



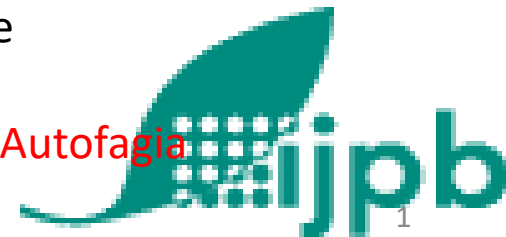
# Role of autophagy in leaf senescence and nutrient management at the whole plant level

Céline Masclaux-Daubresse  
[celine.masclaux-daubresse@inrae.fr](mailto:celine.masclaux-daubresse@inrae.fr)

INRAE Versailles France  
Institut National Recherche Agronomique

Plenary talk. SEFAGIA 2020 Sociedad Espanola de Autofagia  
4-6 of April 2020 Caceres Spain

**INRAE**  
la science pour la vie, l'humain, la terre



# Institut Jean-Pierre Bourgin, INRAE Versailles, FRANCE







Animals can move  
when thirsty, hungry, bad weather  
or dangers

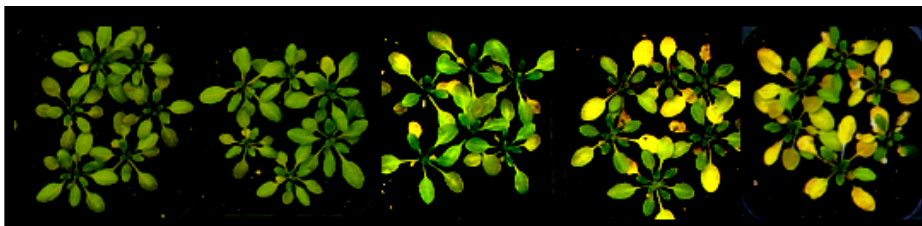
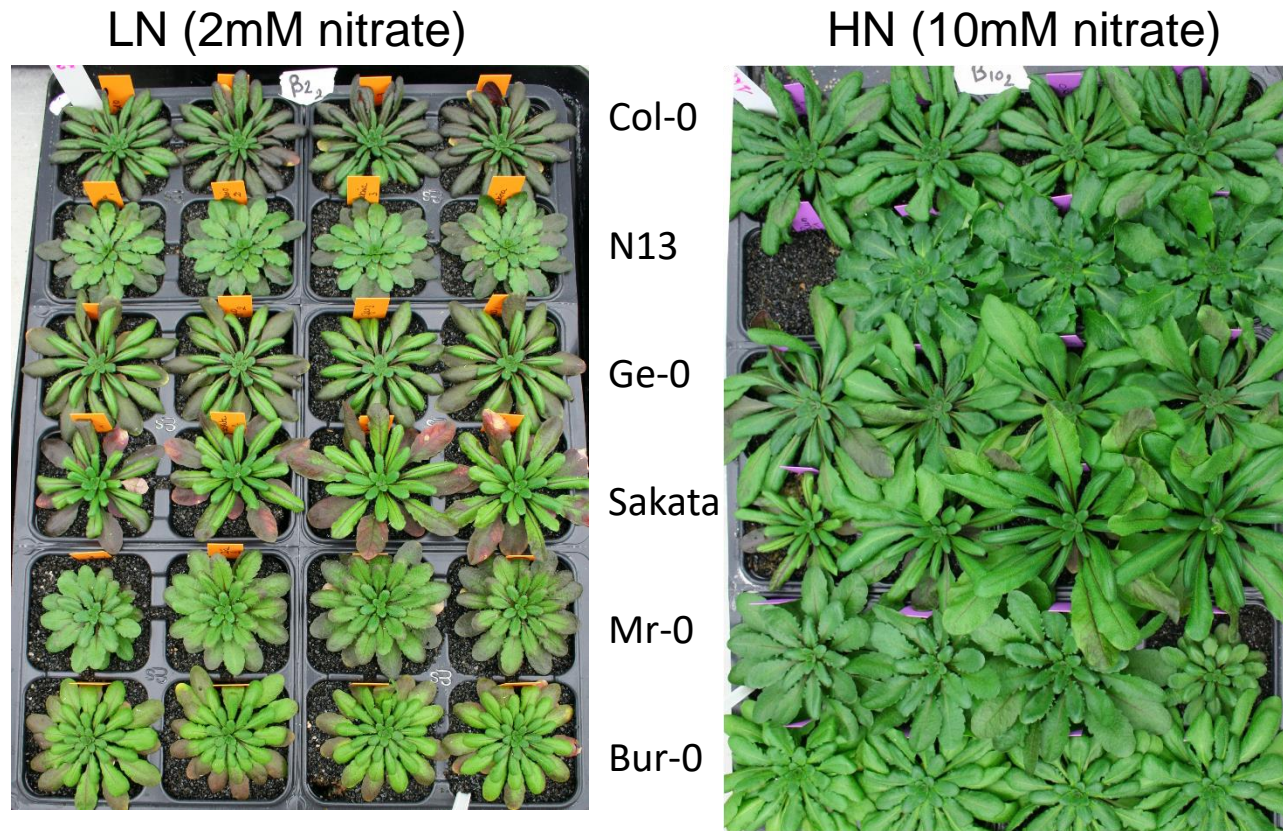


Plants cannot



I have a dream...

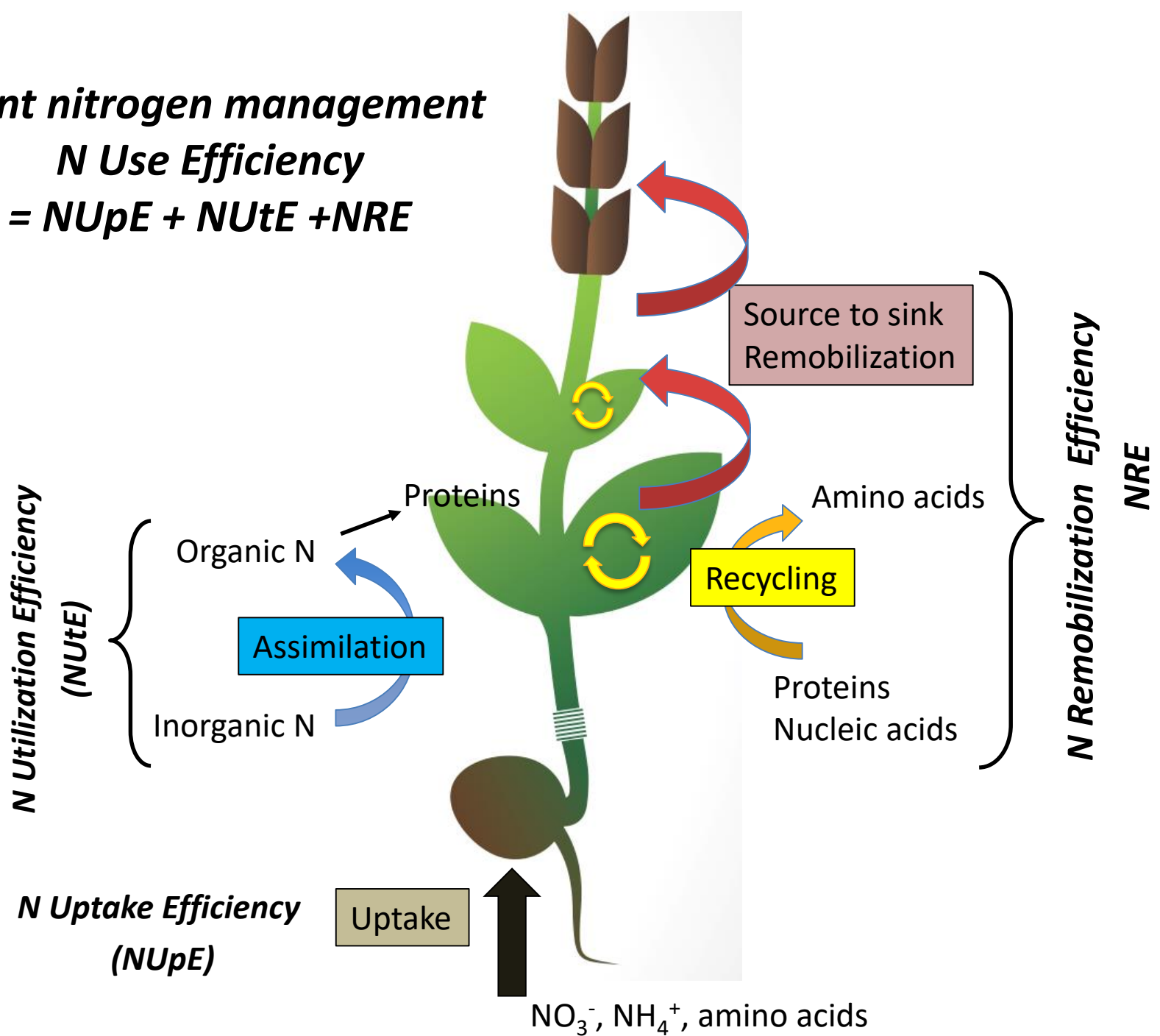
# N is one of the main macronutrients limiting plant growth



# Plant nitrogen management

## N Use Efficiency

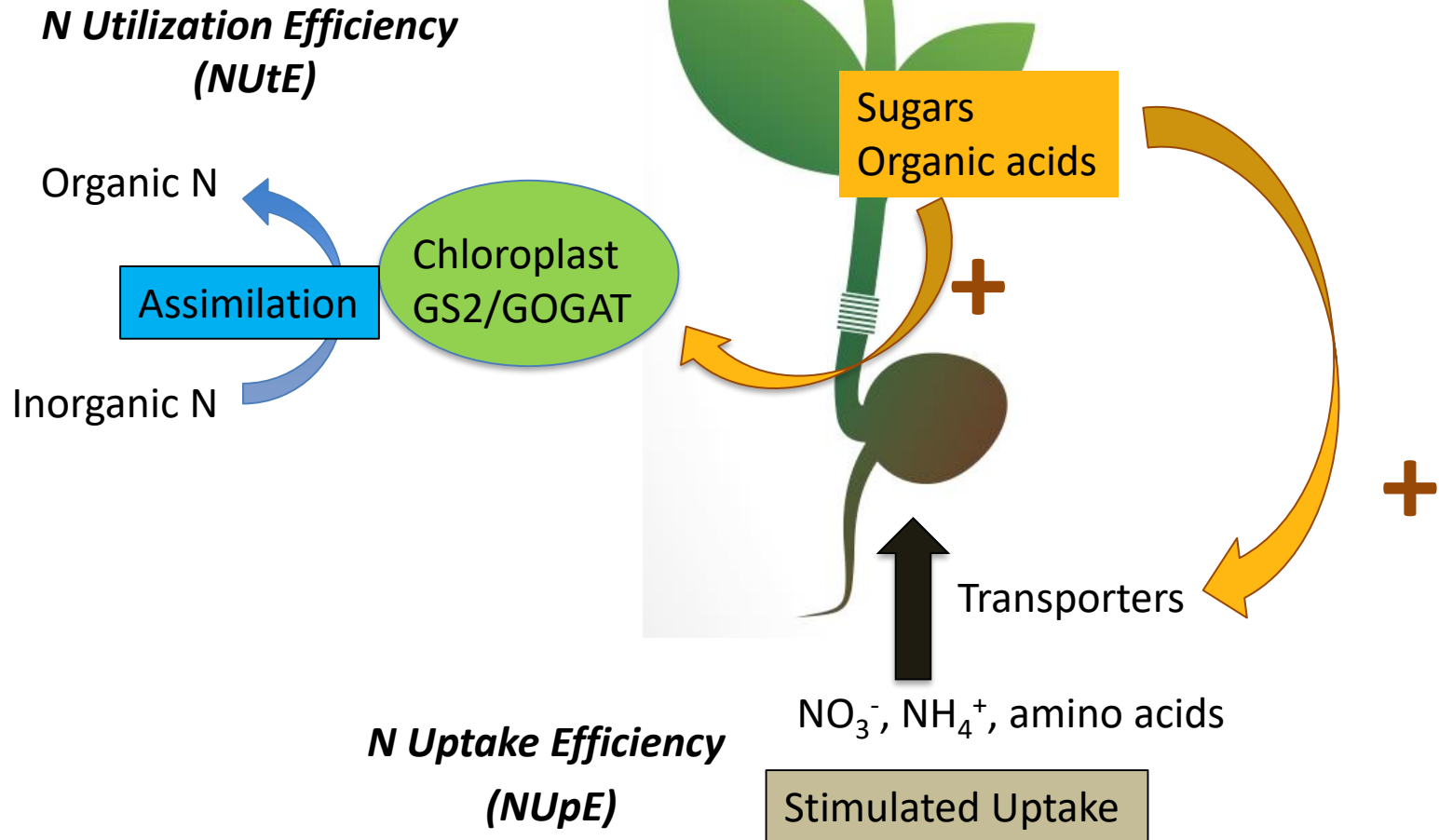
$$= NUpE + NUtE + NRE$$





# Young plant

Active Photosynthesis



# Nutrient recycling and remobilization mainly occurs during senescence

Monocarpic leaf senescence

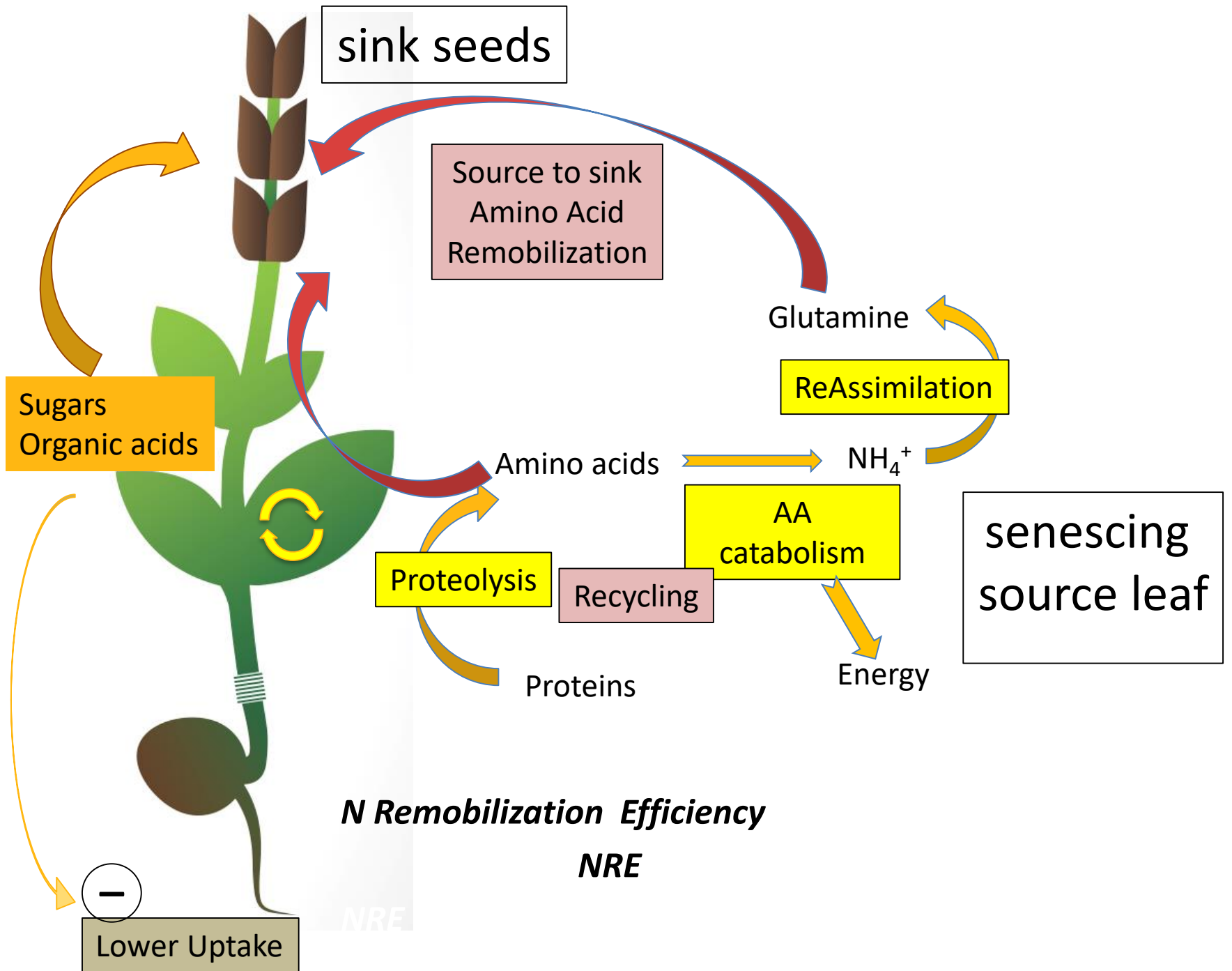


*Magnolia grandiflora*, Versailles INRA

Sequential leaf senescence



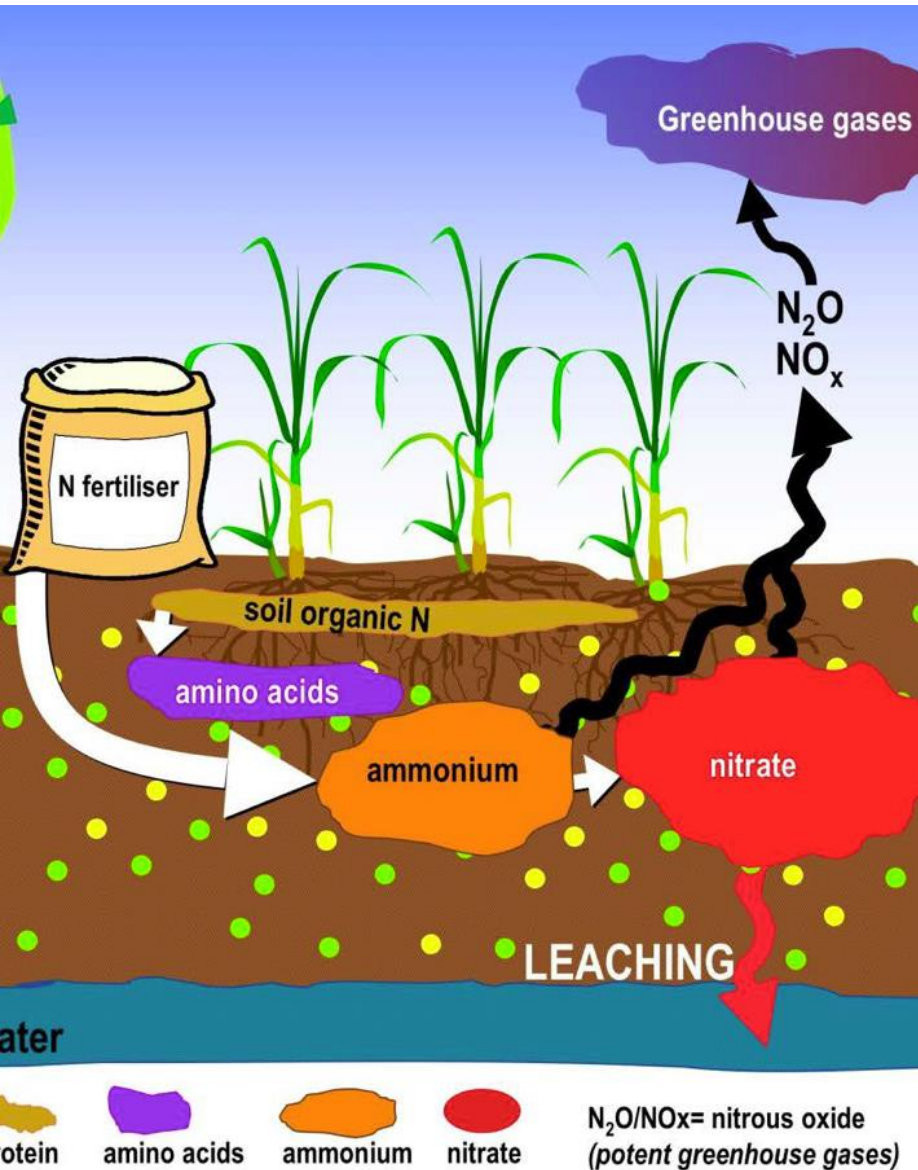
Liu et al. Biol. Res. vol.49 2016



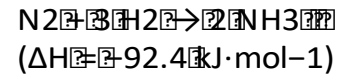


Why to study nitrogen management  
during leaf senescence?

