The regulation of autophagy in susceptible tobacco under TMV infection

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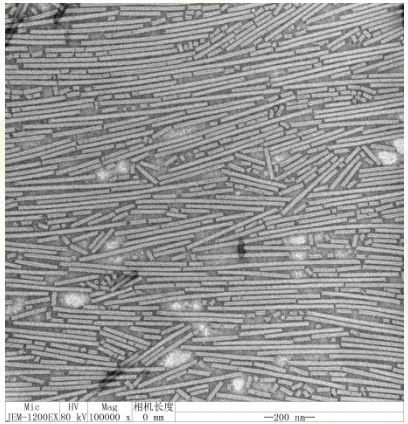
1. Intruduction

1.1Tobacco mosaic virus (TMV)

TMV, a single-strandes RNA virus, is a member of the genus *Tobamovirus*.

TMV spreads via mechanically and infects several crops in the family *Solanaceae*.

Reportedly, TMV is a serious threat to tobacco and decrease tobacco yields in China.



1.2 Autophagy is an evolutionarily conserved process

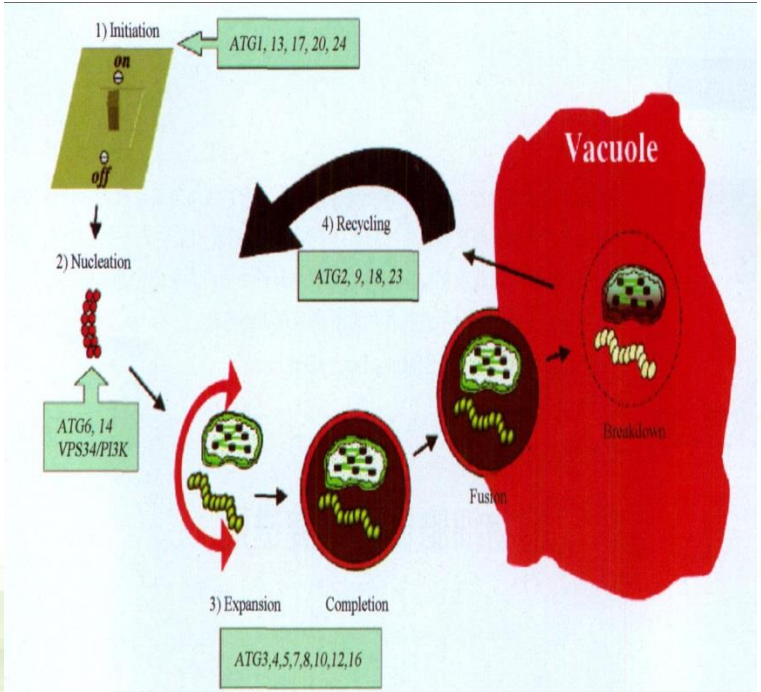
Autophagy is an evolutionarily conserved process by which cytoplasmic constituents, and delivered to lysosomes or vacuoles for degradation.

Three pathways:

