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Fisiologia Vegetal

2ª aula TP Nutrição

2018-2019

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Fisiologia Vegetal

2ª aula TP Nutrição

- **Que podemos concluir da aula prática?**

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O Que podemos concluir da aula prática?

- Que devíamos ter lido os protocolos para saber o significado do que estávamos a fazer
- Que devíamos ter feito as tarefas com mais cuidado para ter menos erros
- Que o número de réplicas por turma não é suficiente para tirar conclusões
- .....

Mas há efeitos que são de tal forma robustos que mesmo com todos estes erros os conseguimos detectar!

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O Que podemos concluir da aula prática?

Delineamento experimental

Inoculação	Principal Forma de N	Biofertilizante
Preto	Nitrato	Sem
Azul	Nitrato	Com
Branco	Amónio	Sem
Vermelho	Amónio	Com

Inoculantes: Lactobacillus

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O Que podemos concluir da aula prática?

Delineamento experimental

Inoculação	Principal Forma de N	Biofertilizante
Preto	Nitrato	Sem
Azul	Nitrato	Com
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Vermelho	Amónio	Com

Que hipóteses podemos testar com este delineamento experimental?

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O Que podemos concluir da aula prática?

Delineamento experimental

Inoculação	Principal Forma de N	Biofertilizante
Preto	Nitrato	Sem
Azul	Nitrato	Com
Branco	Amónio	Sem
Vermelho	Amónio	Com

Que resultados esperamos?

Porquê?

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O Que podemos concluir da aula prática?

Parâmetros a analisar

Biomassa: é uma medida integrativa do desenvolvimento das plantas em determinadas condições.
É normalmente avaliada pelo **peso seco** da planta ou de determinadas partes da planta.

Porque **não** se calcula a **biomassa** com base no peso fresco?

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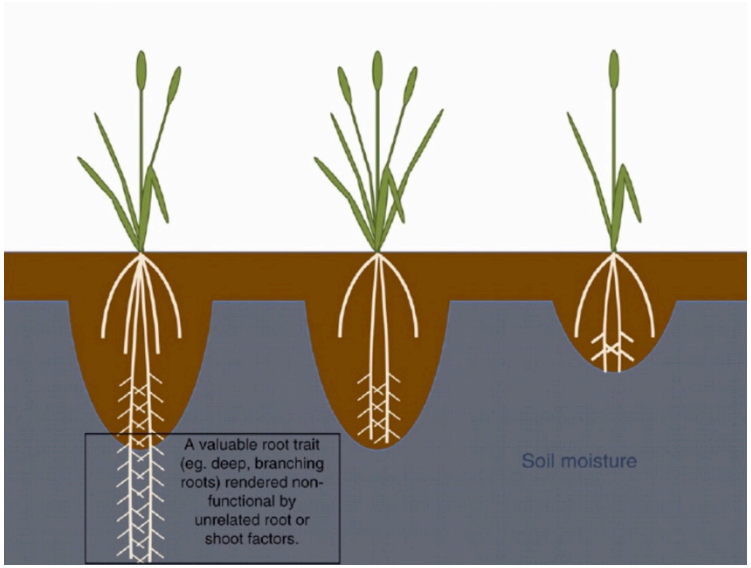
O Que podemos concluir da aula prática?

Parâmetros a analisar



Partição de Biomassa: Razão entre a biomassa da parte aérea e da parte radicular

Altera-se em resposta às condições do meio

nutrientes
luz
temperatura

Qual a partição ideal?

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O Que podemos concluir da aula prática?

Parâmetros a analisar

Relação peso seco/peso fresco:

Pode ser uma medida aproximada do conteúdo hidrico da planta

Particularmente importante quando se trabalha com stress hidrico

Qual a relação ideal?

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O Que podemos concluir da aula prática?

Parâmetros a analisar

Soil Plant Analysis Development (*SPAD*)

The meter works by emitting two frequencies of light, one at a wavelength of 660 nm (red) and one at 940 nm (infrared). Leaf chlorophyll absorbs red light but not infrared, the difference in absorption is measured by the meter and termed "**Optical Density Difference**," ODD. Therefore, the unit of measurement is ODD, a ratio that is provided by the meter. The value does not give an actual chlorophyll or nitrate count

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O Que podemos concluir da aula prática?

Parâmetros a analisar

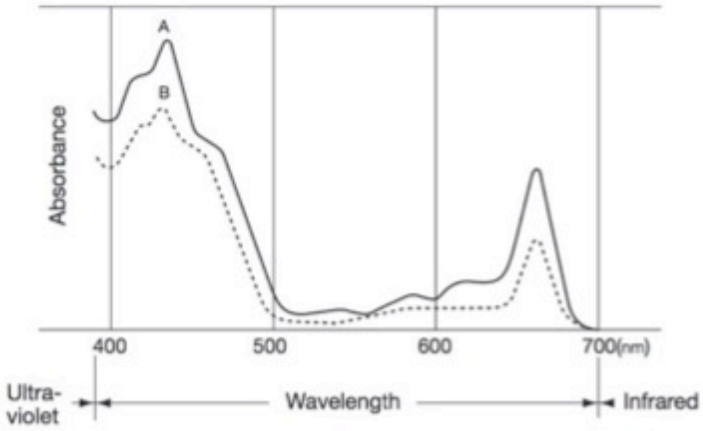
Theory

The SPAD-502Plus determines the relative amount of chlorophyll present by measuring the absorbance of the leaf in two wavelength regions.

The graph below shows the spectral absorbance of chlorophyll extracted from two leaf samples using 80% acetone.

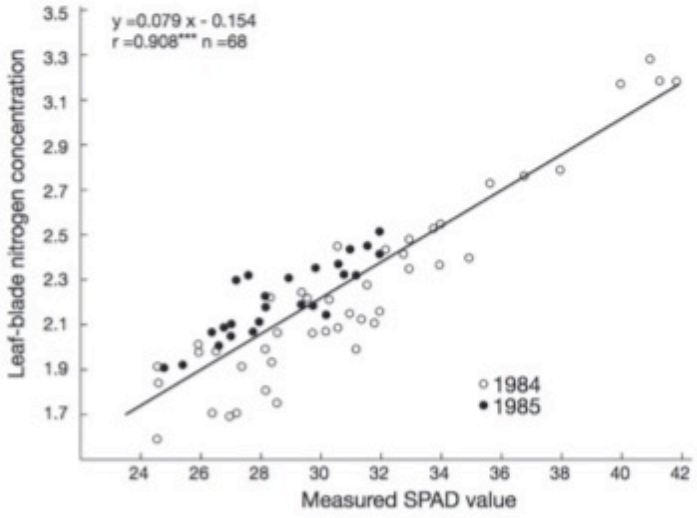
From the diagram, it can be seen that chlorophyll has absorbance peaks in the blue (400-500 nm) and red (600-700 nm) regions, with no absorbance in the near-infrared region.

To take advantage of this characteristic of chlorophyll, the SPAD-502Plus measures the absorbances of the leaf in the red and near-infrared regions. Using these two absorbances, the meter calculates a numerical SPAD value which is proportional to the amount of chlorophyll present in the leaf.



Checking the nutritional condition of plants

The chlorophyll present in the plant leaves is closely related to the nutritional condition of the plant. As can be seen from the graph below, the chlorophyll content (represented by the measured SPAD value) will increase in proportion to the amount of nitrogen (an important plant nutrient) present in the leaf. For a particular plant species, a higher SPAD value indicates a healthier plant.



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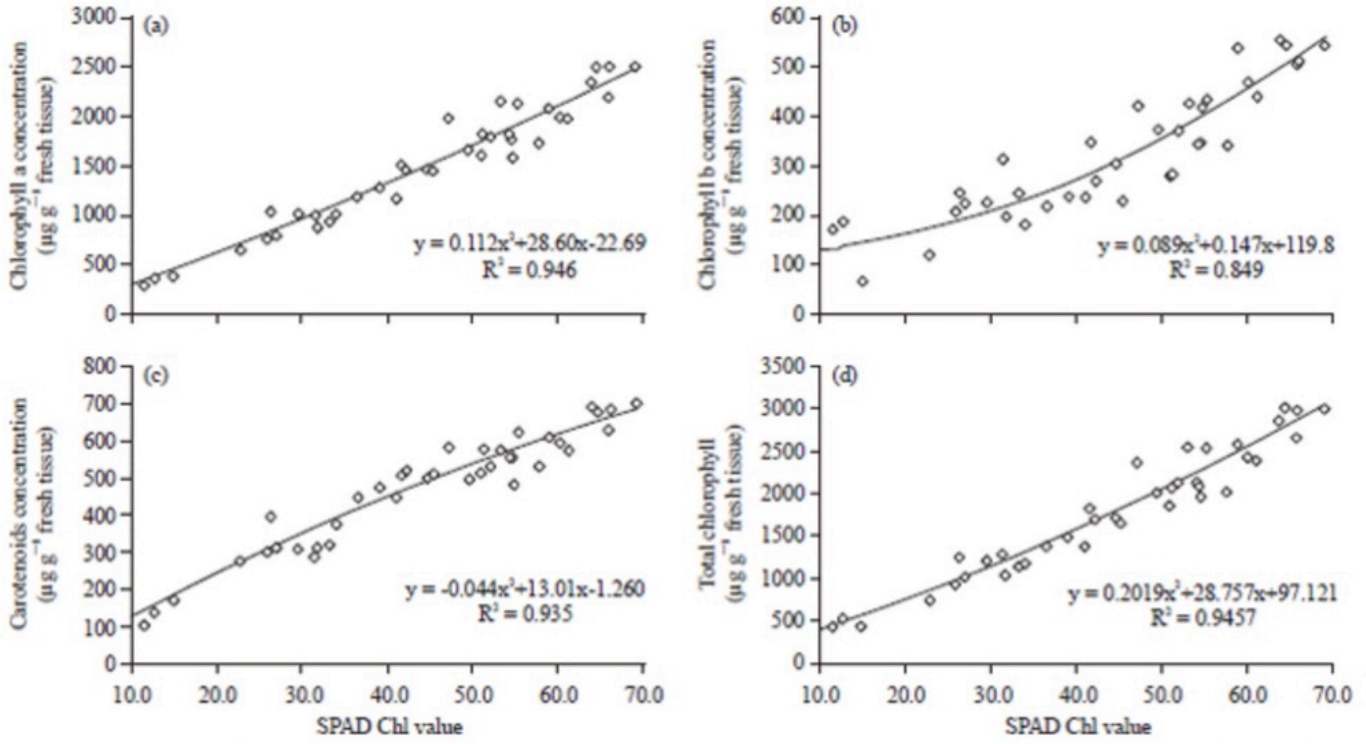
O Que podemos concluir da aula prática?

Parâmetros a analisar



Se esta curva representar a relação entre o valor de SPAD e a concentração de azoto na folha de acelga (*Beta vulgaris*), qual é a cocentração de azoto das folhas dos distintos tratamentos experimentais da aula Prática?

Há diferenças entre os tratamentos?
Será necessário proceder a fertilização?
Será um valor de alerta para nitratos?

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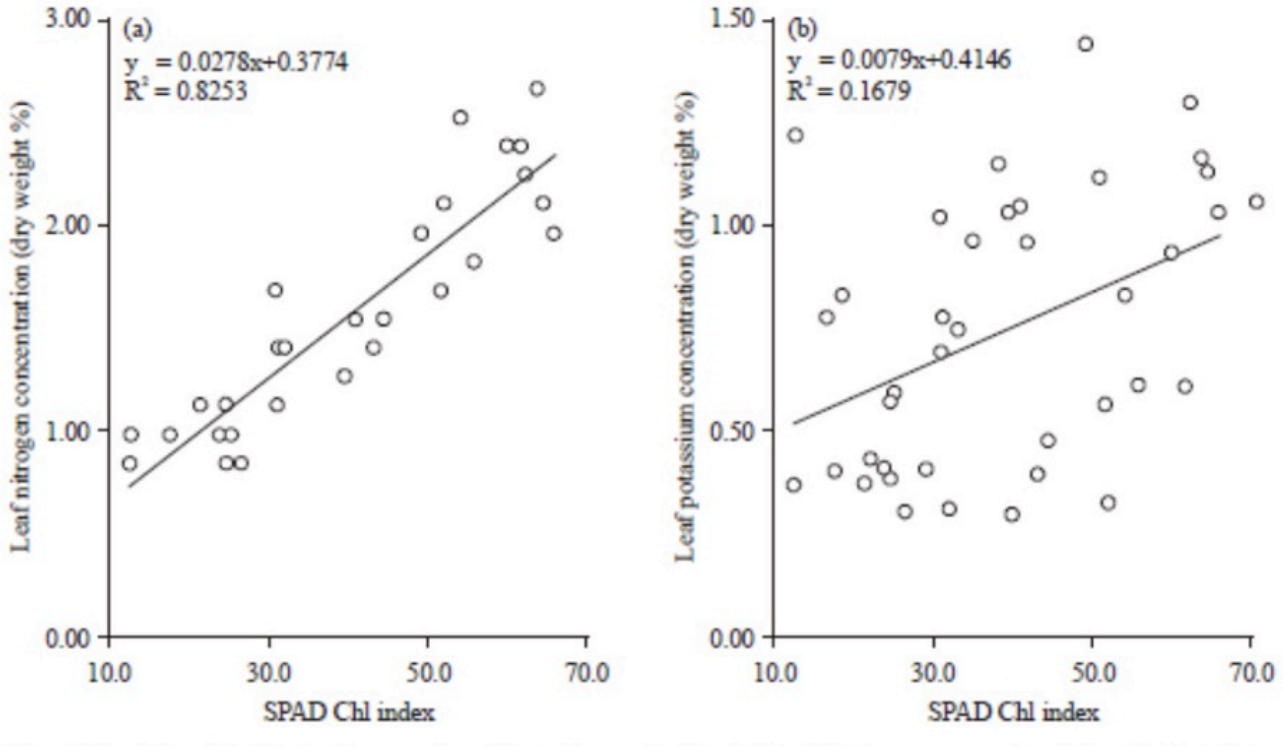
O Que podemos concluir da aula prática?

Parâmetros a analisar



O SPAD relaciona-se com o N mas não com o K.

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O Que podemos concluir da aula prática?

Parâmetros a analisar

Z970 PolyPen

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O Que podemos concluir da aula prática?