# Too much fertilizers in agriculture



Haber-Bosch



Consumes 1% to 2% of the world energy

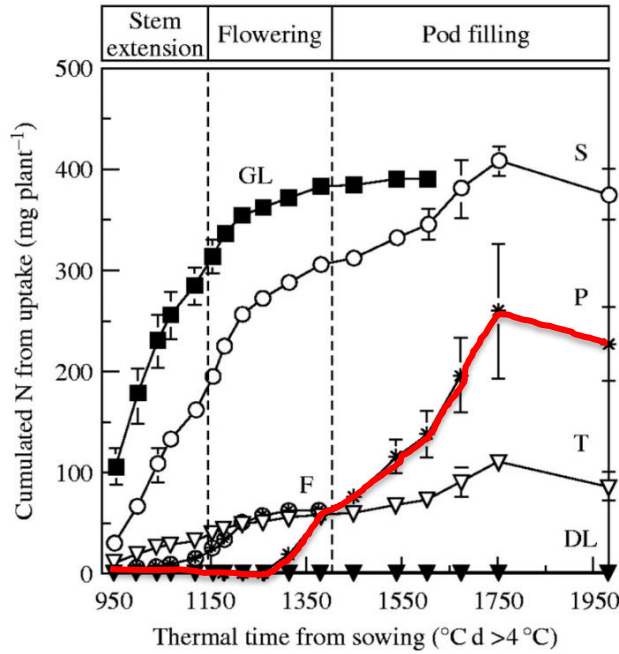


## BONNES VACANCES

L'élevage industriel des porcs et les engrais génèrent des algues vertes. Leur décomposition dégage un gaz mortel pour l'homme.



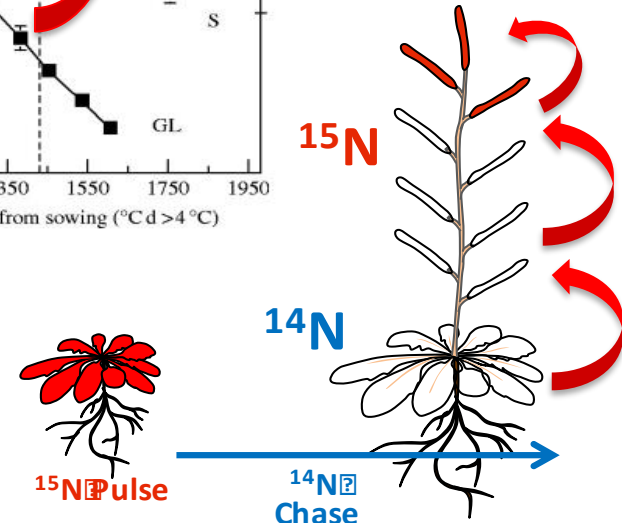
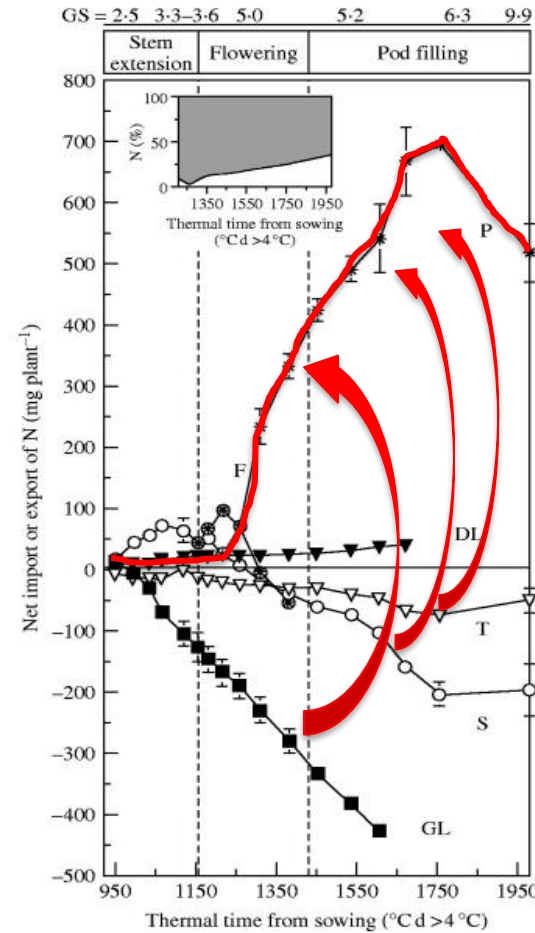
# N uptake vs. N recycling for grain filling



<sup>15</sup>N pulse



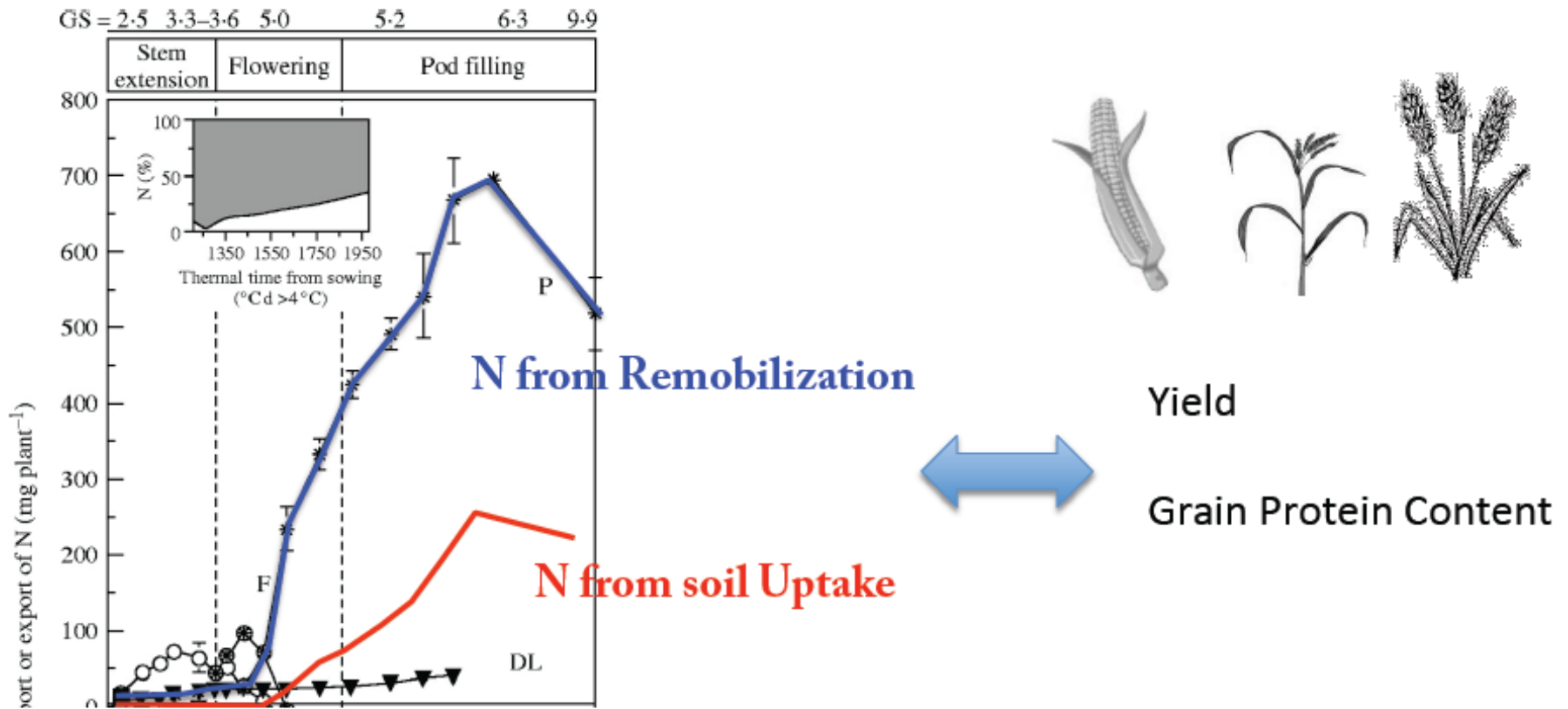
N Uptake from the soil during grain filling



N Remobilization from other organs for grain filling



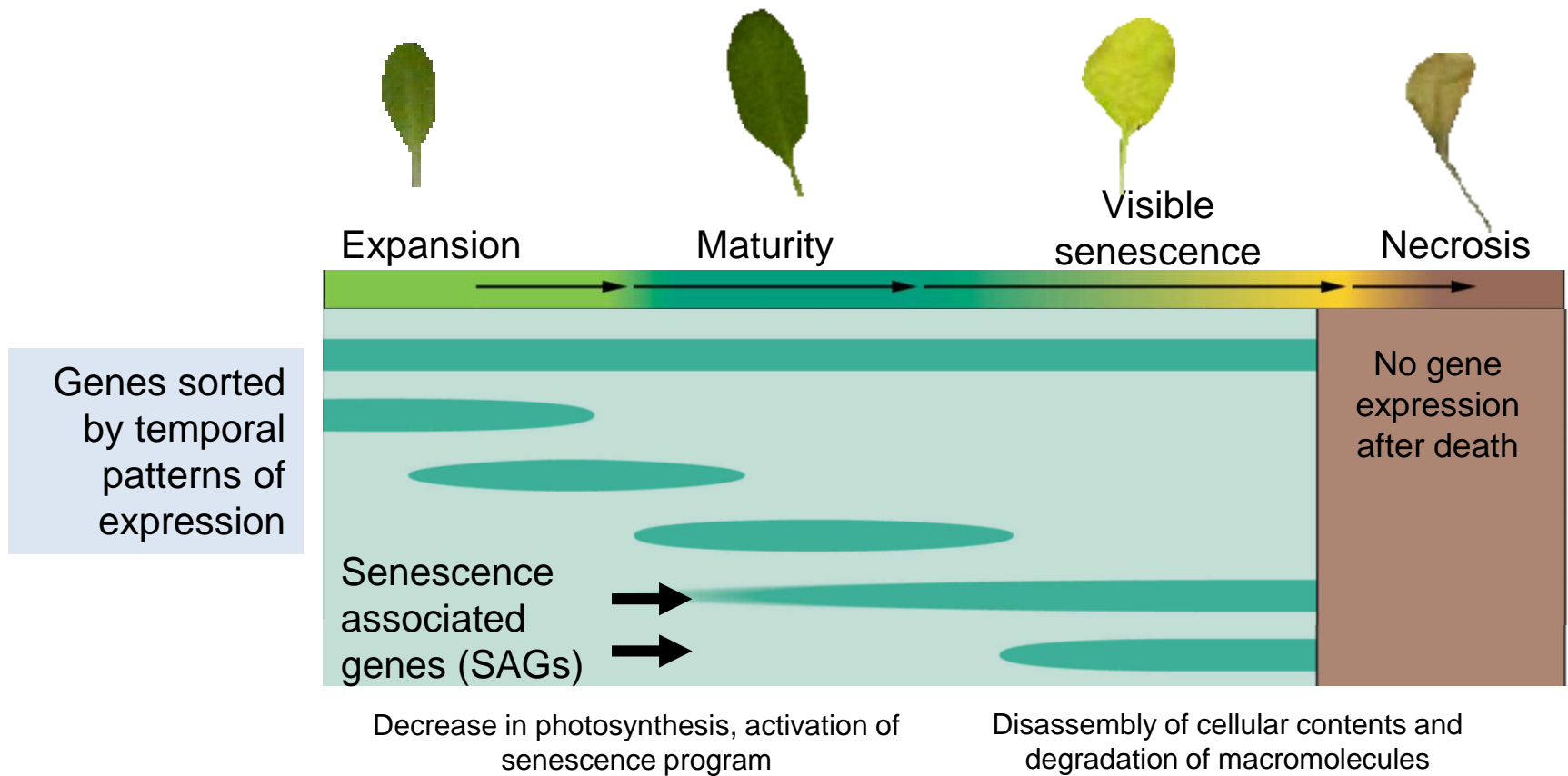
# Seed protein content mainly relies on N-remobilization from senescing source organs



Oilseed rape. Malagoli et al. 2005

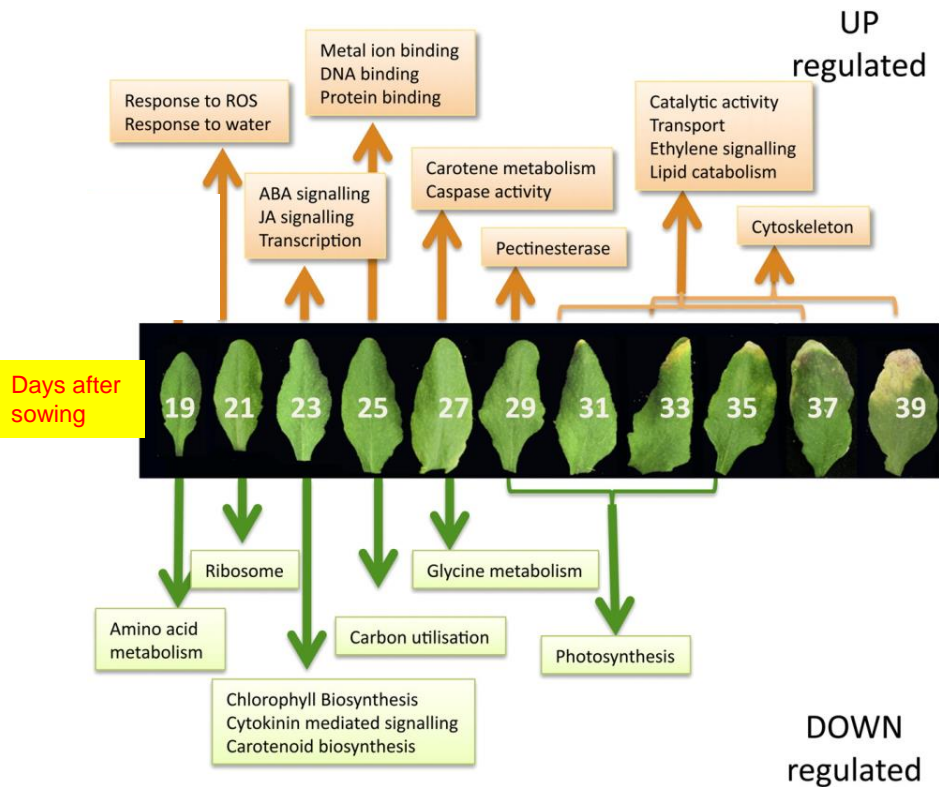
How do leaves senescence  
and remobilize nitrogen?

# Many transcriptome analyses to identify Senescence Associated Genes SAGs

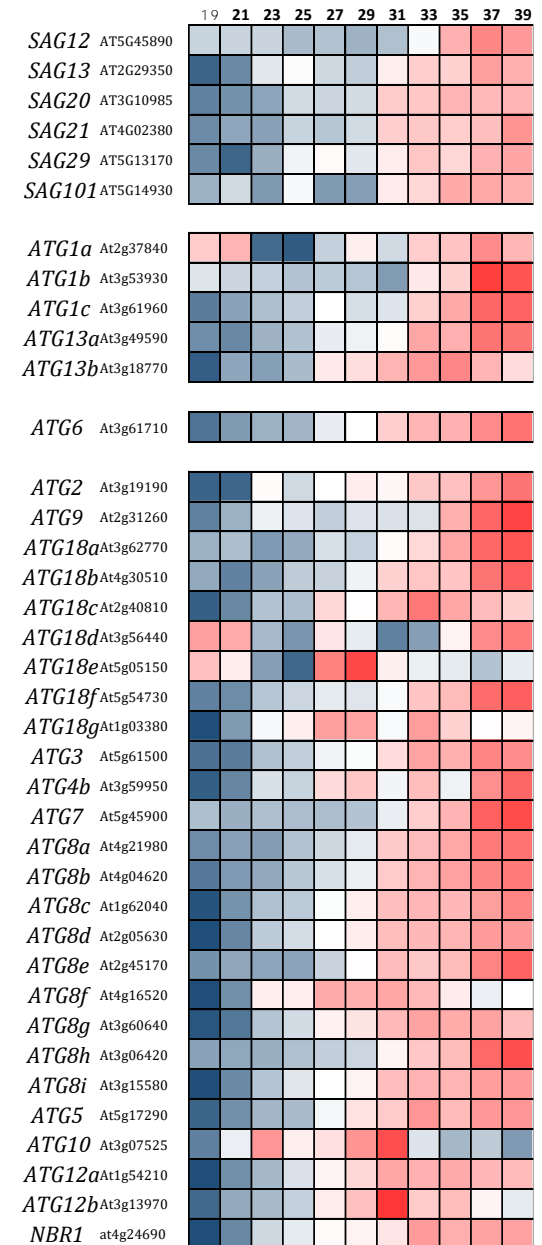




# Proteins encoded by SAGs reveal senescence processes



## Autophagy genes are SAGs





Leaf expansion



Chloroplast 10  $\mu\text{m}$   
70% of leaf proteins

Senescence



Gerontoplast 4  $\mu\text{m}$

Autophagy?

