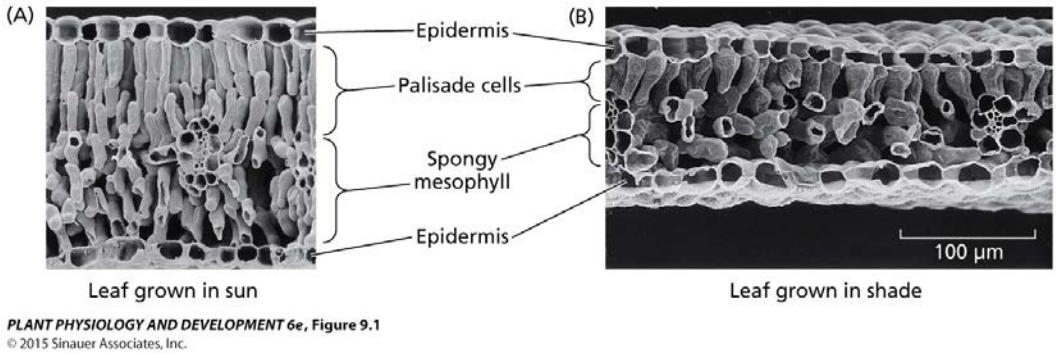
EXPERIMENT 1

PLANTS GROWN UNDER HIGH LIGHT AND LOW LIGHT CONDITIONS



Scanning electron micrographs of the leaf anatomy of a legume (*Thermopsis montana*) grown in different light environments.

Instructors:
BOVR2A:
Marjon Kuiper (KJON
Famke Brouwer
Mark Sibbald (SIMA)
Patricia van der Veek (VEEP)

BOVR2B
Janet Komduur (KJAN)
Jurre Hageman (HJUR)
Lennart Meijerink (MLEN)

Adapted and modified from Protocol from Ecophysiology of Plants, RuG, NL by Janet Komduur and Désirée den Os

Version 7: 27 January 2021

EXPERIMENT 1: PLANTS GROWN UNDER HIGH AND LOW LIGHT CONDITIONS

1.1 INTRODUCTION/THEORY

Light is an environmental factor that strongly affects the growth and development of plants. Plant species that are acclimated to shade (low light) differ morphologically and physiologically from species that are acclimated to high light conditions. However, most plants are neither extremely shade-avoiding nor shade-tolerating and are able to adjust to varying light conditions physiologically (see cover page of this manual) and metabolically. Since plants are sessile and access to photosynthetic active radiation (PAR) is essential for plant growth and nutrition, this ability to respond to high and low light plays a very important role in plants to optimize photosynthesis.

Several physiological adjustments to optimize growth in low and high light conditions have been identified. For instance, in general, the leaf areas of plants grown in low light will be larger to provide a larger area for capturing light energy for photosynthesis (2). Additionally, leaves from plants grown in high light conditions will have multiple layers of palisade parenchyma, loaded with chlorophyll, since there is sufficient radiation present to reach the lower layers (see figure cover page). Furthermore, in order to reach full sunlight, plants grown in low light are regularly inclined to induce length growth. This results in long internodes. Accordingly, the shoot system will contain larger vacuoles and less biomass. Also, plants grown in light conditions will have more evaporation from their leaves, which justifies investments in larger root systems (3).

Metabolic adjustments, to optimize growth in low light and high light, are also made. Plants grown in low light tend to have lower chlorophyll a / chlorophyll b ratios. This acclimation optimizes the harvesting of light for photosynthesis. The pigment Chl*a* is found in the reaction centers. Besides that, chlorophyll a (chl*a*) and b (chl*b*) are both associated with the antennae of the light-harvesting complexes (LHC) of photosynthesis and are so-called accessory pigments. A lower Chl*a*/*b* ratio reflects an increase in antenna complexes relative to reaction centers. Adjustments to the amounts of other pigments are also made as a reaction to the amount of light that is available. Examples of these other pigments are carotenoids like ß-carotene and xanthophylls. ß-Carotene is, just like Chl*a* and –*b,* an accessory pigment associated with the light-harvesting complex. Next to being an accessory pigment, ß-carotene plays a role in photoprotection. Xanthophylls are involved in the dissipation of light energy in the antenna complex as heat. During high light, the xanthophyll zeaxanthin is thought to bind to the antenna of LHC causing a conformational change lowering the harvest of light. Under low light conditions zeaxanthin is converted back into the xanthophyll violaxanthin (4).

In this experiment you will grow bean plants (*Phaseolus vulgaris* 'Saxa') under different light intensities. Once grown, you will assess several physiological and metabolic parameters of these plants and evaluate whether there are (significant) effects of light intensity on these parameters. Physiological parameters to be assessed are:

- Stem length
- Fresh weight (FW) of the shoot system (stem plus leaves), FW of the root system and FW of the whole plant

- Dry weight (DW) of shoot system, DW of the root system and DW of the whole plant
- Shoot/root ratio (FW & DW)
- Leaf area
- Leaf Area Ratio (LAR): leaf area/ DW plant

Metabolic parameters to assess are:

- amount and ratio of chlorophyll *a* and *b* in leaves (spectrophotometrically)
- amount and ratio of chlorophyll *a* and *b*, separation and identification of other leaf pigments (via thin layer chromatography)

For further information about plant anatomical and physiological acclimation towards light stress, and pigments characteristics see your notes from class, the suggested reading and sources at the end of this manual.