Parâmetros a analisar

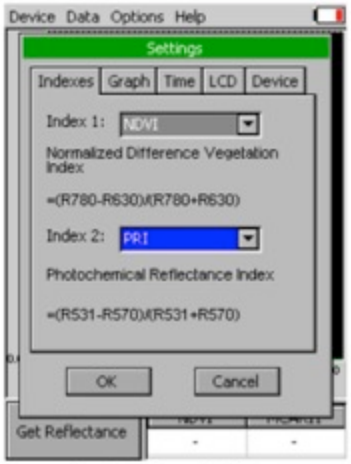

Fig. 9 Settings → Indexes

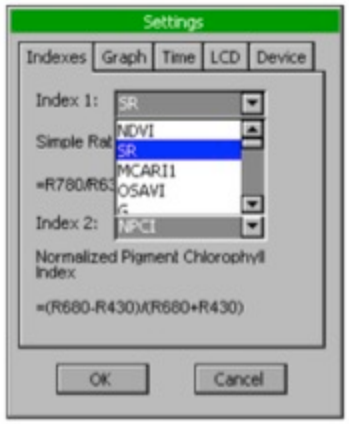

Fig. 10 Index selection

Poly Pen

NDVI

PRI

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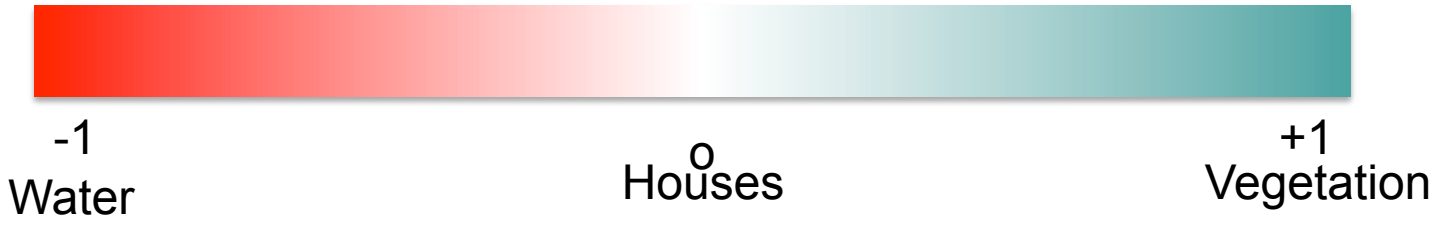
O Que podemos concluir da aula prática?

Parâmetros a analisar

What is NDVI (Normalized Difference Vegetation Index)?

Normalized Difference Vegetation Index (NDVI) quantifies vegetation by measuring the difference between near-infrared (which vegetation strongly reflects) and red light (which vegetation absorbs).

NDVI always ranges from -1 to +1.



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O Que podemos concluir da aula prática?

Parâmetros a analisar

What is NDVI (Normalized Difference Vegetation Index)?

$$NDVI = \frac{(NIR - Red)}{(NIR + Red)}$$

Healthy vegetation (chlorophyll) reflects more near-infrared (NIR) and green light compared to other wavelengths. But it absorbs more red and blue light.

This is why our eyes see vegetation as the color green. If you could see near-infrared, then it would be strong for vegetation too. Satellite sensors like Landsat and Sentinel-2 both have the necessary bands with NIR and red.

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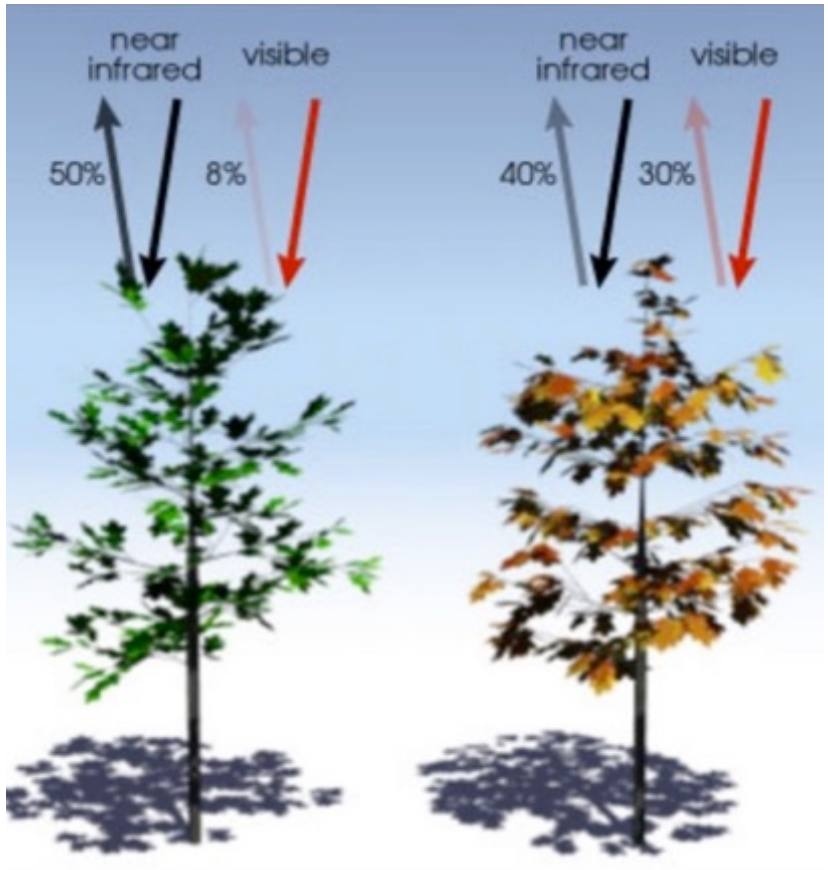
O Que podemos concluir da aula prática?

Parâmetros a analisar

What is NDVI (Normalized Difference Vegetation Index)?



$$NDVI = \frac{(NIR - Red)}{(NIR + Red)}$$

Calcule o NDVI para cada situação

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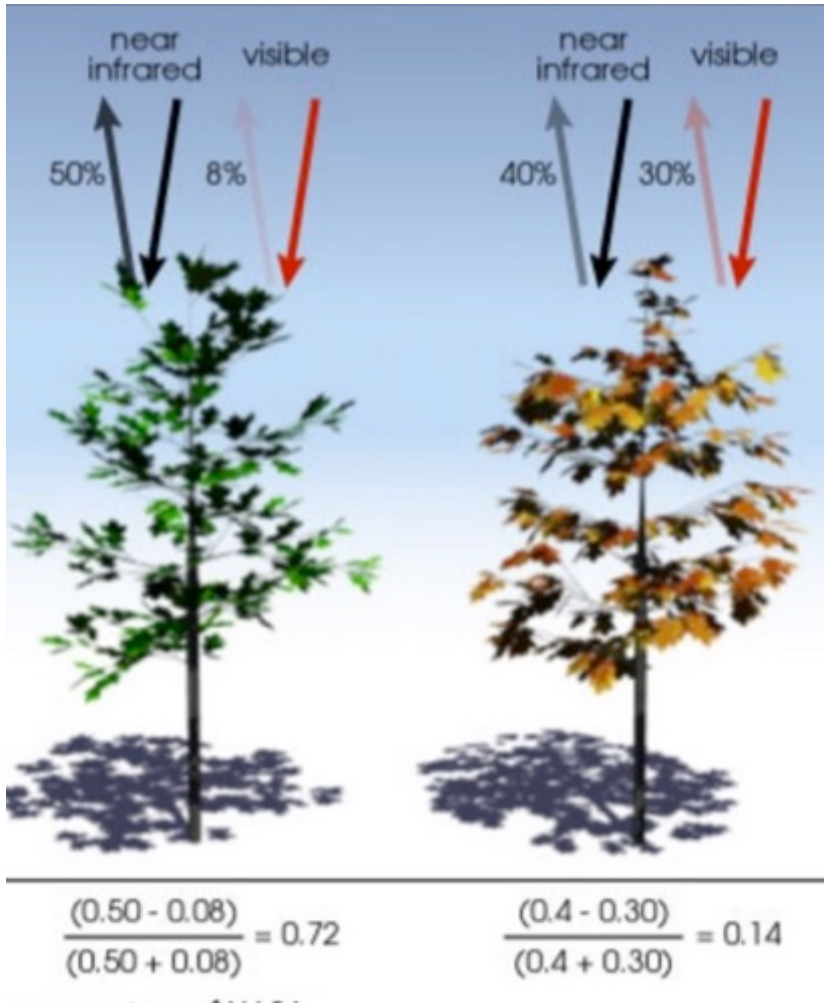
O Que podemos concluir da aula prática?

Parâmetros a analisar

What is NDVI (Normalized Difference Vegetation Index)?



$$NDVI = \frac{(NIR - Red)}{(NIR + Red)}$$

Calcule o NDVI para cada situação

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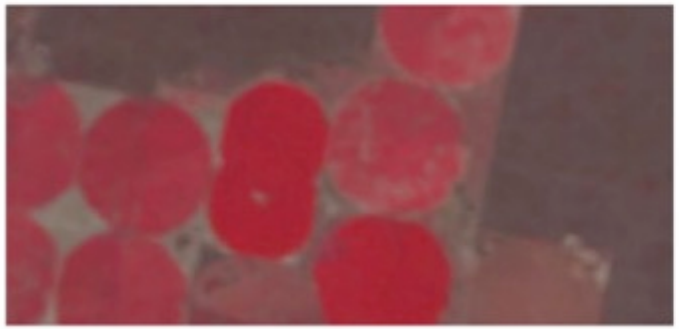
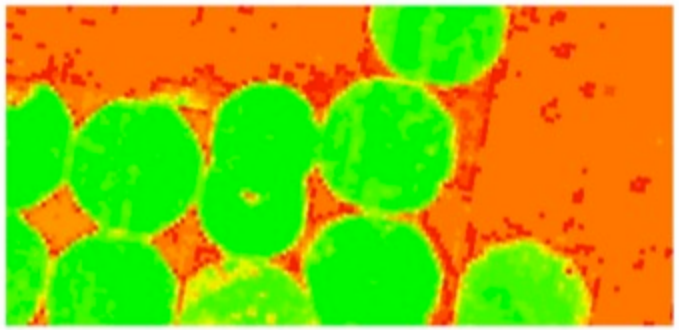
In **true color**, here's how it looks for red, green and blue bands. We say *true color* because it is the same as how our eyes see.

In the formula, you can see how NDVI leverages near-infrared (NIR). So when we put NIR band to display as red, we get **color infrared**. We say color infrared because near infrared is in the red channel. As you can see below, the pivot irrigation vegetation should already be shouting out at you in bright red!

$$NDVI = \frac{(NIR - Red)}{(NIR + Red)}$$



When you apply the formula, bright green indicates high NDVI. Whereas red has low NDVI. So it's quantifying vegetation by measuring the difference between near-infrared (which vegetation strongly reflects) and red light (which vegetation absorbs).



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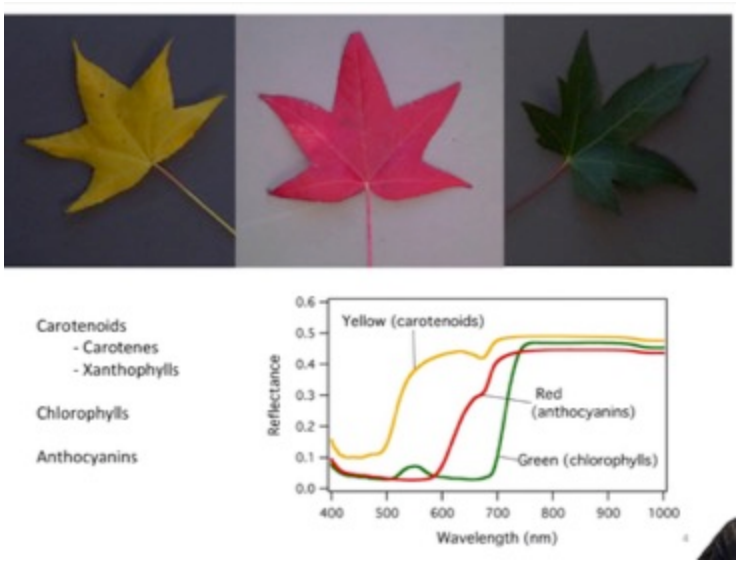
O Que podemos concluir da aula prática?

Parâmetros a analisar

What is PRI (Photochemical Reflectance index)?

The Photochemical Reflectance Index is a measure of the light-use efficiency of foliage and thus is primarily used as an indicator of water stress and for the assessment of carbon-dioxide uptake by plants.



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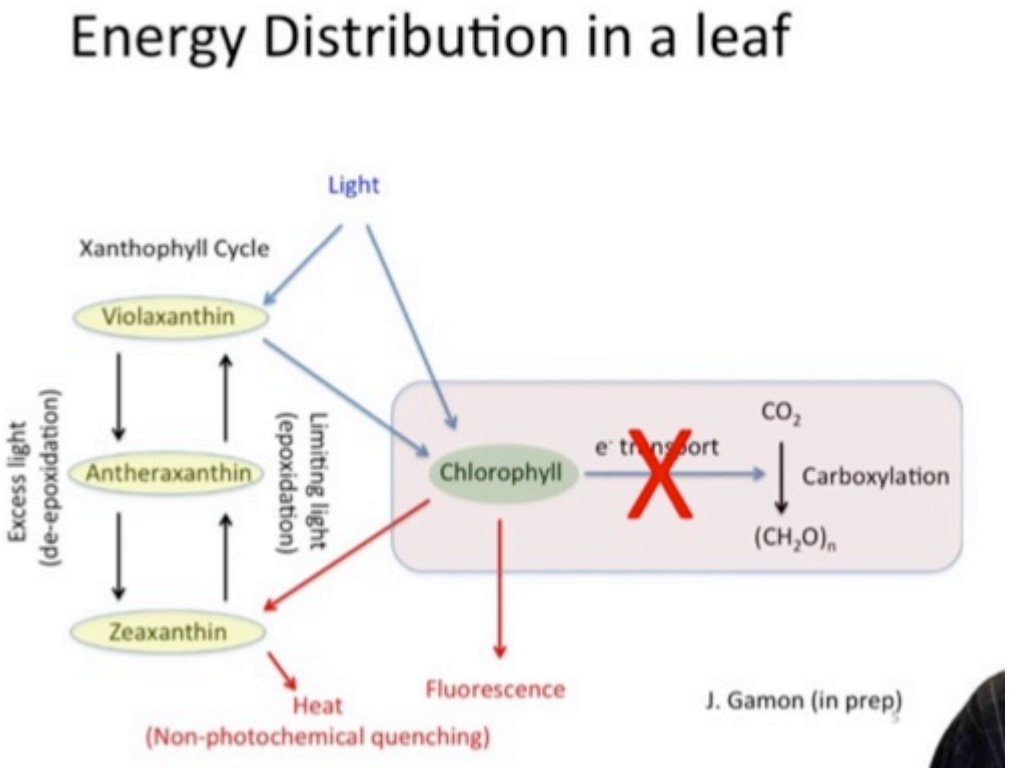