Chloroplast dismantling



Proteolysis, hydrolysis

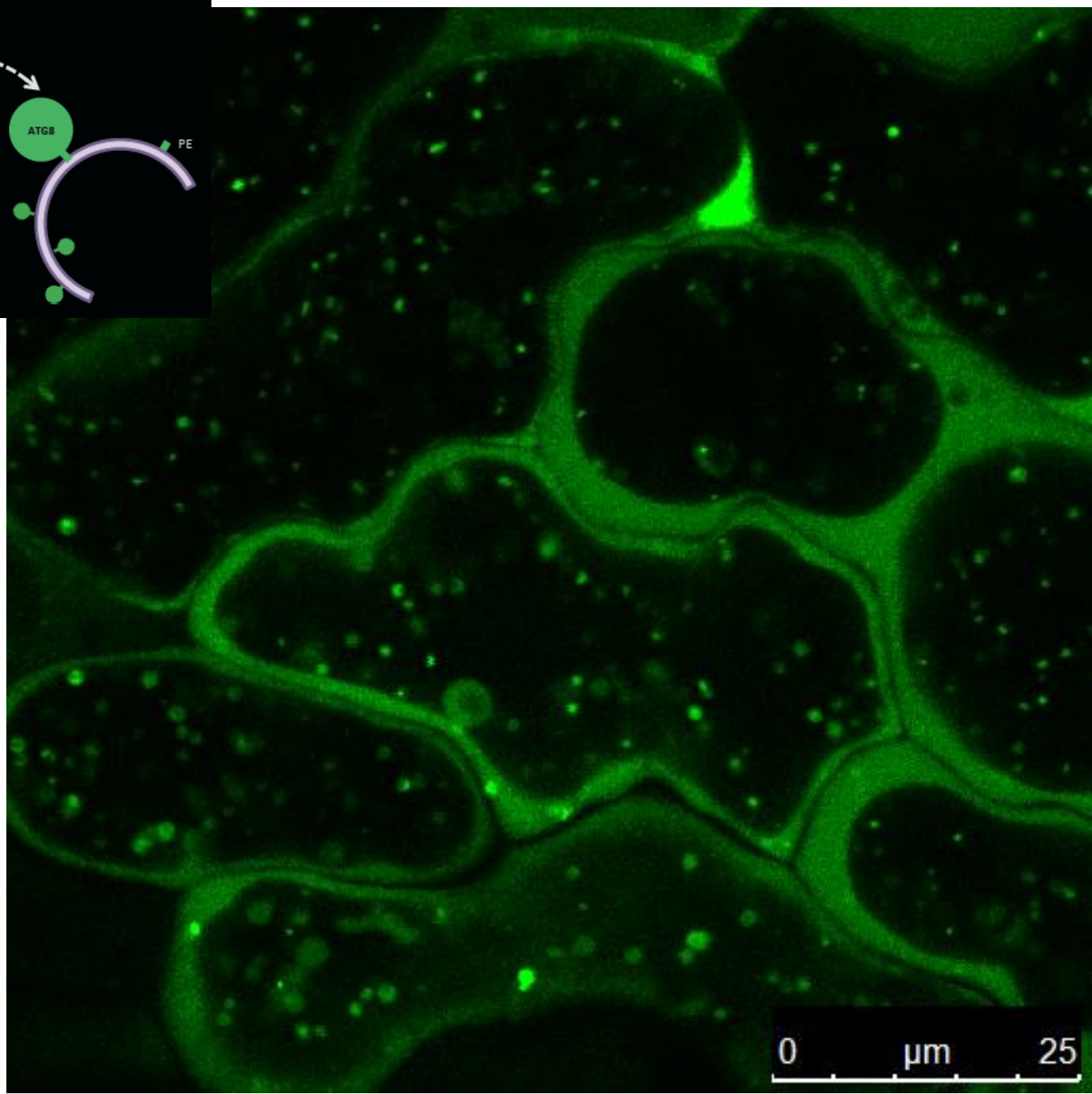
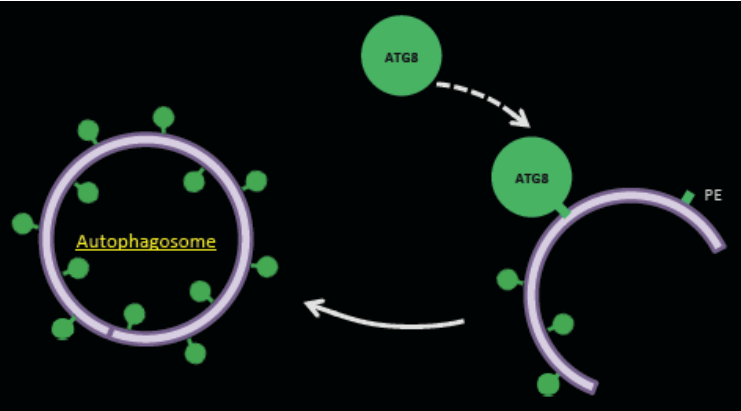


N re-assimilation/interconversion



N translocation to seeds

Senescence associated proteases  
mainly in the central vacuole

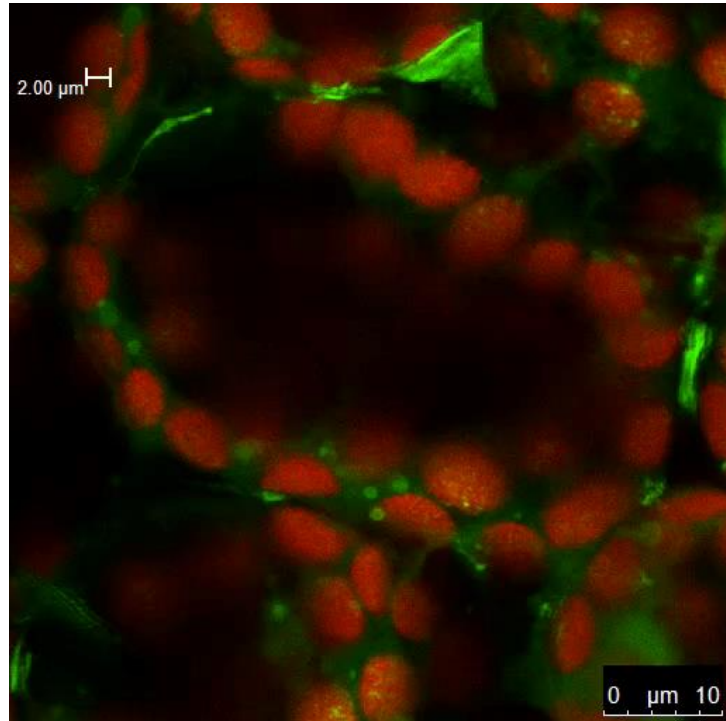


GFP-ATG8 tools  
Leaf epidermis

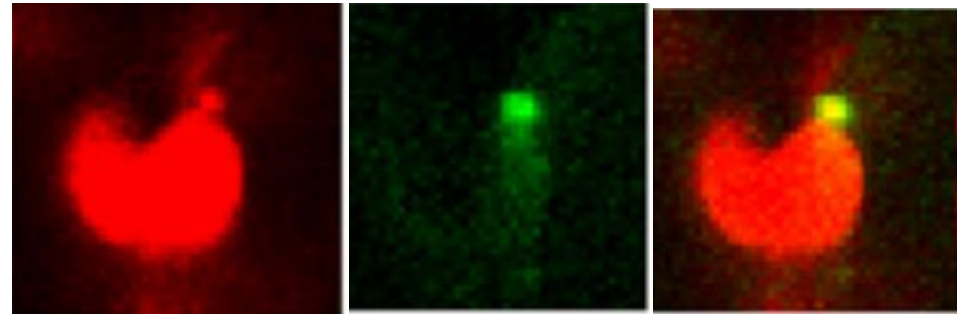
With concanamycin A



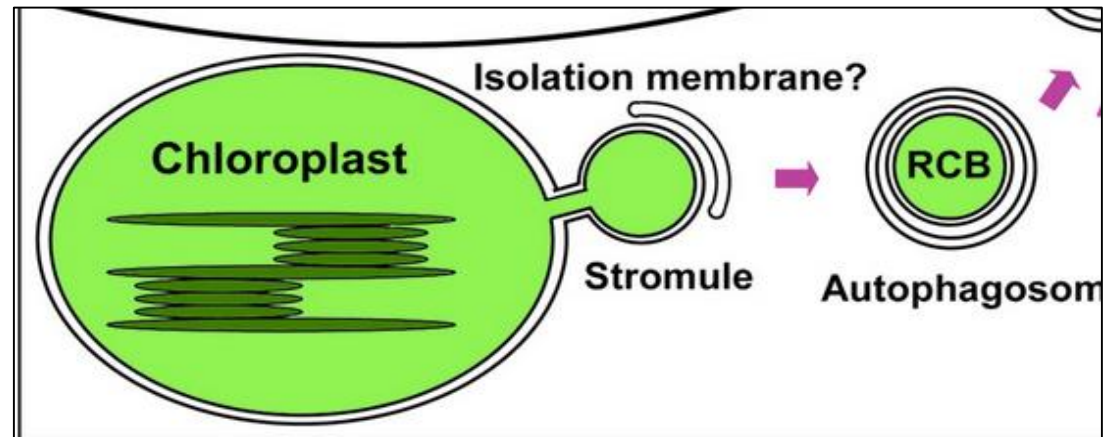
# Autophagy participates to chloroplast degradation



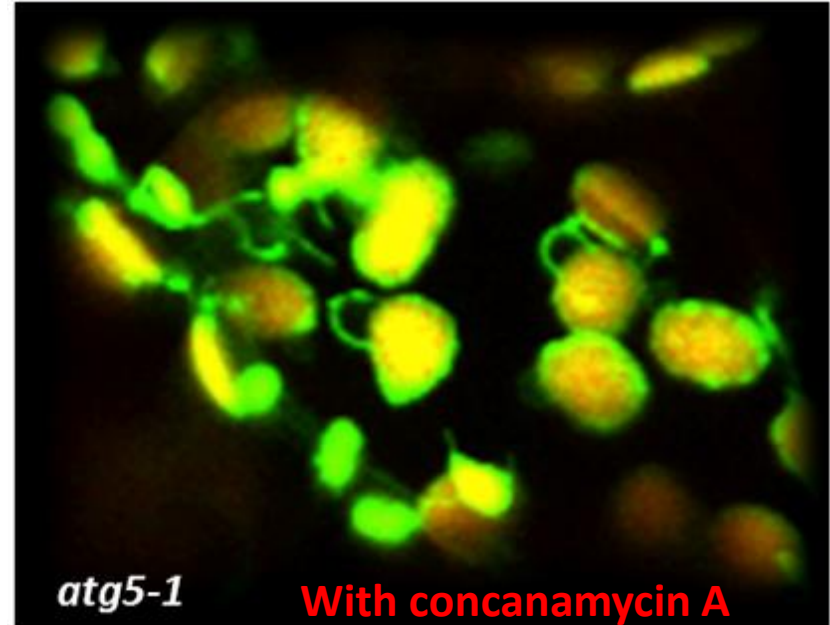
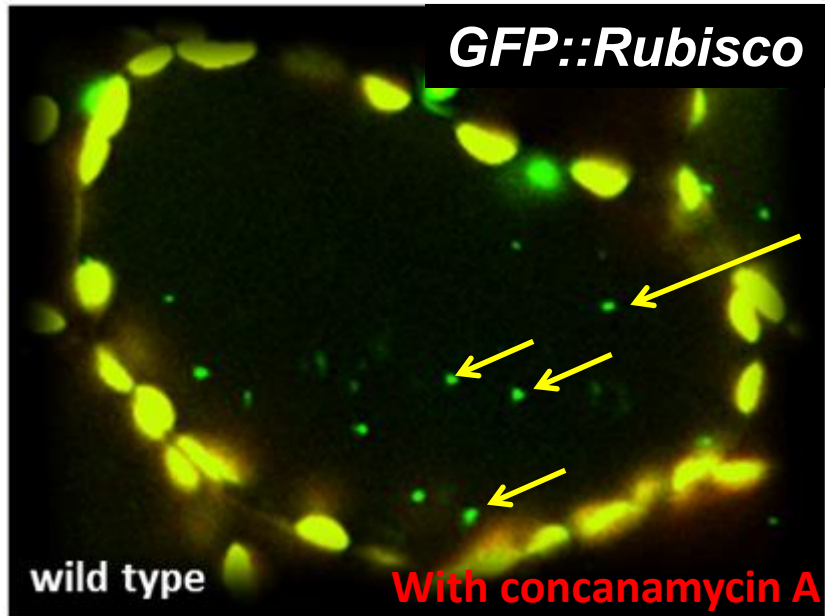
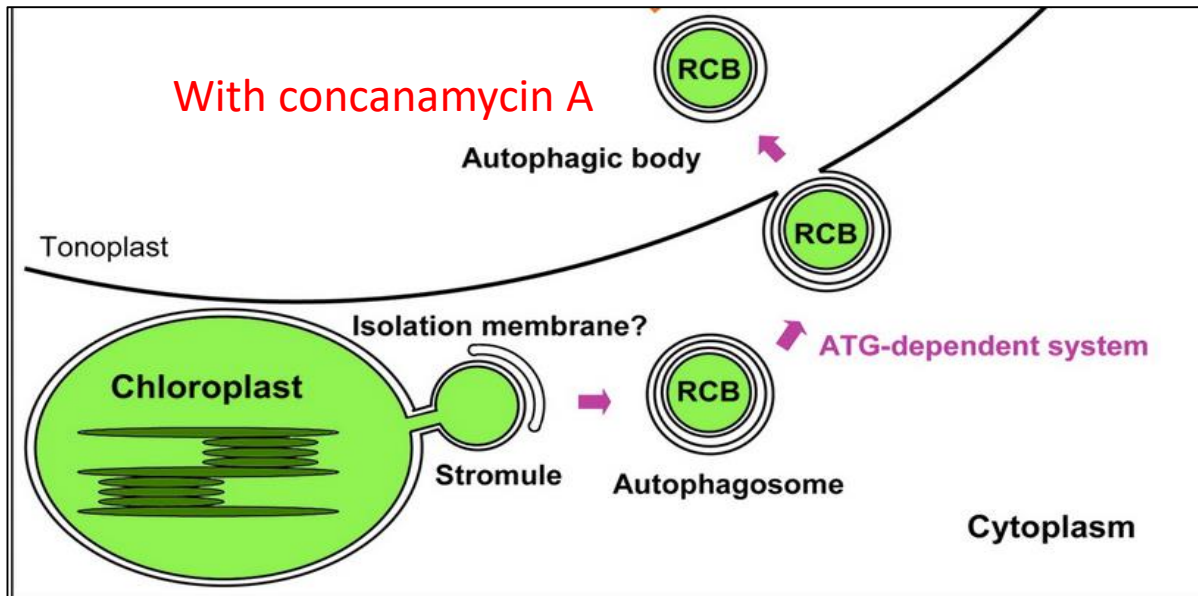
Mesophyll cells



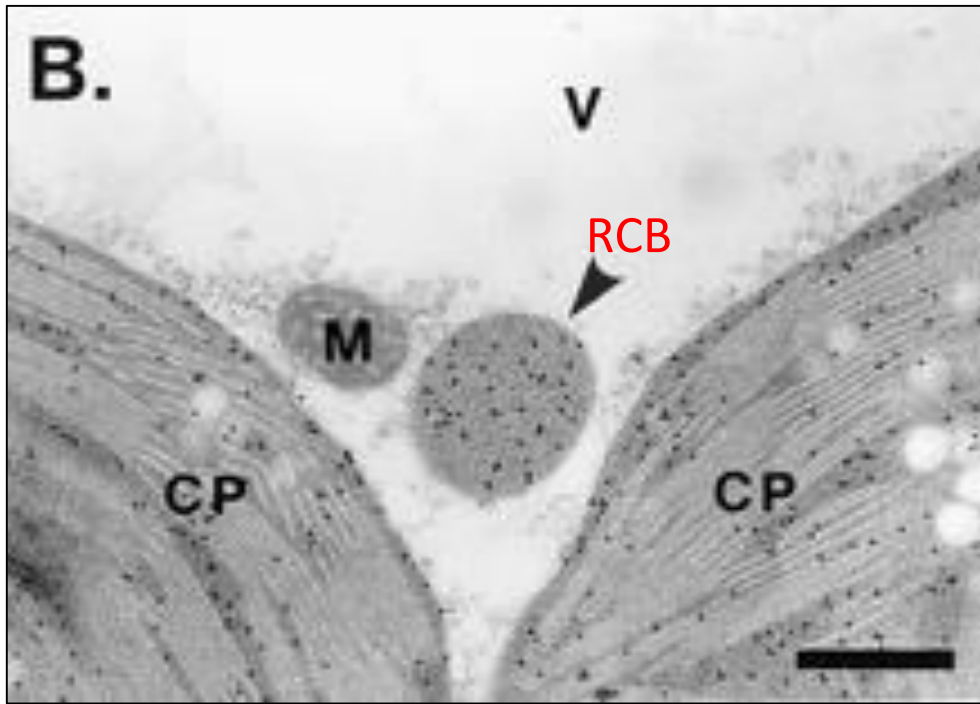
Stroma-DsRed GFP-ATG8 merge  
Ishida et al. Plant Physiol. (2008)



# Trafficking of RCB to the vacuole is autophagy dependent

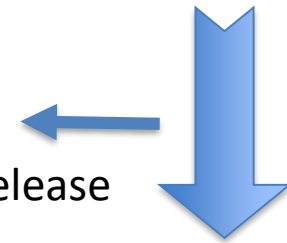


# ImmunoGold localization of Rubisco and GS2 in wheat senescing leaves

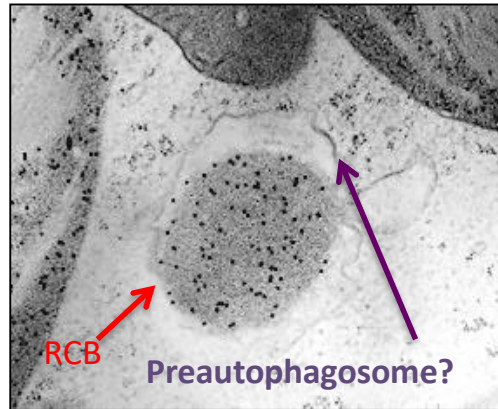
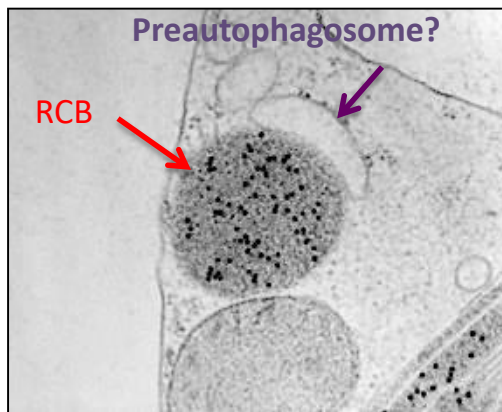


