BIOLOGIA DO DESENVOLVIMENTO EM PLANTAS

ACADEMIC YEAR 2025/26 **PRACTICAL 2** Dep. Biologia, FCUL

Model Organisms in Plant Biology

We will start by having a look at several model plants commonly used in plant biology and plant development research. These are considered models because they gather a set of unique characteristics: from being easy to manipulate in the laboratory to being easily characterised biochemically, physiologically, and genetically. We will have some examples in the classroom: *Arabidopsis thaliana* and *Nicotiana benthamiana*. Take a couple of minutes to observe these plants. We will mostly focus on the reasons for their use as model systems for plant biology and morphology. The correct interpretation and good understanding of the typical organs, structures, tissues, and cell types found in any plant body is essential in the study of genes and development in plant biology. It is especially important when you want to compare the typical structures found in wild-type plants to the mutant phenotypes.

In the first part of this class, your challenge will be to identify the mutated gene when you are given the mutant plant to analyse.

Activity. Imagine you are working in a Plant Biology Lab, your job is to study the function of genes involved in Plant Development, and you have just done a mutagenesis screen using *Arabidopsis* as the model system. A mutagenesis screen (also named genetic screen) is an experimental technique used to identify and select for individuals who possess a phenotype of interest in a mutagenized population (Hartwell *et al*., 2008). Therefore, a genetic screen is a form of phenotypic screen and provides valuable information on gene function and insight into the molecular events involved in a biological process or pathway. Basically, it is a good way to find out how the mutagenized genes function. You may come across an interesting phenotype in the plant body and the challenge is to find out which gene is affected in your mutant plants. So, follow the protocol and instructions below.

A. Preparing a plate for Arabidopsis seedling growth

1. Obtain a square Petri dish, an alcohol lamp, and a Schott flask of growth media for your group.
2. Pour the liquid media into the Petri dish in aseptic conditions and let it cool down and solidify.
3. Distribute the Arabidopsis seedlings in the Petri dish so they are 0.5 cm apart.

B. Observing differences between wild-type and mutagenized population

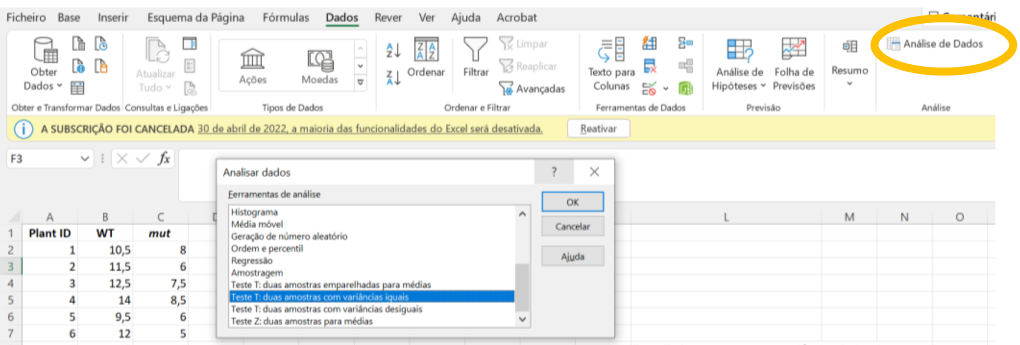
1. Open the picture file with a photo of a Petri dish containing seedlings of two genotypes: wild-type (WT) and mutant ('mut') as indicated. Observe the seedlings in the plate and draw and annotate, in your lab book, the most distinctive characteristics of each genotype. In the drawing identify the organs in the seedling: cotyledons, hypocotyl, primary roots, lateral roots (if present).
   Q1: What qualitative differences do you observe in the seedlings (cotyledons and root organs, other) from WT and 'mut' mutant? Detail the differences you observed.
2. In the picture of the Petri dish, you can find a ruler for measurement guidance. Open the file using the Image J program.
3. Draw a line between two points of known distance such as the ruler on the photo. Go to **Analyze → Set Scale.** In the Set Scale window, the length of the line, in pixels, will be displayed. Type the known distance and units of measure in the appropriate boxes and click OK. Measurements will now be shown using these settings.
4. Draw a line between the hypocotyl root border (at the base of the cotyledons) and the root tip (the most distal part of the root that protects the root apical meristem, RAM). The status bar will show the angle (from horizontal) and the length. **Analyze → Measure** (or Ctrl+M or simply type M on the keyboard) transfers the values to a data window.

