTCGGTGACGGGC	54.5°C
Bar-AS	ATGAGCCCAGAACGACGC	

Table S2: Damage rating scales and categories for evaluation of maize whorl stage resistance to Asian maize borer

Leaf damaged		Rating scales	Stalk cavities	Resistant type
Pinholes	Rare or sporadic on few leaves	1	0~2	1 + R
	Intermediate on few leaves	2		
	Many on several leaves	3		R
Match-head size holes	Rare or sporadic on few leaves	4		
	Intermediate on few leaves	5	3~5	MR
	Many on several leaves	6		
Holes bigger than match head size	Rare or sporadic on few leaves	7		S
	Intermediate on several leaves	8		
	Many on most leaves	9	≥6	HS

Note: The wormhole tunnel of 2.5 cm was 1 hole. HR: highly resistance; R: resist; MR: moderate resistance; S: susceptibility; HS: highly susceptibility.

Table S3: Standard for resistance evaluations

Level	Description	Resistance evaluation
1	No disease spots on the leaves or a small amount of disease spots on the leaves at the lower part of the ear, and the disease spots account for less than 5% of the leaf area.	High resistance (HR)
3	There are a few disease spots on the leaves at the lower part of the ear, accounting for 6% to 10% of the leaf area, and there are sporadic disease spots on the leaves at the upper part of the ear.	Resistance (R)