Chloroplast 10  $\mu\text{m}$

RCB material release

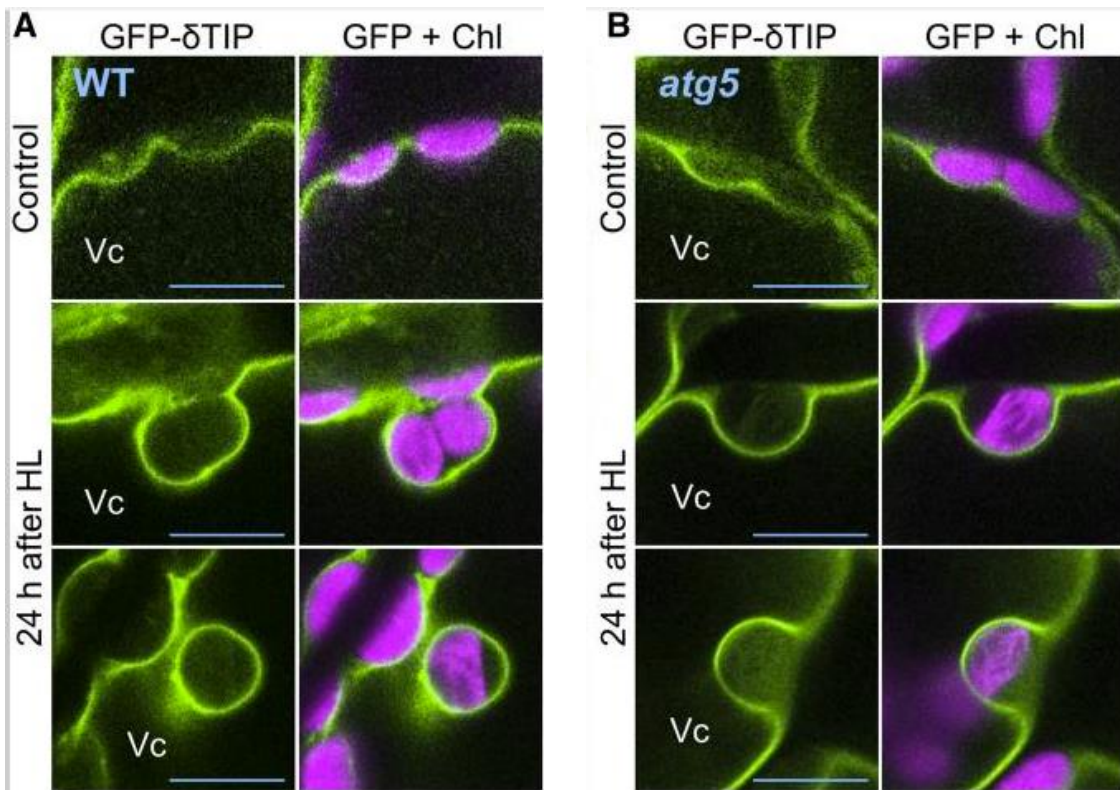


Shrunken Gerontoplast 4  $\mu\text{m}$





# Microautophagy of UV-damaged chloroplasts in Arabidopsis

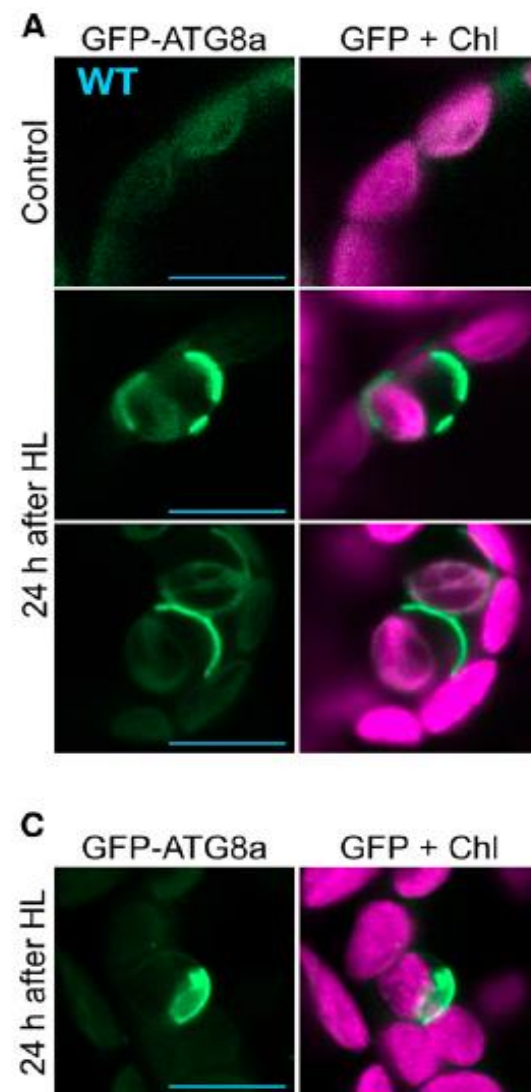


Wild type seedlings

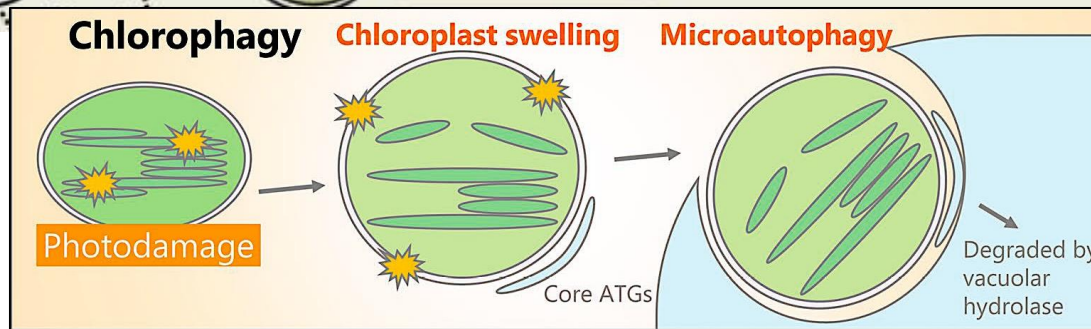
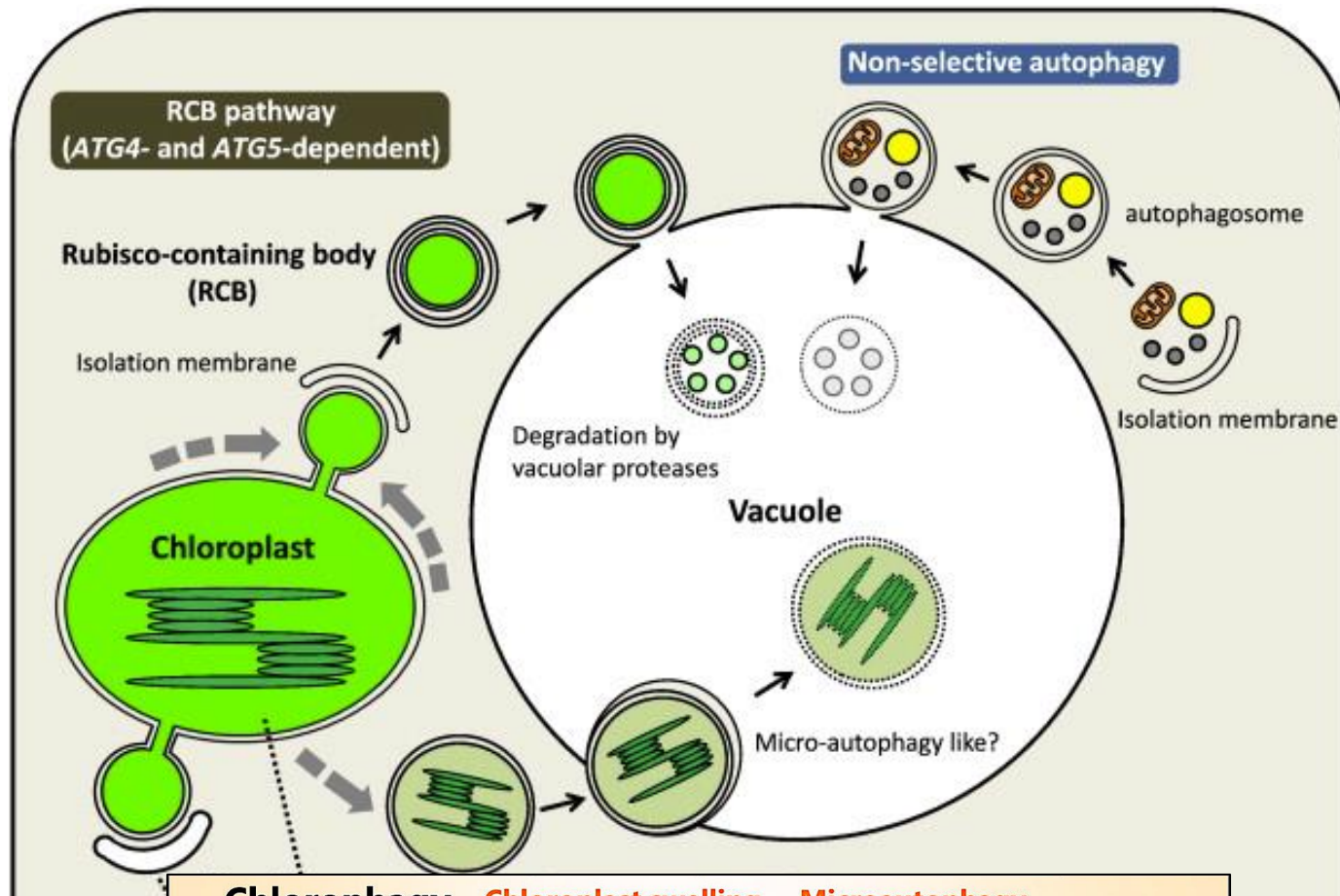
*atg5* seedlings

**No release inside the vacuole**

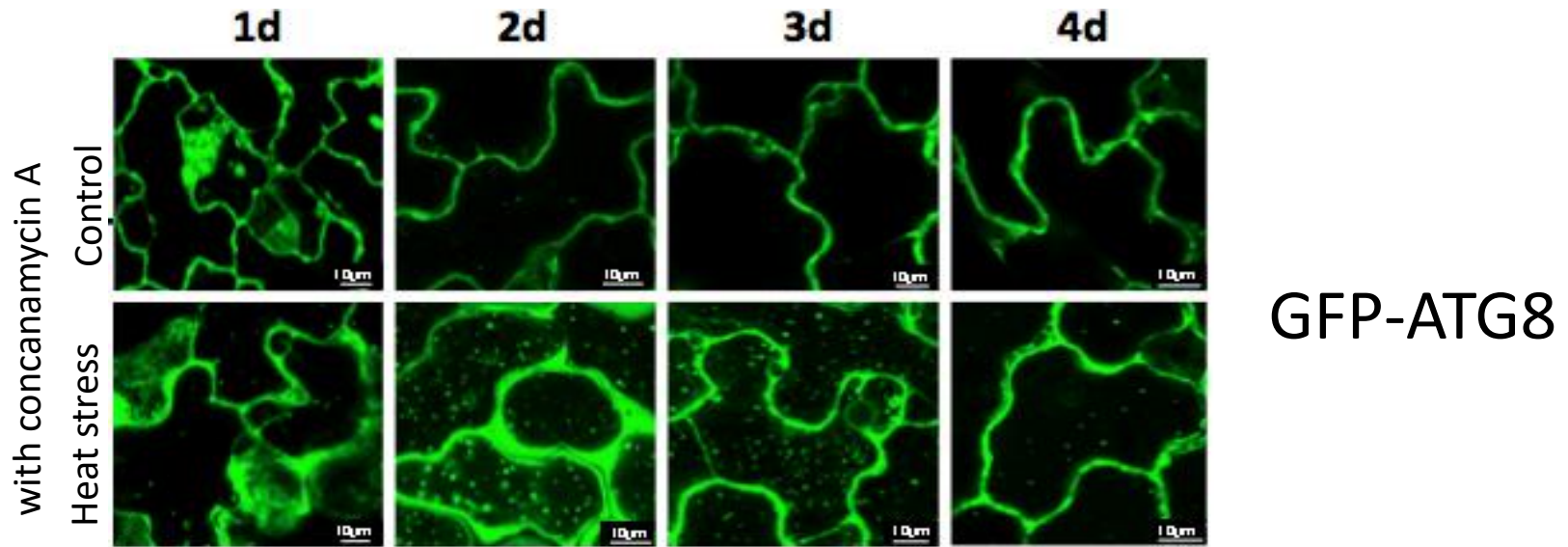
## Wild type seedlings



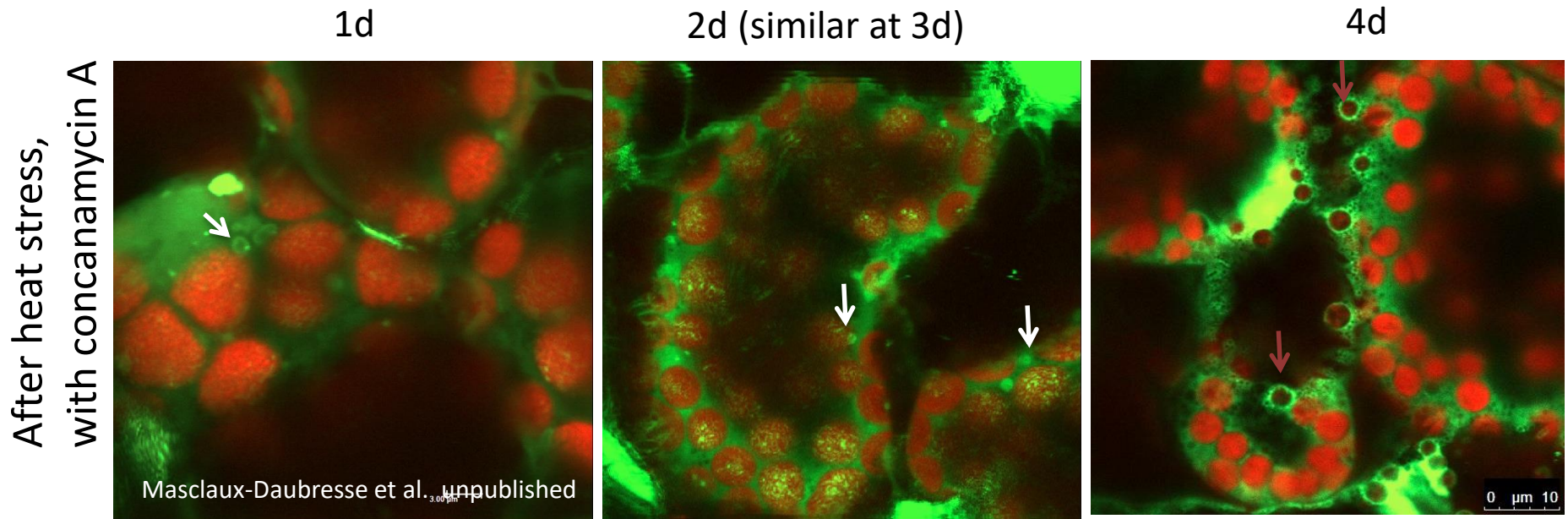




# Chlorophagy after heat shock treatments (Col. S. Balazadeh)



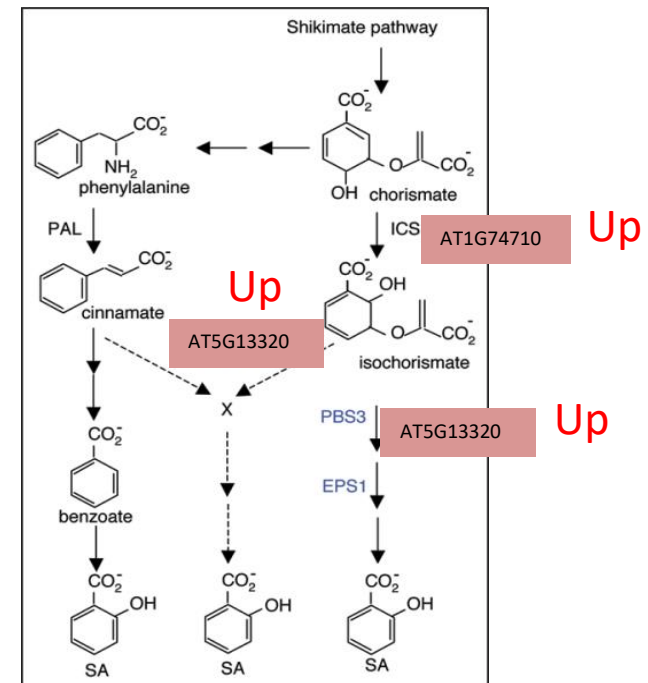
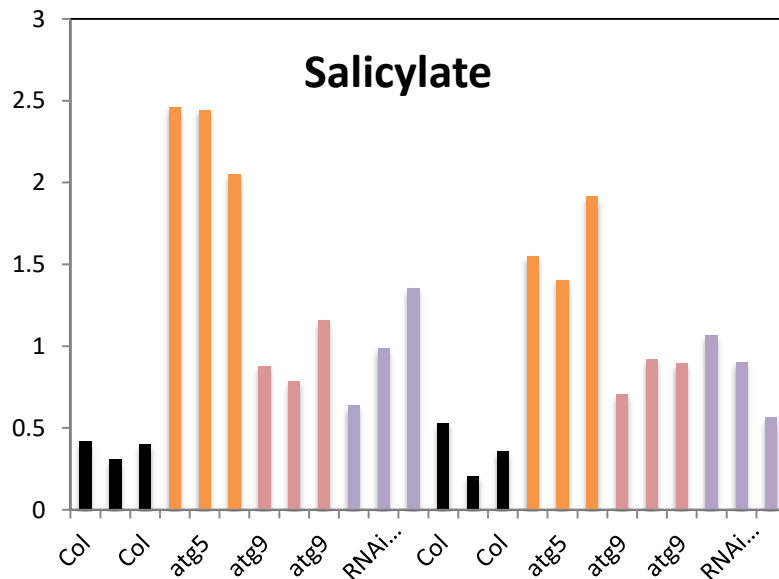
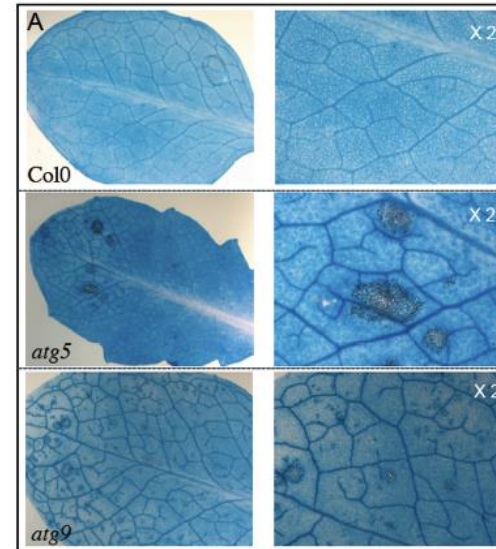
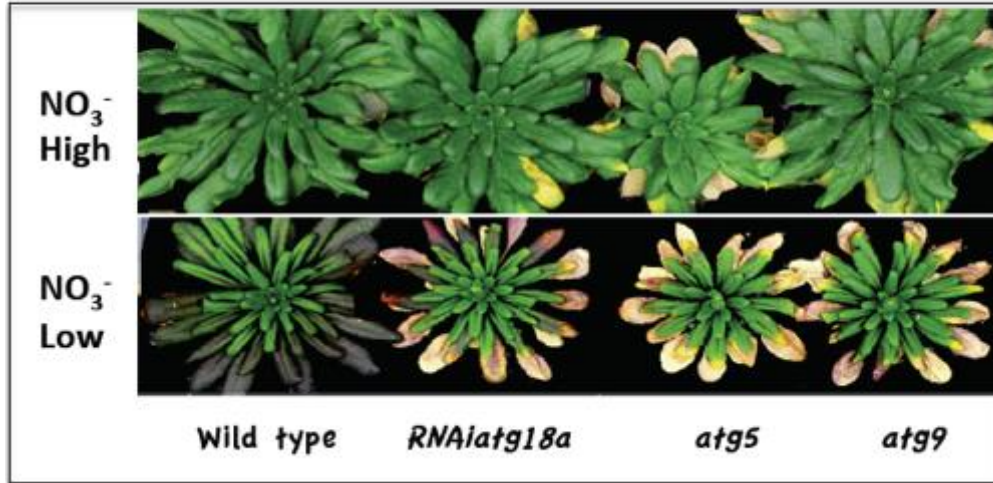
Sedaghatmehr et al. 2018