1.2 DETERMINING CHLOROPHYLL A AND B BY SPECTROPHOTOMETRY

In order to assess the amount of chlorophyll a and b in leaf material (chl*a* and chl*b* respectively), you will use spectrophotometry. Chl*a* and chl*b* absorb light in overlapping spectral regions (5, Fig. 1). Chl*a* and chl*b* are jointly present in the samples that you will use for your measurements. Thus, the absorbance of chl*b* contributes to the absorbance of chl*a* at the chlorophyll a maximum, and vice versa. This complicates the measurement.

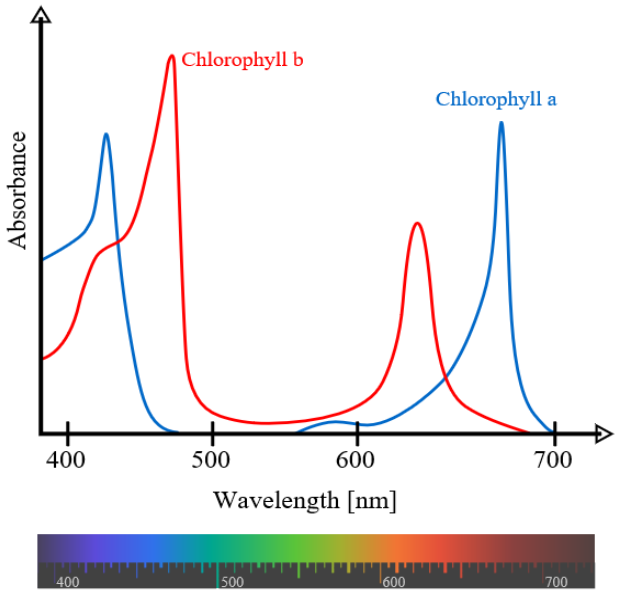


Figure 1. Absorption spectra of chlorophyll a and chlorophyll b (6)

The basis for spectrophotometric quantification of pigments is the Lambert-Beer law (5). This original Lambert-Beer law can only be applied for one isolated pigment. When

the concentration of chl*a* and chl*b* is determined from a pigment extract containing both chlorophylls, the equation derived from the Lambert-Beer law becomes more complex. The absorbance is then expressed as the sum of the absorbances of chl*a* and chl*b*. The concentrations for chl*a* and chl*b* are then given by a different equation, where the specific contribution of chl*b* to the chl*a* maximum and of chl*a* to the chl*b* maximum are subtracted. When ethanol is used as the extraction fluid, the amount of chl*a* and chl*b* and be can be calculated as follows:

Chlorophyll a: $13.36 \times A_{664.2} - 5.19 \times A_{648.6}$ (mg / l)
Chlorophyll b: $27.43 \times A_{648.6} - 8.12 \times A_{664.2}$ (mg / l)

1.3 DETERMINING PIGMENTS BY THIN LAYER CHROMATOGRAPHY (TLC)

Another way to compare the amounts of pigments (chl*a*, chl*b*, carotenoids etc) that are present in leaves, is by using thin layer chromatography (TLC). Chromatography in general is used to separate mixtures of substances into their components. All forms of chromatography are based on the same principle. There is a stationary phase (a solid, or a liquid supported on a solid) and a mobile phase (a liquid or a gas). The mobile phase flows through/over the stationary phase and carries the components of the mixture with it. Different components travel at different rates, depending on their affinity with the stationary phase and the mobile phase (usually determined by polarity issues).

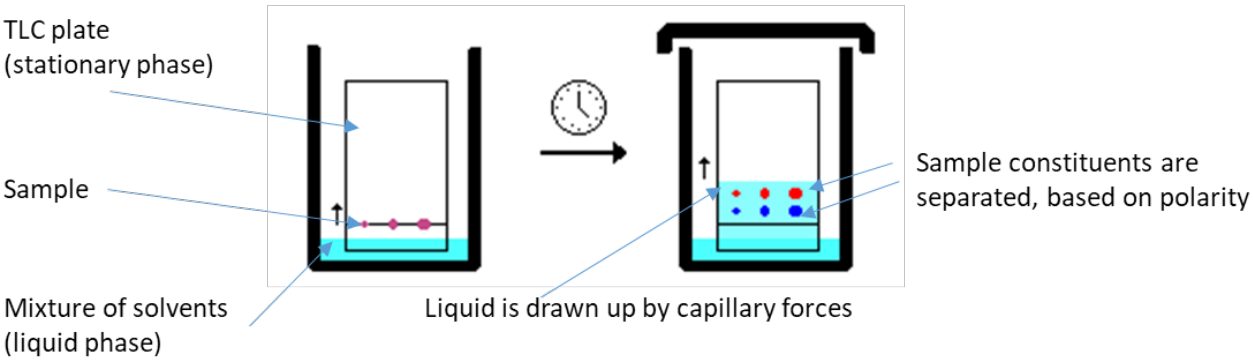


Figure 2. Schematic representation of thin layer chromatography. Leaf extract (pink spots) is spotted at the bottom of a TLC plate and the plate is placed into solvent (cyan). The solvent travels up the TLC plate via capillary action. The pigments travel with the mobile phase and separate (red and blue spots) based on their interaction with the stationary and mobile phase. The pigments can then be identified based on the distance traveled in a given solvent and period of time (7).

Thin layer chromatography is performed using a thin, uniform layer of silica gel or alumina, coated onto a piece of glass, metal or rigid plastic (Fig. 2). The silica gel (or the alumina) is the stationary phase. The mobile phase is a suitable liquid solvent or mixture of solvents. Silica gel is a form of silicon dioxide (silica). The silicon atoms are joined via oxygen atoms in a covalent structure. At the surface of the silica gel, the silicon atoms are attached to -OH groups (Fig. 3). This makes the surface of the silica gel highly polar (8).