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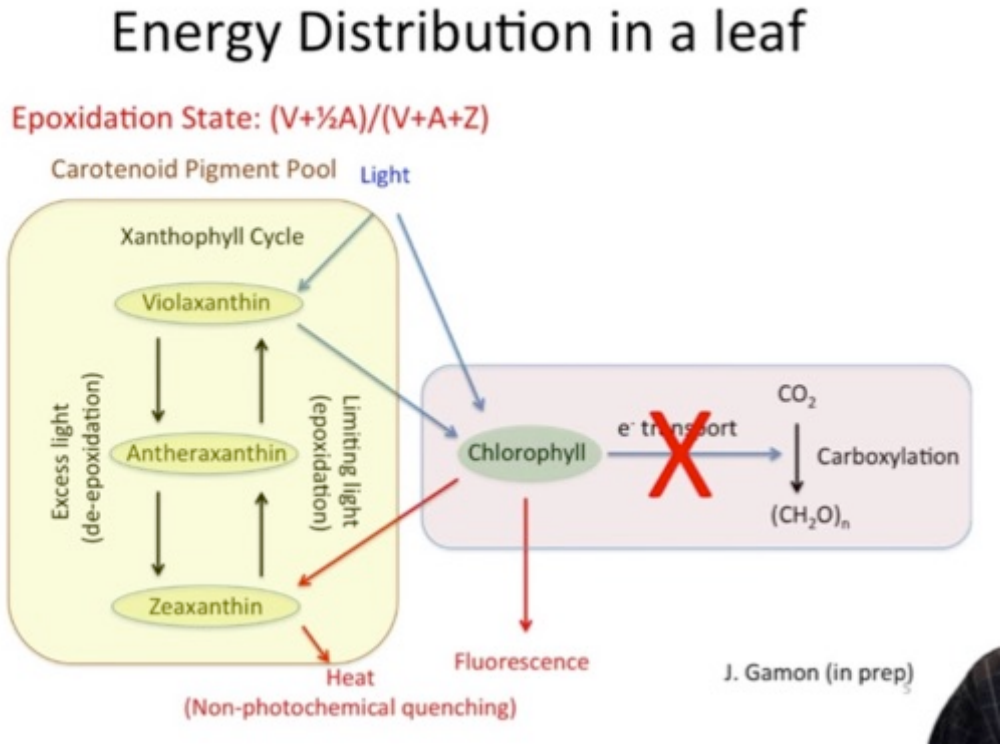

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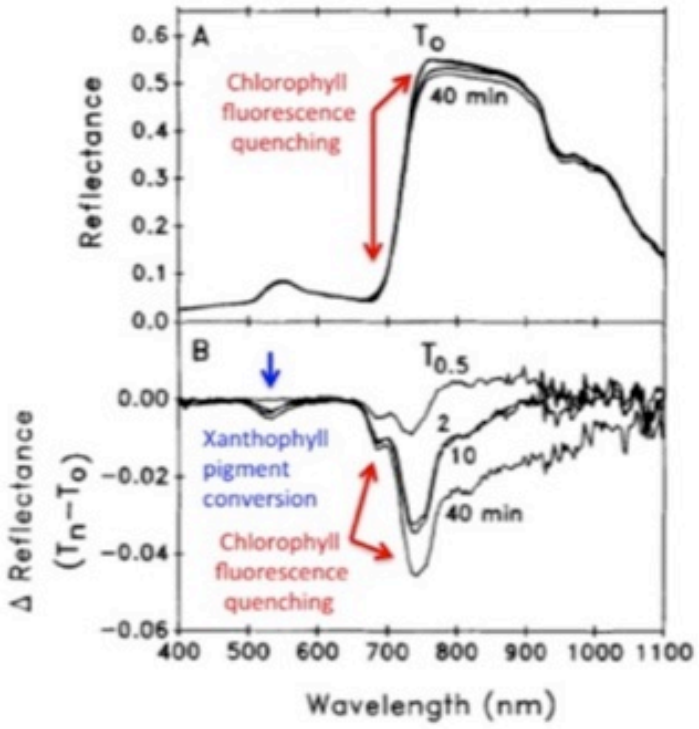
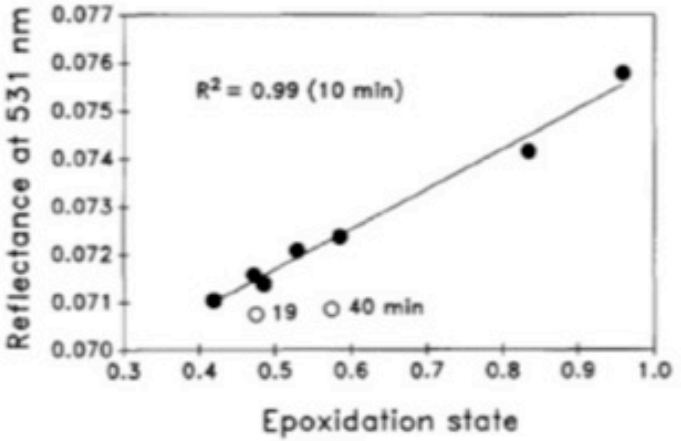
O Que podemos concluir da aula prática?

Parâmetros a analisar

Leaf Optical Changes upon Shade Removal





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Parâmetros a analisar

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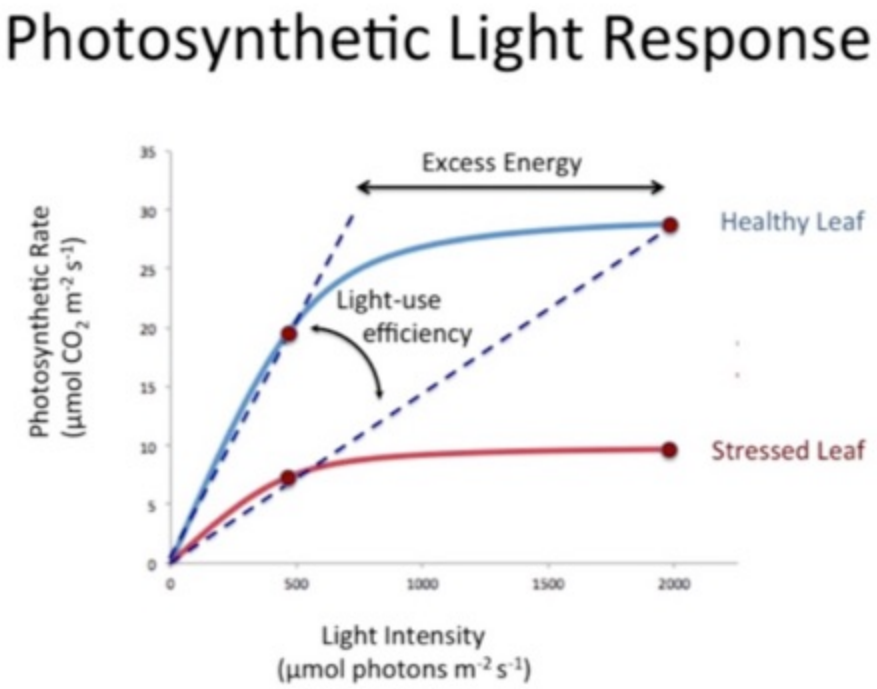

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O Que podemos concluir da aula prática?

Parâmetros a analisar

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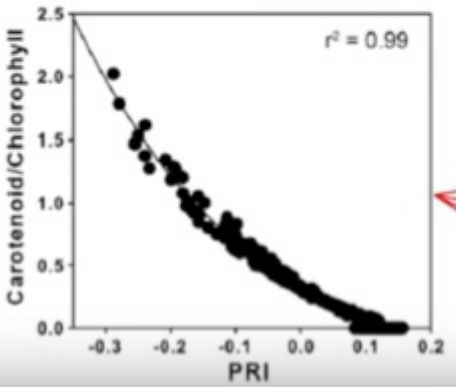
Photochemical Reflectance Index (PRI)

$$PRI = (R_{531\,nm} - R_{570\,nm}) / (R_{531\,nm} + R_{570\,nm})$$

As estimator of photosynthetic activity

PRI Applications: Seasonal Dynamics

- When measured over longer time periods, PRI has been found to vary with several physiological variables related to photosynthetic performance, as well as pigment content (xanthophylls, other carotenoids, chlorophyll).



Pearson correlations

	PRI
PRI	1
NPQ	−0.894
PQ	0.767
Φ_P	0.915
Total Car	−0.597
Chl a + b	0.733
Total VAZ	−0.925
DEPS	−0.856
Car Chl^{-1}	−0.917
VAZ Chl^{-1}	−0.952

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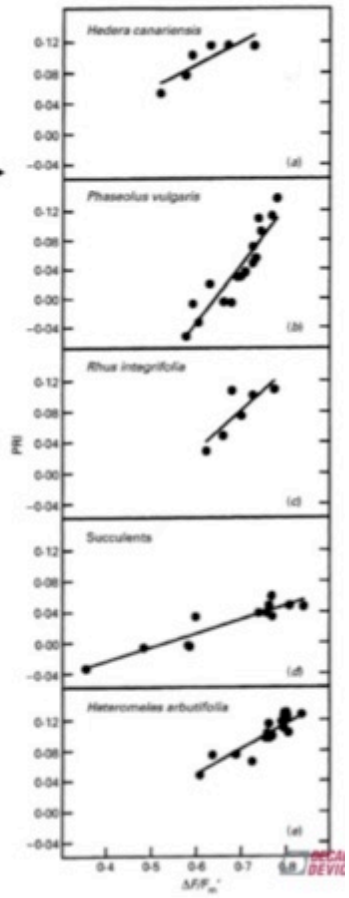
O Que podemos concluir da aula prática?

Parâmetros a analisar

What is PRI (Photochemical Reflectance index)?

PRI Limitations

- Relationship to light use efficiency is site specific, making it necessary to develop correlative relationships for new study areas or species.
- Across long time periods (e.g., growing season), PRI responds to changes in carotenoid/chlorophyll ratios and potentially to structural changes inside leaves of overwintering plants, making direct comparison of measurements from one time period to another difficult.
- During extreme stress (e.g., extreme drought) or senescence, PRI can become decoupled from LUE, typically leading to overestimates of LUE
- PRI does not account for photorespiration.



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O Que podemos concluir da aula prática?

Parâmetros a analisar

Combining NDVI & PRI

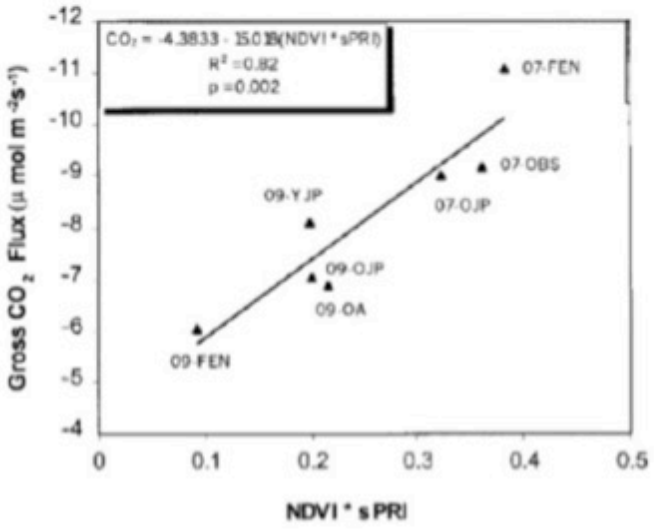
- Monteith Light Use Efficiency Model (Monteith, 1977 *Philos. Trans. R. Soc. London*)

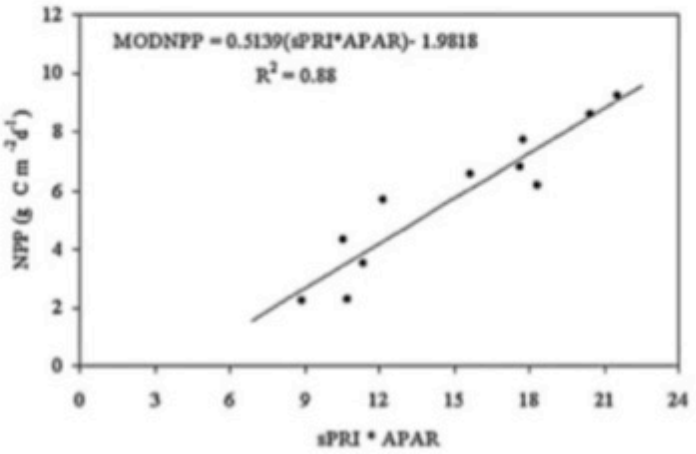
$$GPP = PAR \times fPAR \times \varepsilon$$

GPP – Gross primary productivity
PAR – Photosynthetically active radiation
fPAR – fraction PAR absorbed
ε – Light use efficiency

- Spectral Monteith Light Use Efficiency Model (Rahman *et al.*, 2001, *J. Geophys. Res.*)

$$GPP = PAR \times NDVI \times PRI$$





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O Que podemos concluir da aula prática?

Parâmetros a analisar

Quais os tratamentos com maior
Gross Primary Productivity (GPP?)

Inoculação	Principal Forma de N	Biofertilizante
Preto	Nitrato	Sem
Azul	Nitrato	Com
Branco	Amónio	Sem
Vermelho	Amónio	Com

Era o que esperava?

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O Que podemos concluir da aula prática?

Parâmetros a analisar

Como se relaciona a razão Clorofila total /carotenoides com o PRI?

Inoculação	Principal Forma de N	Biofertilizante
Preto	Nitrato	Sem
Azul	Nitrato	Com
Branco	Amónio	Sem
Vermelho	Amónio	Com

Era o que esperava? Como explica os resultados?

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O Que podemos concluir da aula prática?

Parâmetros a analisar

What is Brix (Solidos totais solúvies)?

The Photochemical Reflectance Index is a measure of the light-use efficiency of foliage and thus is primarily used as an indicator of water stress and for the assessment of carbon-dioxide uptake by plants.

