

COURSE “PLANT STRESS PHYSIOLOGY”

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15:15 - 16:00

Common methods to assess plant stress

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- Brief overview of some laboratory methods for assessing plant stress (examples from Department of Molecular Stress Physiology)
- Hands-on practical examples of cheap, fast and easy methods
 - Electrolyte leakage assay (protocol walk-through and video)
 - Root length measurement (ImageJ)
 - Surface area measurement (ImageJ)

Majority of most common methods in Plant bio-sciences: morphology, physiology, Ecology, molecular biology, biochemistry, etc.
(knowledge of particular stress-related changes is crucial)

➤ Phenotypic monitoring:

Growth rate, physical stature & habitus, mass and size of organs (Index of depression), Damages/lesions, growth phase progression, coloration (chlorosis, anthocyanin accumulation),
Specific size, shape and color of fruits and leaves (automated analyzers)

➤ Presence and activity of stress-related genes:

DNA-level (PCR, sequencing), transcription (qRT-PCR, RNA-seq), protein (Western blot)

➤ Proteins, activity and degradation:

Induction of specific sub-units, activation cleavage, digestion break-down, binding, etc.

➤ Photosynthesis evaluation:

Chlorophyll fluorescence - Pulse Amplitude Modulation (PAM) fluorometry,
Gas analyses (GA), Infra-red GA (IRGA)

Continues...

➤ Biochemical methods:

(many colorimetric, mainly spectrophotometric)

Lipid peroxidation - Malondialdehyde (MDA), Antioxidants (ferric reducing antioxidant power (FRAP) assay, Glutathione, Ascorbic acid)

Enzymatic activity (native gels, spectrophotometry) – CAT, SOD, PerOX, Glutathione Reductase, Ascorbate PerOX, etc.

➤ Metabolomics:

(stress-related metabolites, metabolomic profiles)

Mass spectrometry – Gas & Liquid chromatography GC- & LC-MS

➤ Histochemical staining for tissues and organs:

Diaminobenzidine (DAB) - H_2O_2 , Nitro Blue Tetrazolium (NBT) - Superoxide ($\text{O}_2^{\cdot-}$),

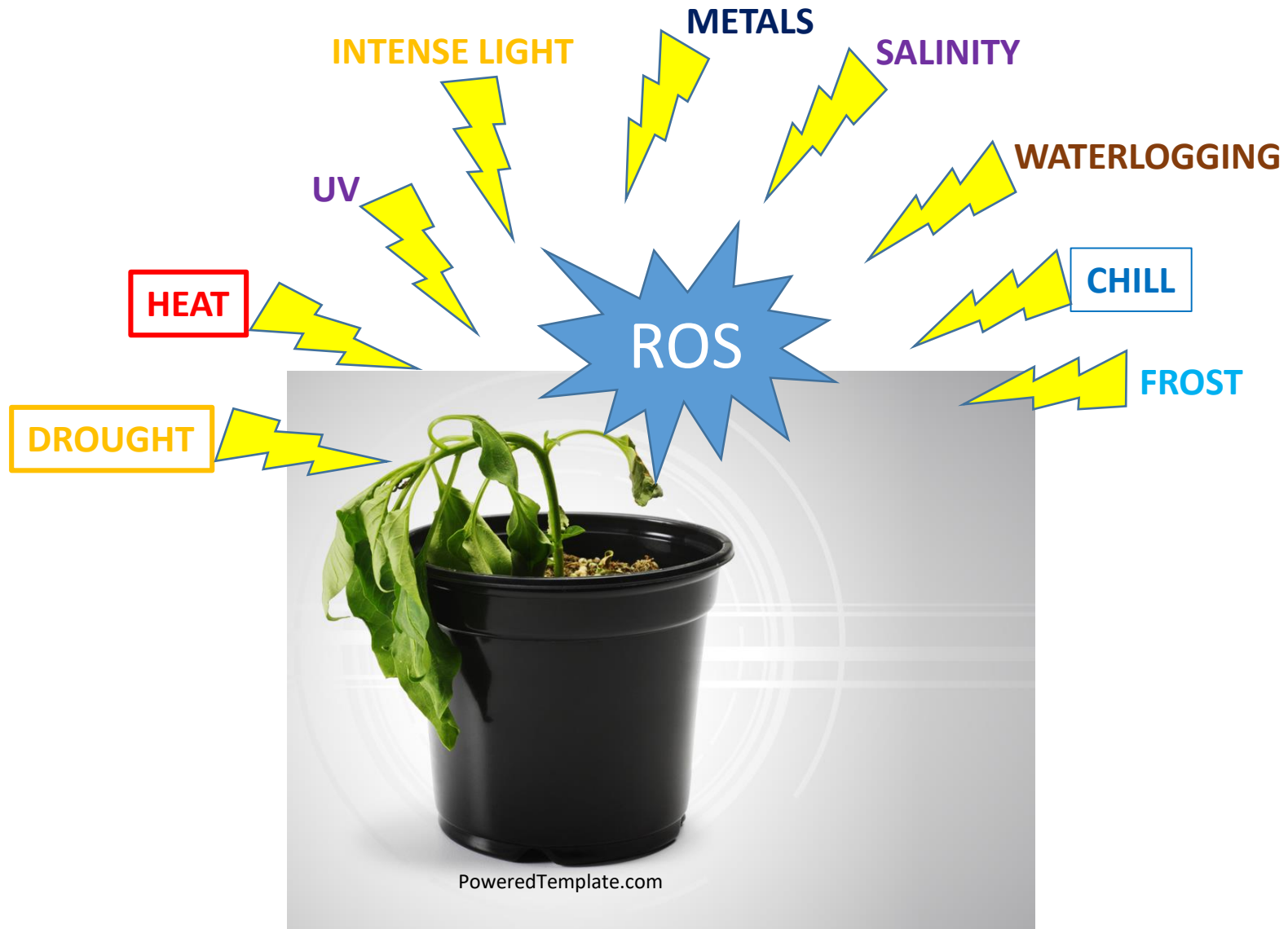
Trypan blue – dead tissue, etc.