Macroautophagy

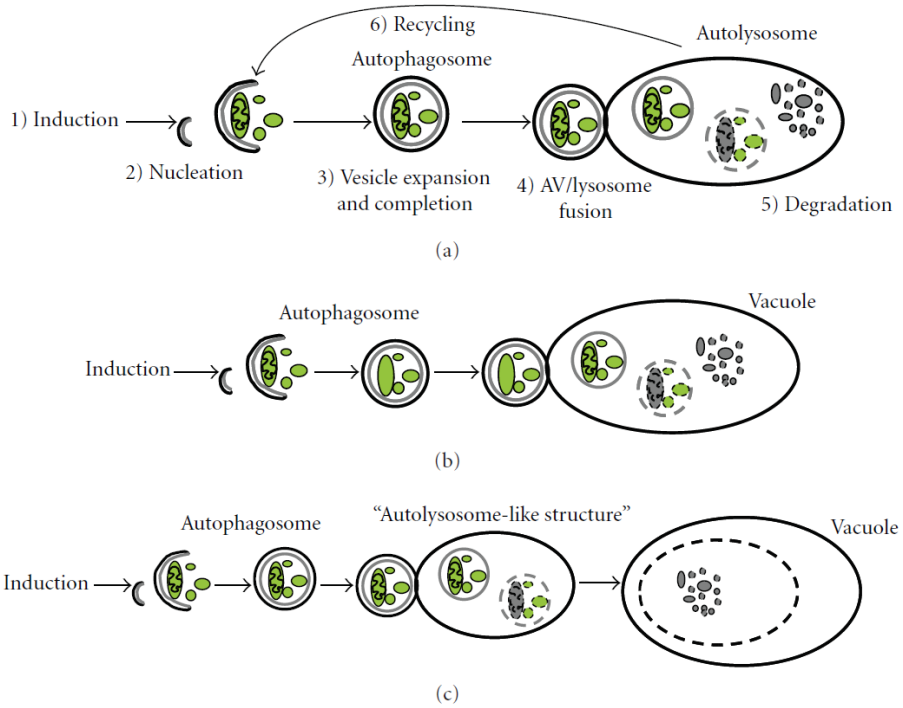
Chaperone-mediated autophagy

Microautophagy



1.3 Autophagy mechanism and alternative pathways for autophagosomes in plants

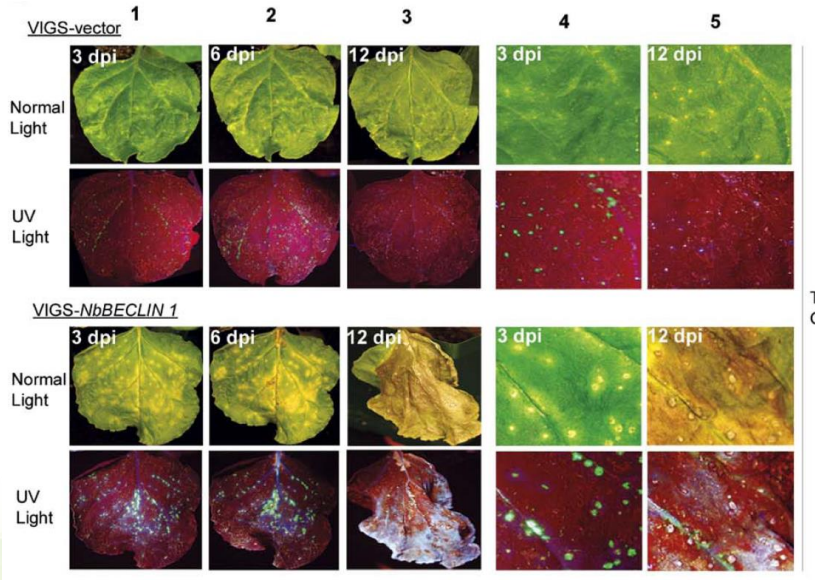
- A：Double membrane vesicle appear, engulf portion, fuse with vacule and autophagosomes degradation.
- B :Autophagosomes fuse directly with vacule.
- C:First fuse with lysosome-like structure or endosomes to form autolysosome-like structures, fuse with the vacule.



Mitou G. et al., 2009

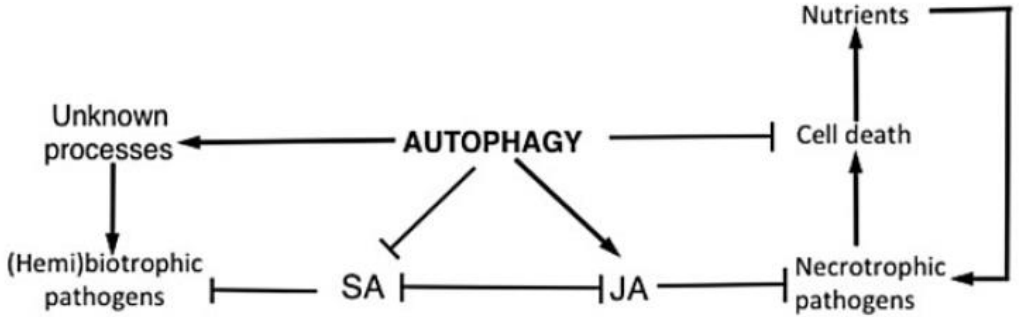
1.4 Autophagy in pathogen-induced hypersensitive cell death

Liu et al. (2005) discovered that the silencing of the tobacco BECLIN1/ATG6 gene resulted in the spread of cell death to uninfected tissues and leaves distal to the TMV-inoculated leaf.



Liu et al. , 2005

1.5 Autophagy in plant basal resistance to biotrophic pathogens



Autophagy generally has a negative role in plant basal resistance to biotrophic pathogens by suppressing salicylic acid (SA) signalling.

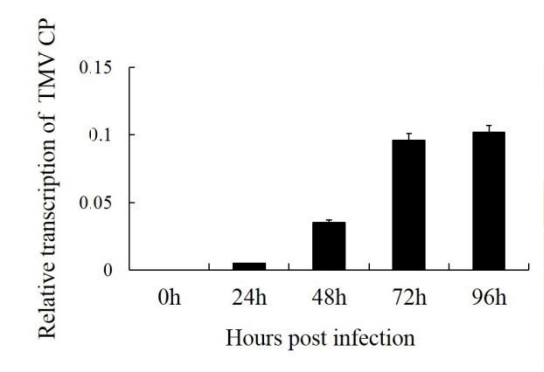
Autophagy plays a critical role in plant resistance to necrotrophic pathogens by inhibiting pathogen-induced cell death and promoting jasmonate (JA) signalling.