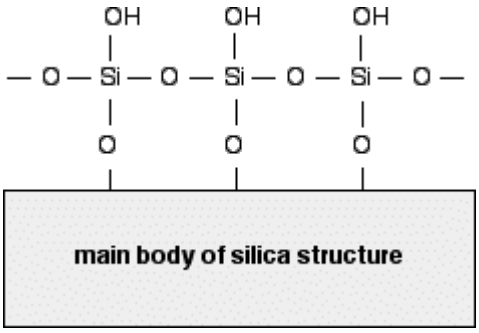


Figure 3. Molecular structure of the surface layer of silica gel. (8)

As the solvent is drawn up the plate by capillary forces, it first dissolves the compounds. The compounds present will then tend to migrate in the solvent up the chromatography plate. How fast/far the compounds get carried up the plate depends on two conditions:

1. How soluble the compound is in the nonpolar solvent.
2. How well the compound is adsorbed to the polar stationary phase.

Adsorption is not permanent - there is a constant movement of a molecule between being adsorbed onto the silica gel surface and going back into solution in the solvent. Obviously the compound can only travel up the plate during the time that it is dissolved in the solvent. While it is adsorbed on the silica gel, it is temporarily stopped - the solvent is moving on without it. That means that the more strongly a compound is adsorbed, the less distance it can travel up the plate. And that the better the substance will dissolve in the solvent, the faster it will move up.

Sometimes it may be that the compounds do not separate out very well on a chromatogram. In that case, changing the solvent may well help - including perhaps changing the pH of the solvent.

As the solvent slowly travels up the plate, the different components of the mixture travel at different rates and the mixture is separated into different components. The solvent is allowed to rise until it almost reaches the top of the plate. That will give the maximum separation of the components for this particular combination of solvent and stationary phase. The R_f value (retention) for each dye is then worked out using the formula:

$$R_f = \frac{\text{distance travelled by component}}{\text{distance travelled by solvent}}$$

To separate and identify the different plant pigments involved in photosynthesis in high and low light conditions, different characteristics are important to consider for TLC. In figure 4 the different chemical structures of the pigments investigated in this experiment are given. Chl*a* and chl*b* have almost identical structures. However, chl*a*

has a methyl group where chl*b* has a formyl group. Chl*a* is slightly more nonpolar compared to chl*b*. Another pigment that can be found is pheophytin. Pheophytin is the first pigment in the electron transport of the light reaction of photosynthesis, but can also be a breakdown product of chlorophyll. Pheophytin has a similar structure as chlorophyll though lacks the central Mg^{2+}. It has more affinity for nonpolar solvents compared to chl*a* and chl*b*. On the TLC plate chl*a* is expected to color blue-green, chl*b* yellow-green and pheophytin grayish. Other colors to be expected are yellow-orange from ß-carotene and yellow from the xanthophylls. Compared to ß-carotene, xanthophylls contain more oxygen atoms making these more polar and giving xanthophylls less affinity for a nonpolar solvent (9).

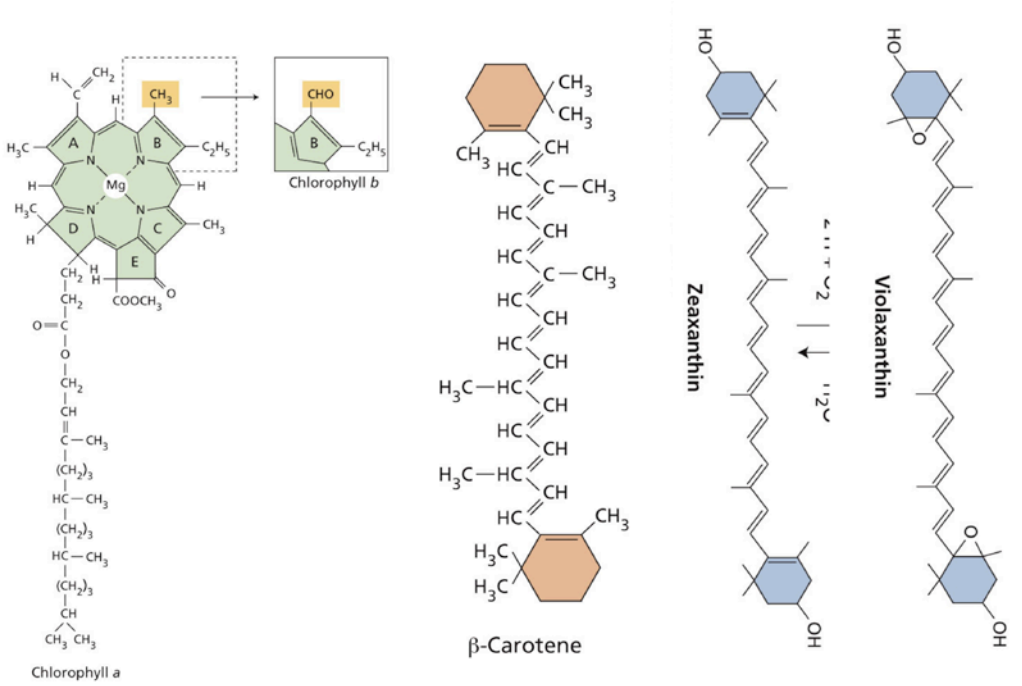


Figure 4. Chemical structures of plant pigments chlorophyll *a* and chlorophyll *b* and closely associated pigments ß-carotene, zeaxanthin and violaxanthin (5)

2.1 PREPARING E-LAB JOURNAL

Subjects that should be covered in the theory section of your E-lab journal are:

1) Explain the rationale behind each assessment. What are your expectations for each assessment (A-H)?
2) Thorough information about the techniques to be used:
    i) extraction (used for quantification of pigments with spectroscopy and TLC)
    ii) spectroscopy: describe the following notions: absorbance, extinction, transmission, the Beer-Lambert law (show your understanding by presenting your calculations involving these notions), cuvet cut off, cuvet matching.
    iii) TLC with the pigments involved

Incorporate the pre laboratory questions in your theory section.
As always, end your theory section with your expectations.