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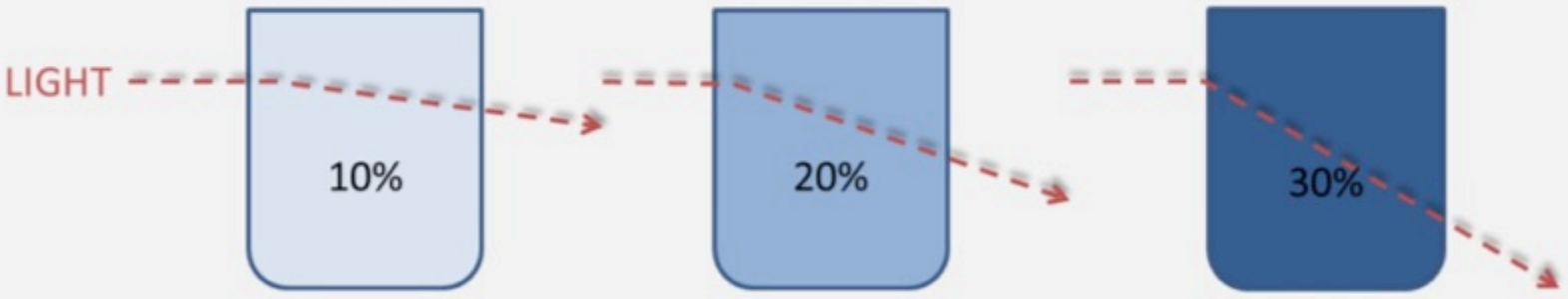
O Que podemos concluir da aula prática?

Parâmetros a analisar

What is PRI (Photochemical Reflectance index)?

Refractive Index

Refractive Index (RI) is the tendency of light to bend as it passes through a liquid. A beaker of pure water will bend light that passes through it. As solids are dissolved in a beaker of water, light will increasingly bend as the concentration increases.



BRIX: History and Application

BRIX is a unit of measurement of refractive index, in the same way Fahrenheit or Celsius is a measurement of temperature. In both circumstances the properties of real world materials were used to devise a continuous scale of measurement. In the case of Fahrenheit, the properties of water were used to come up with a scale based on its thermal behavior. The two ends of the scale were divided by 180°F, yielding 32°F for water's freezing point and 212°F for its boiling point.

In the case of BRIX, sugar (sucrose) solutions were used to develop the scale. Arbitrarily, the refractive index of pure water (RI=1.333) was simply defined as "0" BRIX. The rest of the scale was calibrated to be read directly as percent Sucrose (i.e. BRIX). This was a convenient reference, as it was used to monitor when fruit had ripened and was ready for harvesting. Today, the BRIX scale is commonly used in the food industry for measuring the approximate amount of sugars in fruits, vegetables, juices, wine and soft drinks. It also finds use in the metalworking industry as a convenient way to check water-based lubricant concentration.

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O Que podemos concluir da aula prática?

Parâmetros a analisar

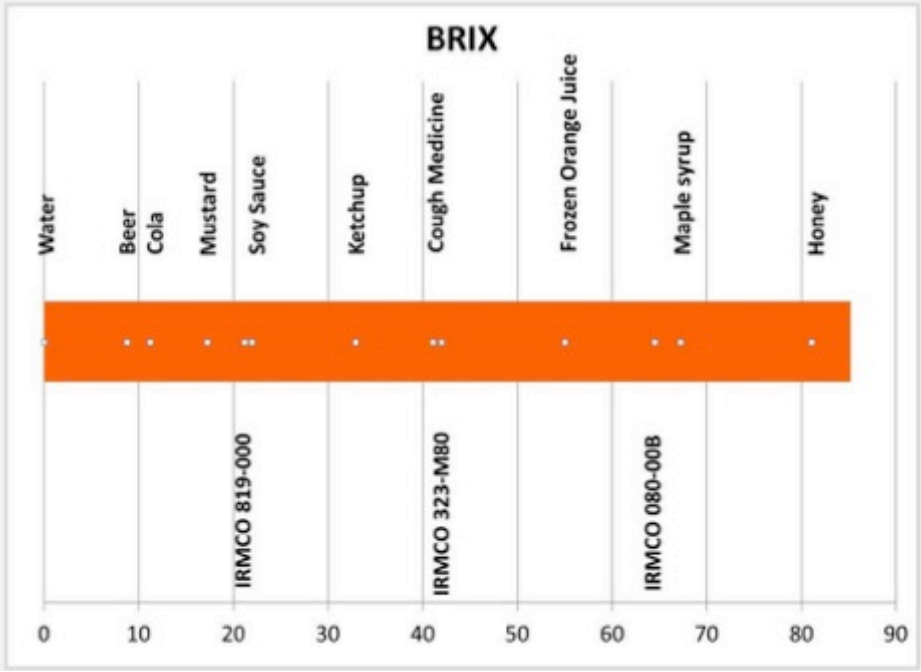
Measurement

Because of the widespread availability of handheld refractometers, measuring the BRIX value of a fluid is easy. However, not all solids bend light to the same degree. The actual concentration of a given fluid must be calibrated to relate to BRIX by the manufacturer. A conversion factor or Brix table should be provided for each product.

The first major deadline was December 1, 2013. All Employers that handle chemicals were required to train their employees about the upcoming changes being made to the 29 CFR 1910.1200 Hazard Communication Standard. MSDSs and labels were not required to change at this time. It is expected to take approximately 18 months after the initial training deadline for chemical manufacturers and distributors to fully reformat their current product labels and MSDSs to the GHS format.

Interpretation

Not all solids are equal! In metalworking, BRIX is quite handy for monitoring dilutions but it is less useful for comparing two different fluids. Depicted to the right are several fluids and their BRIX values. It is important to understand that although BRIX can be related to concentration, it says nothing about the nature of the solids. Put another way, a 64 BRIX could be a highly additized synthetic lubricant concentrate - or, it could be maple syrup. A 32 BRIX could be a 1:1 mixture of that same lubricant - or it could be ketchup. One would be suitable for working metal - and the other would crash your die.



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O Que podemos concluir da aula prática?

Parâmetros a analisar

BRIX º

Como se relaciona o Brix do material com os tratamentos efectuados?

Inoculação	Principal Forma de N	Biofertilizante
Preto	Nitrato	Sem
Azul	Nitrato	Com
Branco	Amónio	Sem
Vermelho	Amónio	Com

Era o que esperava? Como explica os resultados?

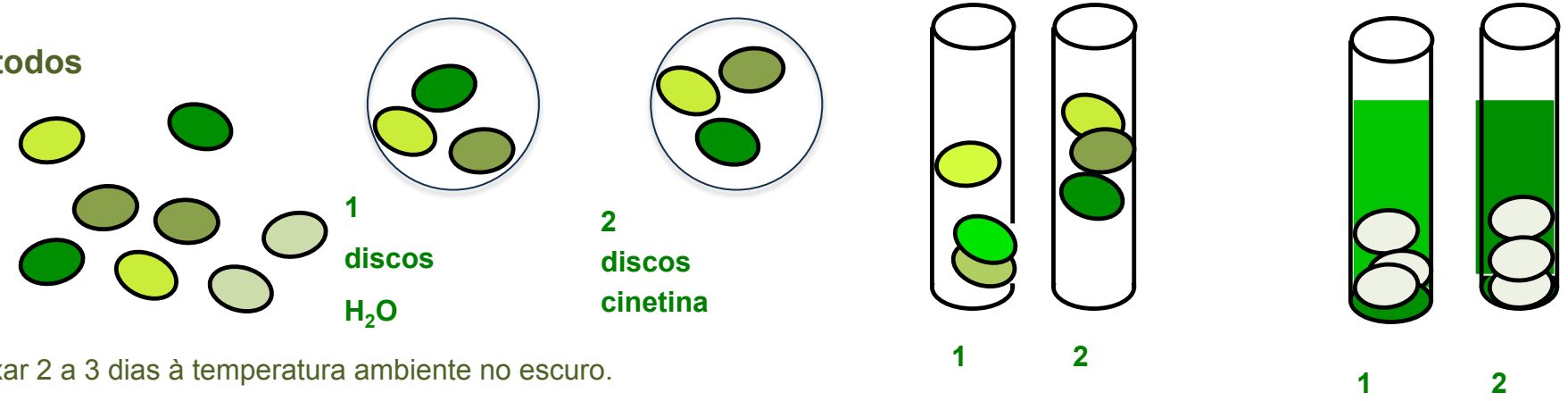
Citocininas – Efeito anti-senescente

Material vegetal

Folhas de tomateiro, *Licopersicum esculentum,* ou de outras plantas:

- com fertilização
- inoculadas
- controlo, sem qualquer inoculo ou fertilização.

Métodos



Deixar 2 a 3 dias à temperatura ambiente no escuro.

Colocar em metanol, para extração dos pigmentos, manter no escuro a 4ºC.

Citocininas – Efeito anti-senescente

Redução da taxa de degradação da clorofila

em folhas destacadas

Doseamento espectrofotométrico dos pigmentos
(clorofilas e carotenoides)
após extração em metanol

Lei Lambert-Beer

$$A = \varepsilon \cdot c \cdot l$$

Doseamento dos pigmentos

$A = \varepsilon . c . l$

A_{665} & A_{652}& A_{470}

(Metanol a 100%)

$C_a = 16.72 \times A_{665} - 9.16 \times A_{652}$ ($\mu g\ mL^{-1}$)

$C_b = 34.09 \times A_{652} - 15.28 \times A_{665}$ ($\mu g\ mL^{-1}$)

$C_{total} = 1.44 \times A_{665} + 24.93 \times A_{652}$ ($\mu g\ mL^{-1}$)

Carotenoides= $(1000 \times A_{470} - 1{,}63\ C_a - 104{,}96\ C_b)/211$ ($\mu g\ mL^{-1}$)

(Lichtenthaler, 1987, *Methods in Enzymology,* 148: 351-372)

a) Determine o conteúdo em clorofila *a*, clorofila *b* e carotenoides existente num mL de solução.

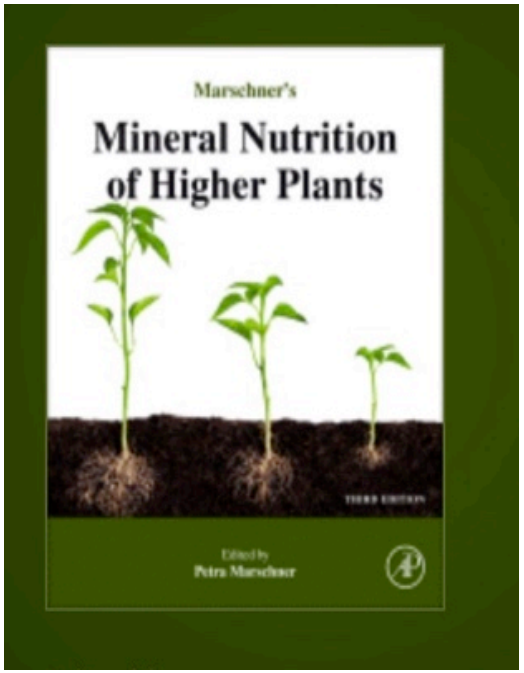
b) Qual o conteúdo desses pigmentos existente por cm^2 de folha?

c) Qual a razão clorofila *a*/clorofila *b*?

d) Qual a razão clorofila total/carotenoides?

e) Comente os resultados obtidos.

Livro importante para o estudo da nutrição mineral em plantas recomendado para as aulas teóricas e práticas.:

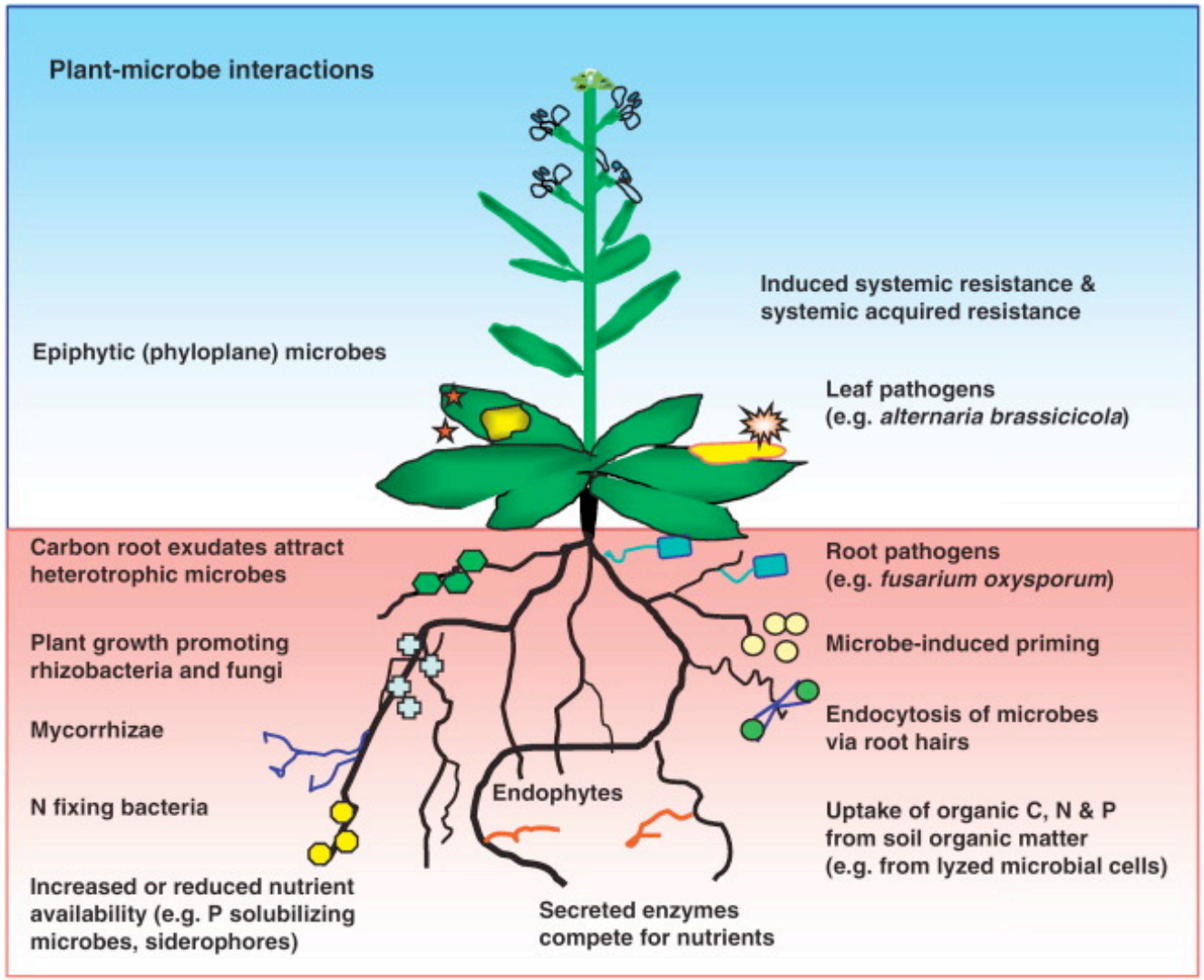


Outras sugestões de leitura, além da bibliografia referida:

- *Physiol Mol Biol Plants*, 2011, Seema et al., 17:247-253
- *J. Exp. Bot.*-2002-Hörtensteiner-927-37.
- http://www.carbonzeroplanet.org/science/chlorophyll.php

FV- nutrição

Nutrição Vegetal



Aula Prática de Fisilogia Vegetal

Nutrição

Poplars given microbes (top) survived better in drought conditions, compared with plants with no added microbes (bottom).