5	There are more lesions on the leaves at the lower part of the ear, accounting for 11% to 30% of the leaf area, and there are more lesions on the leaves at the upper part of the ear.	Moderate resistance (MR)
7	There are a large number of diseased spots on the upper leaves, and the diseased spots are connected, accounting for 30% to 70% of the leaf area, and the lower diseased leaves die.	Susceptibility (S)
9	The leaves of the whole plant are basically covered with disease spots, and the leaves die.	Highly susceptibility (HS)
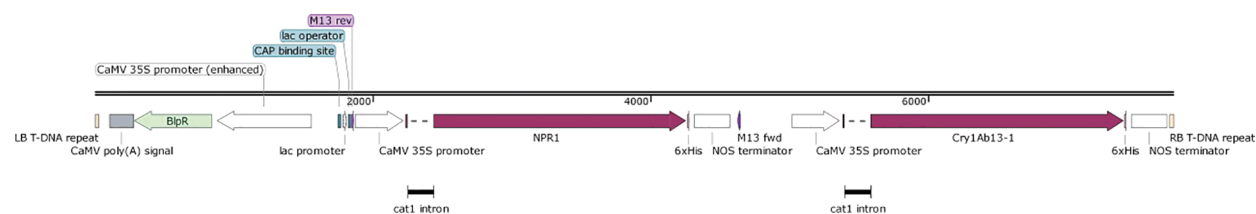
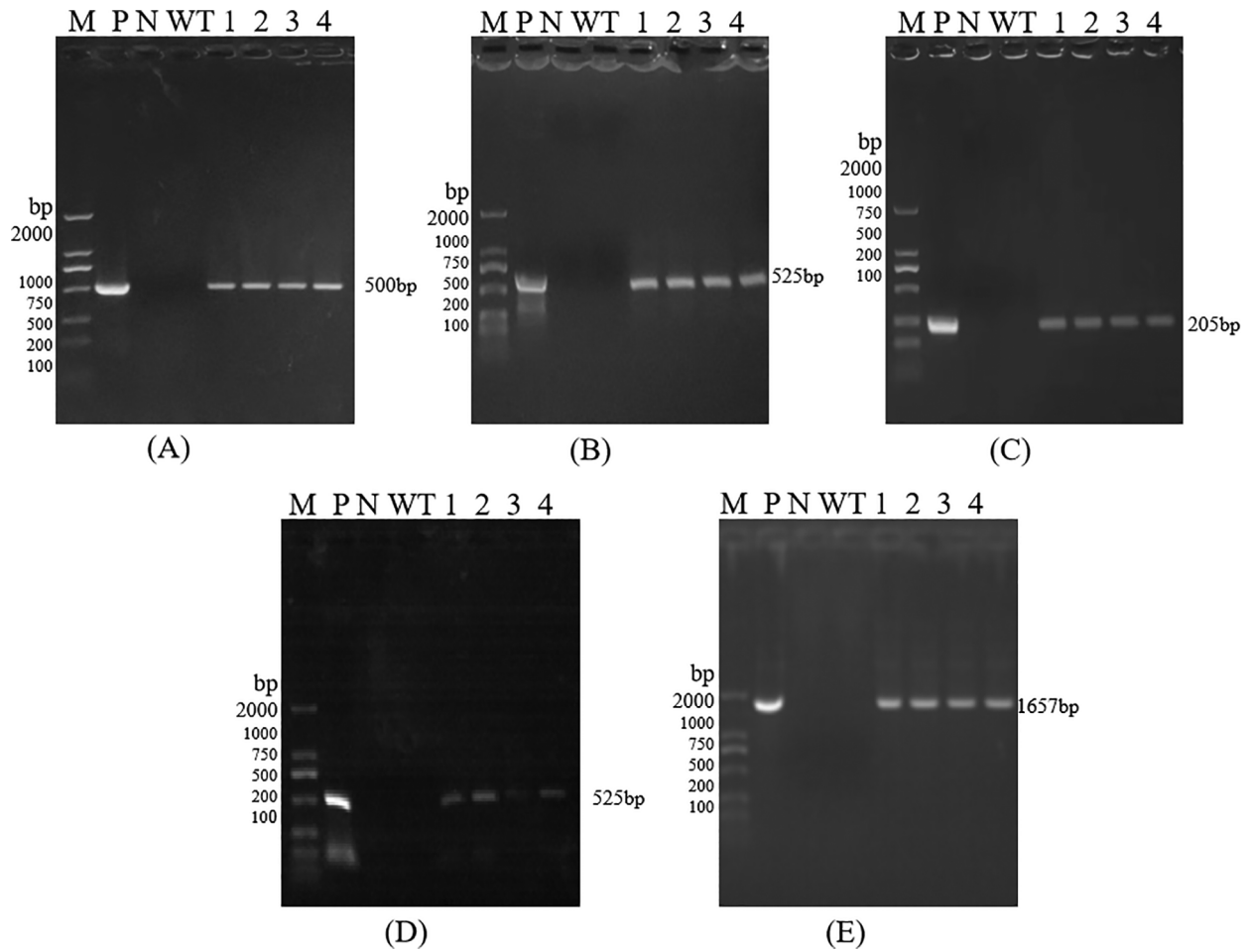**Figure S1:** Structure of recombinant plant expression vector PCAMBIA3301-Cry1Ab13-1-NPR1
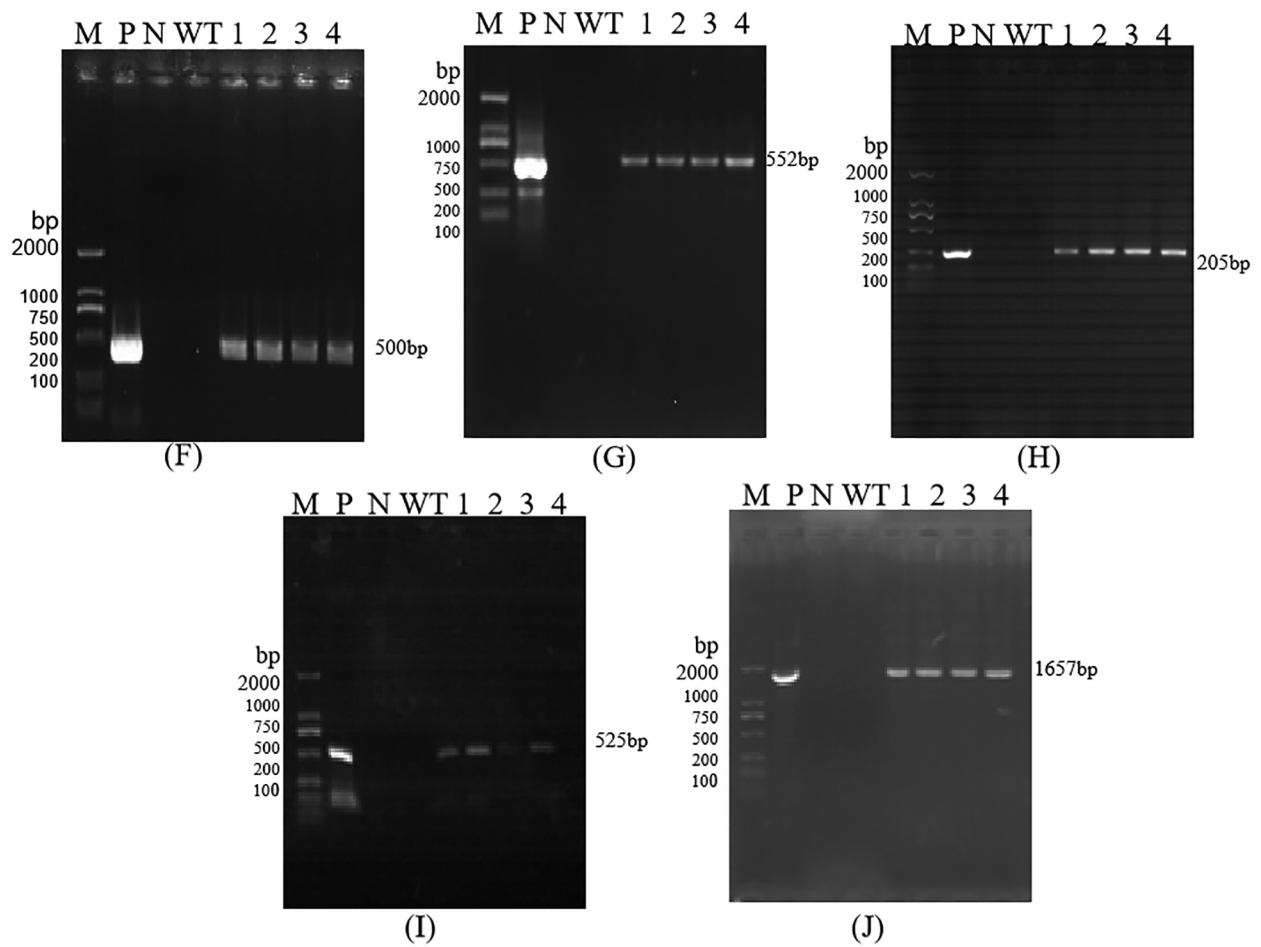
