Increasing Japonica MPB rice resistance to R3 virus using CRISPR gene editing

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Abstract

Rice is one of the principle crops throughout the world. Laodelphax Striatellas (small brown planthoppers) are present throughout much of the Afro-Eurasian land mass and are carriers of the R3 virus. This virus when exposed to Japonica rice often results in severe plant stunting and reduced yield. This is thought to happen due to an increase of reactive oxygen species (ROS), byproducts of metabolism critical in cell signaling, which can cause oxidation of cells and damage the organism. This project studied whether CRISPR technologies could be used to create mutant plants that were resistant to this virus. Specifically, this project monitored the DNA, RNA, and protein concentrations in mutant samples altered with “Gene X”.

Methods

All wet lab work was conducted in the Life Sciences building of PKU. Plant samples were grown at Tsinghua University. DNA presence was tested through extraction/isolation, PCR, and gel electrophoresis. Samples of the PCR solution were sent to Rubio Biotech for full sequencing. Protein presence was tested through the Western Blot process, proteins were transferred from the PAGE gel to the nitrocellulose membrane via semi dry blotting and conjugated with primary and secondary antibodies for enhanced chemiluminescence. Additional samples were set aside for a protein quantification assay using the electron microscope in the Analytical Instrumentation Center of the College of Chemistry. RNA was isolated from SF9 cells for transfection using TRIzol, and the cells were cultivated in Grace's unsupplemented insect cell medium. Samples were planted at Nanjing Agricultural University in Nanjing and in the Chinese Agriculture University experiment fields in Shang Zhuang to examine the plant development in different climates.

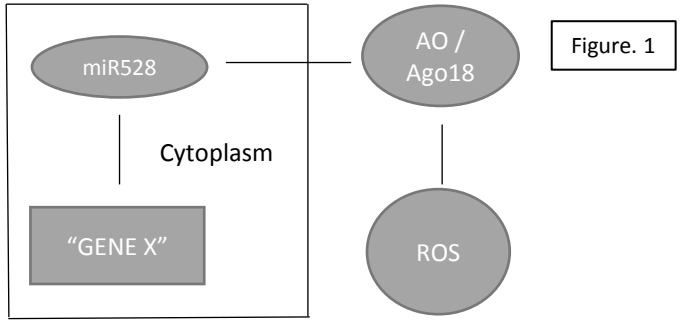

Figure. 1

Results

A B C D E

Figure. 2

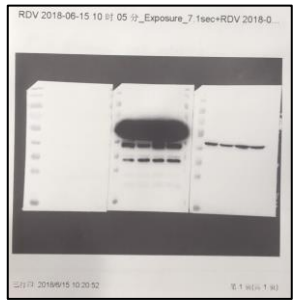

Figure. 3

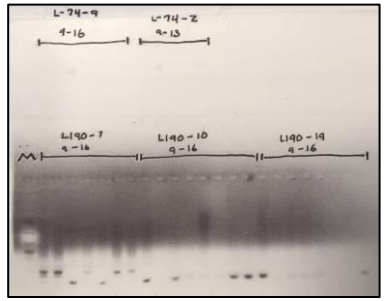

Figure. 4

Conclusions

No concrete conclusion can be drawn at this time. While initial results are hopeful, more research needs to be done to establish the interactions between “Gene X”, Ago18, miR528, and ROS. Additionally, any mutant plant altered by CRISPR would have to undergo further tests as to its effects on environmental health, and its relative advantage compared to other rice strains and crops, before it would be considered for larger scale agriculture.

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Figure. 1- Hypothesized connections between “Gene X” and ROS

Figure. 2-
A- Healthy wild type
B- RSV wild type
C,D,E- Transgenic over expression lines

Figure. 3- Results of Western Blot, after enhanced chemiluminescence

Figure. 4- Results of Gel Electrophoresis