# Senescence-associated phenotypes of autophagy mutants

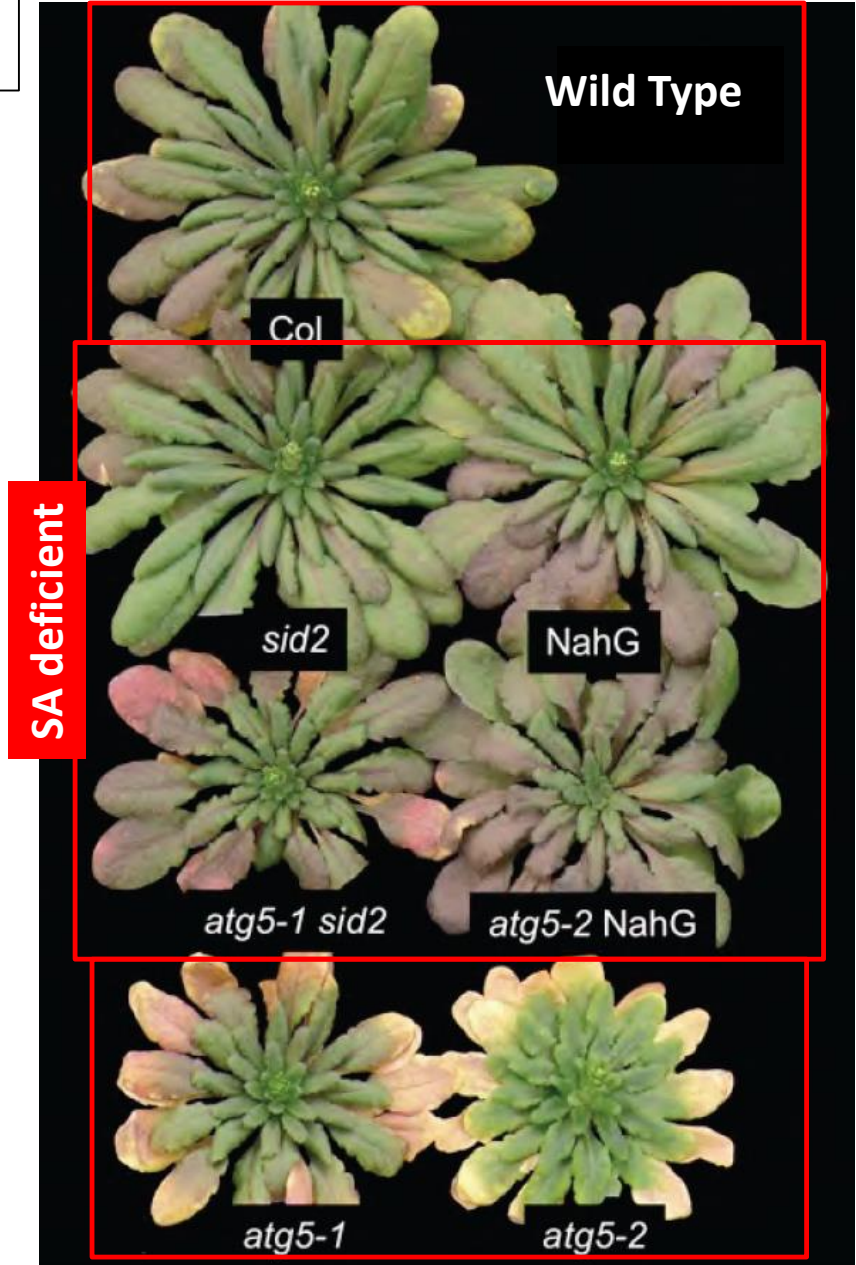
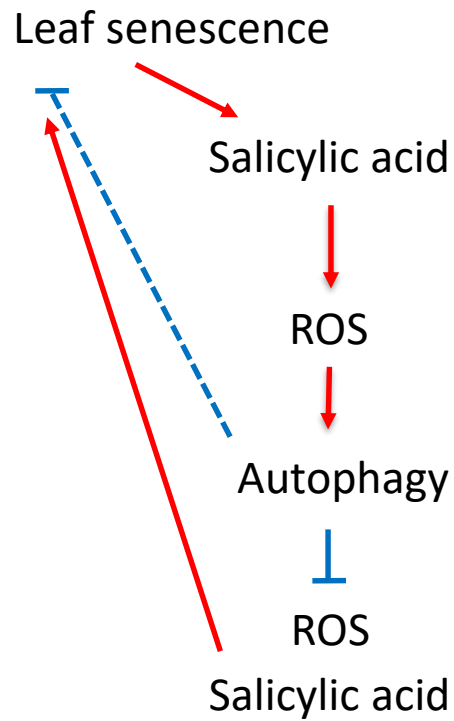


# Lef yellowing and spontaneous cell death in autophagy leaves are salicylic acid dependent





Lef yellowing and spontaneous cell death in autophagy leaves are salicylic acid dependent

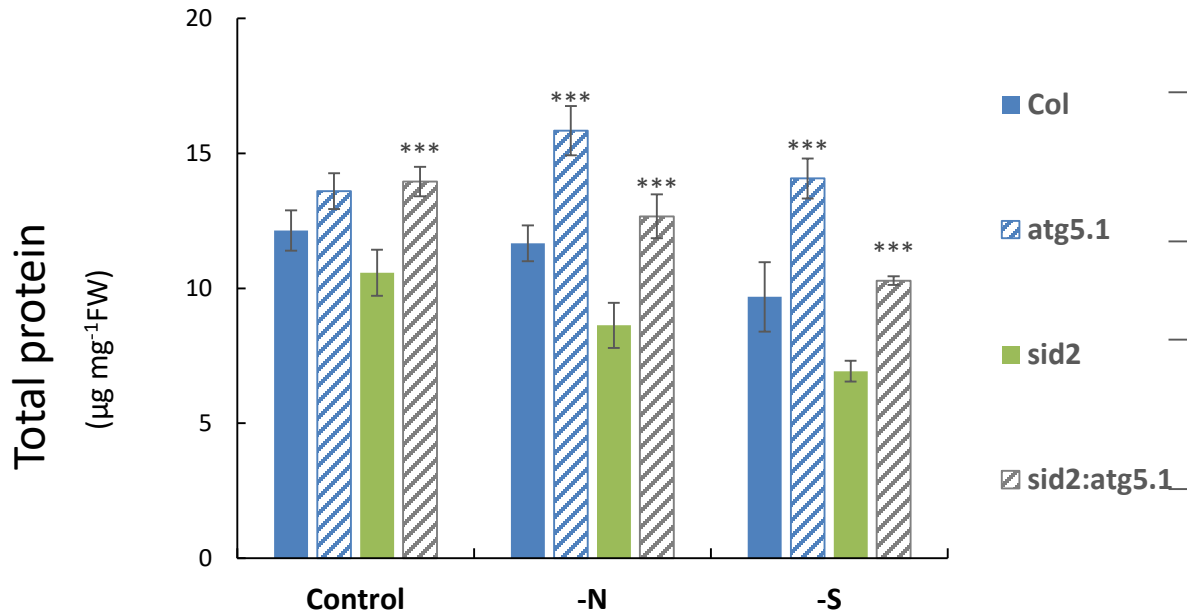


Yoshimoto et al. Plant Cell 2009  
Guiboileau et al. New Phytol 2012

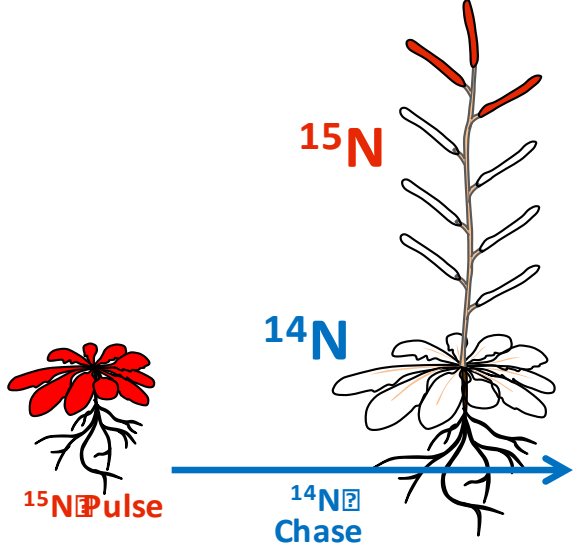
# N-recycling and remobilization in autophagy mutants?



Anne Guiboileau



Rosette leaves of autophagy mutants over-accumulate nitrogen and proteins with ageing



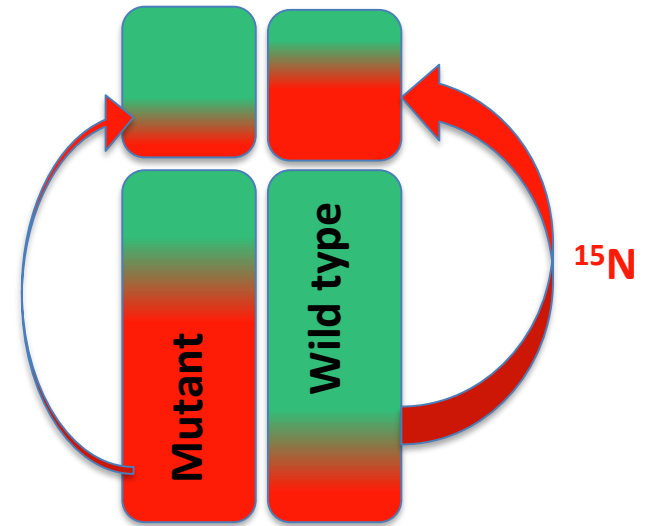
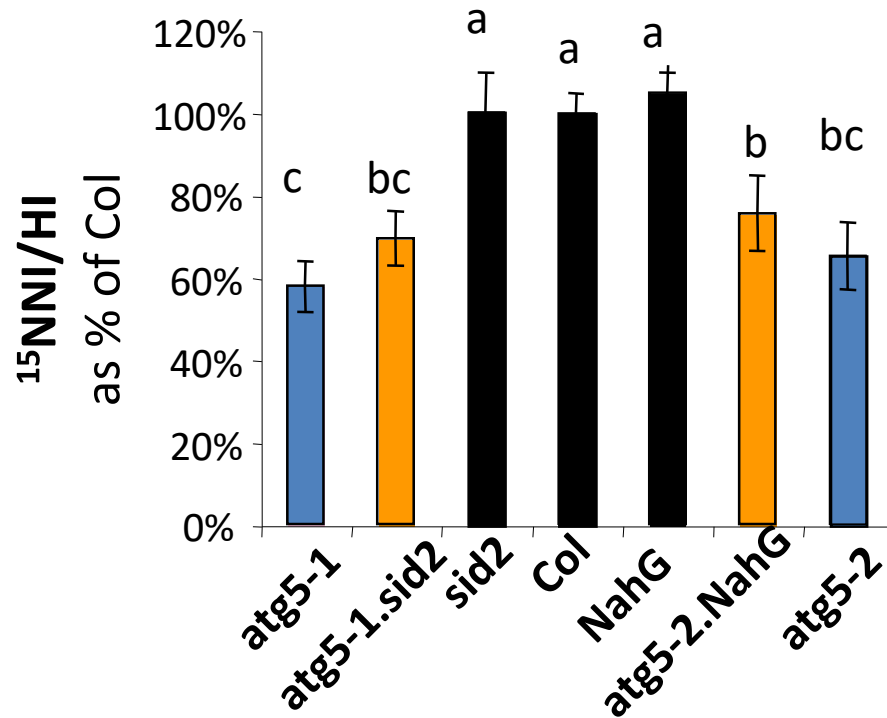
Rosette/Stem/Seeds sampling, weight and analysis in IRMS

Biomass allocation

$$HI = \left[ \frac{\text{Dry Seeds}}{\text{Dry Remain DR} + \text{Dry Seeds}} \right]$$

Nitrogen remobilization

$$^{15}\text{NHI} = \left[ \frac{\text{Dry Seeds}}{\text{Dry Remain DR} + \text{Dry Seeds}} \right]$$



N remobilization is reduced by 40% in autophagy mutants irrespective of salicylic acid effect

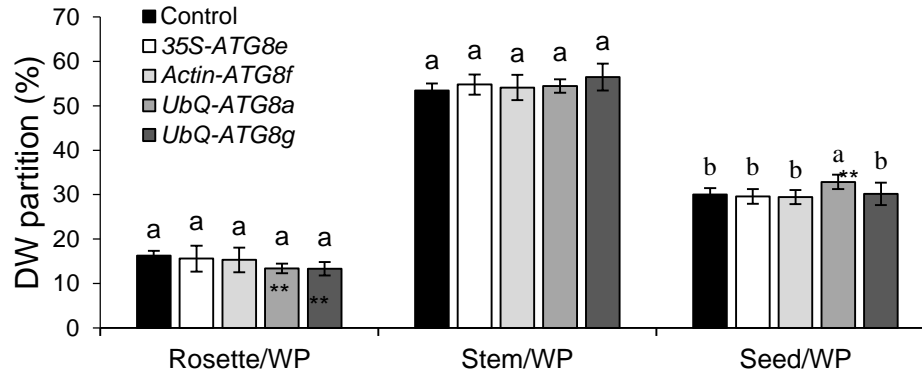
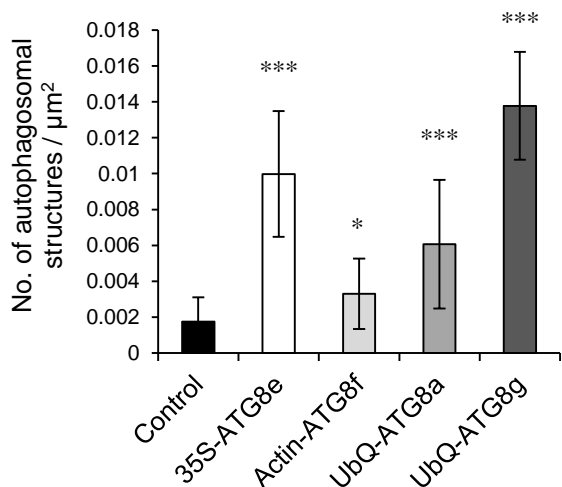
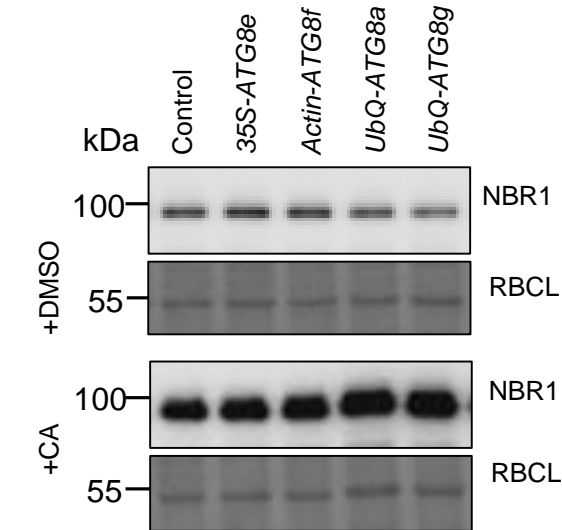


# Increasing Autophagic activity in plants ?

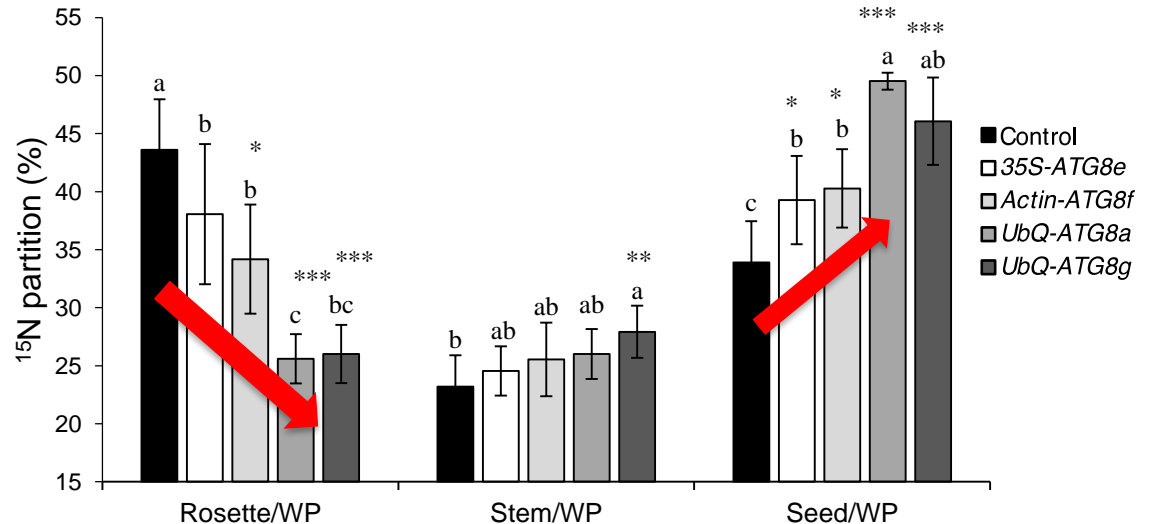


Qinwu Chen

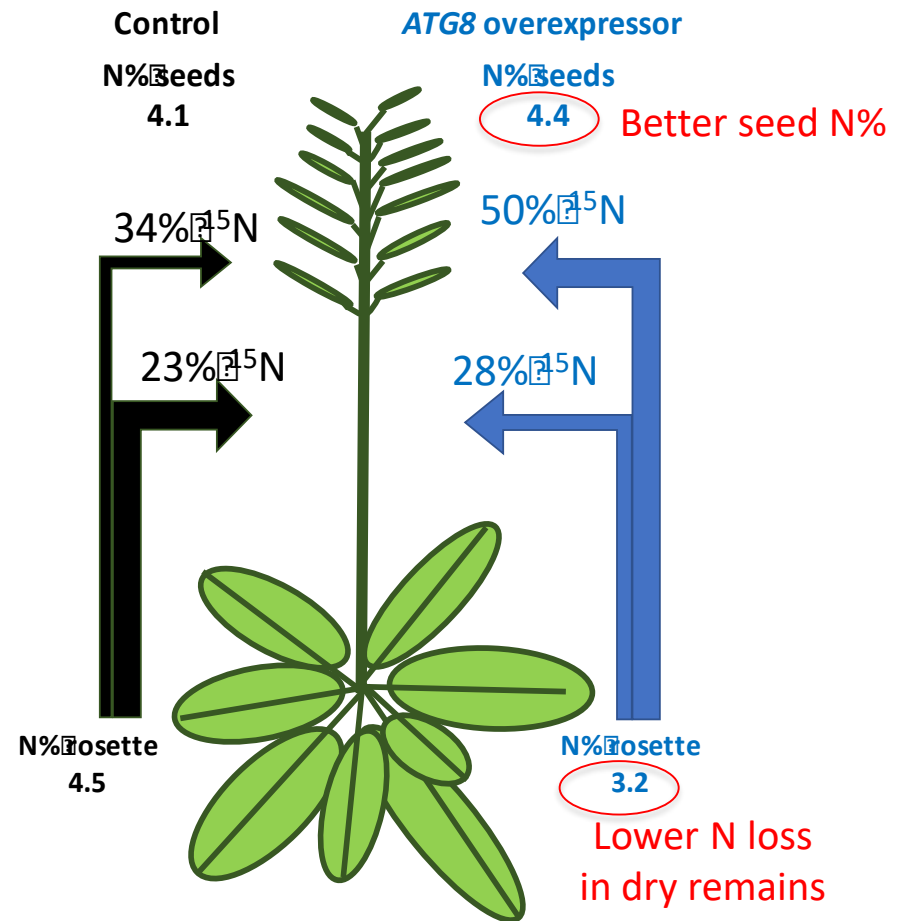
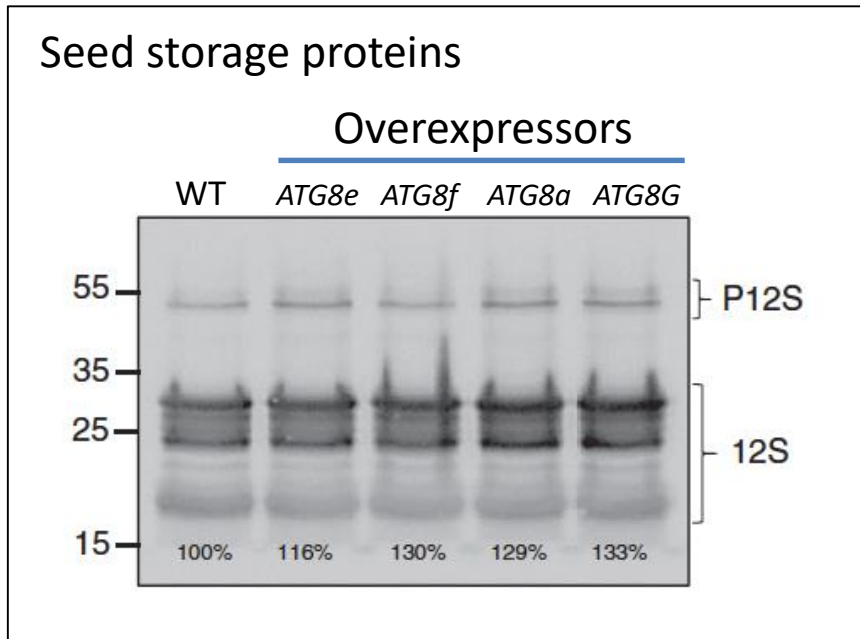
## GFP-ATG8 overexpressors