Figure S2: PCR detection of T_0 Transgenic Maize; Each part of electrophoretogram is *35S* (3A), *Bar* (3B), *Nos* (3C), *NPR1* (3D), *Cry1Ab13-1* (3E). Note: A–E are PCR detection of *35S*, *Bar*, *Nos*, *NPR1*, *Cry1Ab13-1*; M: DL 2000 DNAmarker; N: H_2O; P: Positive control; WT: Untransformed plants; 1–4: Samples

Figure S3: PCR detection of T_2 Transgenic Maize; Each part of electrophoretogram is *35S* (3F), *Bar* (3G), *Nos* (3H), *NPR1* (3I), *Cry1Ab13-1* (3J). Note: F–J are PCR detection of *35S*, *Bar*, Nos, *NPR1*, *Cry1Ab13-1*; M: DL 2000 DNA marker; N: H_2O; P: Positive control; WT: Untransformed plants; 1–4: Samples
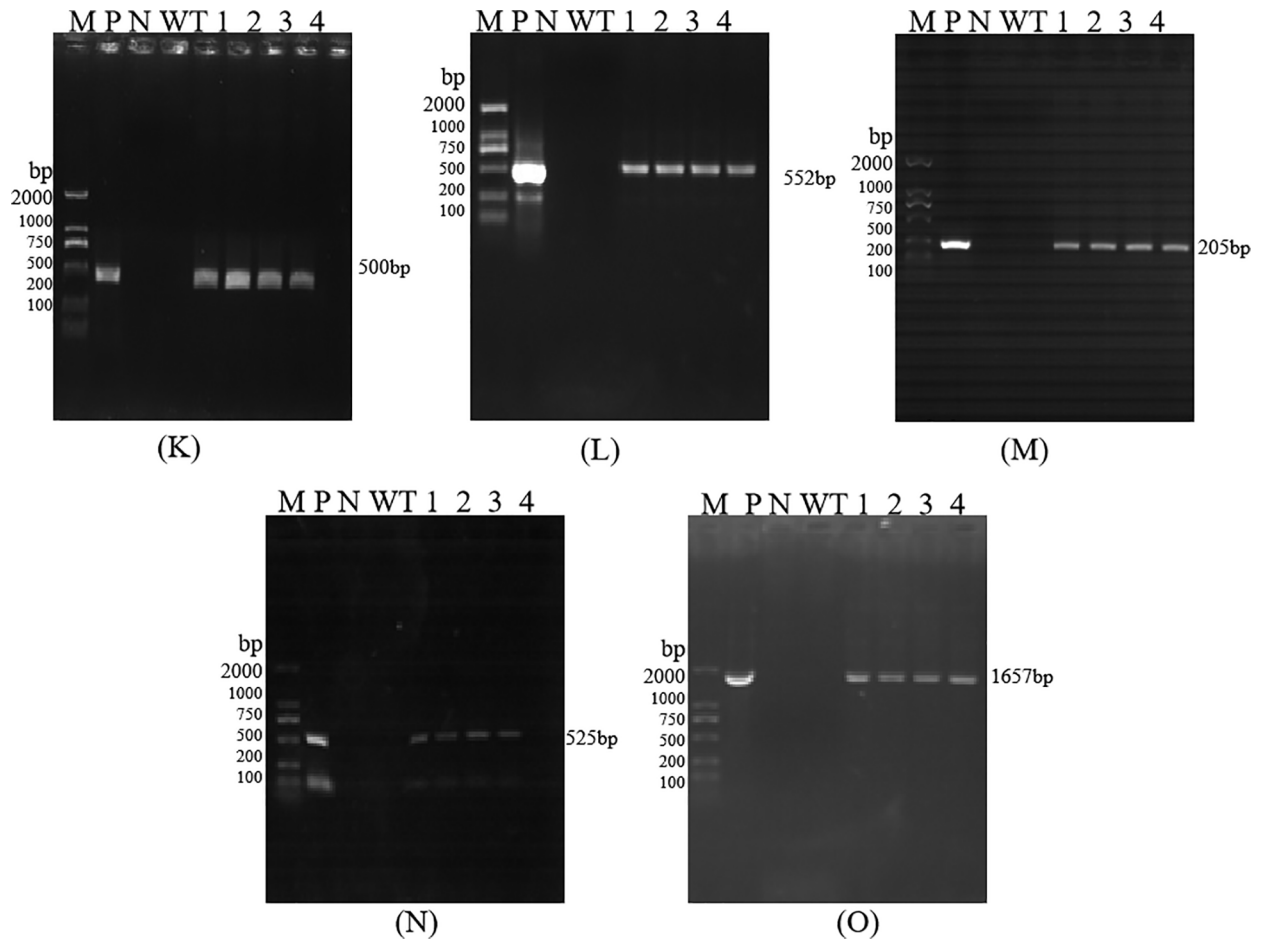