Mandatory: prepare **an easy-to-use excel sheet** to record/ log your individual measurements during the experiments and. Consider especially the measurements of i.e. weight of aluminum trays with/ without material, the averages, the standard deviations and the results of your statistical analyses (t-test)

2.2 PRE LABORATORY QUESTIONS

1. Plants grown in high light conditions will most likely have:
A. longer internodes than plants grown in low light.
B. shorter internodes than plants grown in low light.
C. equally long internodes as plants grown in low light.

2. The fresh weight of the shoot system of plants grown in high light conditions will most likely be:
A. lower than in plants grown in low light.
B. higher than in plants grown in low light.
C. the same as plants grown in low light.

3. The fresh weight of the root system of plants grown in high light conditions will most likely be:
A. lower than in plants grown in low light.
B. higher than in plants grown in low light.
C. the same as plants grown in low light.

4. The leaf surface of plants grown in high light conditions will most likely be:
A. smaller than in plants grown in low light.
B. larger than in plants grown in low light.
C. similar as that of the leaf surface of plants grown in low light.

5. The amount of chlorophyll *a* in plants grown in high light conditions will most likely be:
A. lower than in plants grown in low light.
B. higher than in plants grown in low light.
C. similar as that of the amount of chlorophyll *a* of plants grown in low light.

6. The amount of chlorophyll *b* in plants grown in high light conditions will most likely be:
A. lower than in plants grown in low light.
B. higher than in plants grown in low light.
C. similar as that of the amount of chlorophyll *b* of plants grown in low light.

7. The amount of ß-carotene in plants grown in high light conditions will most likely be:
A. lower than in plants grown in low light.
B. higher than in plants grown in low light.
C. similar as that of the amount of ß-carotene of plants grown in low light.

3. THE EXPERIMENT

In this experiment you will investigate the effects of light intensity on bean plants *Phaseolus vulgaris* 'Saxa'. 24 bean seeds will be grown on vermiculite to germinate and cultivate in either high light (10 plants) or low light (10 plants) for 17-20 days. Once grown, you will assess several physiological and metabolic parameters of these plants and evaluate whether there are (significant) effects of light intensity on these.

3.1 GROWING BEAN PLANTS (EXECUTED BY TEACHERS AND PEOPLE FROM THE STOCKROOM)

- 20 seeds of *Phaseolus vulgaris* 'Saxa'
- Place **on top** of a layer of vermiculite and slighty push them in: 10 seeds to be grown in high light conditions, 10 seeds to be grown in low light conditions (20 seeds per tray = two couples). Make sure that the seeds are slightly pushed into the vermiculite to take up water from the vermiculite, but can still receive light.
- Germinate and grow the plants in the Mobile LED Research Growth Lab (ZAP facility, access only with your instructor) at high and low light intensities for 17-20 days.
- Temperature: 22 ^{O}C / 17 ^{O}C (day / night)
- Light period 14 hours, relative humidity 60%

3.2 PHYSIOLOGICAL PARAMETERS

From each light treatment, plants are harvested. Per student 3 plants of one condition, that were sown a few weeks ago and assess the parameters stated below. **For detailed instructions see §3.2 A-D.**

- Fresh weight (FW) of the shoot system (stem plus leaves), root system and whole plant
- Leaf area
- Stem length: distance from the stem base to the first leaf
- Dry weight (DW) of shoot system, root system and whole plant and shoot root ratio
- Leaf Area Ratio (LAR): leaf area/ DW plant
- Shoot/root ratio (FW & DW)

In order to prevent drying, it is advised to work fast through §3.2 A-D until the fresh weights and the leaf areas have been determined!

Log all your results in the excel-sheet that you prepared.

ACTIONS ON DAY 1

3.2 ASSESSMENT A: FRESH WEIGHT (FW) SHOOT SYSTEM AND ROOT SYSTEM

- Annotate three aluminum trays for each treatment and carefully determine the empty weight of the trays (do this before you take the plants from the vermiculite).
- Take 3 plants from one growth conditions.
- Carefully rinse away all vermiculite and place the plants in a vessel of water.
- Separate the root systems from the shoot systems with a knife.
- Carefully pat-dry the root system.
- Swiftly determine the total fresh weight of the 3 root and shoot systems, using the predetermined trays.
- Place the shoot systems back into the original vessels with water, to prevent drying and continue with assessment B.

3.2 ASSESSMENT B: LEAF AREA PER PLANT AND LEAF AREA RATIO (LAR), USE OF IMAGEJ

- For all the 3 plants: carefully pull all leaves from 1 plant and put these together on a graph paper. Make sure to indicate the treatment and plant number.
- Take 1 CLEAR picture of all leaves of 1 plant. To get a clear picture make sure you take the picture straight from above the leaves, not oblique.
- Safe as JPG file
- Repeat for all separate plants per treatment
- Add the leaves to the appropriate aluminum tray with the stem to dry overnight and continue with assessment C.

3.2. ASSESSMENT C: STEM LENGTH: DISTANCE FROM THE STEM BASE TO THE FIRST LEAF

- Return to the stems of each plant.
- Measure the distances (cm) between the stem base and the first leaf (stem length).
- Immediately after taking effective measurements, place the trays with plant material in a stove of 60 ^{O}C overnight.
- Make sure that the hatch on top of the stove is open.