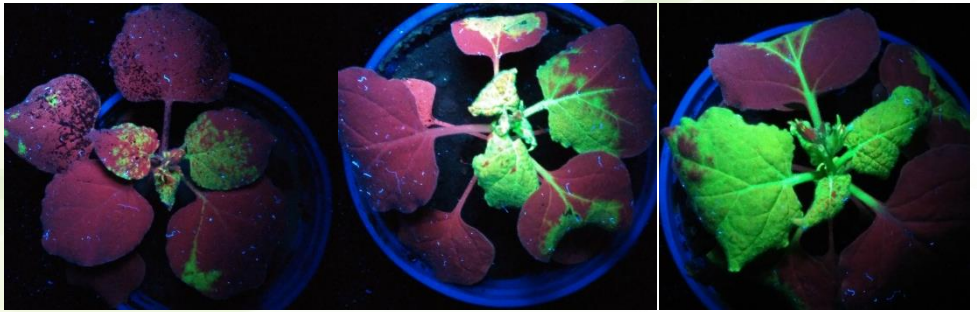
2. Result and Discussion

2.1 Amount of virus in TMV-infected tobacco plants

The amount of virus in TMV-infected leaves was quantified using RT-qPCR. The value showed a peak at 72 h post-inoculation and maintained the high peak value, and it was higher than in controls.

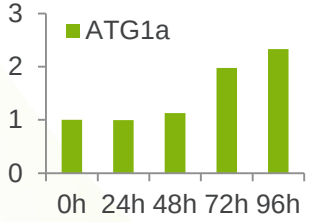
The virus particles (TMV-30B) were analyzed based on the leaf position from the inoculated leaf (1st leaf) to the distal 5th leaf of 2-week-old plants post-inoculation. The number of particles increased significantly in the 2nd leaves, and further increased in the 3rd and 4th leaves from the inoculated leaf and decreased slightly in the 5th leaves, but it was higher than in the controls.

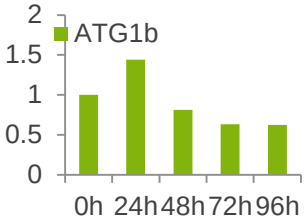


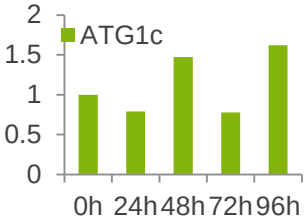


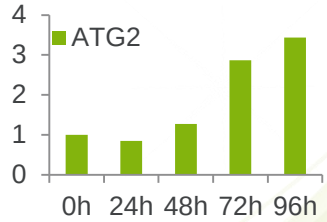
3 days post-inoculation 6 days post-inoculation 12 days post-inoculation

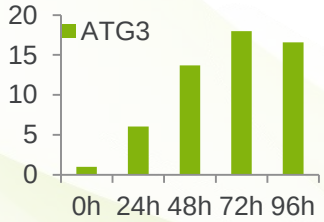
2.2 Partial autopahgy-related gene expression levels were increased as a result of TMV infection

