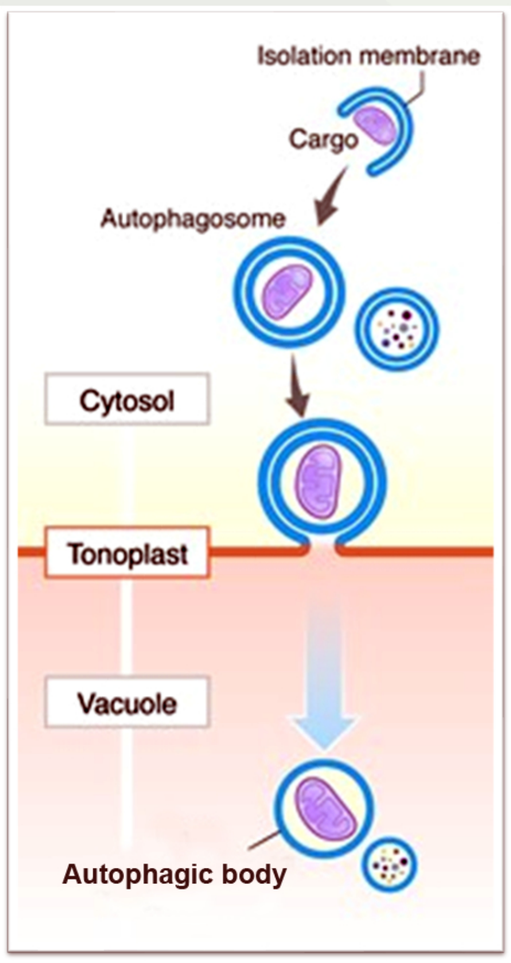
NGS screening for identification of novel pexophagy-related mutation in *Arabidopsis thaliana*

Katarzyna Sieńko 1, Kacper Żukowski 2,3, Kenji Yamada 1, and Shino Goto-Yamada 1
1 Małopolska Centre of Biotechnology, Jagiellonian University, Cracow, Poland; 2 National Research Institute of Animal Production, Balice, Poland;
3 Nicolaus Copernicus University in Torun, Poland;
katarzyna.sienko@uj.edu.pl

Figure 1
Autophagy in plants (Sieńko *et al.* 2020, Cells)



Background of the study

Autophagy (Figure 1) is a cellular degradation process of:
a) particular organelles e.g. peroxisomes (selective autophagy) or
b) cellular components in the cytosol (non-selective autophagy).

Arabidopsis thaliana *peup* mutants:
- *peroxisome unusual positioning* (*peup*) mutants have been identified based on different distribution of peroxisomes in a cell.
- *peup* mutants demonstrated increased number of peroxisomes, which formed aggregates containing damaged peroxisomes (indicated by arrows, Figure 2).
- *peup* mutants turned out to be defective in autophagy (mutations in *ATG* genes).

Autophagy – defective phenotype of *peup* mutants:
- accumulation of abnormal peroxisomes.
- E64-d induced vessicles accumulation is suppressed in *peup* mutants (Figure 4ac).
- dark-induced senescence (*peup* mutants are not able to survive in the dark) (Figure 3).

Figure 2
The peup mutants have mutations in *ATG* genes (Shibata *et al.* 2013, Plant Cell).

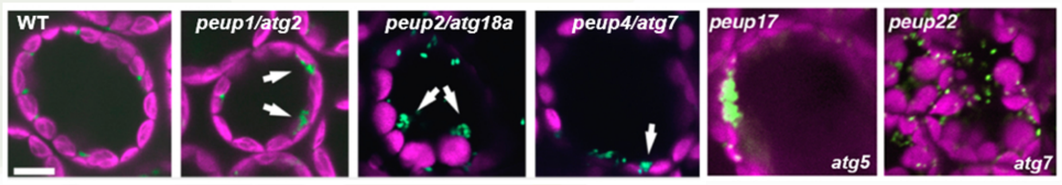
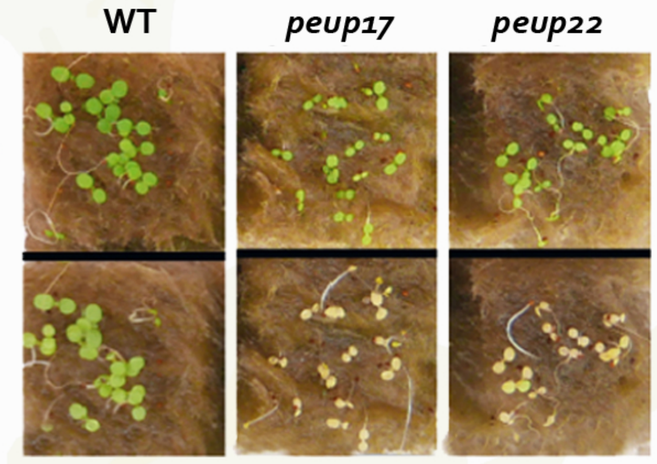


Figure 3
Dark treatment (Goto-Yamada *et al.*, 2017, poster session at Plant Science Workshop, Lyon).



Aim of the study

Application of next-generation sequencing to identification of pexophagy-related mutation in *peup33* mutants of *Arabidopsis thaliana*.