Credit: University of Washington

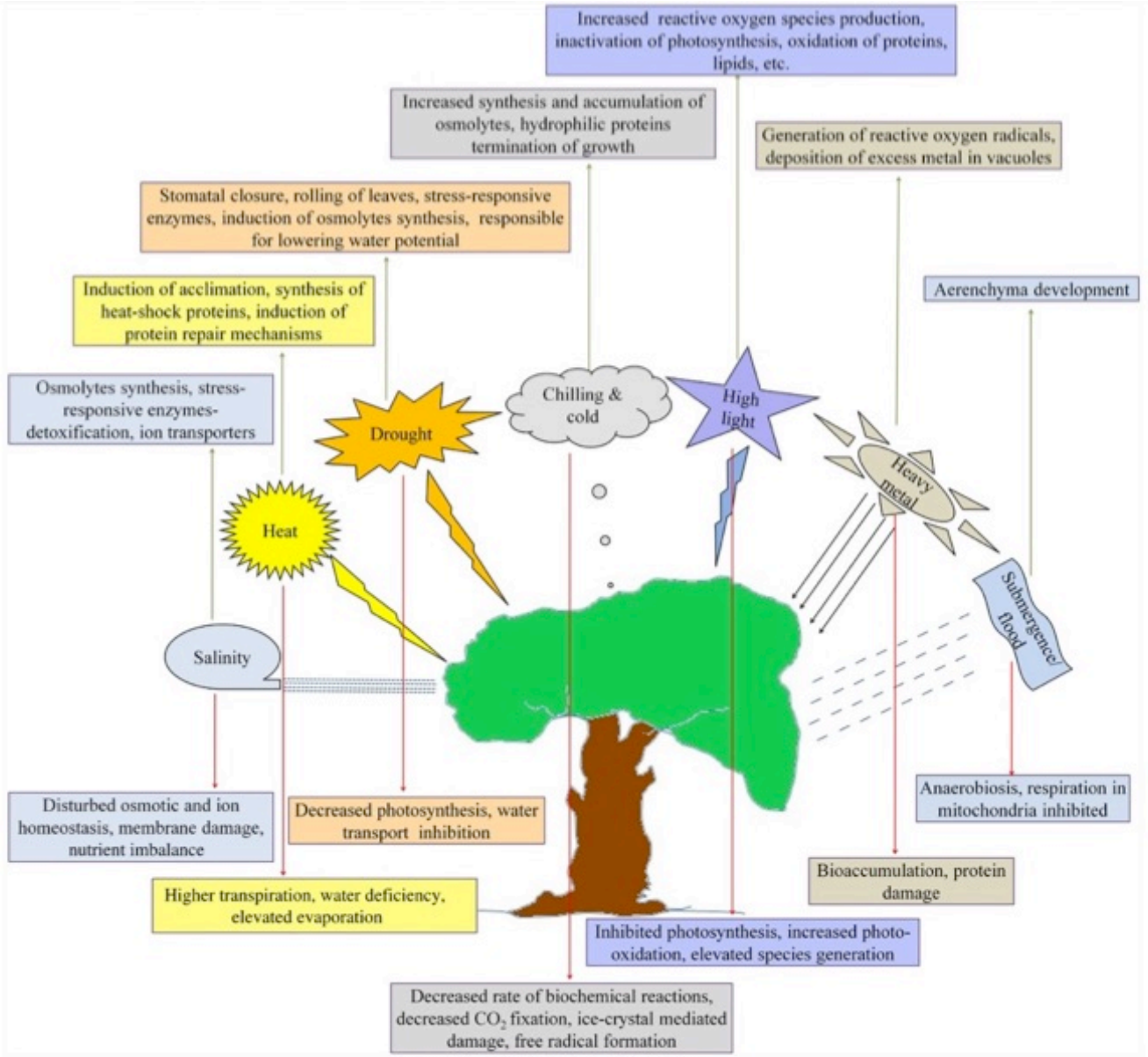


FIGURE 1 | Diverse abiotic stresses and the strategic defense mechanisms adopted by the plants. Though the consequences of heat, drought, salinity and chilling are different, the biochemical responses seem more or less similar. High light intensity and heavy metal toxicity also generate similar impact but submergence/flood situation leads to degenerative responses in plants where aerenchyma are developed to cope with anaerobiosis. It is therefore, clear that adaptive strategies of plants against variety of abiotic stresses are analogous in nature. It may provide an important key for mounting strategic tolerance to combined abiotic stresses in crop plants.

Aula Prática de Fisilogia Vegetal

Nutrição

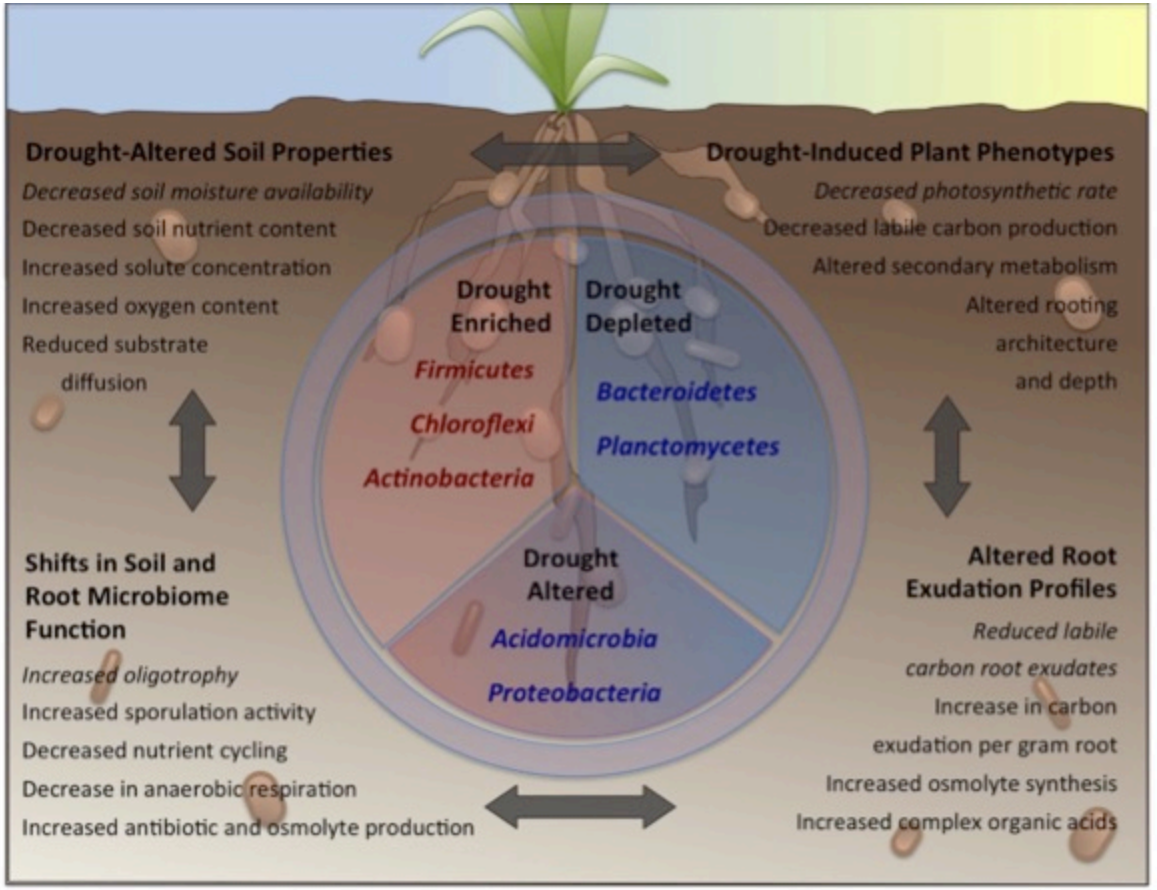


FIGURE 1 | The effects of drought on soils, plants and their associated bacterial communities. Drought induces shifts in soil physicochemistry (upper left), plant phenotype (upper right), root exudation (lower right) and soil and rhizosphere microbiome function (lower left). These shifts are capable of influencing one other; for instance decreases in soil moisture availability (upper left) leads to a decrease in the rate of plant photosynthesis (upper right), which in turn leads to a reduction in the rate of labile carbon exudation to the rhizosphere (lower right) and a greater prevalence in bacteria with oligotrophic life-strategies (lower left), who are less reliant on such simple carbon sources. These shifts lead to a selection for specific phyla (center panel) within the soil, rhizosphere and root microbiome, including enrichment for many Gram-positive, oligotrophic (middle left) phyla, and concurrent depletion of many Gram-negative, copiotrophic (middle right) phyla. Members of other phyla exhibit a more balanced mixture of enrichment and depletion (middle bottom).

Naylor et al 2018; doi: 10.3389/fpls.2017.02223

Aula Prática de Fisilogia Vegetal
Nutrição

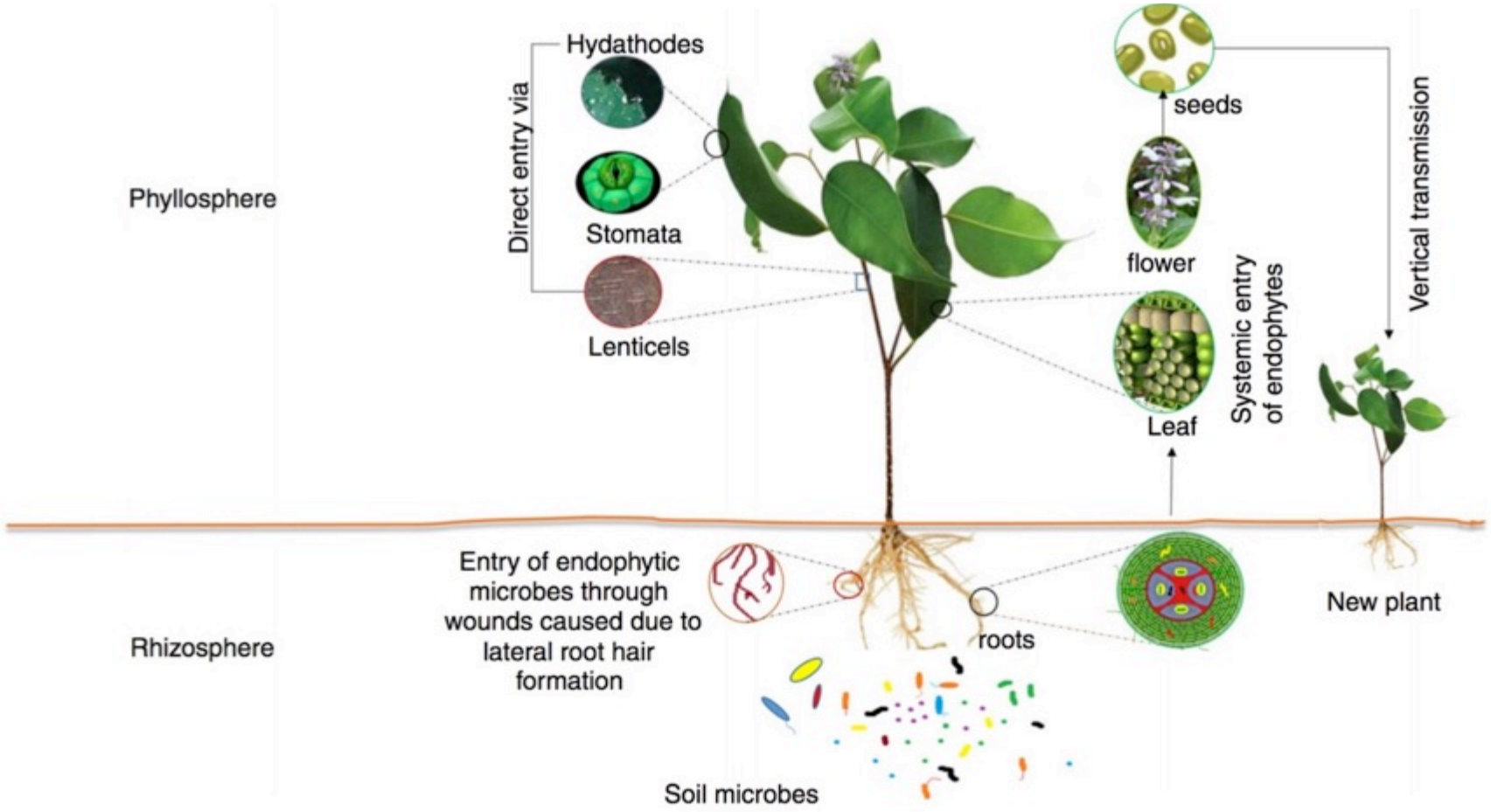


Figure 1 Entry, establishment and transmission of endophytes in plant. [Colour figure can be viewed at wileyonlinelibrary.com]

Lata et al, 2018; doi:10.1111/lam.12855

FV- nutrição

Nutrição Vegetal

Plant-microbe interactions or how can beneficial microorganisms compatible with crop plants be identified and further developed to support sustainable crop production by improving stress management & avoiding agrochemicals.