5. Measure all the roots in the Petri dish and then, copy and paste your measurements to an Excel file as the following example:

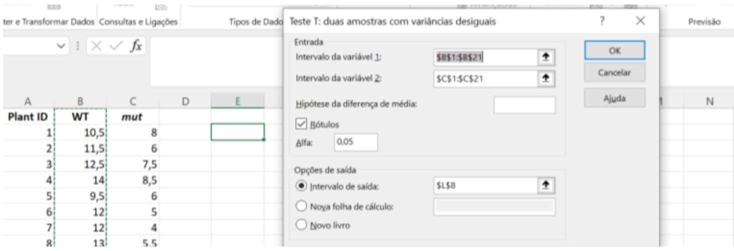
Plant ID	WT	*mut*
1	10,5	8
2	11,5	6
3	12,5	7,5
.	.	.
.	.	.
20	11	7
Mean	MÉDIA()	
StaDev	DESVPAD.P(2:21)	

6. Calculate the Mean Root Length for WT and mut mutant (MEAN/MÉDIA function on excel) and the standard deviation (S.D.) - a measure of how dispersed the data is in relation to the mean (by using the DESVPAD.P function on excel). If values of S.D. are similar, we can assume variances in the two populations are equal for statistical testing purposes.
7. Verify whether you have a normal distribution of your data by doing a histogram for WT and mut root length data. First select the WT data. Go to **Insert** > **Graph** > **Histogram** (for N=20, choose to have 3-5 classes in the horizontal axis). Do the same for 'mut' data.

   Q2: Is the root length data normally distributed?
8. Let's assume the distribution of your root length data is normal (Note: it will never be evenly distributed as biological data usually is not so boring!). Now you need to test whether the differences in the mean values between WT and mut are significant. For that, perform a t-test on excel (or alternatively go online to https://www.graphpad.com/quickcalcs/ttest1.cfm if you don't have excel toolbox up and running. Note: in GraphPad online tool, use dots instead of commas to separate decimal numbers). On Excel, go to Data and select the "Data Analysis" ("Análise de Dados") tool. Then choose "t-test: two sample assuming equal variances", as shown below.



9. Select the cells for WT (range interval variable 1) and ‘mut’ (range interval variable 2), include labels, and select any cell in the worksheet to deliver the results of the t-test, as shown below.



10. The result of the t-test is read in the Stat t value and in the P-value. You may consider the one-tail statistic, as we are just aiming at checking for differences between means. Check the example below to help you draw your own conclusions from your phenotyping experiment.

Teste T: duas amostras com variâncias desiguais		
	WT	*mut*
Média	11,8	6,35
Variância	1,536842	1,713158
Observações	20	20
Hipótese de diferença de média	0	
gl	38	
Stat t	13,51979	
P(T<=t) uni-caudal	2,13E-16	
t crítico uni-caudal	1,685954	
P(T<=t) bi-caudal	4,25E-16	
t crítico bi-caudal	2,024394	

11. In the example, P(T <= t) one-tailed is 2,13E-16, which is smaller than our alpha, set at 0.05 (95% confidence interval), we can therefore assume that there is a significant difference in the root length mean value between WT and mutant ‘mut’. If the P-value is greater than our alpha, then we must accept the null hypothesis, i.e., assume there are no statistically significant differences between the genotypes/phenotypes quantified.

    Q3: Is root length significantly different between the two genotypes? (Based on your experiment data analysis, including statistics)

You translated qualitative information into quantitative information by taking the root length measurements and calculating the statistical significance to the differences observed in the two populations/genotypes. This is extremely valuable in Biology –it is important in research that you get quantifiable data for your qualitative observations. By observing at least 20 plants per genotype, your initial observations are considering the variability among biological replicates. This statistical robustness will ensure biological significance to your observations.

1. The *'mut'* mutant you just phenotyped is a loss-of-function mutant for a given gene. Very often genes are named after the phenotype that is observed in the loss of function mutants' phenotypes.

   Q4: If you were asked to name this mutant, how would you name it? And why.

2. Your lab colleague observed the *mut* mutant and WT roots (Figure 1) under light and confocal microscopes. Figure 1 depicts your colleague observations.

   Q5: Can you identify the cell types/layers in the root apical meristem of Arabidopsis pictures. Explain in which way has disruption of this gene altered the typical organization of RAM, when comparing WT and *mut*.