Methods and Materials

Samples preparation

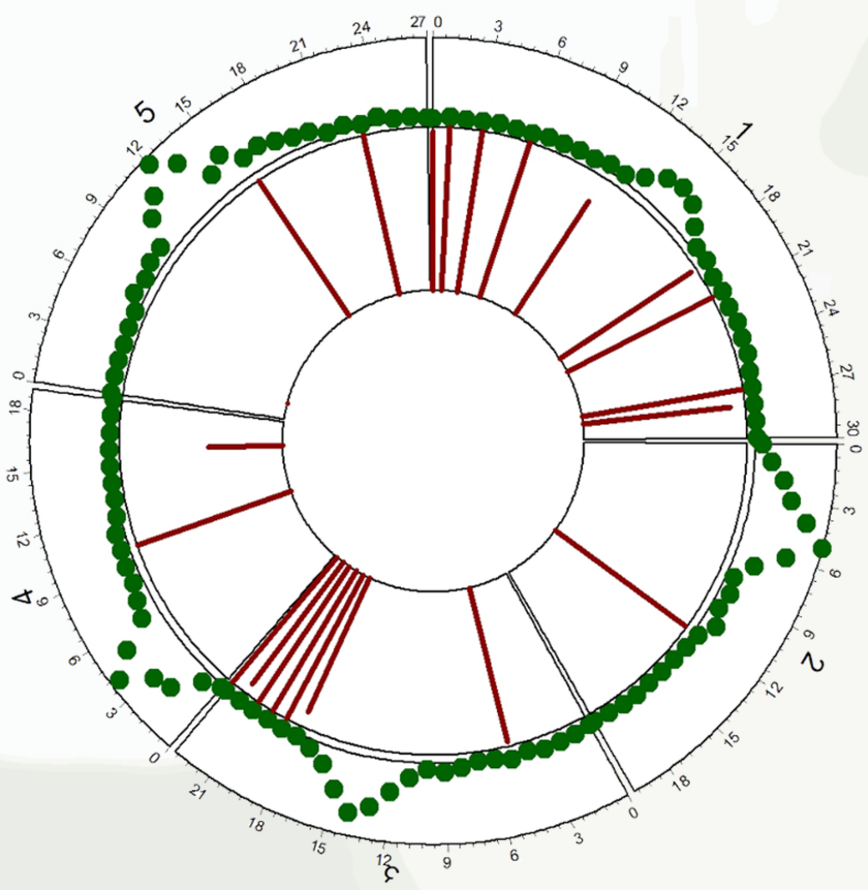
- EMS mutagenesis of WT (Col-0) seeds.
- Phenotype screening with FM4-64/E-64d
  ~ 400 seeds of L*er* x *peup33* F2.
  ~ 100 seeds of WT.
- Library preparation and next-generation sequencing
- Sequencing of pooled: *peup33* and WT.

Bioinformatics analysis

The analysis was conducted on Prometheus HPC cluster.

Workflow steps:
1) quality control of the raw sequence (FastQC).
2) cleaning and trimming (Flexbar).
3) alignment of the *Arabidopsis thaliana* reference genome TAIR10, Ensembl release (BWA).
4) SNPs identification (SAMtools with BCFtools).
5) filtration and annotation (Snpeff, Biomart, R Project).
6) gene ontology (PANTHER).

Figure 5
Circos plot showing sequencing results for the identified SNP counts (green point track) per chromosome Mbp with the indication of autophagy candidate genes (brown bars track) across the *Arabidopsis thaliana* TIAR10 genome. The shown results were unbiased by the Landsberg *erecta* ecotype.



Results

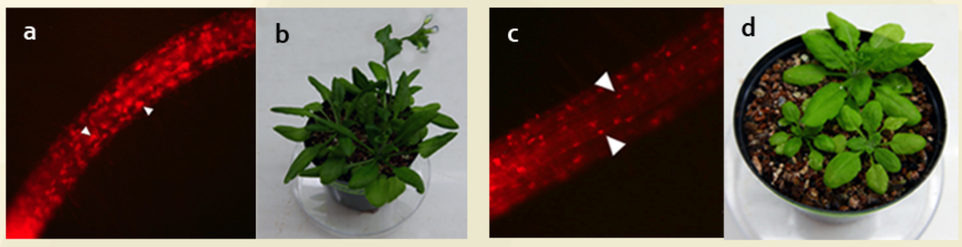
Phenotype screening

- *peup33* mutants (Figure 4c) shows autophagy-defective phenotype *i.e.* formation of vessicle aggregates after FM4-64/E-64d treatment is suppressed and the size of these aggregates decreased in comparison to WT (Figure 4a).
- *peup33* plants are slow in growth and display dwarf phenotype (Figure 4d) compared with WT (Figure 4b).

NGS identification of *peup33* causative mutation

- twenty-seven polymorphisms, located mainly on chromosome 1 and 3, has been linked with 26 candidate genes involved in autophagy processes (Figure 5).
- most of the variants were missense, several splice site variants, and one stop-gained mutation.

Figure 4
The phenotypes of (a) wild-type (WT) plant and (c) *peup33* mutant after the treatment with FM4-64 and E-64d for 24 h. White triangles indicate E-64d vesicle accumulation. WT (b) and *peup33* mutant (d) plant phenotypes; FM4-64 – membrane fluorescence dye; E-64d – protease inhibitor.



Conclusions

- next-generation sequencing can be applied to identification of autophagy-related causatve mutation instead of standard mapping procedure.
- identification procedure requires prior optimization to shorten the time of analysis.
- genetic and molecular background knowledge is required to link the phenotype of interest with a proper SNP variant.
- additional analyses are required to confirm the mutation *e.g.* direct sequencing, real-time PCR or allelism test

Future plans

- analyze the function of PEUP33 protein by profiling gene expressions in the *peup33* mutant.
- observe the phenotypes of *peup33* under a confocal laser-scanning microscope during inducing of pexophagy/general autophagy.
- apply this technique to other autophagy/pexophagy mutants.

Funding
This research was funded by the National Science Centre, Poland:
UMO-2019/34/NZ3/00299 to SG-Y and UMO-2016/23/B/NZ1/01847 to KY and KS.
This research was supported in part by PLGrid Infrastructure.