Pathogen

↓

HR-resistant plant

(hypersensitive reaction)

↓

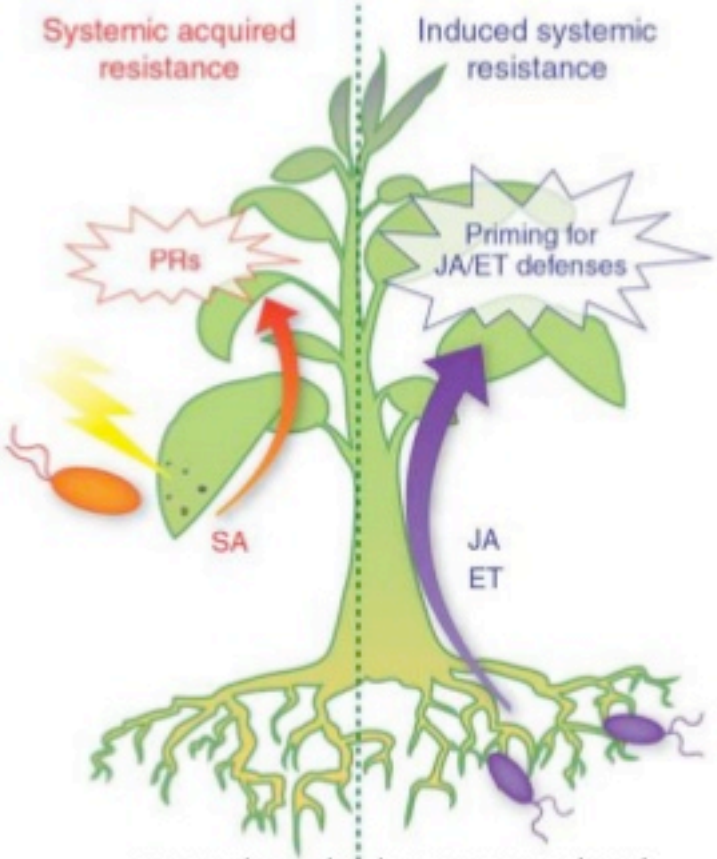
SA

(salicylic acid)

↓

SAR – protection

(PR-proteins)



Nature Chemical Biology 5, 308 - 316 (2009)

Beneficial microbe

(PGPB/PGPR)

↓

Priming (latent defense)

Growth promotion

stress ↓ abiotic-biotic

JA (jasmonic acid)

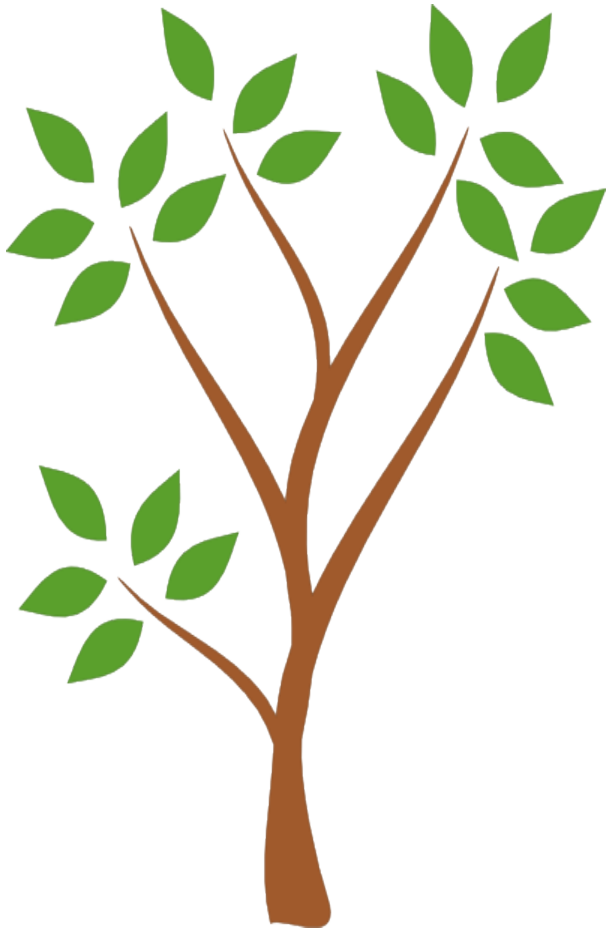
↓

ISR - protection

(??????????)

Plant hormone

(a chemical produced in one part of the body that has a target elsewhere in the body).

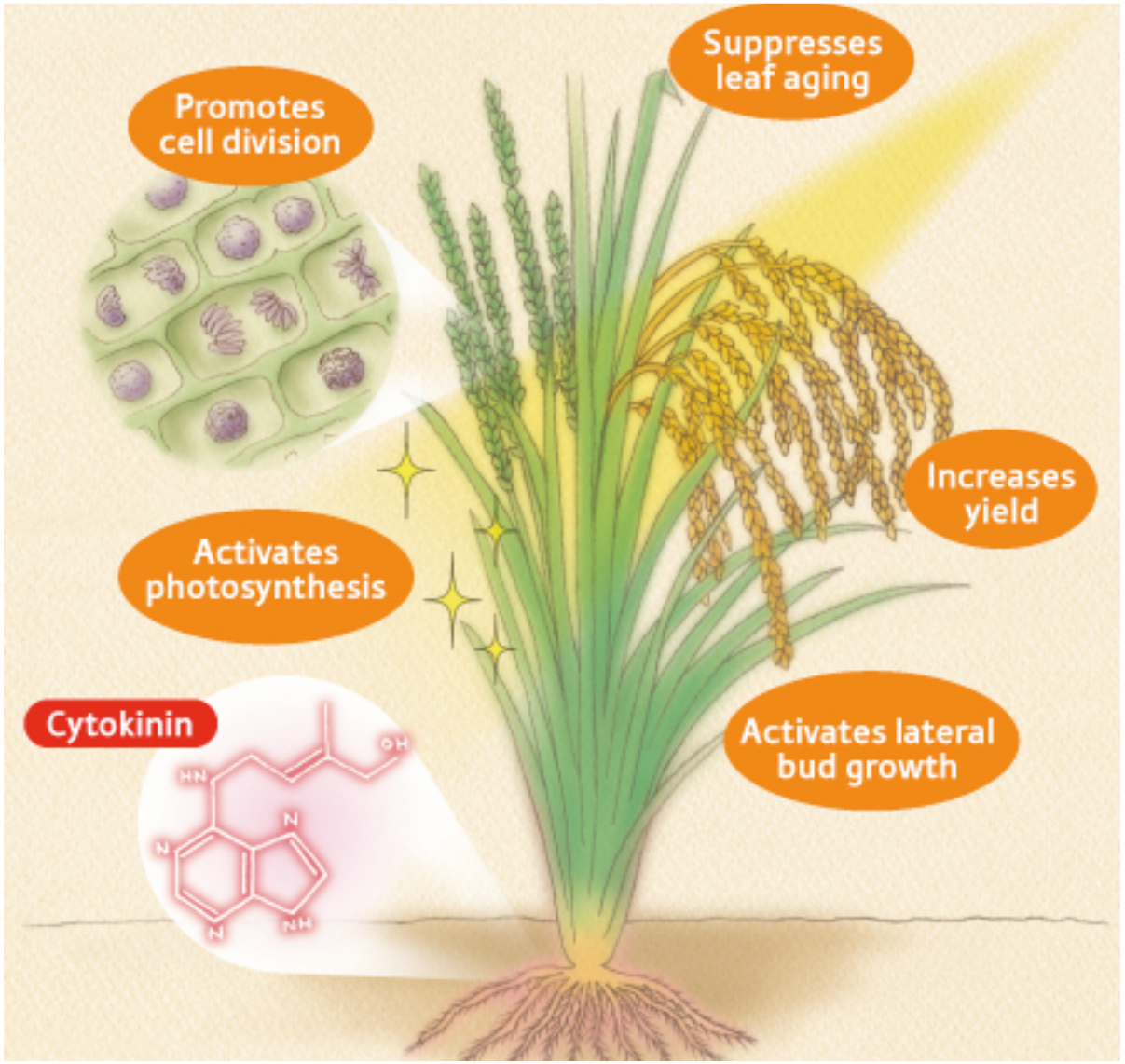


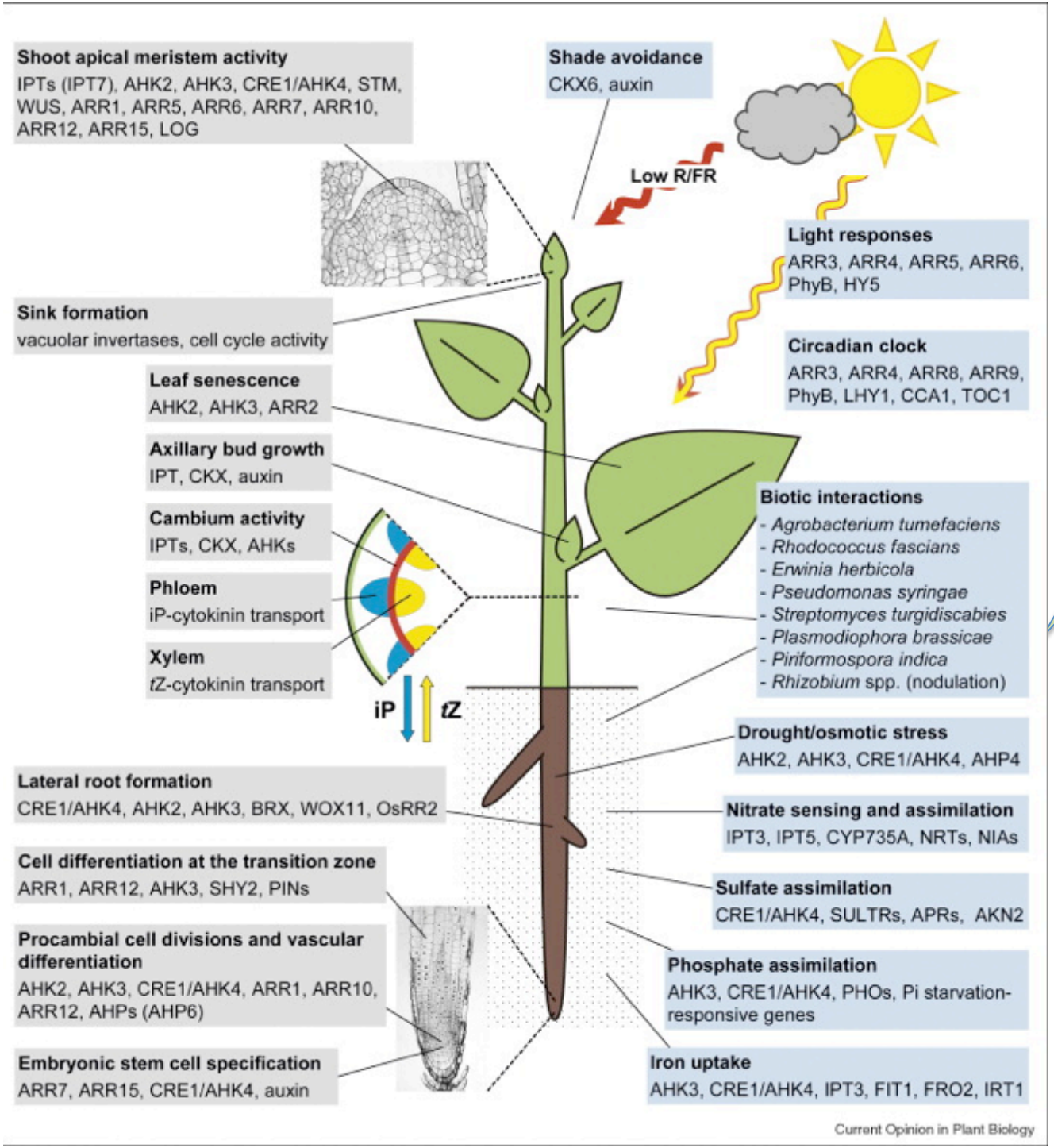
- Auxins - (IAA). Produced in the stem tip that promotes cell elongation. Responsible for photopropism. Maintain apical dominance. begin to grow.
- Gibberellins promote stem elongation.
-
- Cytokinins promote cell division. (Produced in growing areas, such as meristems at tip of the shoot. Zeatin)
- Abscisic acid promotes seed dormancy. Involved in opening and closing of stomata as leaves wilt.
- Ethylene is a gas produced by ripe fruits.