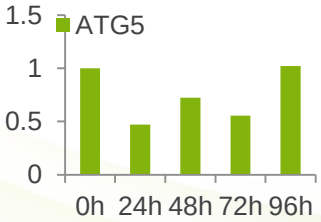


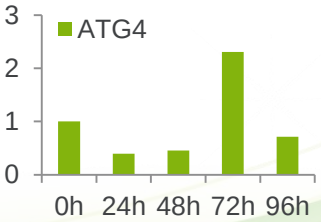


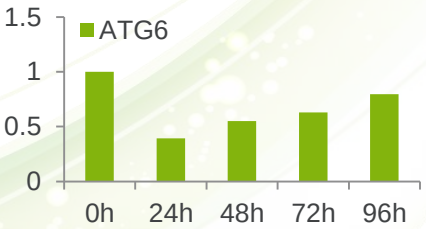


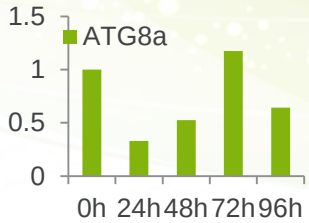


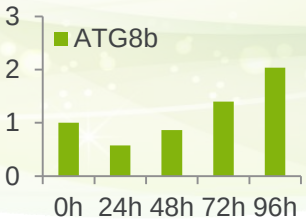


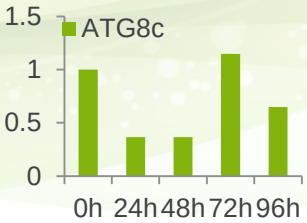


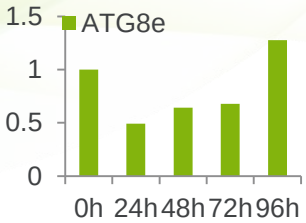


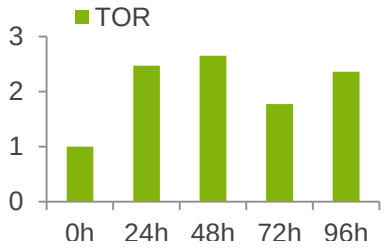


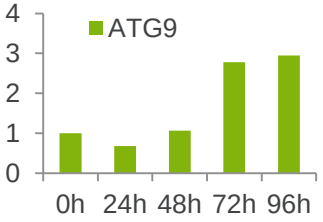


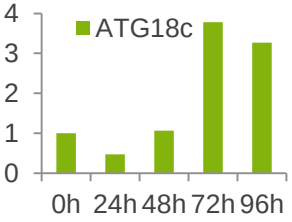


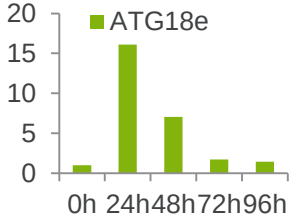


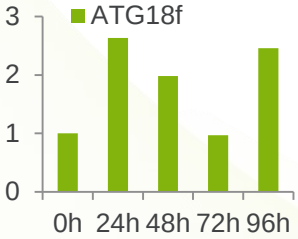


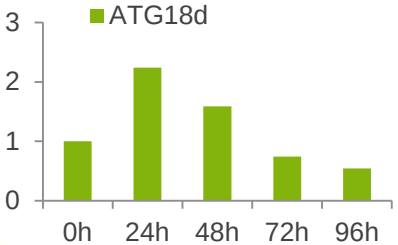


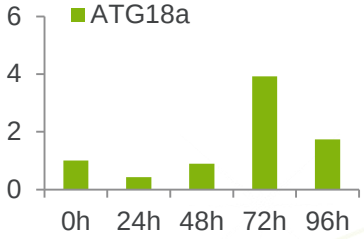


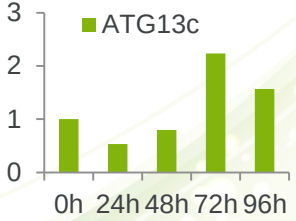


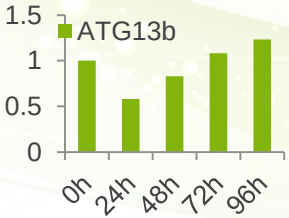


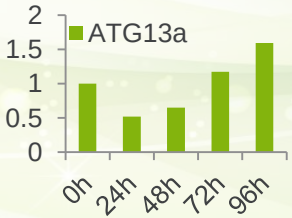


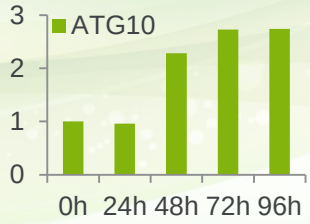


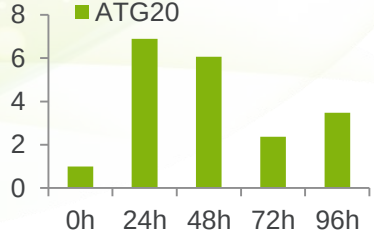












2.3 Monodansylcadaverin (MDC) staining

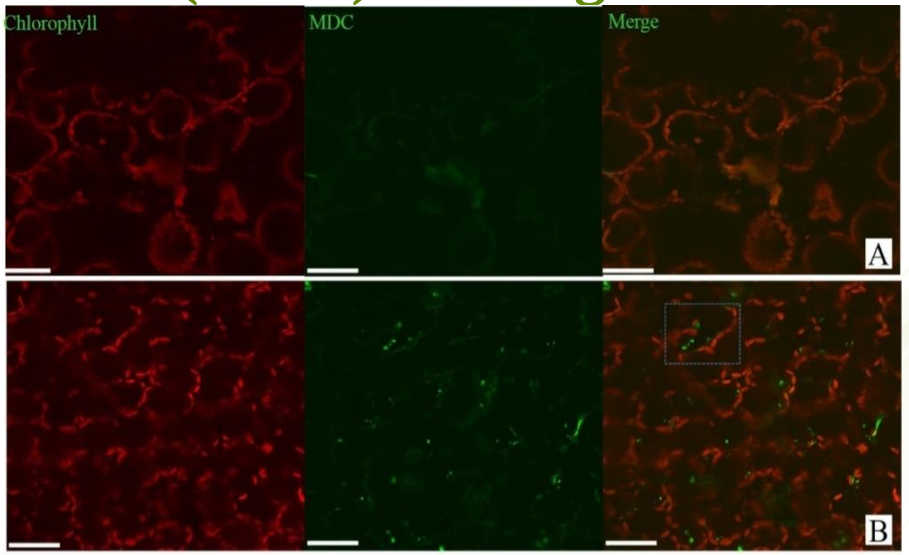
After 72 h post-inoculation of TMV, the leaves were stained with MDC and observed by CLSM. Bar=100μm.