## Better $^{15}\text{N}$ remobilization under N+



# Autophagy overexpression improves NUE under **high N**



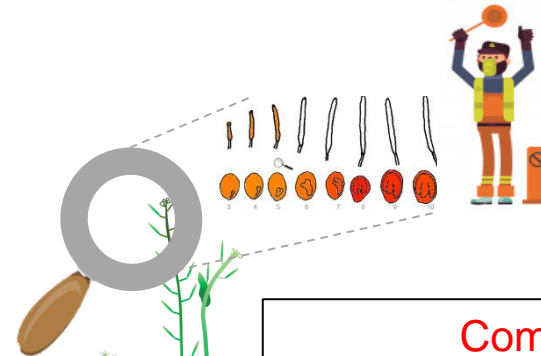
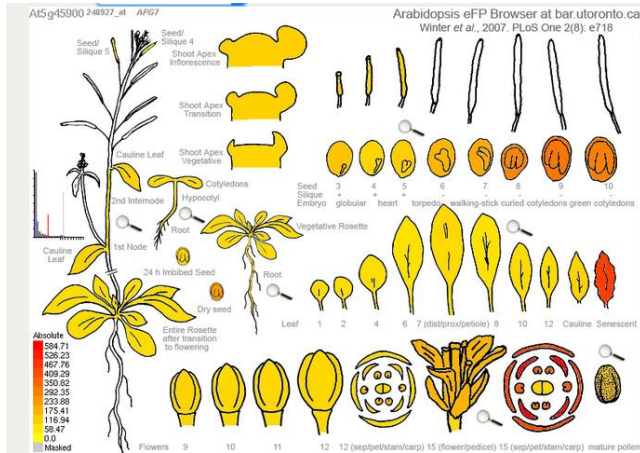
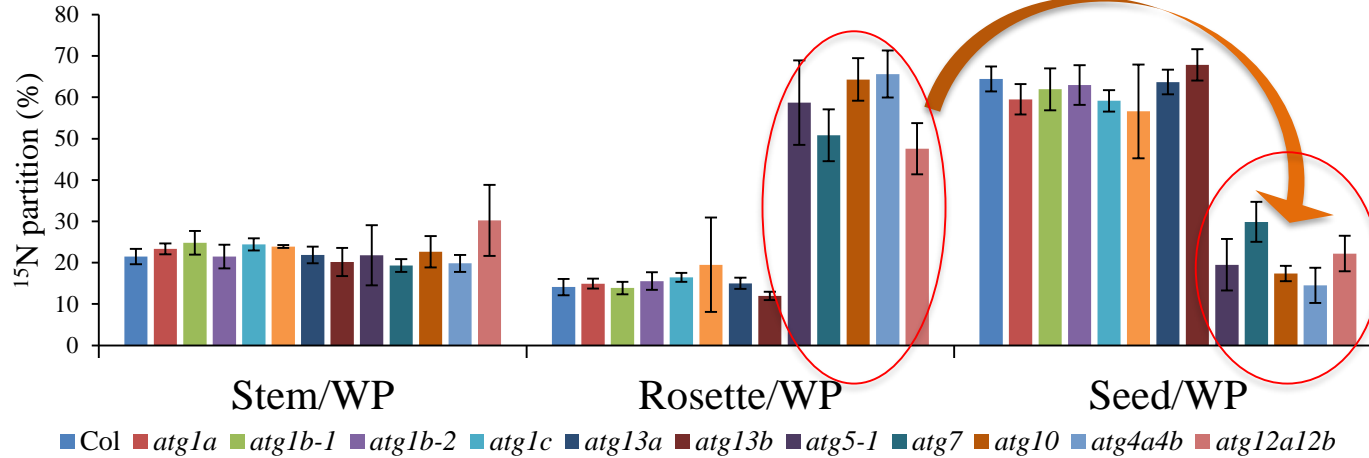
Autophagy  
is induced by N limitation  
thus not a limiting factor  
under low N conditions

No phenotype  
under low N

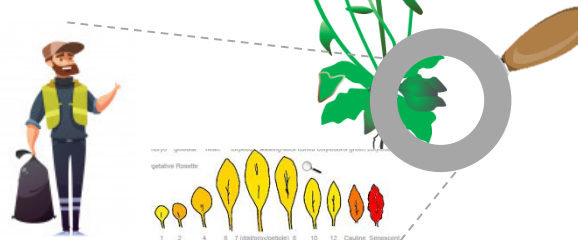
# Autophagy effect on seed filling: Sink effect or source effect?



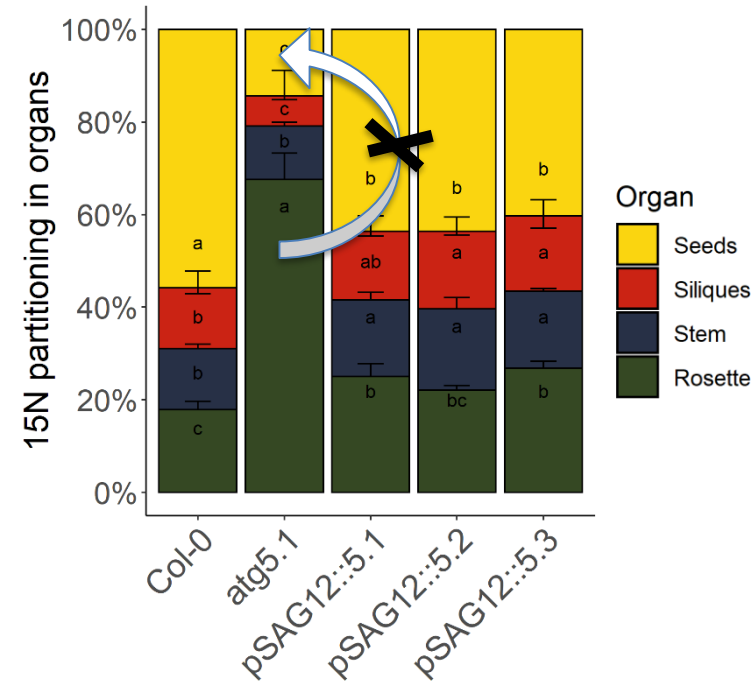
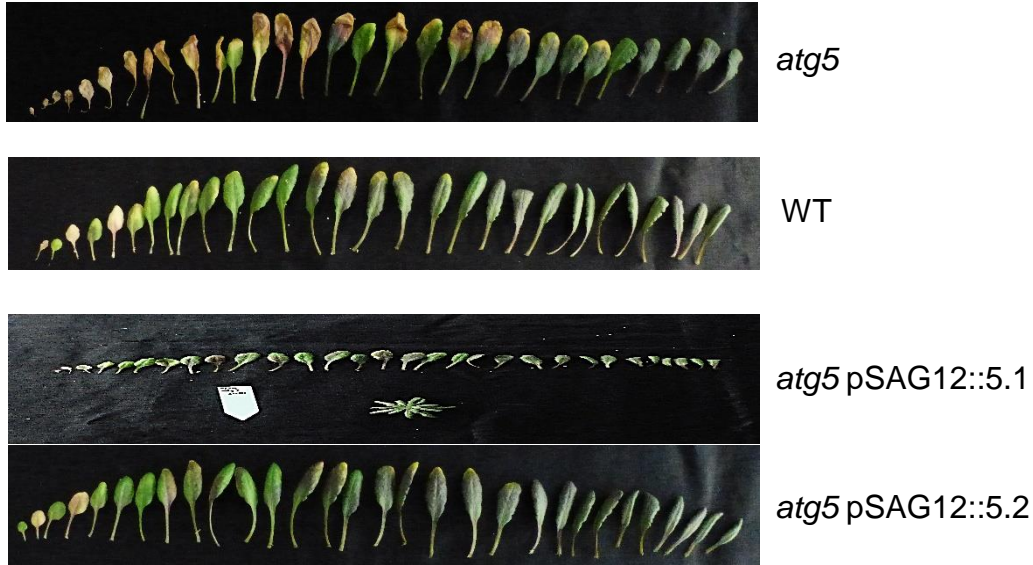
Anne Marmagne



Complementation of *atg5* mutants with pSAG12::ATG5



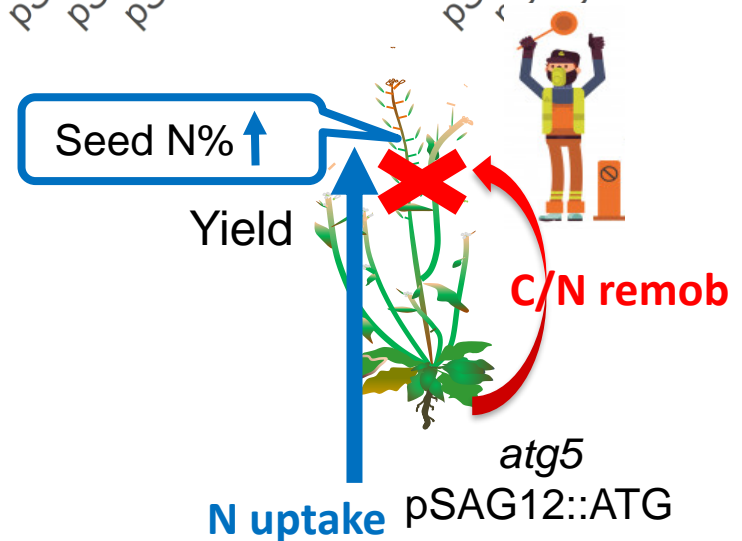
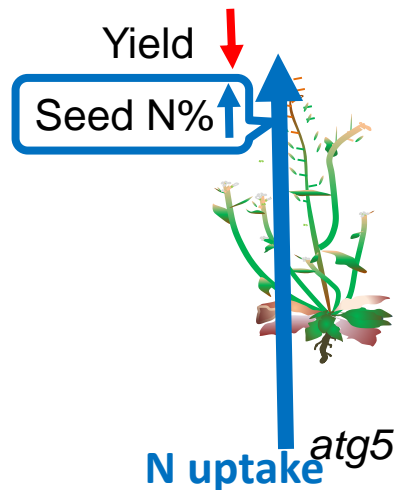
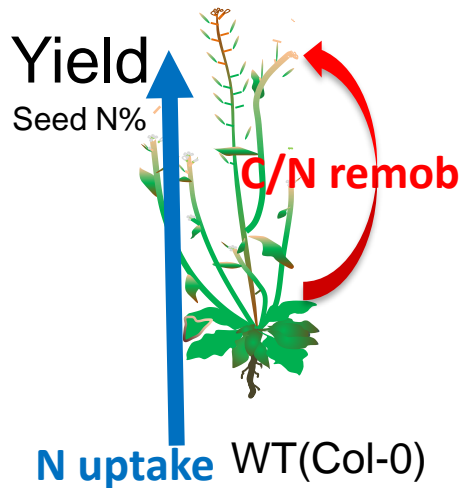
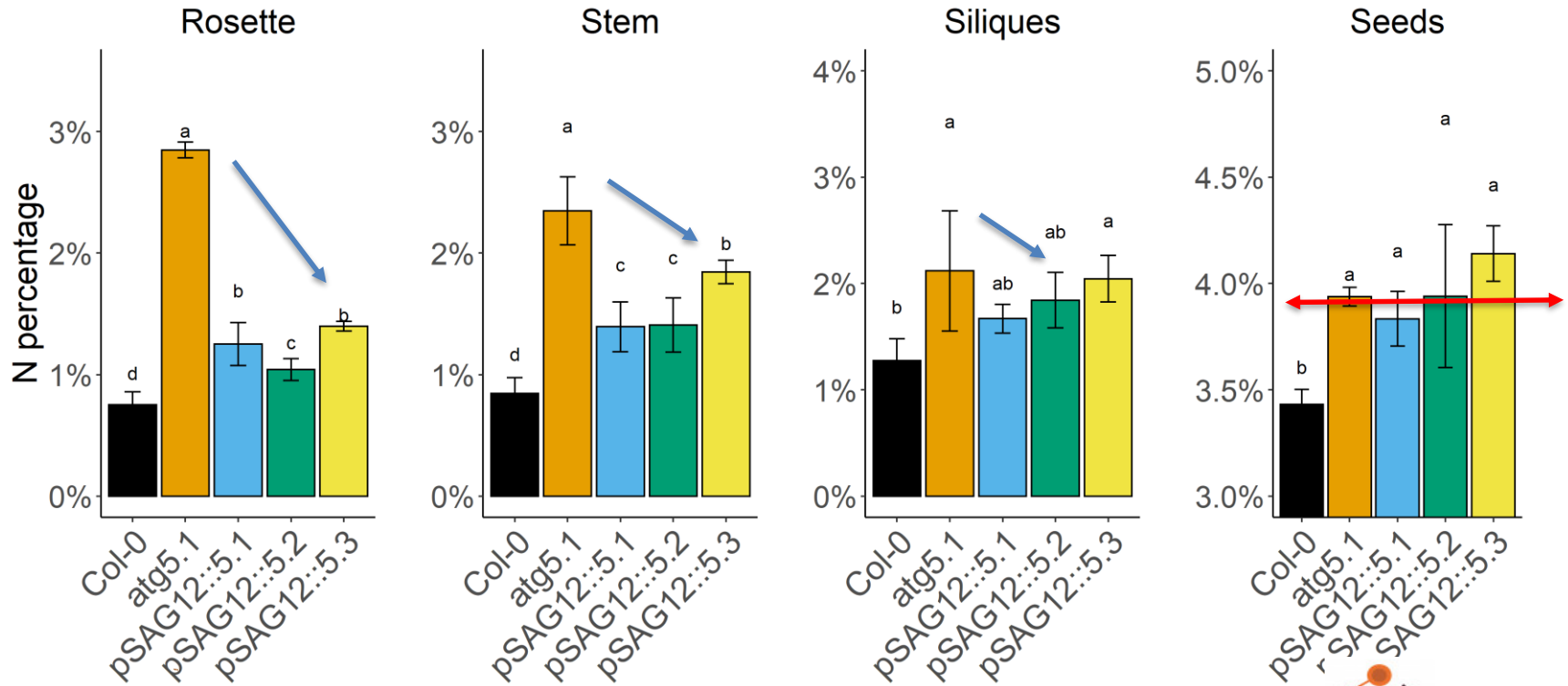
# Complementation of *atg5* mutants with pSAG12::ATG5



Reverse early leaf yellowing and restaure N-remobilization



# But does not restore WT seed composition

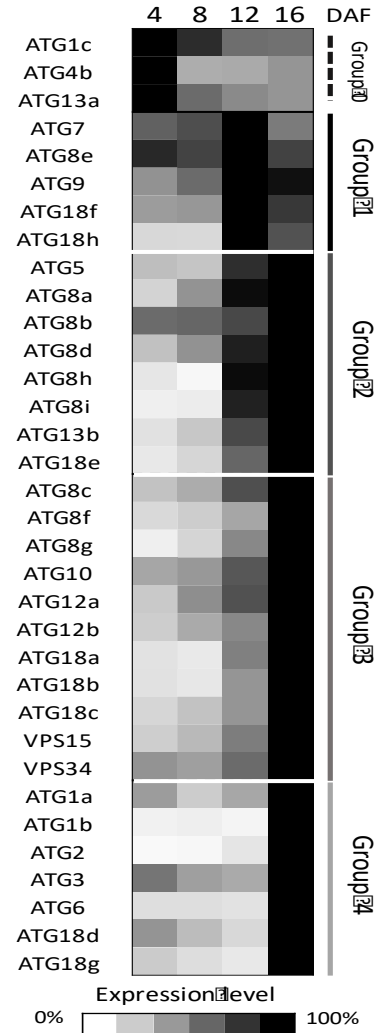


# Role of autophagy in the seeds

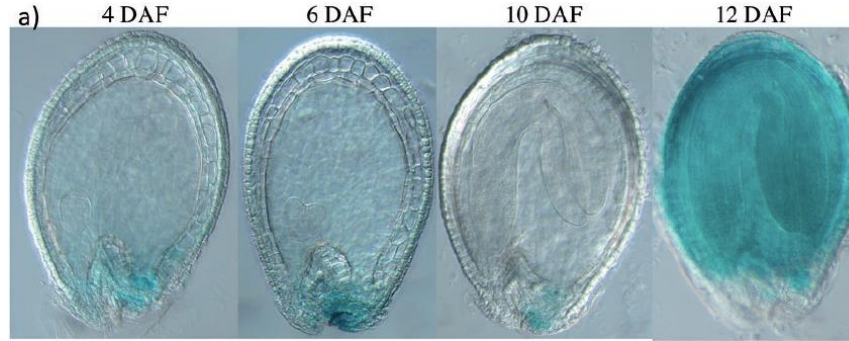


Julien di Bernardino

Relative expression



promATG8f-ATG8f::GUS

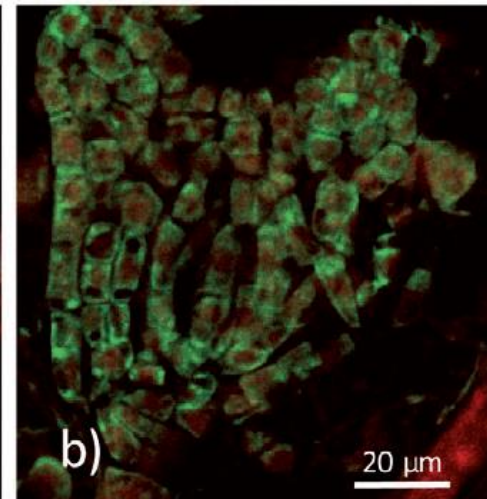
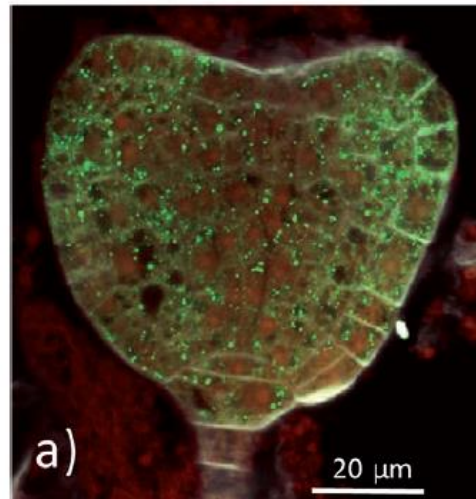


16 DAF  
dessication

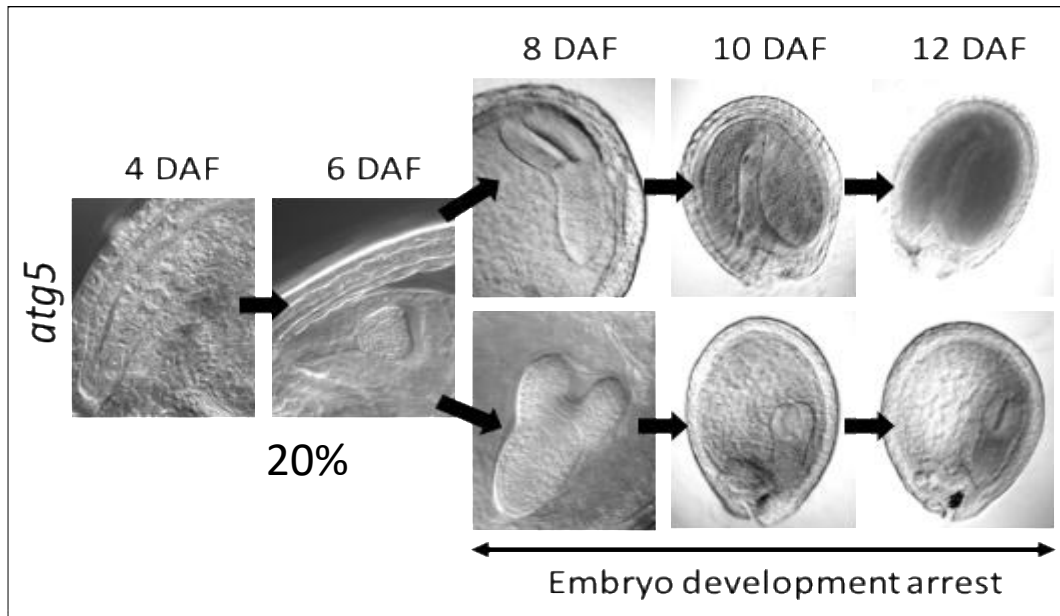
Immunodetection of GFP-ATG8f in embryos during development, 6 DAF