FV- nutrição

Aula Prática de Fisilogia Vegetal

Nutrição



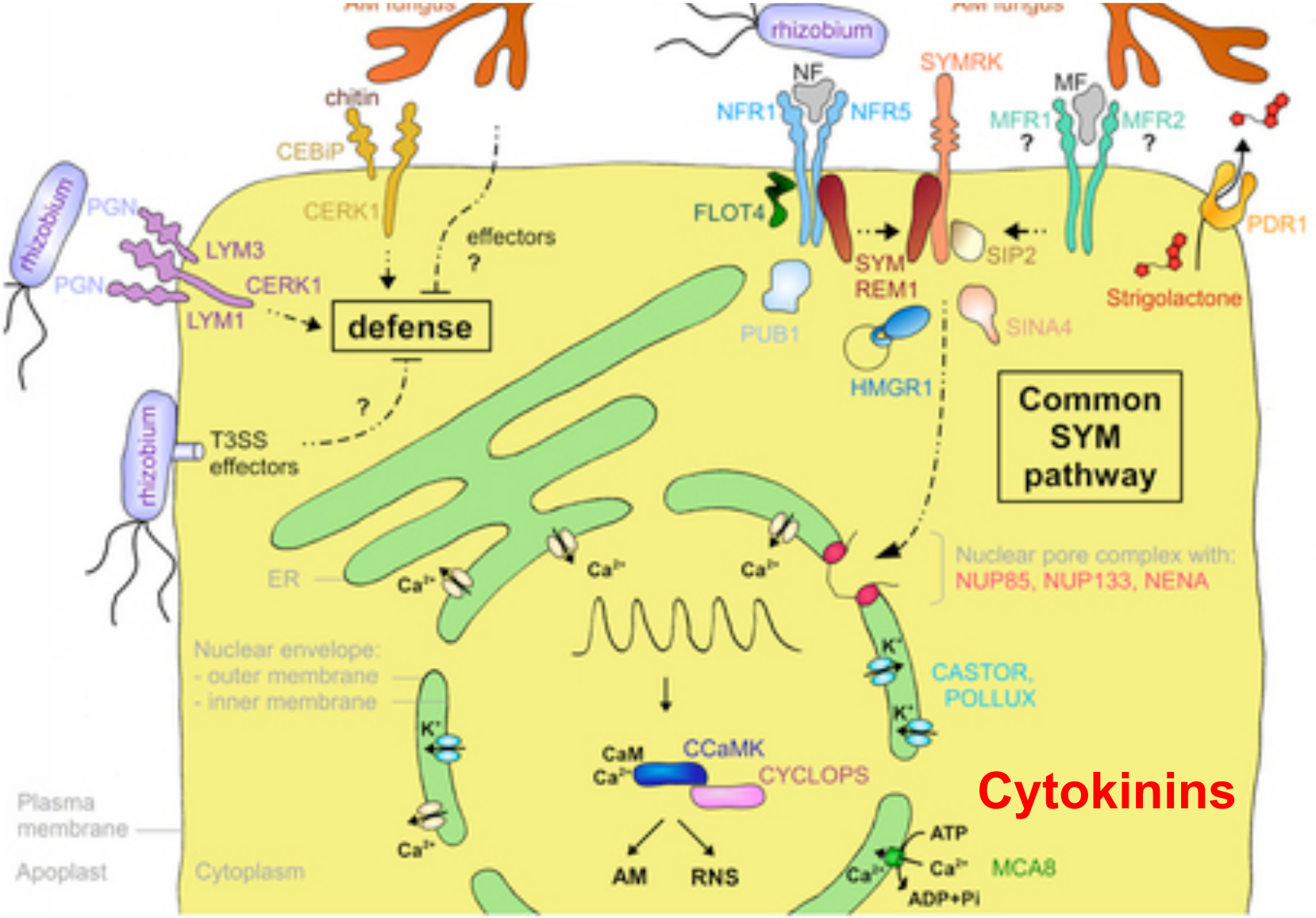

Shoot apical meristem activity
IPTs (IPT7), AHK2, AHK3, CRE1/AHK4, STM, WUS, ARR1, ARR5, ARR6, ARR7, ARR10, ARR12, ARR15, LOG
Shade avoidance
CKX6, auxin
Low R/FR
Light responses
ARR3, ARR4, ARR5, ARR6, PhyB, HY5
Circadian clock
ARR3, ARR4, ARR8, ARR9, PhyB, LHY1, CCA1, TOC1
Sink formation
vacuolar invertases, cell cycle activity
Leaf senescence
AHK2, AHK3, ARR2
Axillary bud growth
IPT, CKX, auxin
Cambium activity
IPTs, CKX, AHKs
Phloem
iP-cytokinin transport
Xylem
tZ-cytokinin transport
iP
tZ
Biotic interactions
- Agrobacterium tumefaciens
- Rhodococcus fascians
- Erwinia herbicola
- Pseudomonas syringae
- Streptomyces turgidiscabies
- Plasmodiophora brassicae
- Piriformospora indica
- Rhizobium spp. (nodulation)
Drought/osmotic stress
AHK2, AHK3, CRE1/AHK4, AHP4
Nitrate sensing and assimilation
IPT3, IPT5, CYP735A, NRTs, NIAs
Sulfate assimilation
CRE1/AHK4, SULTRs, APRs, AKN2
Phosphate assimilation
AHK3, CRE1/AHK4, PHOs, Pi starvation-responsive genes
Iron uptake
AHK3, CRE1/AHK4, IPT3, FIT1, FRO2, IRT1
Lateral root formation
CRE1/AHK4, AHK2, AHK3, BRX, WOX11, OsRR2
Cell differentiation at the transition zone
ARR1, ARR12, AHK3, SHY2, PINs
Procambial cell divisions and vascular differentiation
AHK2, AHK3, CRE1/AHK4, ARR1, ARR10, ARR12, AHPs (AHP6)
Embryonic stem cell specification
ARR7, ARR15, CRE1/AHK4, auxin
Current Opinion in Plant Biology

Cytokinins

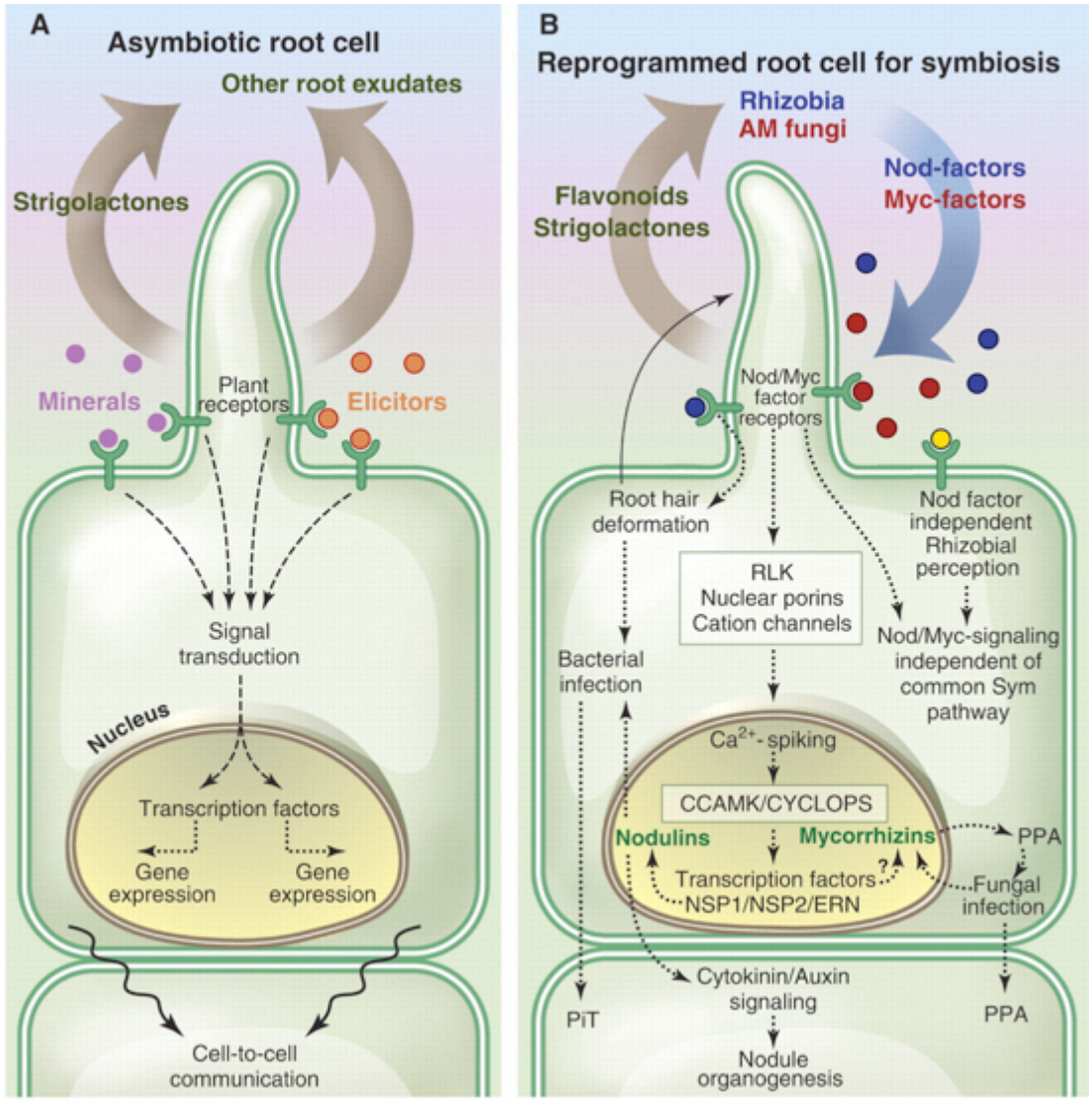
FV- nutrição

Aula Prática de Fisilogia Vegetal

Nutrição



Cytokinins


A
Asymbiotic root cell
Other root exudates
Strigolactones
Minerals
Plant receptors
Elicitors
Signal transduction
Nucleus
Transcription factors
Gene expression
Gene expression
Cell-to-cell communication
B
Reprogrammed root cell for symbiosis
Rhizobia
AM fungi
Nod-factors
Myc-factors
Flavonoids
Strigolactones
Nod/Myc factor receptors
Root hair deformation
Nod factor independent Rhizobial perception
RLK
Nuclear porins
Cation channels
Nod/Myc-signaling independent of common Sym pathway
Bacterial infection
Ca2+-spiking
CCAMK/CYCLOPS
Nodulins
Mycorrhizins
PPA
Transcription factors ?
NSP1/NSP2/ERN
Fungal infection
Cytokinin/Auxin signaling
PiT
PPA
Nodule organogenesis

Tarefas a realizar na aula:

2 - Preparação das amostras para determinação do efeito das citocianinas

Cada grupo prepara 2 caixas de Petri por tratamento com papel de filtro e adiciona 2 ml de solução de cinetina (10^{-2} g/L), ou 2 ml de água.

Cada caixa deve ser marcada (no bordo) com a seguinte informação:

Experiência

Tratamento da cultura

Água ou cinetina

Data

Operador/grupo/turma

Cada grupo corta de cada tratamento dois grupos de 3 discos com 4 mm de diâmetro

Um dos grupos é colocado na caixa com água, o outro na caixas com cinetina. Todos os discos são colocados com a **face abaxial para cima**.

No final as caixas devem ser colocadas num tabuleiro no escuro (coberto com papel de alumínio)

Passados 3-4 dias os discos devem ser transferidos para tubos de ensaio com 3 mL de metanol (fazer na câmara de fumos).

Os resultados serão avaliados na aula prática seguinte

FV- nutrição

O Que podemos concluir da aula prática?

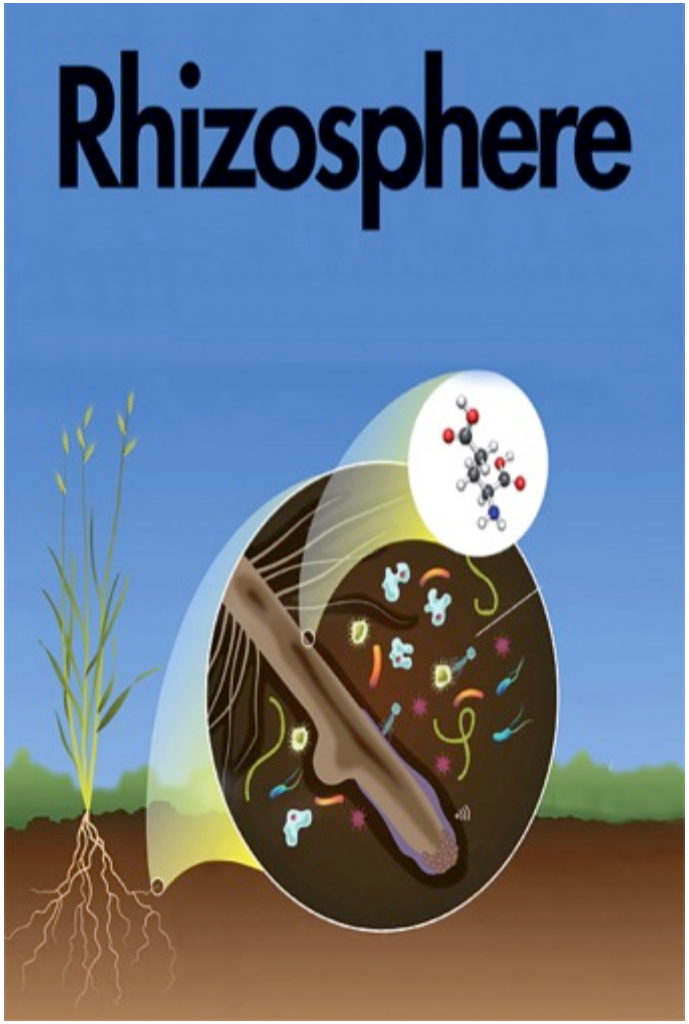
Parâmetros a analisar

PIGMENTOS E BALANÇO HORMOANL.
Que relação com os microrganismos?

Inoculação	Principal Forma de N	Biofertilizante
Preto	Nitrato	Sem
Azul	Nitrato	Com
Branco	Amónio	Sem
Vermelho	Amónio	Com

Era o que esperava? Como explica os resultados?

R

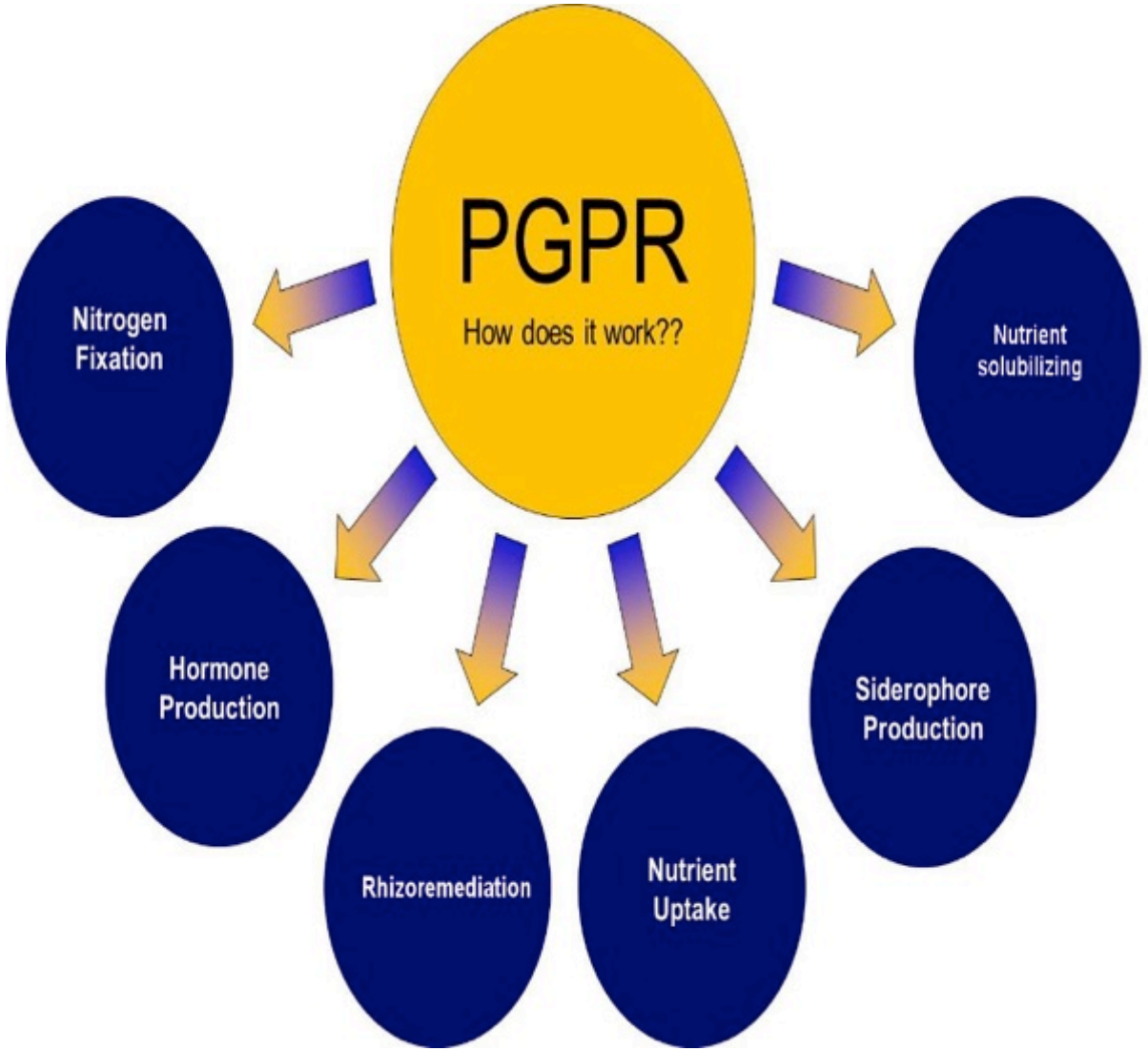


They live in the rhizosphere and can even colonize the plants!!