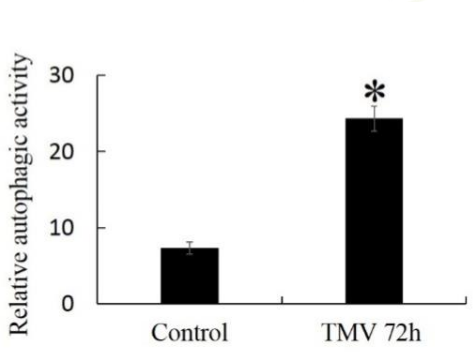
A. PBS treatment

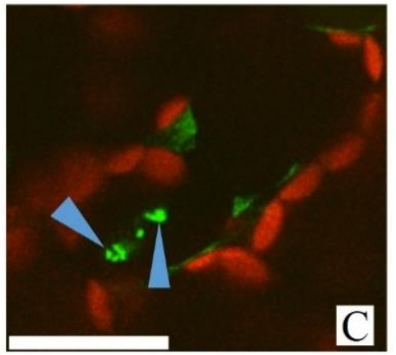
B.TMV infection

The blank control only the central vacuoles were stained and we did not observe any significant alteration, in TMV-infected leaves , MDC stained the autolysosomes in addition to the central vacuole.

These results suggesting that there were autolysosomes in TMV-infected leaves





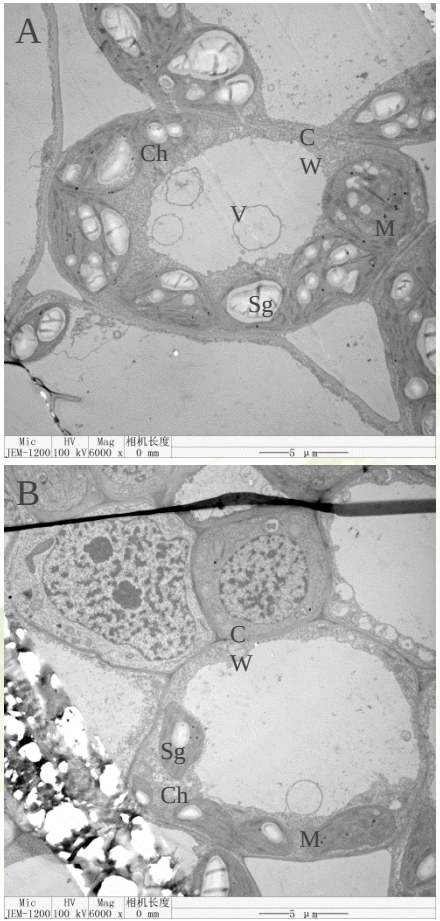


2.4 Cytological changes in TMV-infected leaves

Ultrastructural analysis of normal and TMV-infected leaves using transmission electron microscopy.

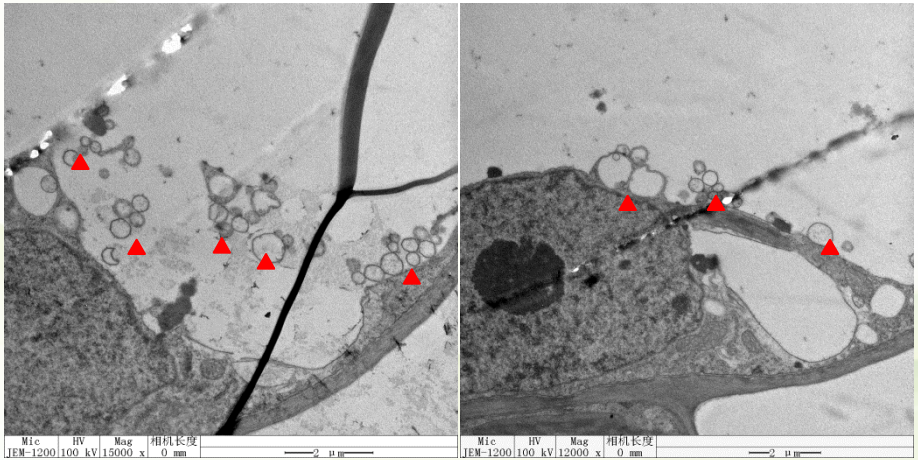
（A）typical fearue such as chloroplasts, including many starch granules, vacuole were observed in the chloroplasts (Ch) of normal healthy leaves. The cell wall (CW) was intact.

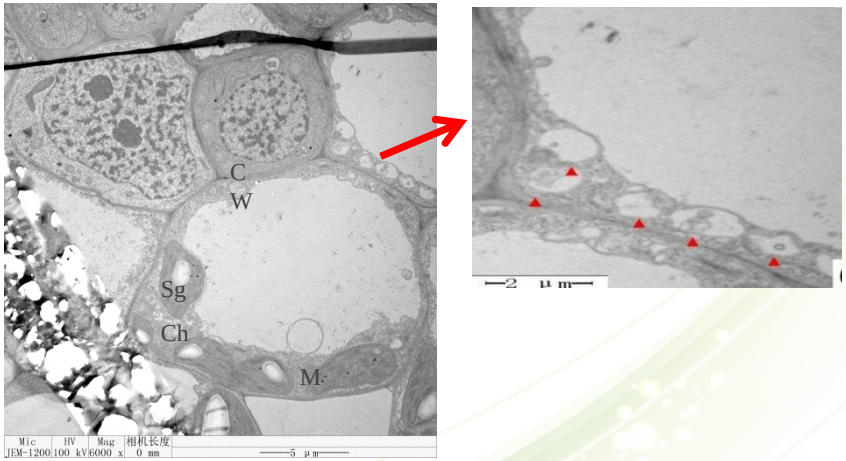
(B) Shrunken chloroplasts, irregular and a reduced number of starch granules (Sgs) were visible in the Ch of tobacco leaves infected with TMV.