Post laboratory assignments of Assessment A

1. Use your own measurements and that of another student who studied a different light condition.
2. Calculate the average fresh weight of one shoot system, one root system and one integral plant per growth condition.
3. Calculate the shoot / root ratio, based on fresh weight.

Put these results in **a Result overview excel-sheet table**.

	HL			LL			
Parameter	average	std	n	average	std	n	t-test
Stem length (cm)							
etc.							

Post laboratory assignment of Assessment B

4. Determine the surface area of the leaves per plant. Do this for each plant per condition. You can semi-automate it by using Image J for these analyses. The work flow with detailed instruction is given in Appendix 1.
5. Calculate the average leaf surface area per plant for each growth condition.
6. Calculate, using the dry weight (see below) the "Leaf Area Ratio" (LAR) ($cm^2\ g^{-1}$ plant dry weight).

Put these results in **a Result overview excel-sheet table**.

Post laboratory assignments of Assessment C

7. Calculate the average stem lengths and standard deviation (SD) of both light conditions.
8. Calculate, by using the two-sample t-test, whether the difference of the mean stem lengths between plants grown in high or low light intensity is significant. List the attained p-value. Use your manual of Statistiek 2 (BB, theme 4, paragraph 4) if necessary.

Put these results in **a Result overview excel-sheet table**.

ACTIONS ON DAY 2

3.2 ASSESSMENT D: DRY WEIGHT OF SHOOT SYSTEM, ROOT SYSTEM AND WHOLE PLANT

- After drying (the material should be completely brittle), weigh the trays.

Post laboratory assignment

9. Calculate the total dry weight of the shoot systems and root systems.
10. Determine the dry weight of the root and shoot system per plant.
11. Calculate the average weight of the root system, shoot system and complete plant per growth condition.
12. Calculate the shoot / root ratio, based on dry weight.

Put these results in **a Result overview excel-sheet table**.

Post laboratory assignment as part of your result section in your e-labjournal

13. Make a bar chart for stem length, fresh weight (FW) of the shoot and root, leaf area per plant, LAR and total dry weight (DW) of the plant and indicate the standard deviation and whether this parameter is significantly different between HL and LL conditions. When significant, indicate this with a '*' in the graph.
14. Ad proper legends and titles etc. to your graphs.
15. Give a correct *Results* description applying the WHO rule (Why How Outcome).

3.3 METABOLIC PARAMETERS

From a light treatment harvest 3 plants and assess the parameters stated below. **For detailed instructions see §3.3 E-G.** Make sure to work with the plant material at 4˚C and protect the material from light to prevent the pigments from bleaching.

- Cuvette cut-off
- Spectrophotometrical assessment: amount and ratio of chlorophyll *a* and *b* in leaves
- Thin layer chromatogram (TLC) of leaf pigments: ratio of chlorophyll *a* and *b*, detection of other pigments

ACTIONS ON DAY 2

3.3 ASSESSMENT E: PERFORMING SPECTROPHOTOMETRY – CUVETTE CUT-OFF

Before you measure the concentration of chlorophyll in your samples, you need to convince yourself of the quality of the spectrophotometer and determine which cuvettes to use. Three types of cuvettes are used in general practice: quartz, glass and plastic cuvettes. Not each type is applicable for all wavelength areas. In the following experiment you will determine which of the three types of cuvettes is most suitable for your experiment. The wavelength that results in an extinction of 1.0 A is called the cuvette cut-off, beneath this wavelength, the cuvette is not applicable.

- ☐ Place an empty cuvette in the measuring area of a spectrophotometer that can perform a lambda scan (ask you instructor).
- ☐ Record a complete lambda scan.

Post laboratory assignment

16. Determine which cuvette is suitable for your experiment and log your results clearly in e-lab journal.

Now that you have determined which cuvette matches your measurements, continue to extract chlorophyll from the leaves and use the right cuvette to measure the desired concentrations.

3.4 ASSESSMENT F: AMOUNT AND RATIO OF CHLOROPHYLL *A* AND *B*

High temperatures and light easily degrade chlorophyll. Make sure that all reactions (from harvesting to measurement) are performed on ice and that the plant material is protected from light. For the latter, use tubes etcetera wrapped in 1 layer of aluminum foil. In the following procedure you will make one chlorophyll extracts: 1 of a plant grown in high light or 1 plants grown in low light conditions. Exchange your data with a student who studied the other light condition.

CHLOROPHYLL EXTRACTION & SPECTROFOTOMETRY

- ☐ Place a measuring cylinders of 100 ml on ice to cool.
- ☐ Take 2 plants grown in high light, cut all leaves from the plant and cut the leaves into pieces such that you can mix it. Make sure not to make the pieces too small, this to prevent the leaves from getting wet. Wet material will instantly freeze when liquid nitrogen is added and will make grinding impossible.

- Take approximately exactly 0.5 g of leaf material and put it in a mortar (around 0.5 g, though know exactly how much you take. Do not throw away the rest of the leaf material, as it is also needed for Assessment G.
- Snap-freeze the sample with liquid nitrogen.
- Grind the sample with a pestle to powder.
- Add part of 50 ml pre-cooled 96% ethanol (no methanol added!) to your sample in the mortar.
- Homogenize the mixture
- Immediately filter the mixture through a piece of Miracloth that is placed in a funnel that is placed in a 100 ml pre-cooled measuring cyllinder.
- Rinse the mortar and pestle with the remaining 96% ethanol and filter as well through the miracloth.
- Evaluate the absorption of your sample immediately at 648.6 and 664.2 nm against 96% ethanol.

