Plants Fight Back: Understanding The Role of EDS1 Gene in Nicotiana tabacum Pathogen Resistance

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Abstract

Scientists look for ways to understand how plants respond to pathogens. One line of investigation is to identify specific genes that are required to naturally protect plants from pathogen infection. Scientists want to learn how and when these genes are brought into action and how they function to help plants fight off pathogen infection. Once we achieve a better understanding of the genes behind natural plant defense mechanisms, scientists can use this knowledge to engineer enhanced natural plant defense for sustainable and affordable alternatives to pesticides that are currently used to protect crops from pathogen destruction.

Investigations have found that plants have evolved, in addition to physical barriers and chemical toxins as first lines of defense, pathogen induced resistance pathways. One of these pathways is mediated by transcription factors called R genes. EDS1 is a member of the R gene family that can induce an immune response by activating a pattern recognition receptor R (PRR) gene. Once triggered, these PRRs activate signaling pathways that reprogram infected cells for resistance to a wide range of pathogens (viral, bacterial, and fungal pathogens).

Results

• Primers, EDS1_F/EDS1_R, amplified a 200 pg of both in the WT and the silenced plants genomic DNA samples (Figure 3).

Background continue

Figure 3
Silencing

Methods

Plant growth
N. tabacum seeds (wt and silenced plant) were surface sterilized (20% commercial bleach) and sown onto agar plates supplemented with kanamycin (50 mg/ml). Plates were incubated in a growth cabinet and two weeks old seedlings were transplanted onto soil and grown in a greenhouse.

Edwards DNA Extraction
Leaf material was collected into a 1.5 ml tube and 200ul of extraction buffer (200mM Tris-HCl, 250mM NaCl, 0.5% SDS) added. The plant material was ground using a pestle and centrifuged for 1 min at maximum speed. The supernatant containing DNA was transferred to a new tube and precipitated by adding 100% isopropanol and centrifuging for 5 min at maximum speed. The pellet was washed with 70% ethanol air-dried and resuspended in 20ul TE.

Polymerase Chain Reaction (PCR)
We designed primers EDS1_F, 5’-TTG GGA CGG GGA AAG GAA TG-3’ and EDS1_R, 5’-TCA GGT GCG CTT GG-3’ to amplify a 230bp sequence of the EDS1 gene (Acc. Num. 12028572). The DNA extracted from wt plants was used as positive control, while water as negative control. PCR was performed using a TaKaRa PCR system.

Total RNA Extraction
Nucleic acids were purified from leaves following Frosz. The resultant solution contained a mix of genomic DNA, high molecular RNA and low molecular RNA, called total nucleic acid (TNA).

DNase and Retro Transcription and PCR (RT-PCR)
DNase-treated samples were used as template in RT with random primers. PCR was performed with the EDS1 primer pair (EDS1_F/EDS1_R) and primers 30 PCR cycles and products at 27 and 30 cycles to semi-quantify the expression of the targeted genes. PCR products were resolved onto 1% agarose gel.

Discussion

Results indicate first that the designed primers work as expected, binding to the known sequences and allowing primerose to amplify a 200 base pair sequence of the genomic DNA in both the eds1 and WT tobacco. Both bands have the same density (equal concentrations of PCR product) confirming that the DNA used in the preliminary PCR was in fact genomic and that there was no deletion of the EDS1 gene. Controls ruled out contamination and validated accuracy of our PCR.

Second, RT-PCR results confirm that eds1 plants were not only transformed but the EDS1 gene was silenced. Also, we concluded that EDS1 expression was reduced substantially in the transformed plant vs. the WT plants. The difference in the band density shows that the RNAi-EDS1 tobacco plants have reduced levels of EDS1 mRNA (less mRNA and thus less CDNAs) produced by RT-PCR than untransformed wild type tobacco plants. Finally, we conclude that EDS1 gene is silenced in the transformed N. tabacum leading to reduced mRNA levels and products. The finding that the EDS1 expression was transgenic tobacco lines to investigate reduced but not eliminated open the possibility of using these the mechanism of ED51 gene in general resistance and specific R gene mediated resistance.

References


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