Plant proteases mediate 6K1 turnover during *Turnip mosaic virus* infection

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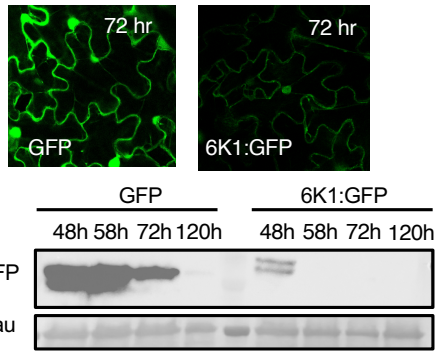
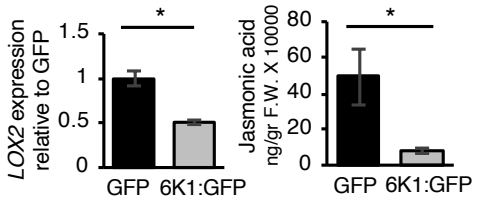
I. Introduction

- Potyviral genomes encode just 11 major proteins with multifunctionality associated to most of these proteins at different stages of virus life cycle.
- The **potyviral protein 6K1** is required for virus replication at the early stages of viral infection and may mediate cell-to-cell movement.
- **6K1** is challenging to study due to instability (See II).
- In this study, we characterise additional functions associated with the 6K1 protein from *Turnip mosaic virus* (TuMV) and the underlying molecular mechanisms of instability using *Nicotiana benthamiana*.

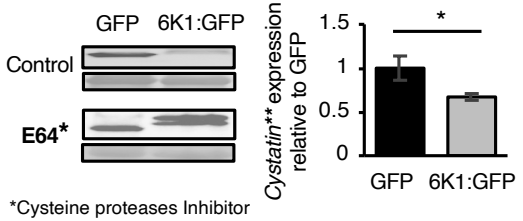
II. The ectopically expressed 6K1 protein is instable



III. 6K1 expression inhibits jasmonic acid accumulation



IV. The 6K1 protein is degraded by cysteine proteases



*Cysteine proteases Inhibitor
**Encodes protein inhibiting cysteine proteases

V. TuMV (Tu) infection induced more protease inhibitor transcripts compared to aphid feeding (Ap)

PROTEASE INHIBITORS TRANSCRIPTS

TAIR ID	Gene	Ap	Tu
AT5G47550	CYS5		
AT4G16500	CYS4		
AT2G31980	CYS2		
AT1G55230	DUF716		
AT2G02120	LCR70		
AT5G48490	DIR1-LIKE		
AT3G53980	N.F		
AT1G17860	ATKTI5		
AT5G43570	K9D7.7		
AT2G38870	T7F6.4		
AT3G04320	T6K12.6		
AT5G55460	LTP4.5		
AT2G37870	T8P21.22		
AT5G43580	UPI		
AT2G02100	LCR69		
AT5G05960	K18J17.13		
AT5G62080	MTG10.3		
AT2G02130	LCR68		
AT1G64020	F22C12.27		
AT1G73325	N.F		
AT3G58550	LTPG22		
AT5G54740	SESA5		
AT1G51330	F11M15.19		

The number of proteases inhibitors transcripts

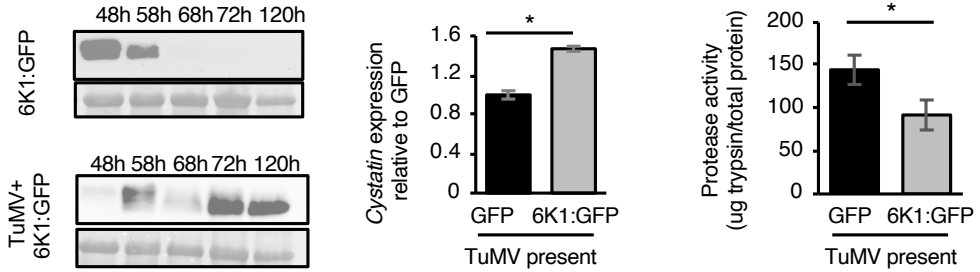
Induced	
Ap	Tu
3	12

Repressed	
Ap	Tu
20	11

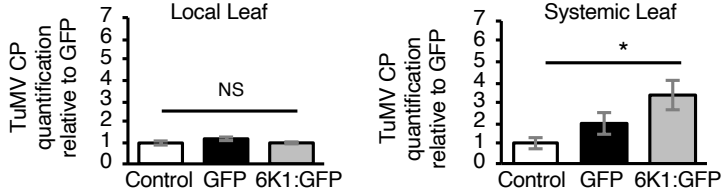
χ^2	*P*
8.01	0.005

Fold change relative to the mock
Lowest — 0 — Highest

VI. 6K1 protein stability increases, and protease activity decreases during TuMV infection



VII. 6K1 expression increases TuMV systemic movement



VIII. Conclusions

- The stability of the 6K1 protein is dynamic and depends on cysteine proteases.
- 6K1 degrades rapidly at early time points in the infection process, whereas at later stages 6K1 becomes more stable when protease activity is reduced, resulting in increased systemic movement of TuMV.

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