Post laboratory assignment

17. Calculate the concentration of chlorophyll a and b in mg / l by using the following formulas:
    Chl*a*: 13.36 x $A_{664.2}$ – 5.19 x $A_{648.6}$ – (mg/l)
    Chl*b*: 27.43 x $A_{648.6}$ – 8.12 x $A_{664.2}$ – (mg/l).
18. Calculate the content of Chl*a*, Chl*b* and Chl*a*+*b* in the leaf from both light conditions in mg/g FW leaf.
19. Compare the amounts of Chl*a*, Chl*b* and the ratio chl *a/b* as measured in leaves from both growth conditions.

ASSESSMENT G: TLC OF LEAF PIGMENTS: COMPARISON OF AMOUNTS OF CHLOROPHYLL A AND B AND DETECTION OF OTHER PIGMENTS

The procedures to prepare a chromatogram are as follows: a pencil line is drawn near the bottom of the plate and a small drop of a solution of the mixture that needs to be separated is placed on the line. When the spot of mixture is dry, the plate is put in a shallow layer of solvent in the development chamber. It is important that the solvent level is below the line with the spot on it.

Make sure that the development chamber is covered tightly. In this way you make sure that the atmosphere in the beaker is saturated with solvent vapor. Saturating the atmosphere in the beaker with vapor stops the solvent from evaporating as it rises up the plate.

PIGMENT EXTRACTION

- Weigh 0.5 g of chopped plant material from each condition (prepared for assessment F) and put in mortar.
- Snap-freeze the sample in liquid nitrogen.
- Grind the sample with a pestle.
- Transfer you sample to a greiner tube of 15 ml.
- Add 5 ml of 100% acetone to each sample.
- Add a little bit of $NaSO_4$ to remove water from your sample.

- Let stand for 1 min.
- Vortex thoroughly for 1 min.
- Centrifuge for 10 min. at 3000 rpm and collect the supernatant.
- This is your final extract.
- Protect your sample from being exposed to light.

SAMPLE APPLICATION AND TLC

- Do not touch the TLC-plate with bare hands.
- Activate the TLC plate for one hour at 120 ^{O}C
- Fill the bottom of the chamber with the running solvent: a mixture of 70% wasbenzine, 30% dioxane, 10% isopropanol (7 : 3 : 1) (work in the fume hood!!)
- Close the chamber and incubate for at least 30 min. in order to attain saturation by the vapor of the eluens.
- Carefully draw a line at 1.5 cm from the bottom of a TLC plate. Make sure that you do not damage the silica surface.
- Load portions of 10 µl of your extracts on each predesignated spot. Allow for the acetone to evaporate. Continue until 50 µl has been loaded on each spot. Make sure that your samples are applied in duplo (this makes 4 spots in total). Allow the TLC plate to dry completely before you proceed to the next step. Protect from light.
- Place the loaded TLC plate in the prepared developing chamber. Make sure that the plate does not stick to the side of the chamber to prevent adhesion of the running solution between your plate and side of the chamber.
- Make sure that the lid is not open for too long.
- Observe as the solvent front moves up the TLC plate. You should also be able to see the pigments separating. Again protect you sample from excessive light: switch off the light in the hood.
- When you are content with the result (the mobile phase is at appr. 4/5th), remove the TLC plate from the chamber. Immediately make a picture (do not inhale the fumes!!) and mark the position of the solvent front with a pencil.
- Allow the plate to dry completely before you remove it from the fume hood.

Post laboratory assignment

20. Identify each pigment based on its color.
21. Calculate the Rf values for each pigment that you observe.
22. Can you determine any differences in pigment composition between the plant grown at high light and grown at low light conditions? Use Image J (see appendix 2) to quantify. Discuss these differences based on the function of the pigment involved.
23. Compare your chl*a* and chl*b* TLC results to your results of the spectrophotometer.

4. SUGGESTED READING

Plant Physiology and Development, 6th edition 2015, eds Taiz, Zeiger, Muller, Murphy

CHAPTER 7:

- §Photosynthetic pigments absorb the light that powers photosynthesis (p.175 + fig. 7.6 and 7.7)
- § Many antenna pigment-protein complexes have a common structural motif (p.183 + fig.7.17)
- § Repair and regulation of the photosynthetic machinery (p.195-p.197 + fig 7.32 and 7.33)

CHAPTER 9:

- § Photosynthesis is influenced by leaf properties (first two paragraphs) (p.246 + fig. 9.1)
- § Leaf anatomy and canopy structure maximize light absorption (p.247 – p.248)
- § Leaves acclimate to sun and shade environments (p.249 –p.250)
- § Leaves must dissipate excess light energy (p.252 – p.253)

5. SOURCES

1. Protocol lichtproef Ecophysiology of Plants, Staal, M. et al. University of Groningen
2. Plants in action- Adaptation in Nature, Performance in Cultivation: Ch. 12.1.1. Light interception and utilisation. Eds Atwell, B.J., Kriedemann, P.E., Turnbull, C.G.N., 1999.
3. Lichtenthaler, H.K., Buschmann, C., Doll, M., Fietz, H.J., Bach, T., Kozel, U., Meir, D., Rahmsdorf, U., 1981. Photosynthetic activity chloroplasts ultrastruture and leaf characteristics of hight-light and low-light plants and of sun and shade leaves. Photosynthesis Research. 2, 115–141
4. Plant Physiology and Development, 6th edition 2015, eds Taiz, Zeiger, Muller, Murphy
5. Lichtenthaler, H.K., Buschmann, C., 2001. Chlorophylls and carotenoids: measurement and characterization by UV-VIS spectroscopy. Current Protocols in Food Analytical Chemistry F4.3.1-F4.3.8
6. https://commons.wikimedia.org/wiki/File:Chlorophyll_ab_spectra-en.svg Jul. 2017
7. https://www.bio-rad.com/en-nl/applications-technologies/introduction-chromatography?ID=LUSMIS7OP
8. http://www.chemguide.co.uk/analysis/chromatography/thinlayer.html
9. Experiments in Plant Physiology: Ch. 3 Separation, identification and quantification of plant pigments. Reiss, C. 1993