Col

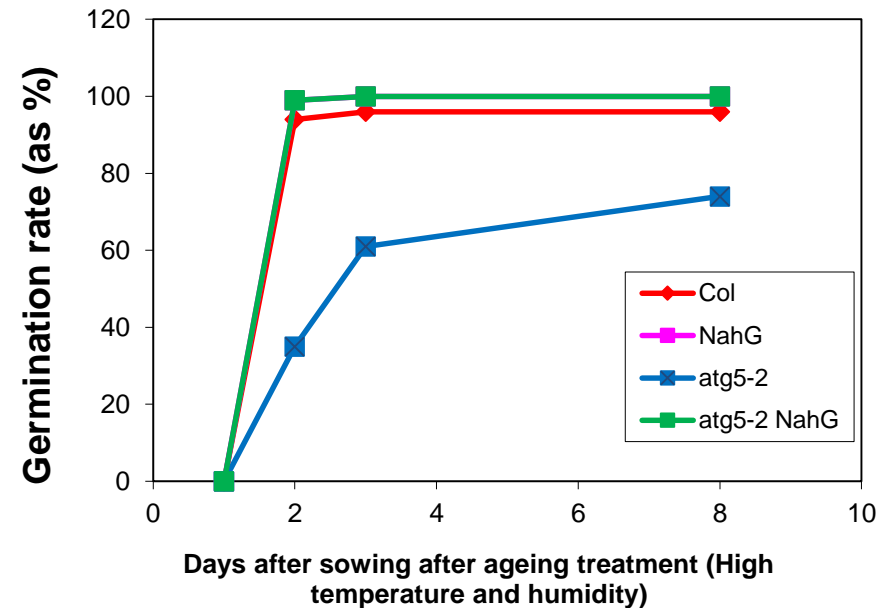
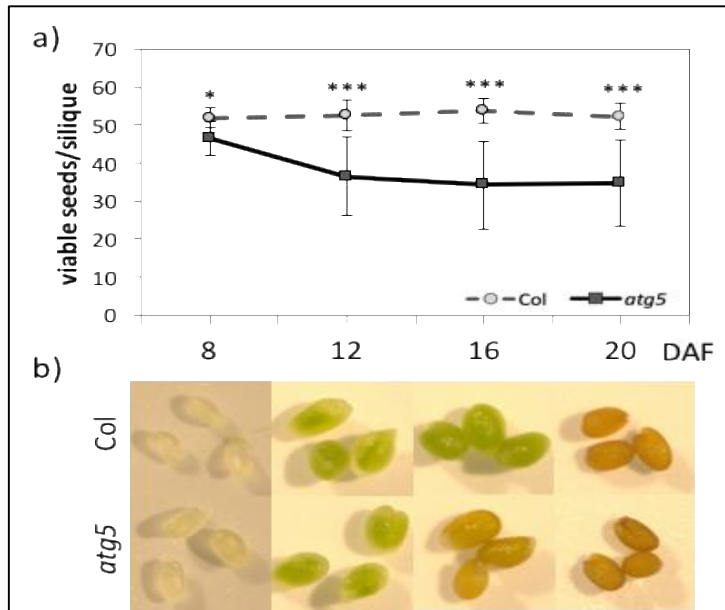
*atg5*



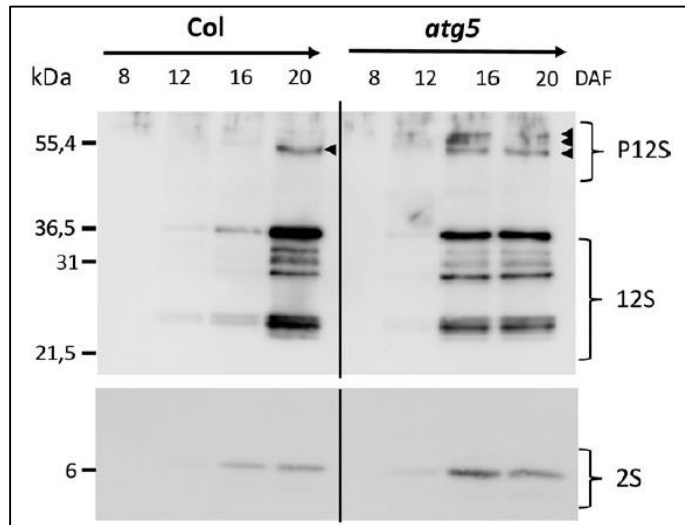
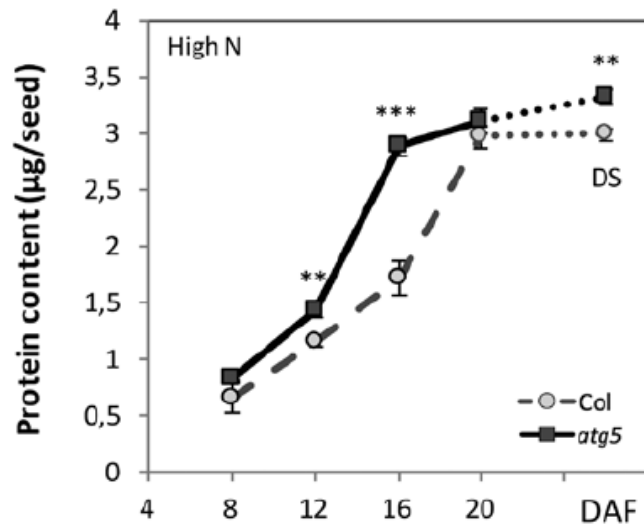
## Less viable seeds in autophagy mutants



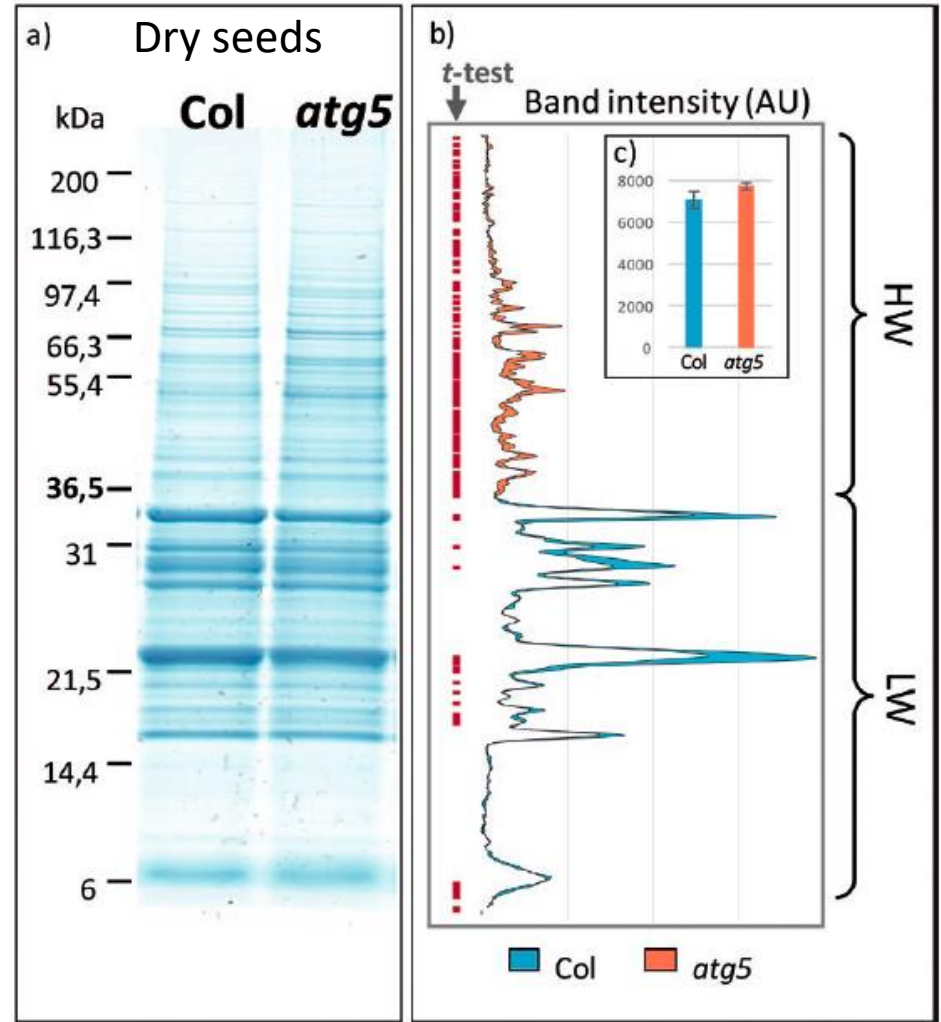
## *atg5* viable seeds are aging faster



# Deposition of the 12S storage proteins is impaired in *atg* seeds



SA-independent (not shown)





Conclusion: Autophagy controls N-recycling and remobilization at the whole plant level for seed filling

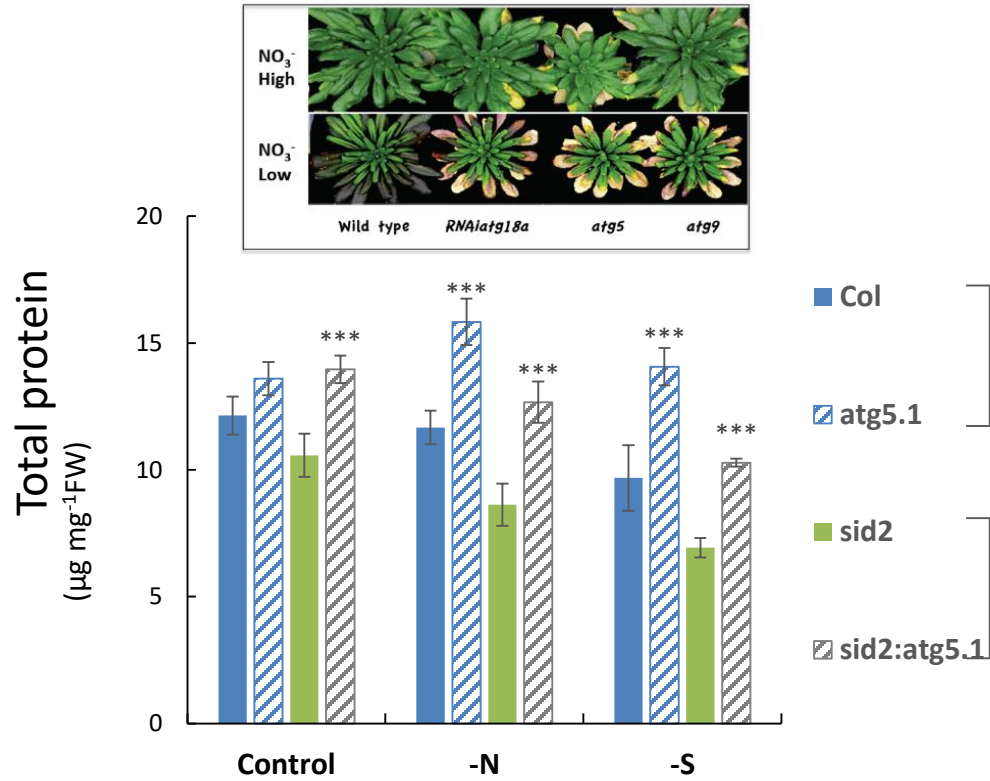
# What are the proteins over-accumulated in the rosettes of *atg* mutants with aging?



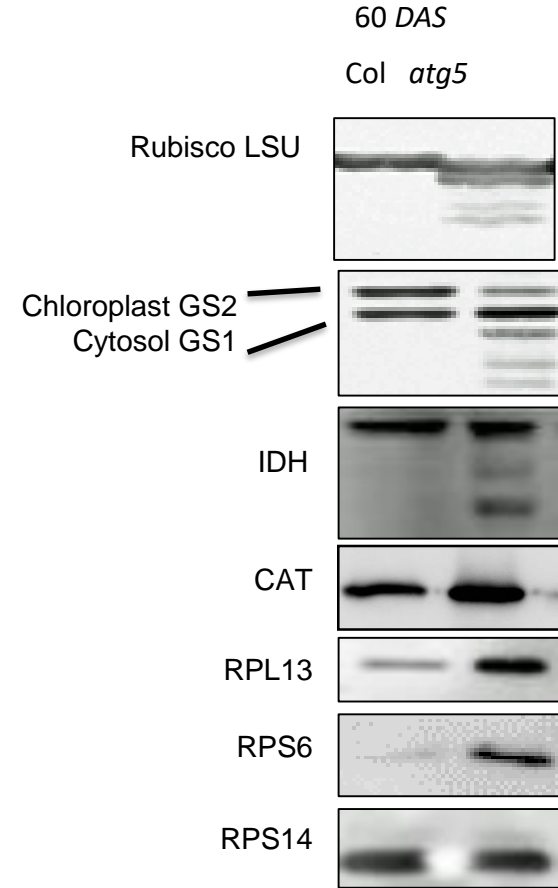
Marien Havé



Anne Guiboileau



Shot gun proteomics LC-MS

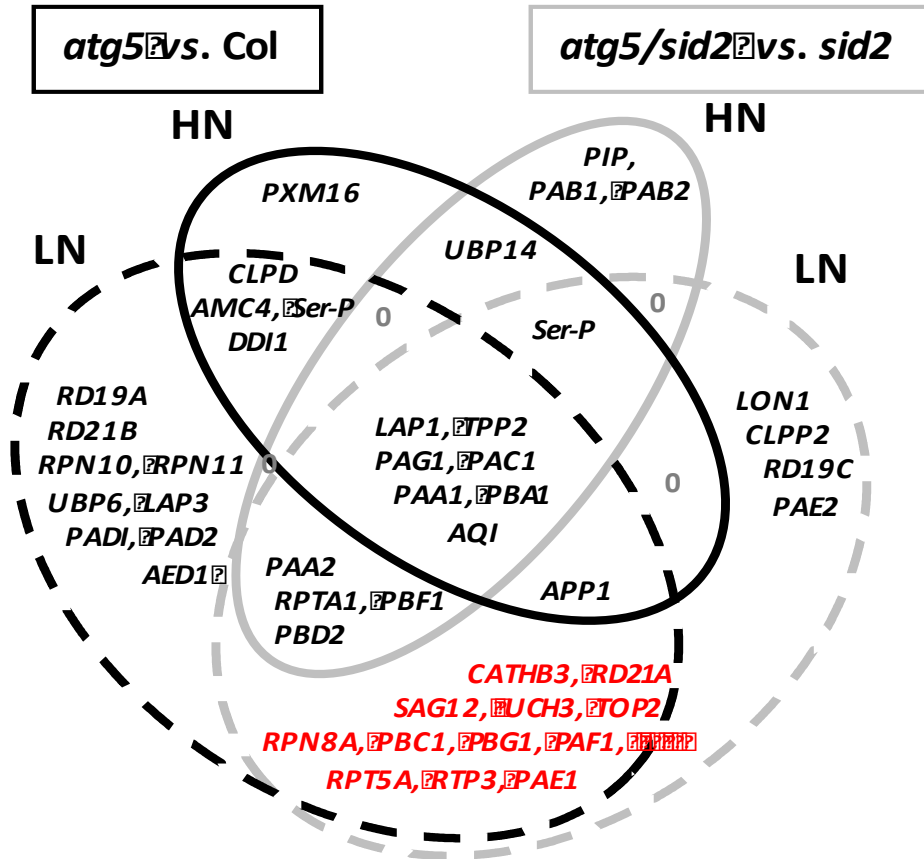


# Many cysteine proteases are more abundant and active in *atg5* vs. WT



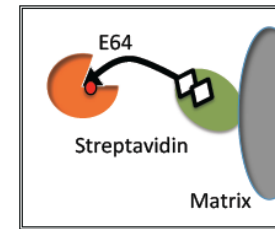
Marien Havé

UP(47)

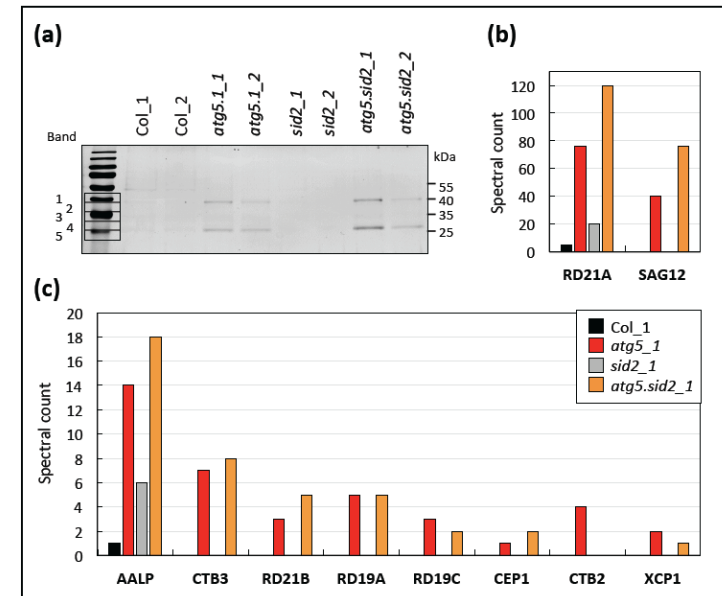


Shotgun proteomics LC-MS

DCG-04 Purification

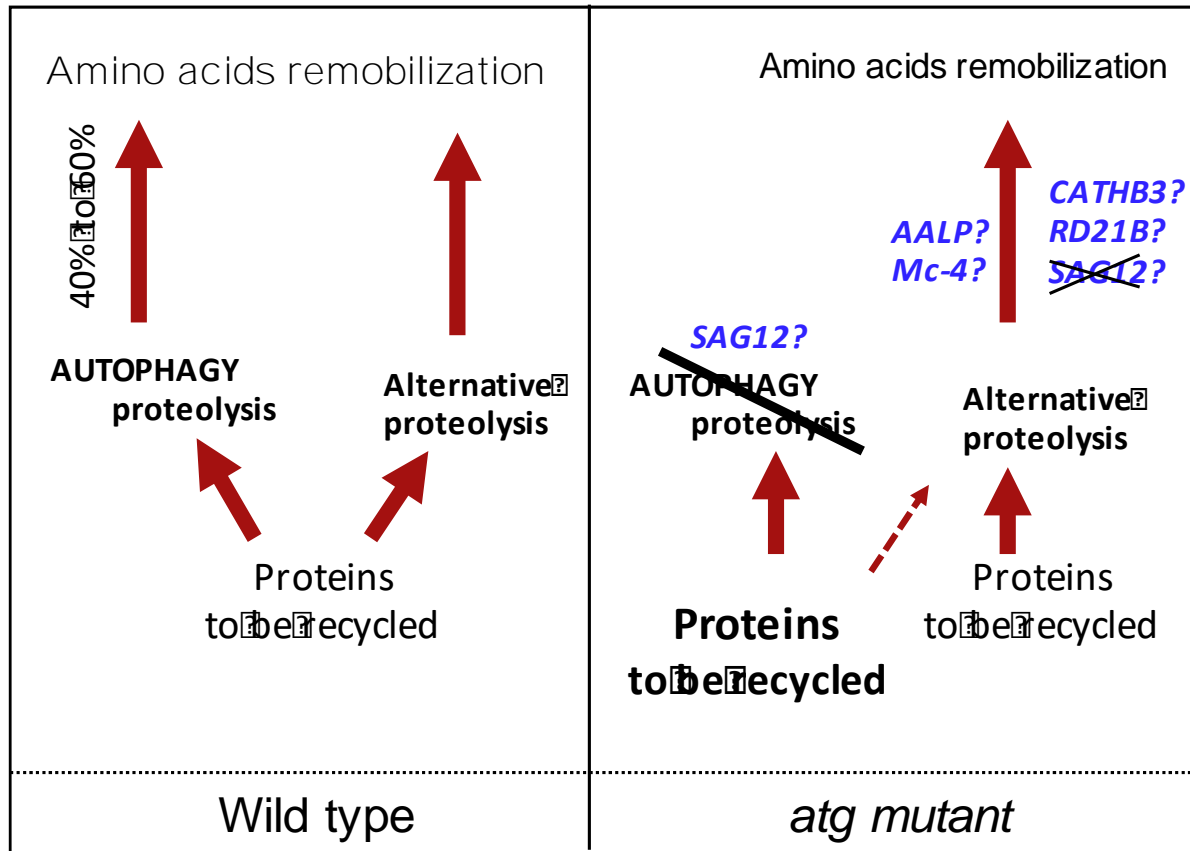
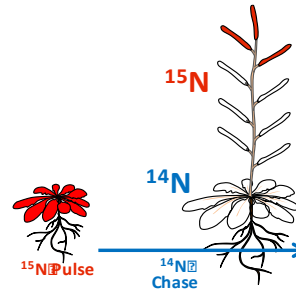


Shotgun LC-MSMS



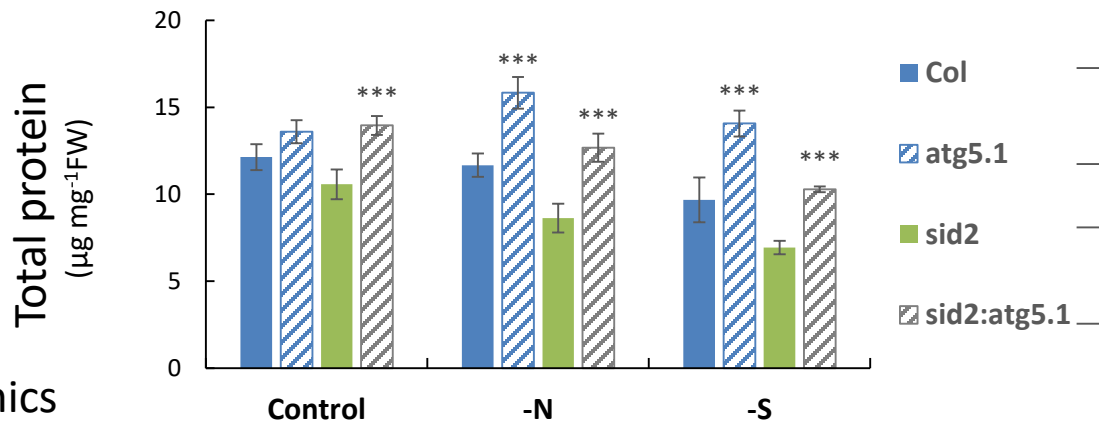
# Autophagy-dependent or independent recycling pathway ?