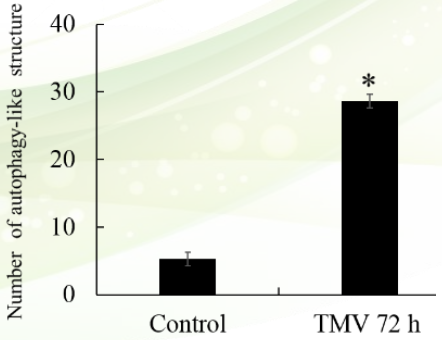


2.5 TMV infection triggered autophagosome and autolysosome formation insusceptible tobacco agianst TMV

Transmission electronic microscripy observation showed that number antophagosome consisting of a double-membarane structure were in the infected cells than in the PBS-treated cells.

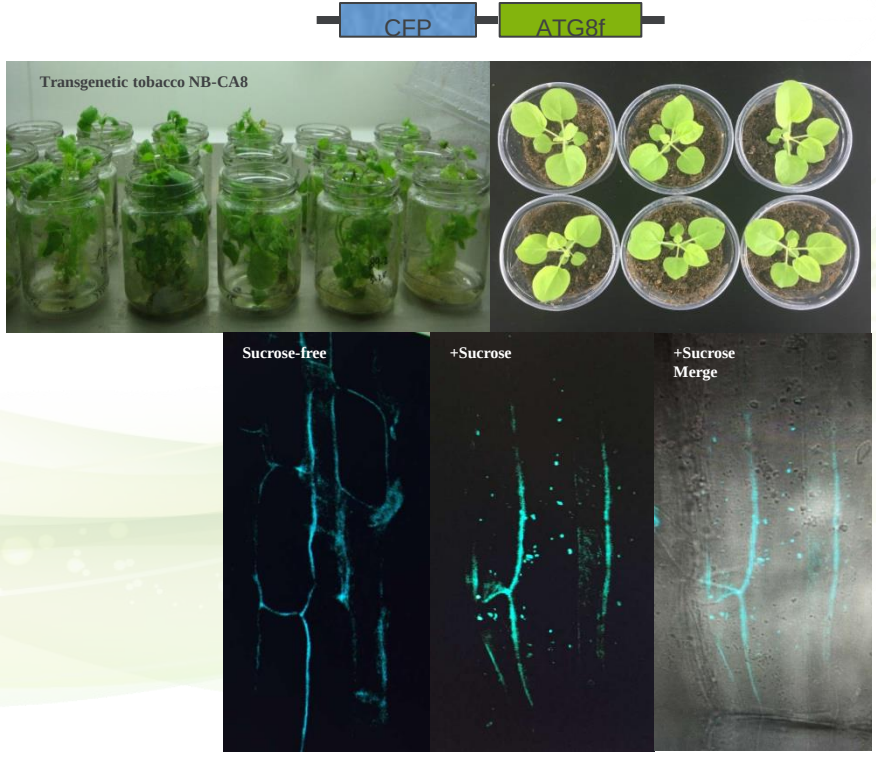






2.6 A non-invasive monitoring system for autophagic activity in tobacco *N. benthamaina* expressing CFP-ATG8

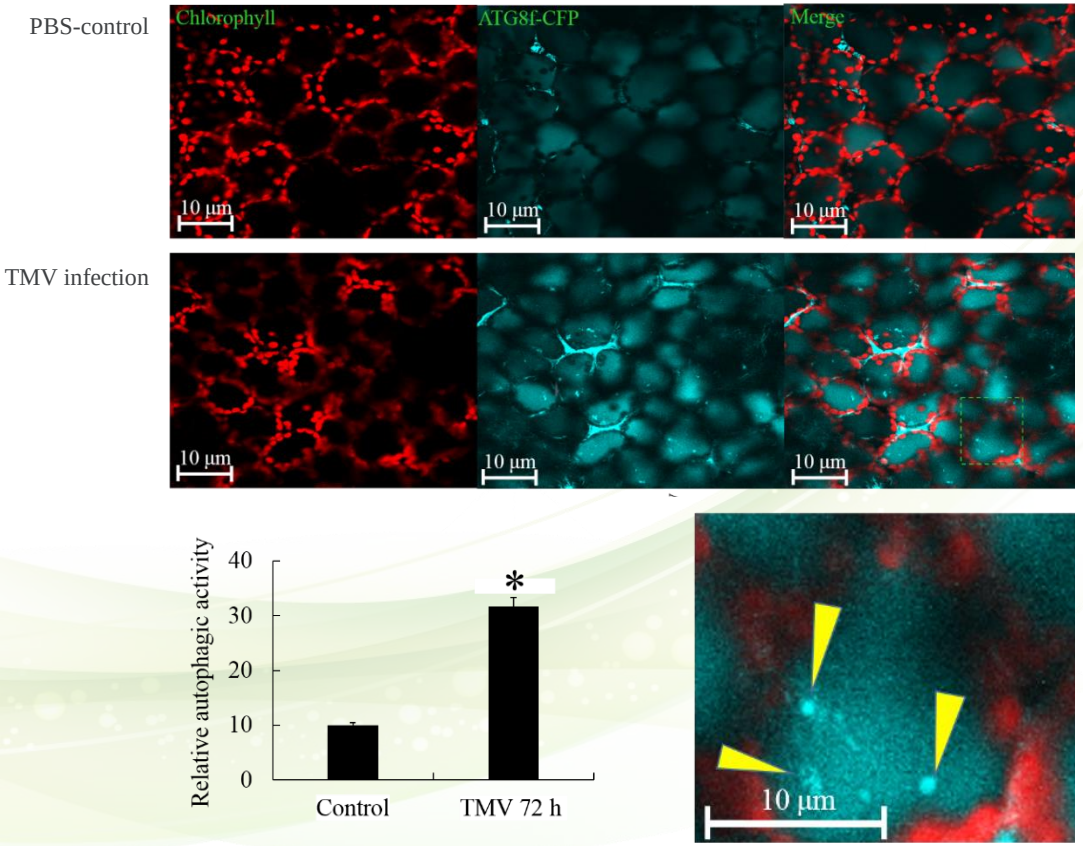
Under normal growth conditions, CFP fluorescence was detected in the cytoplasm and nucleoplasm of NB-CA8 plant. A few punctate signals of CFP-ATG8 were observed in the cytoplasm. When the NB-CA8 plant were transferred to sucrose-free medium, an increase of punctate signals was observed. It reached a plateau at 12 h and did not change until 8 h under sucrose-starved conditions.



2.7 In vivo imaging of autophagic activity under TMV infection

To further monitor autophagic activity of NB-CA8 by TMV infection, the average number of autophagic was measured in ten randomly selected field by CLSM.