➤ Other:

Extraction of pigments: Chlorophyll A+B, Anthocyanin, Carotenoids – spectrophotometric

Electrolyte leakage assay

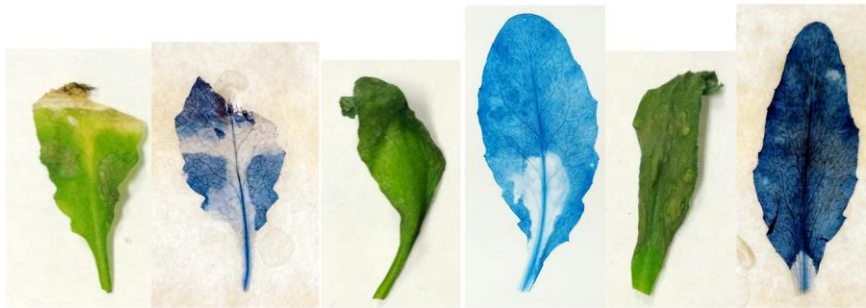
Too intense environmental factors act as abiotic stresses



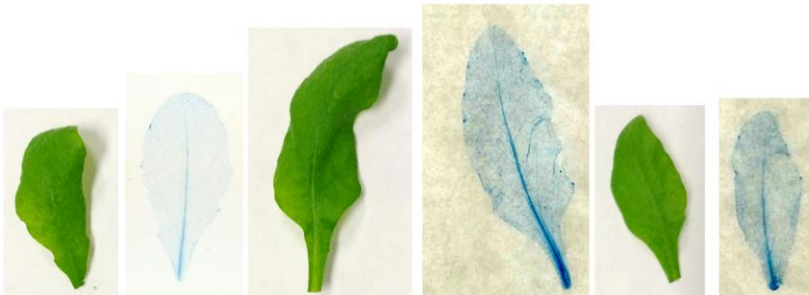
Strategy for detection: assess general stress-markers

Trypan Blue Test Staining

Treated (PQ 15 μ M + Tween 0.1%)

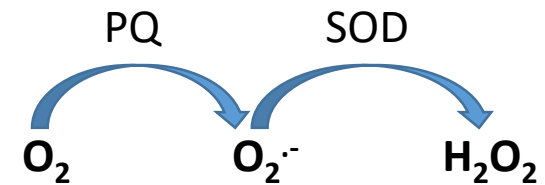


Control (Tween 0.1%)