Figure S4: PCR detection of T$_3$ Transgenic Maize. Each part of electrophoretogram is *35S* (3K), *Bar* (3L), *Nos* (3M), *NPR1* (3N), *Cry1Ab13-1* (3O). Note: K–O are PCR detection of *35S*, *Bar*, *Nos*, *NPR1*, *Cry1Ab13-1*; M: DL 2000 DNA marker; N: H$_2$O; P: Positive control; WT: Untransformed plants; 1–4: Samples
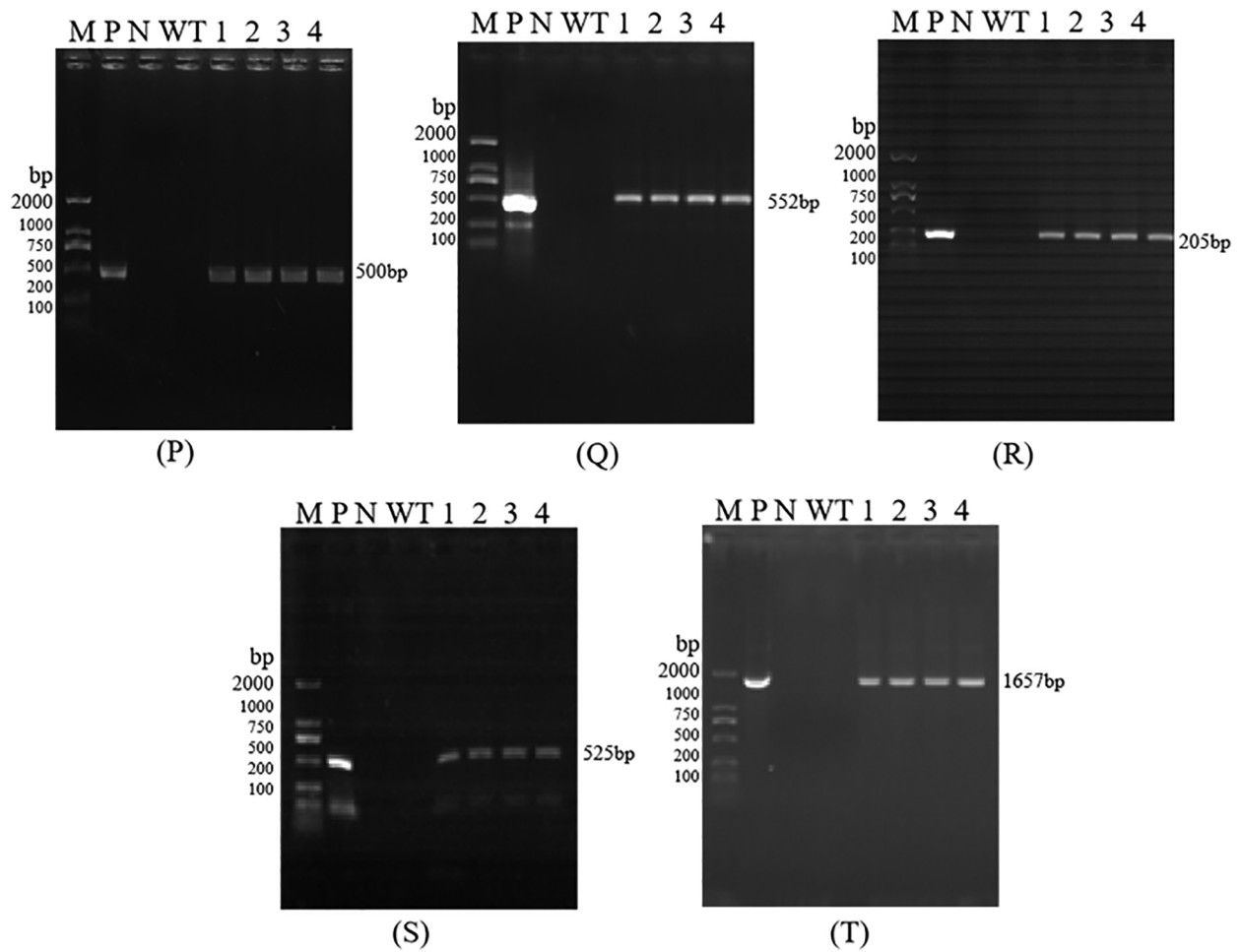
Figure S5: PCR detection of T_4 Transgenic Maize. Each part of electrophoretogram is *35S* (3P), *Bar* (3Q), *Nos* (3R), *NPR1* (3S), *Cry1Ab13-1* (3T). Note: P–T are PCR detection of *35S*, *Bar*, *Nos*, *NPR1*, *Cry1Ab13-1*; M: DL 2000 DNA marker; N: H_2O; P: Positive control; WT: Untransformed plants; 1–4: Samples