APPENDIX 1. WORKFLOW DETERMINING SURFACE AREA USING IMAGEJ BY AUTOMATIC DETERMINING OF OBJECTS

- ☐ Install Image J **http://imagej.nih.gov/ij/** (open source)*
- ☐ Open Image J
- ☐ Open your picture (File > Open).
- ☐ Magnify until you get a clear picture of part of the ruler (1 to 5 cm).
- ☐ Click on the "Straight line" icon.
- ☐ Draw a line of exactly 4 or 5 cm long, on the ruler.
- ☐ Choose "Analyze" > "Set scale"
- ☐ Fill in your distance (known distance) in cm (unit of length).
- ☐ Tick "Global"
- ☐ Zoom out, to find the whole image again.
- ☐ Choose "Image" > "Adjust" > "Color Threshold"
- ☐ Adjust the brightness of the image until the leaves show up in red and the background is white.
- ☐ Choose "Analyze" > "Tools" > "ROI manager" and use the "Wand Tracing Tool" to indicate one of the leaves in view.
- ☐ Click on the top button (Add) in "ROI Manager".
- ☐ Repeat for each leaf.
- ☐ Finish your measurements by clicking "Measure" in "ROI Manager".
- ☐ Go to "Results" and "Summarize" to find the total leaf area
- ☐ Go to: https://www.youtube.com/watch?v=WIrY-egQSS8 if you need help.

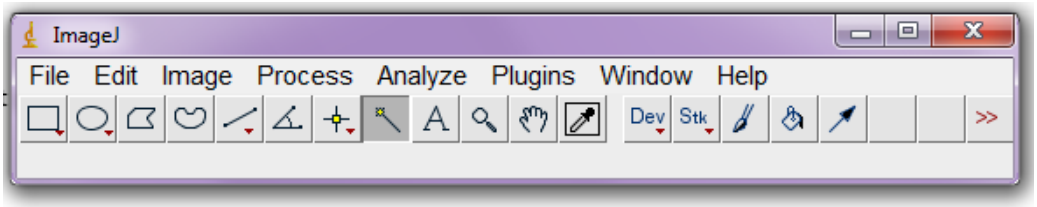
*When you experience problems by applying the calibration using an Apple system, please got to: https://imagej.nih.gov/ij/download.html

APPENDIX 2. COMPARE THE AMOUNTS OF PIGMENTS ON TLC USING IMAGE J

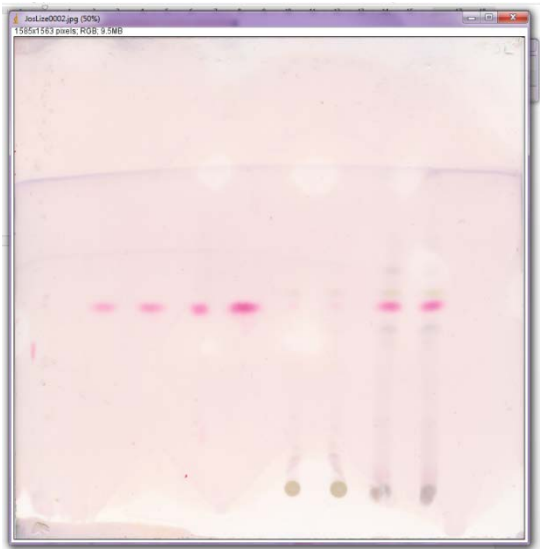
Compare the amounts of pigments by eye or by Image J as well.

Analysis with Image J: **http://rsb.info.nih.gov/ij/docs/menus/analyze.html#gels**

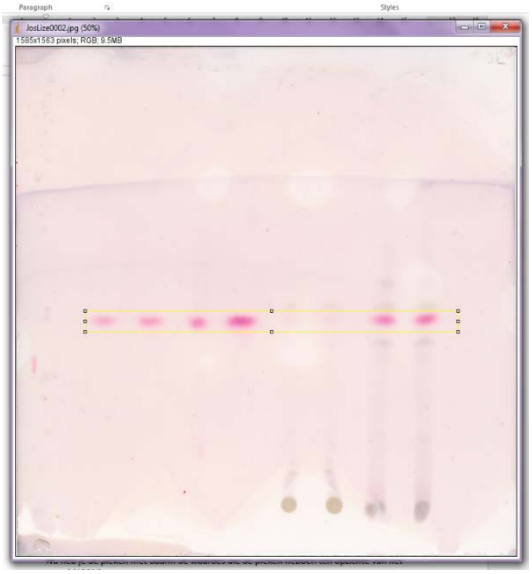
To compare peak intensities of your calibration series to your samples:
Scan your gel and safe as a JPEG
Install Image J. **http://imagej.nih.gov/ij/**
Open Image J



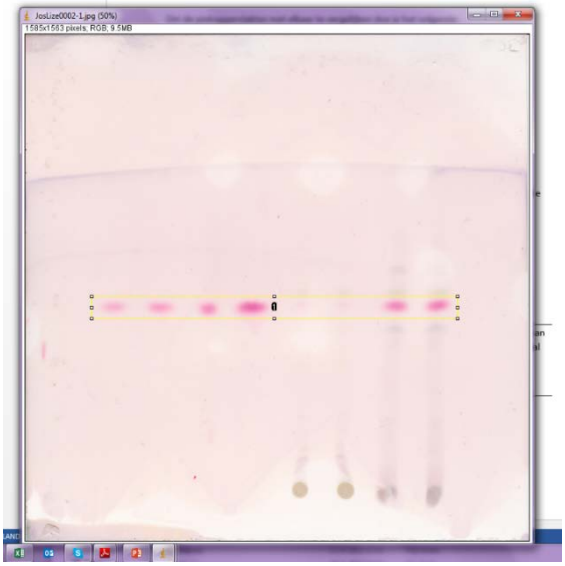
Open your file via Image J (File>Open)