The percentage of CFP-ATG8 punctate-positive cells was significantly higher in the TMV-infection leaves than in the negative control leaves.

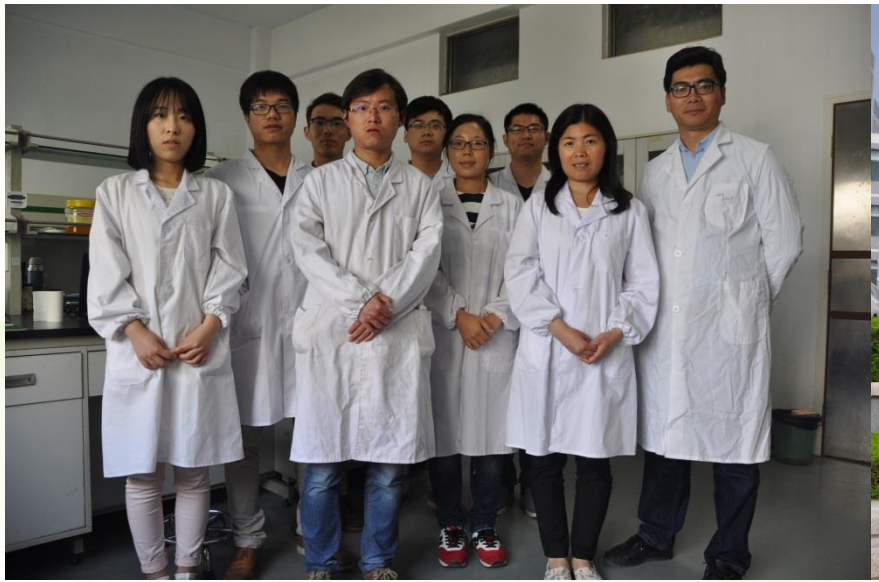


3. Conclusion

- TMV replication was the highest at 3 days post-inoculation in innoculated leaf and higher in leaves distal the inoculated leaf, indicating that TMV moved to young leaves. The expression level of ATGs of tobacco were changed significantly when TMV infection.
- MDC stains assays showed that there are autolysosomes in TMV-infected leaves except to vacoule.
- Autophagosome consisting of a double-membrane structure were observed by transmission electronic microscopy in tobacco under TMV infection.
- The transgenic plant NB-CA8 that stably express CFP-ATG8 fusion protein was generated, which was used to monitor the autophagic activity of tobacco. The percentage of CFP-ATG8 punctage-positive cells was significantly higher in the TMV-infected cells than in the negative control cells.
- **Above all, TMV infection can induce the autophagic activity in susceptible tobacco.**