Crossing  
 $\Delta atg \times \Delta protease$

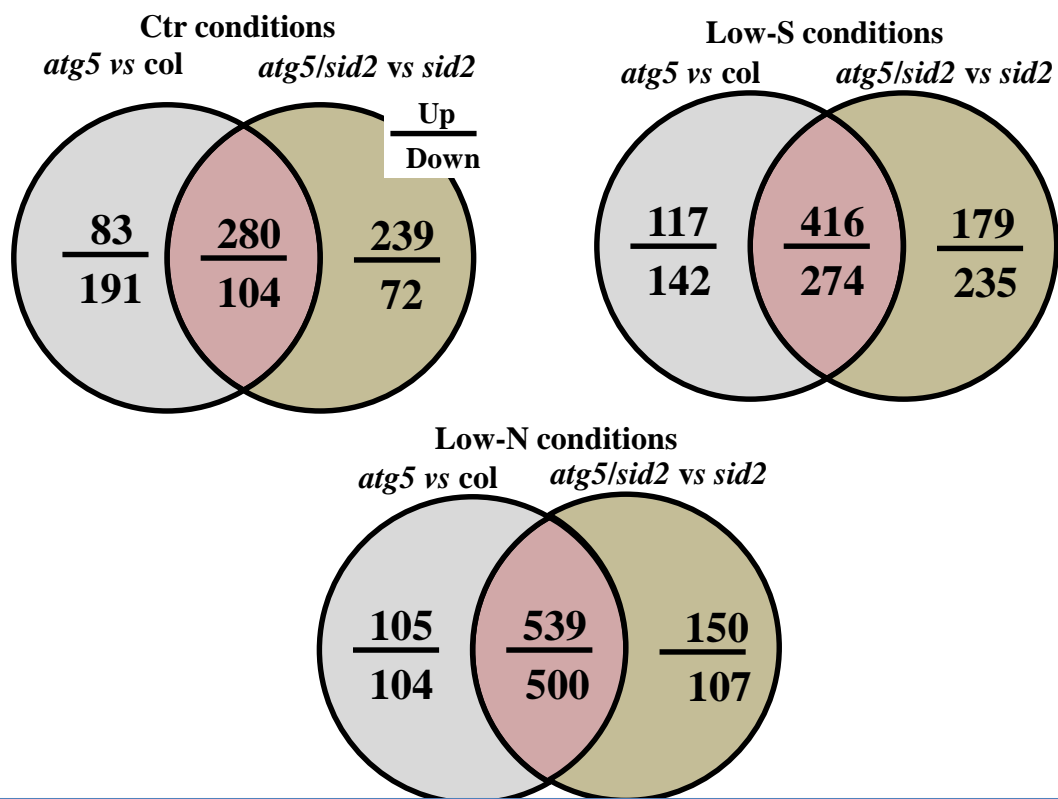




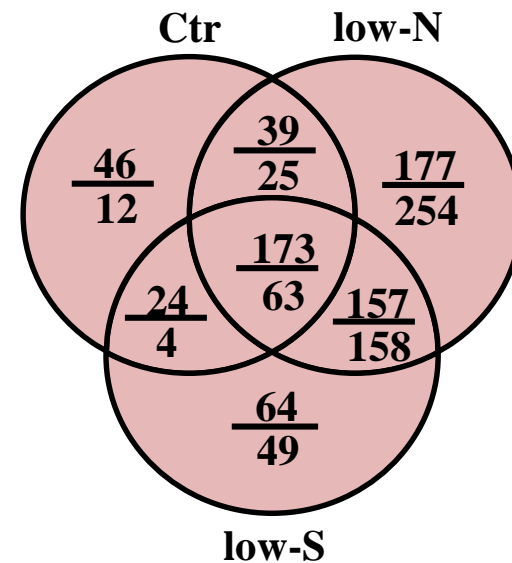
# SA independent effect of *atg5* mutation on the proteome of rosette leaves



Shotgun proteomics  
LC-MS

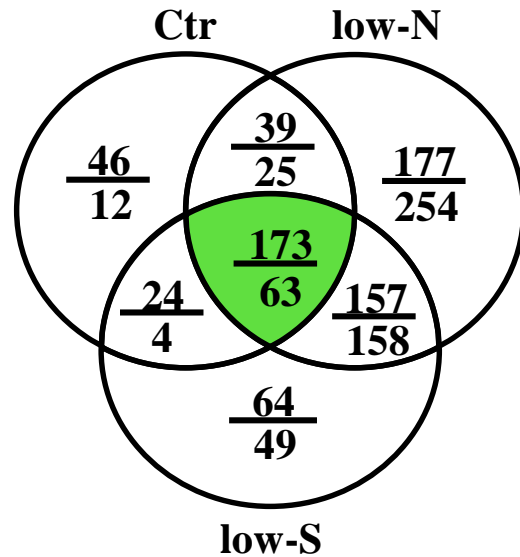


Full List (FL): 1236 proteins

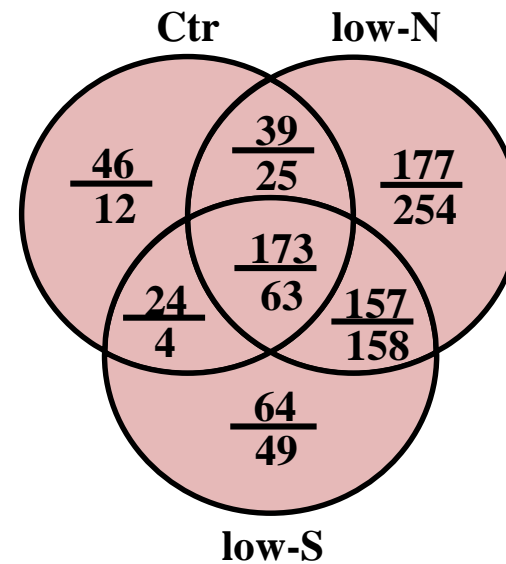


# SA independent effect of *atg5* mutation on the proteome of rosette leaves

Core List (CL): 236 proteins



Full List (FL): 1236 proteins

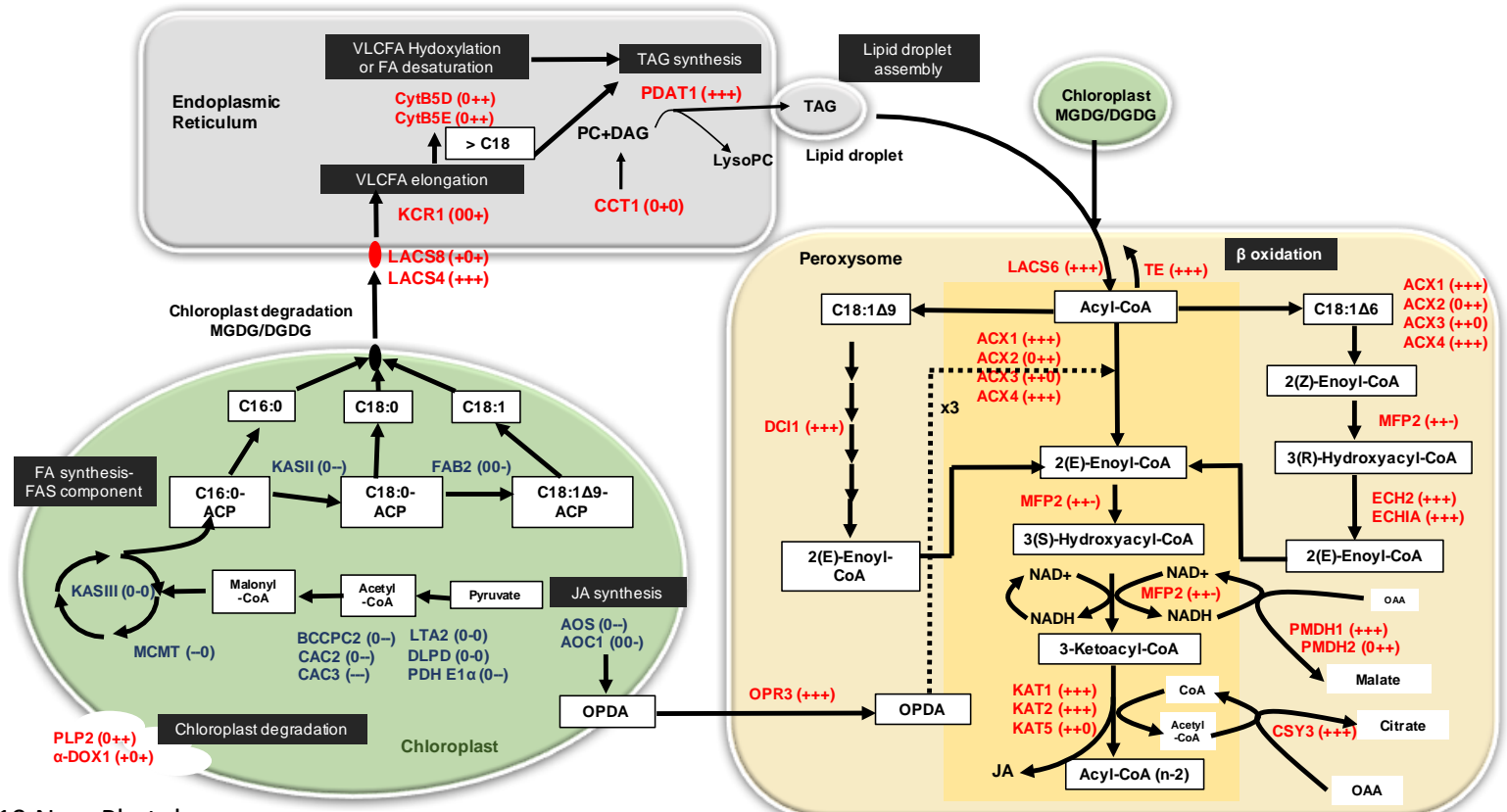


	FL	CL	CL/FL(%)	
extracellular	120	5	4%	●
plasma membrane	71	13	18%	●
cytosol	331	68	21%	●
golgi	42	12	29%	●
endoplasmic reticulum	59	29	49%	●
mitochondrion	63	5	8%	●
peroxisome	72	35	49%	●
plastid	396	58	15%	●
nucleus	59	10	17%	●
vacuole	21	1	5%	●

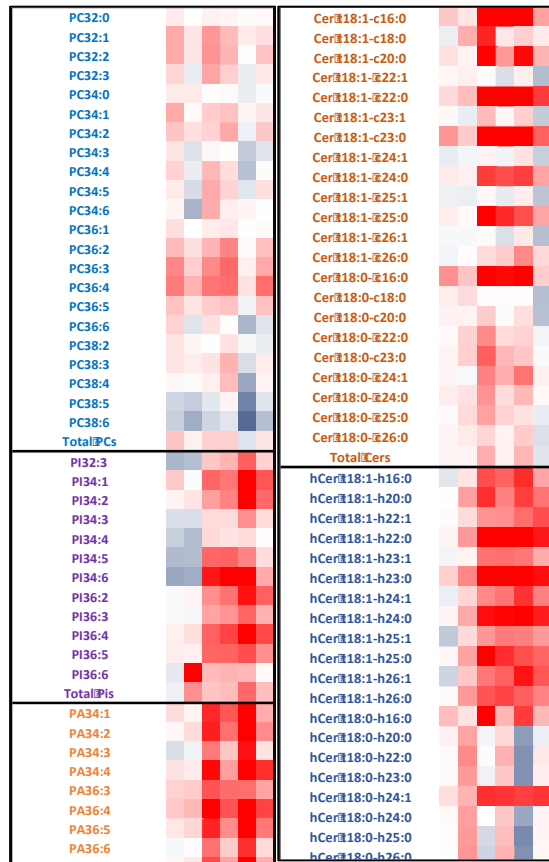
Up  
Down

	FL	CL	CL/FL(%)	
extracellular	120	5	4%	●
plasma membrane	71	13	18%	●
cytosol	331	68	21%	●
golgi	42	12	29%	●
endoplasmic reticulum	59	29	<b>49%</b>	●
mitochondrion	63	5	8%	●
peroxisome	72	35	<b>49%</b>	●
plastid	396	58	15%	●
nucleus	59	10	17%	●
vacuole	21	1	5%	●

**Up: FA catabolism, VLCFA synthesis,  $\beta$ -oxidation**  
**Down: Photosynthesis, FA synthesis**

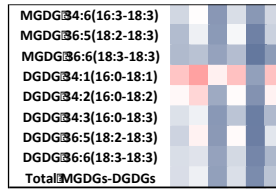


# Phospholipids and Sphingolipids

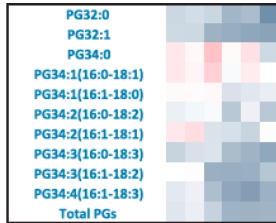


0 down 1 up 3  
FC values

# Galactolipids

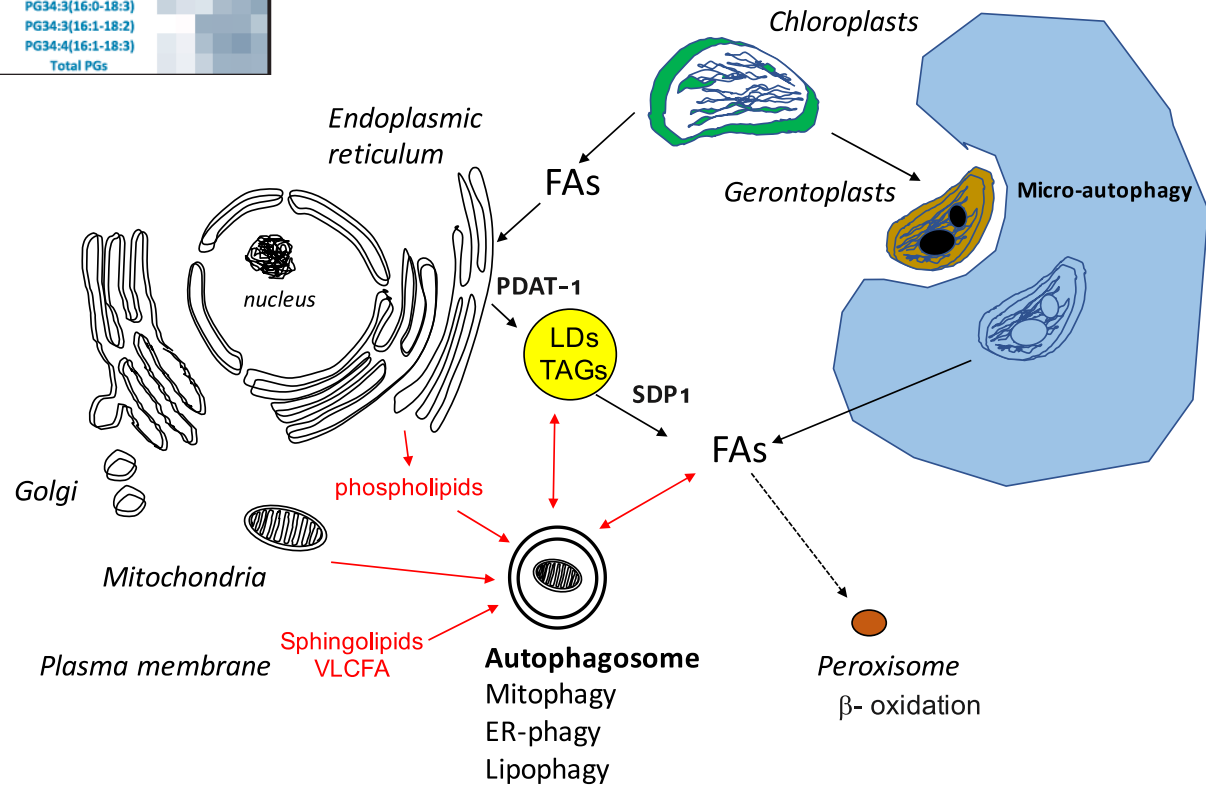


# Phosphatidyl Glycerols



Autophagy is involved in recycling of endomembranes, except chloroplast membranes

Consistent with Fan et al. (2019) Plant Cell





Conclusion: Autophagy controls  
endomembrane recycling (ER, PM) and  
lipid homeostasis

# Take home message

In plant, macroautophagy is a longevity factor enhanced during leaf senescence.

In senescing tissues, autophagy removes altered and dysfunctional organelles and proteins, and contributes that way to N recycling.

Autophagy controls storage protein deposition during seed maturation.

Double checkpoint of autophagy on sink/source relationship:

- in the source for degradation & N export
- in the seeds for N storage

Autophagy also controls endomembrane recycling and lipid homeostasis in leaf tissues.

Remains to be investigated in seeds during maturation/ germination ?

Metabolic control of autophagy in plants TOR, SNRK1 (AMPK like), other ?



Anne Marmagne  
Fabien Chardon  
Michèle Reisdorf-Cren  
Fabienne Soulay  
Laurence Bill  
Nolwenn Bonadé-Bottino  
Manon Lardos  
Qinwu Chen  
Jie Luo  
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