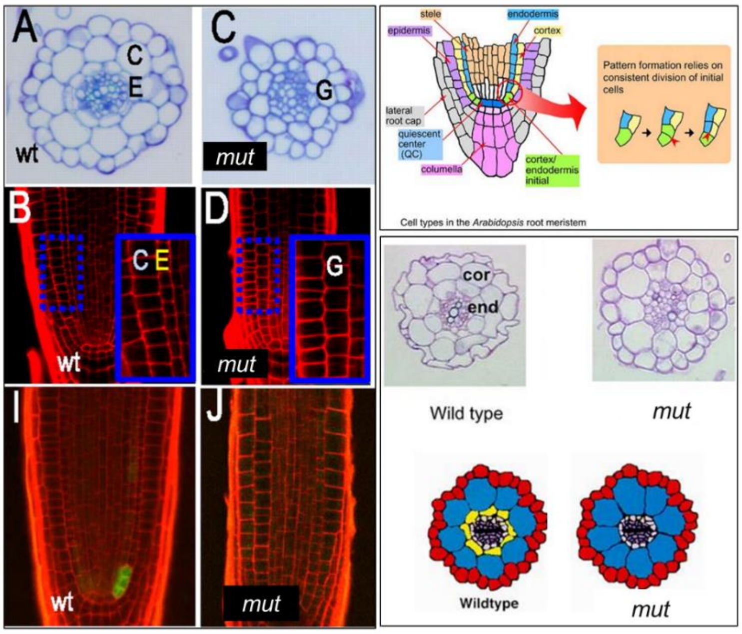


Figure 1. Longitudinal and radial organization of *mutant* (*mut*) and wild-type (WT) Arabidopsis roots. (Images credit: Ben Scheres, Phil Benfey, Liam Dolan, from Scheres *et al*., 2002)

3. Next logical step would be to find out which gene is disrupted in the mutant you phenotyped. Since this mutant is a T-DNA insertional mutant, it is gifted with a *tag* sequence (this can be e.g. an antibiotic resistance marker, or a fluorescent marker) i.e. a sequence that is well known and that allows us to design specific primers that will enable sequencing the flanking region around this insertional T-DNA. This is one of the ways to know which gene you knocked-out in the mutagenesis assay.

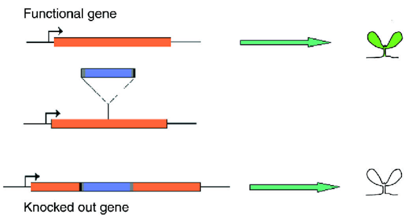


Figure 2. *mut* is the result of insertional mutagenesis (blue depicts T-DNA). (Image credit: Ken Pallet)

After sequencing the flanking region, we found this T-DNA disrupted the gene on chromosome 4 (genome coordinates Chr4:17691871-17693466). Search in *The Arabidopsis Information Resource* TAIR website https://www.arabidopsis.org/ for the name of the mutated gene. Go to Tools > GBrowse> fill out Landmark or Region.

Q6: What is this gene or protein coding names? What is the gene AT identifier (locus)? What is the coding sequence length? How many amino acids are translated into the corresponding protein? Is the mutated gene you found in your screening already studied? What is this gene described function during Arabidopsis root development?

References

Hartwell LH, Hood L, Goldberg ML, Reynolds AE, Silver LM, Veres RC (2008). Genetics: from genes to genomes. Boston: McGraw-Hill Higher Education. ISBN 978-0-07-284846-5.

Plant Model systems

Chang, Caren et al. Field Guide to Plant Model Systems. Cell, Volume 167, Issue 2, 325 – 339. doi: 10.1016/j.cell.2016.08.031. URL: https://doi.org/10.1016/j.cell.2016.08.031

Stefan A. Rensing, et al (2020) The Moss *Physcomitrium* (*Physcomitrella)* patens: A Model Organism for Non-Seed Plants, The Plant Cell, 32: 1361–1376, https://doi.org/10.1105/tpc.19.00828

Identifying mutations

O'Malley, R. C., Barragan, C. C., & Ecker, J. R. (2015). A user's guide to the Arabidopsis T-DNA insertion mutant collections. Methods in molecular biology (Clifton, N.J.), 1284, 323–342. https://doi.org/10.1007/978-1-4939-2444-8_16

The SHR mutation

Ben Scheres, Phil Benfey, Liam Dolan (2002) The Arabidopsis Book, https://doi.org/10.1199/tab.0101

⚠ **Please read the protocol beforehand and bring the following materials to class:**

- laptop computer - 1 per group

Make sure you have Excel on your office or equivalent and **before class** please verify that you have the toolpak "Data analysis" ("*Análise de dados*") in your excel. Check the many videos on Youtube on **Installing Analysis Toolpak in Excel for Statistical Analysis e.g.** https://www.youtube.com/watch?v=FzL84Imopss and more details on t-test basics here: https://statisticsbyjim.com/hypothesis-testing/t-tests-excel/.

Instal the Image J software at https://imagej.nih.gov/ij/download.html.