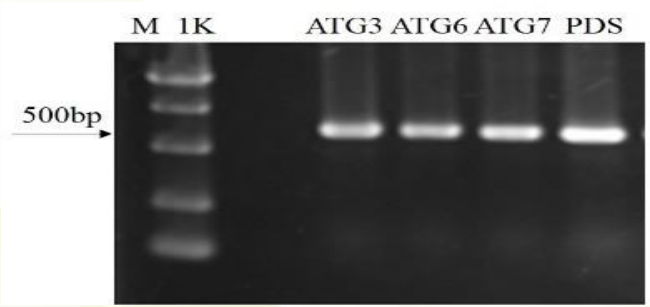
Acknowledgements

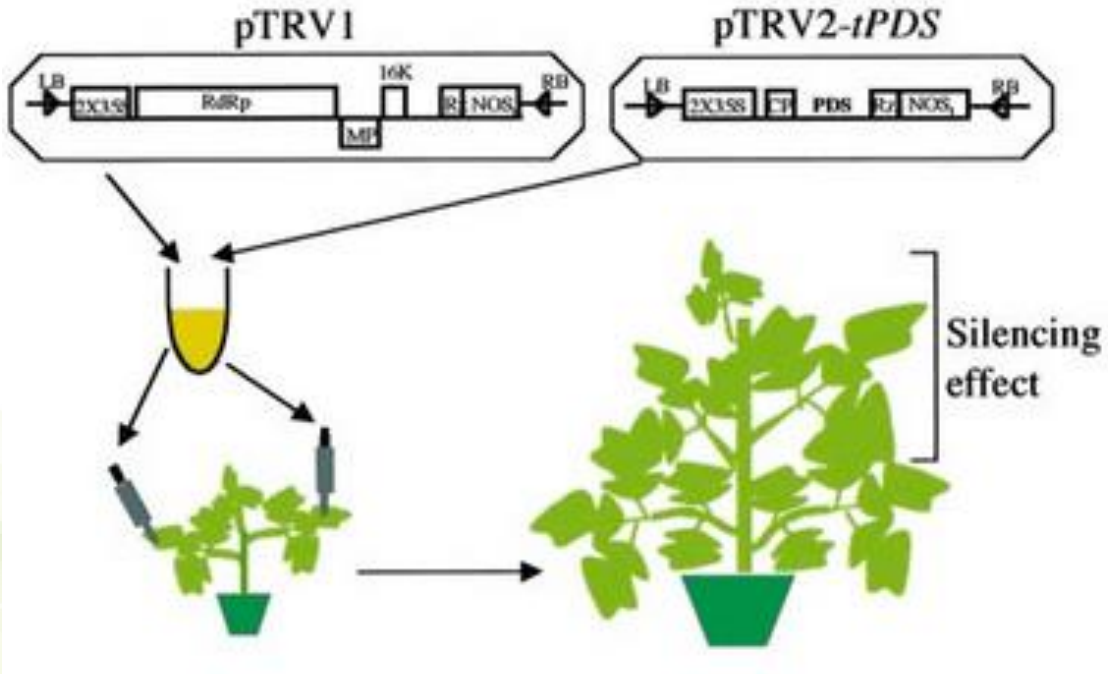
Supported by State Tobacco Monopoly Administration grant.

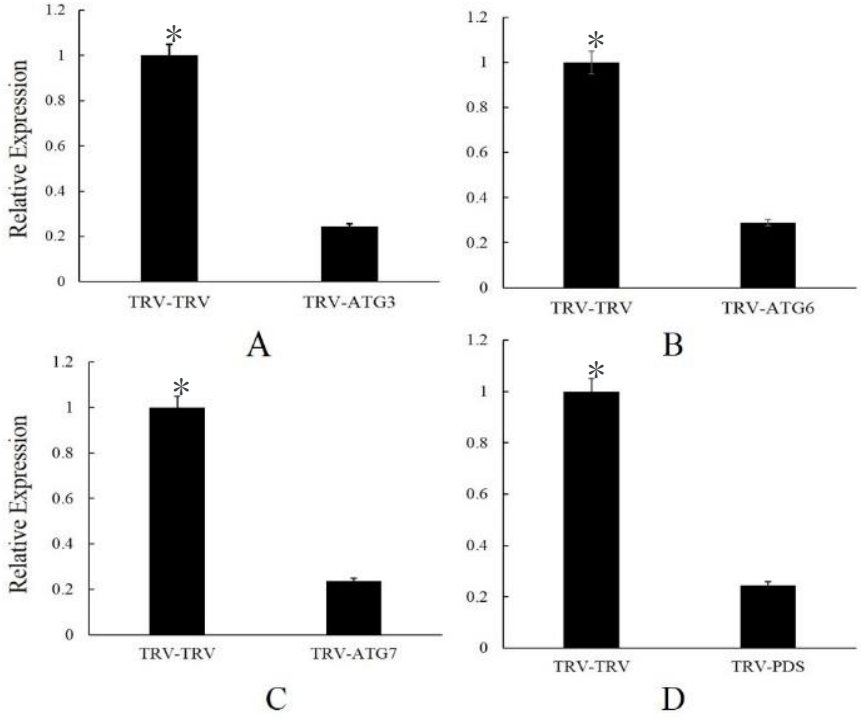


Thank you !

Constructure of ATGs-RNAi based on VIGS







E

Inhibiting autophagy activity of tobacco facilitate TMV infection and replication