Increases crop productivity

Decreases content of harmful elements

Increases content of beneficial nutrients

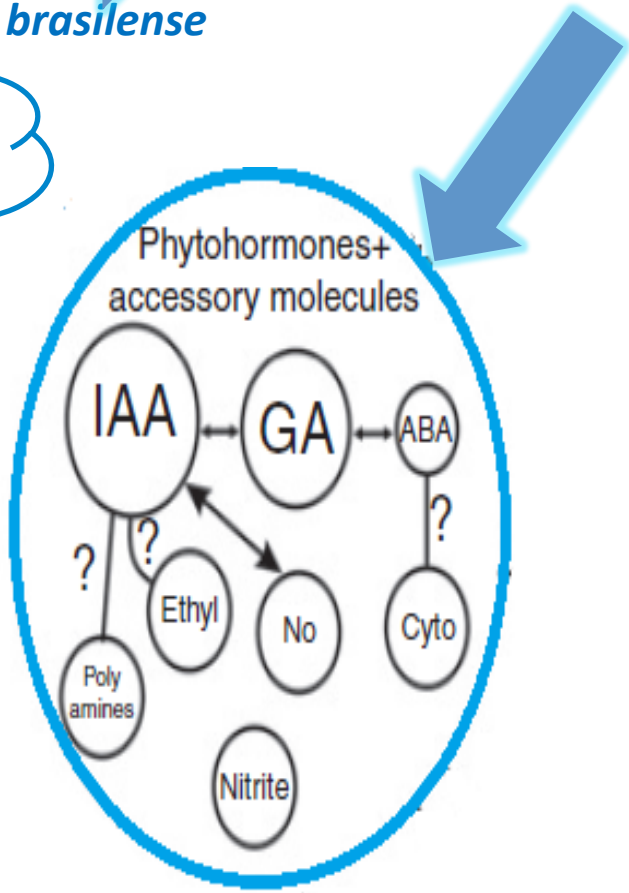

PGPR
How does it work??
Nitrogen Fixation
Nutrient solubilizing
Hormone Production
Siderophore Production
Rhizoremediation
Nutrient Uptake

Promotes plant growth

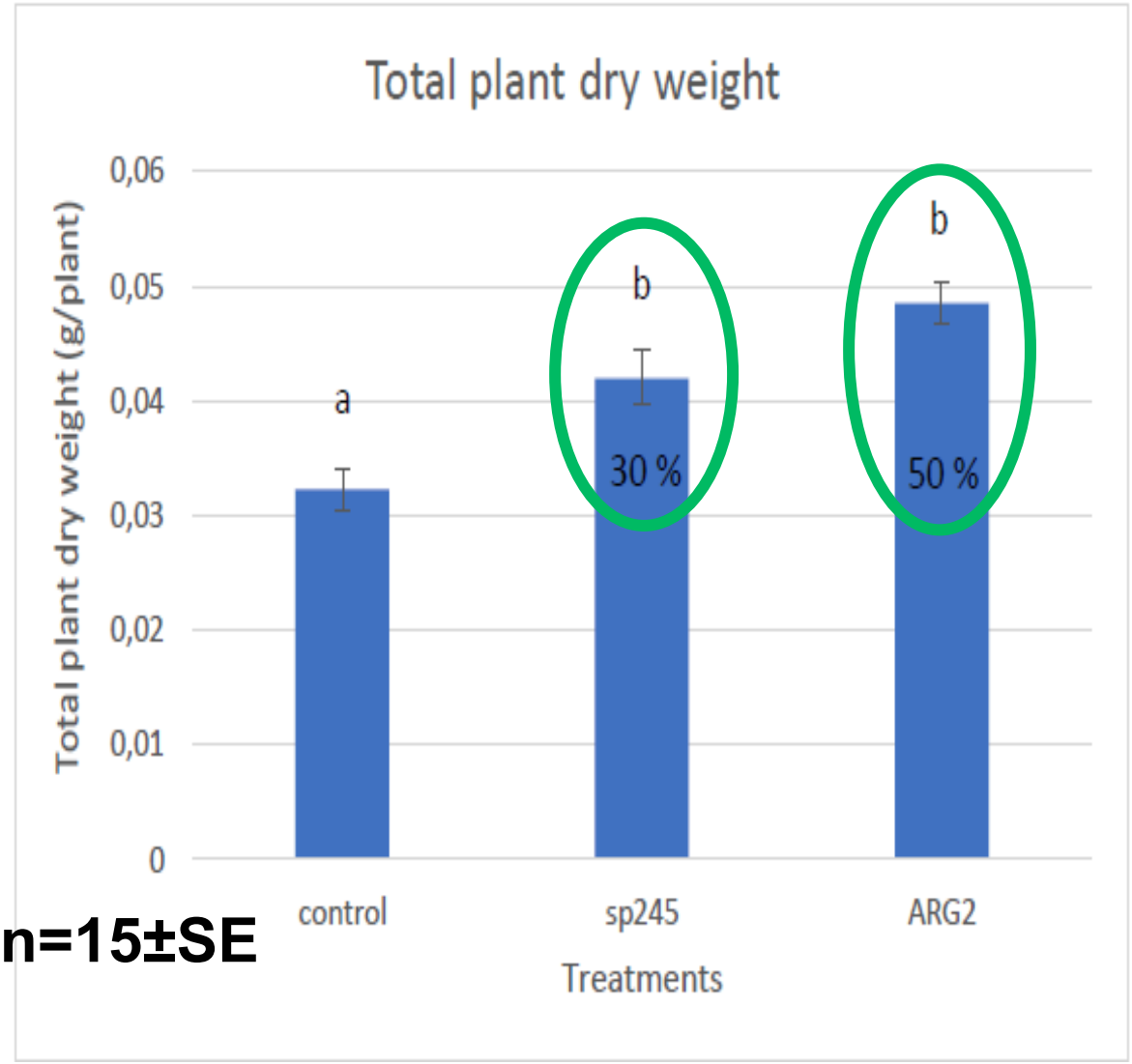
through

Production of phytohormones

Azospirillum brasilense

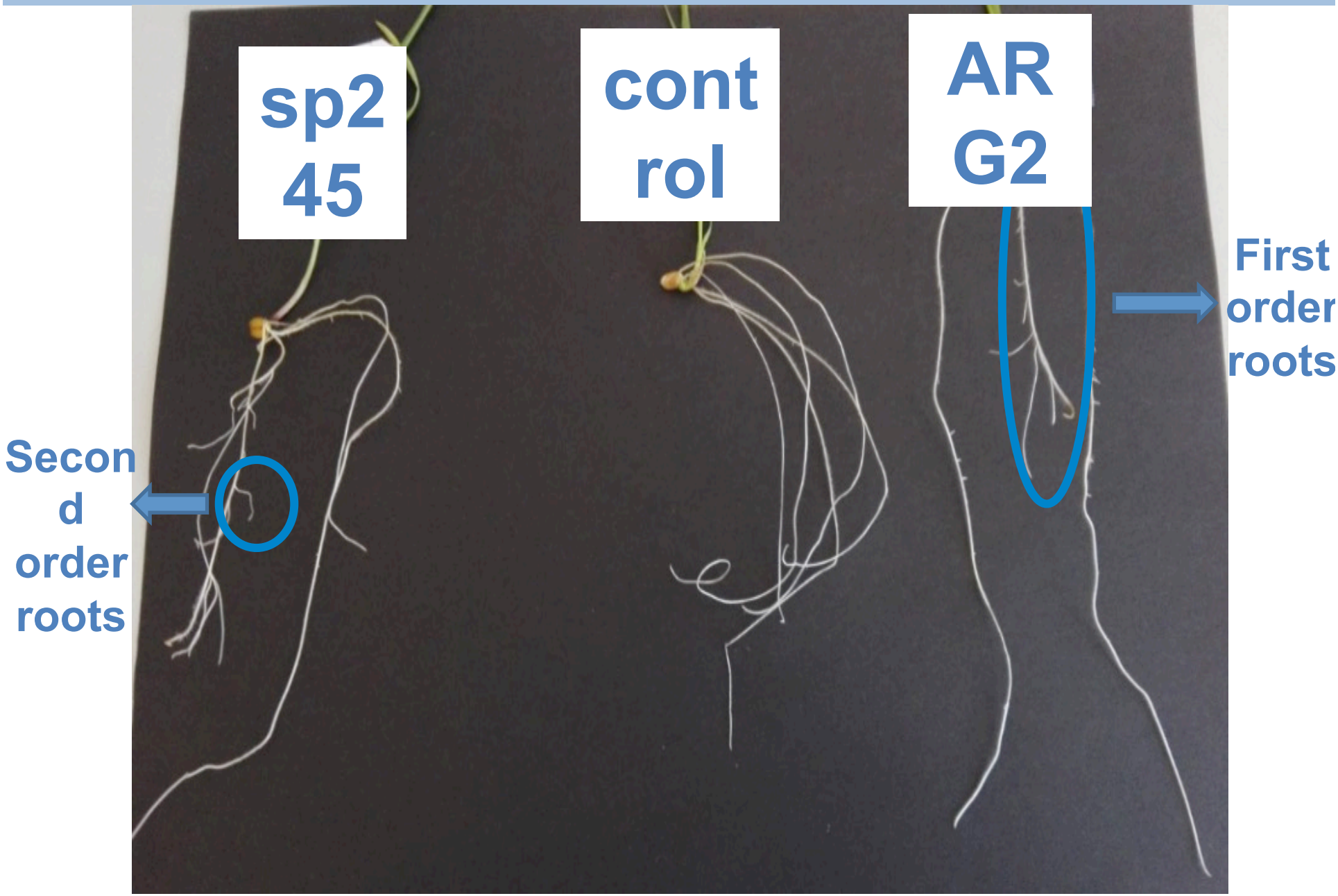


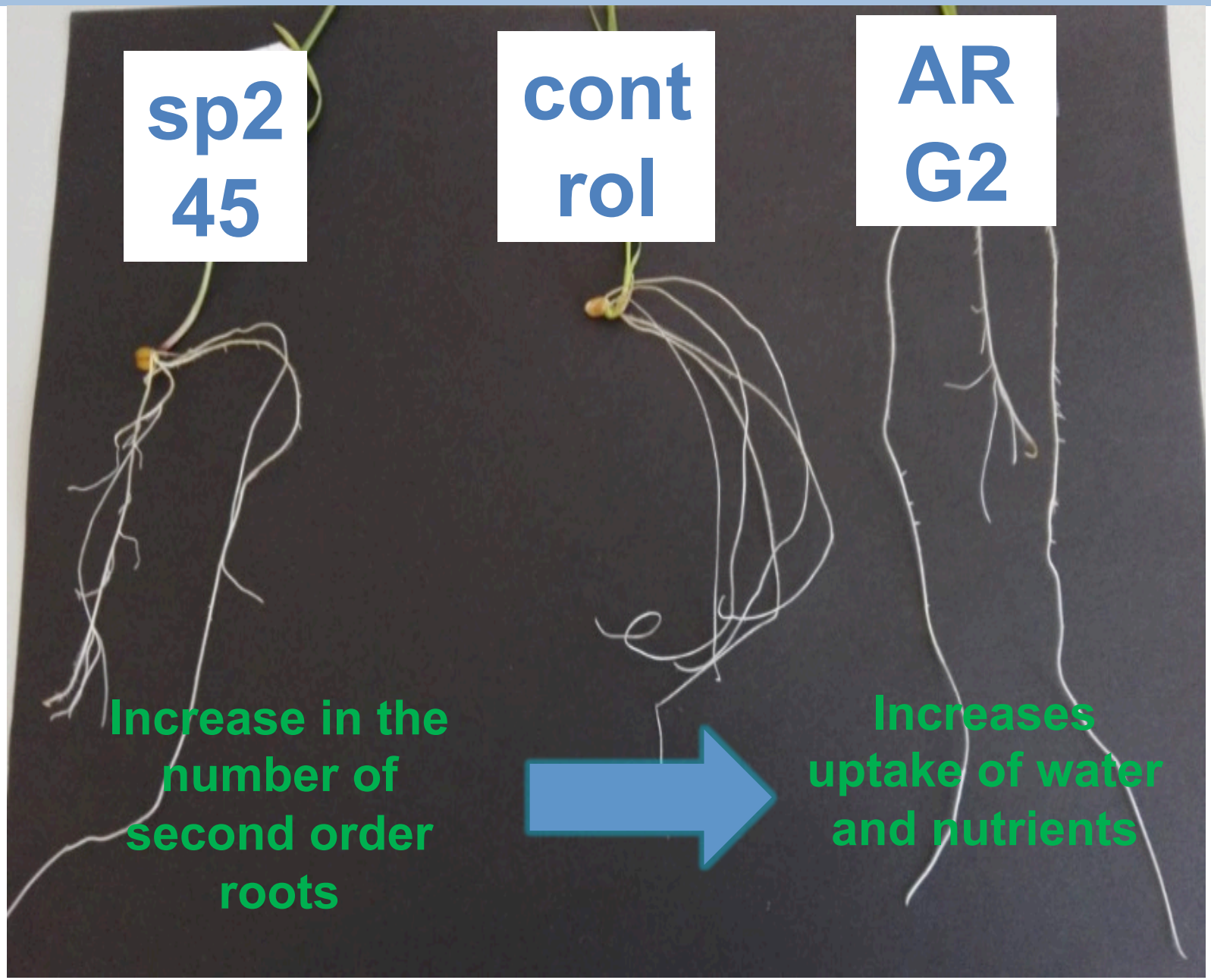

WORK IN
PROGRESS



n=15±SE

40 % increase in wheat biomass production


sp2
45
cont
rol
AR
G2
First
order
roots
Secon
d
order
roots


sp2
45
cont
rol
AR
G2
Increase in the number of second order roots
Increases uptake of water and nutrients

Increase crop productivity

Reduction in the amount of synthetic fertilizers

Reduction of the Ecological Footprint

FV- nutrição

O Que podemos concluir da aula prática?

Parâmetros a analisar