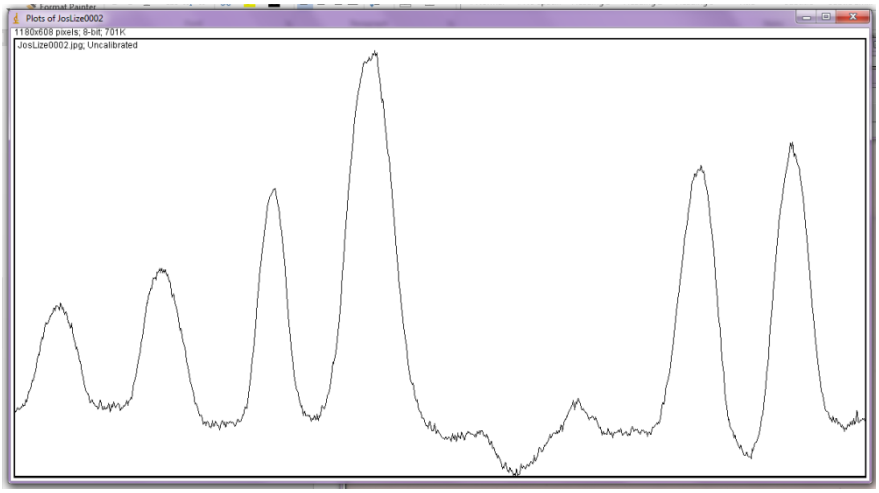
Draw a rectangle around the bands that you want to measure, using the square tool. A yellow rectangle will appear. Make sure that you do not include any other bands.



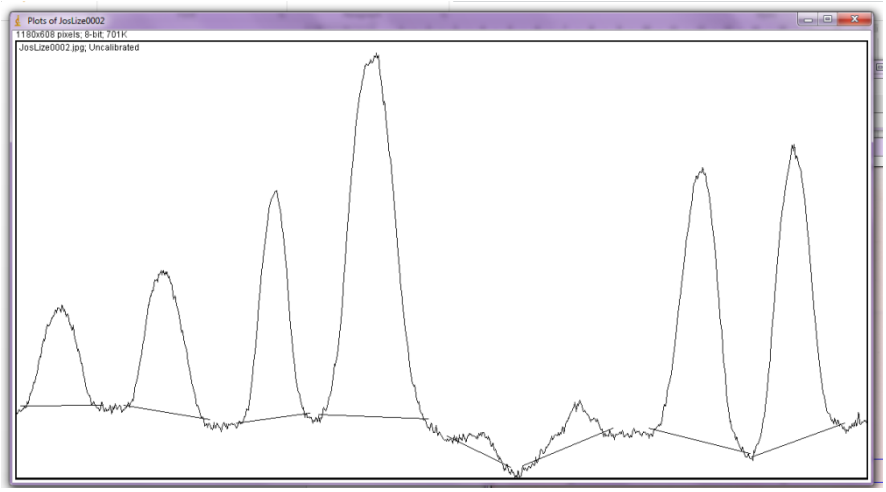
Choose: Analyze>Gels>Select First Lane
Click on the rectangle. A 1 will appear in the selected area.



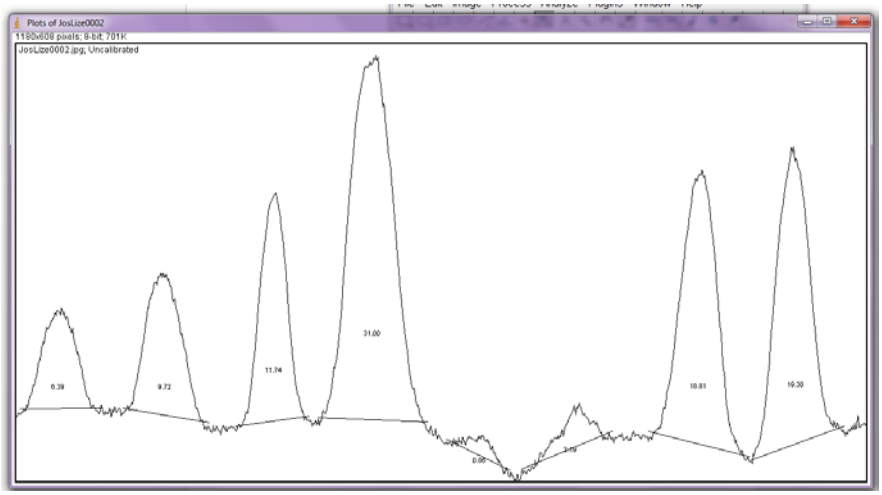
Choose: Analyze>Gels>Plot lanes.
The peak pattern of the spots will appear.



Close all the peaks that have appeared with the tool: Straight. Try to include the whole spot but not the background color.



Click with the “Magic Wand Tool”in all the peaks that you want to analyze. The peaks will turn yellow.
Choose: Analyze>Gels>Label Peaks



The numbers that have appeared inside the peaks represent the values that the peaks have compared to the total peak area (%). Use the values of your calibration series to draw a calibration line in Excel. Determine the amount of chlorophyll in the spots of your sample.