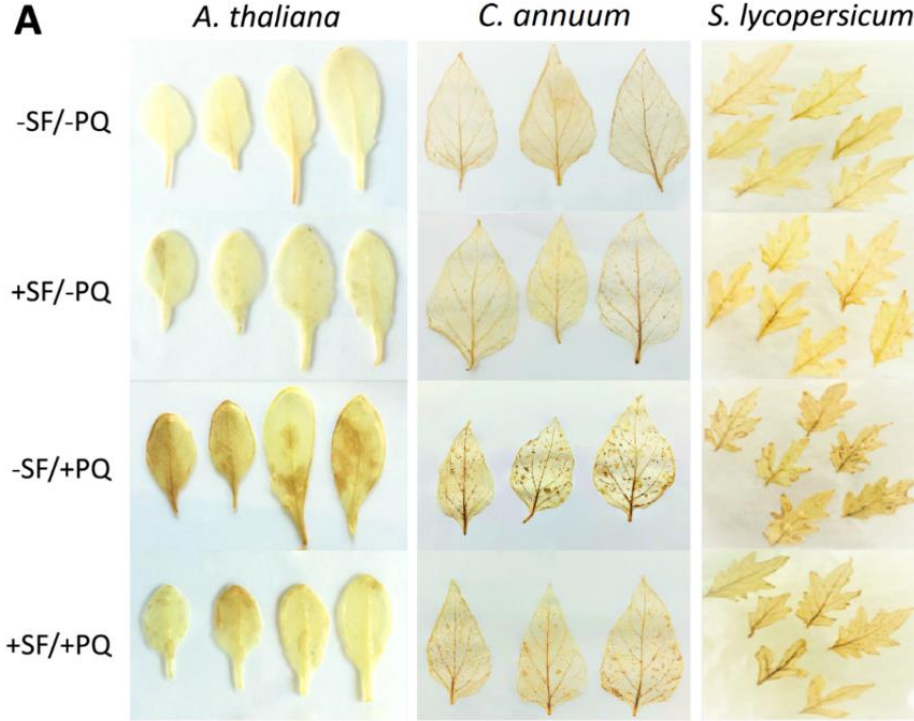
PQ

Paraquat generates free radicals:
Superoxide ($O_2^{\cdot-}$)

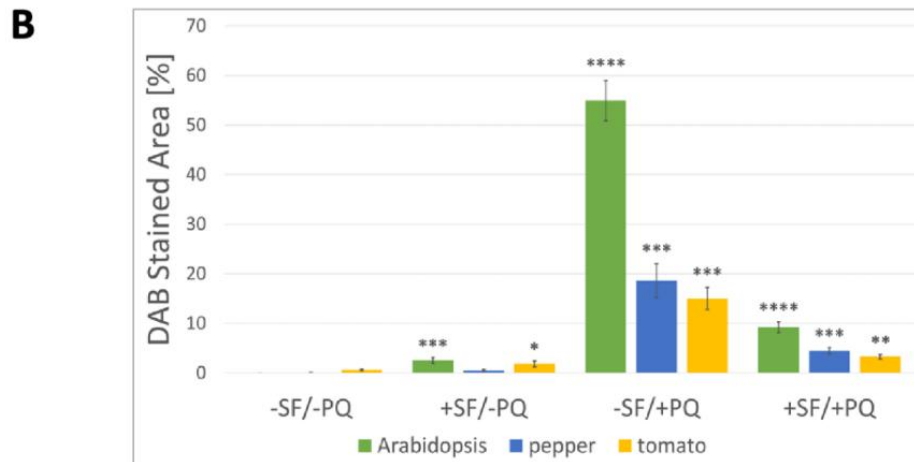


Trypan blue is cell membrane impermeable – stains dead tissues only.

Accumulation of H_2O_2 in PQ treated plants



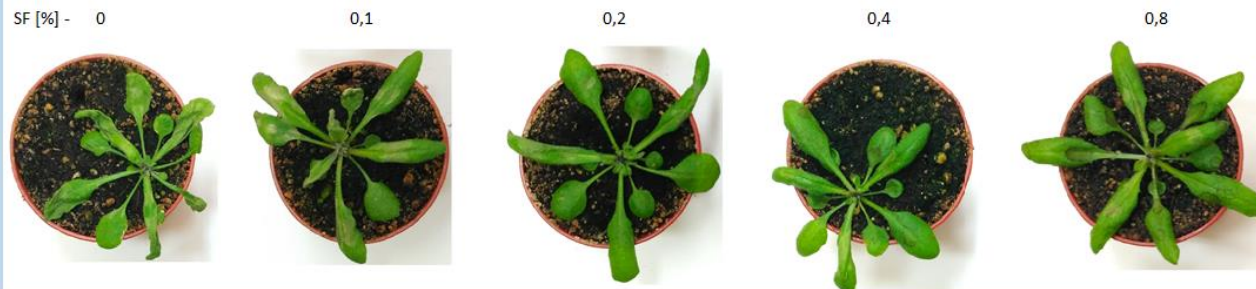
(A) DAB-stained leaves of Arabidopsis, pepper and tomato exposed to different treatments



(B) The percentage of DAB stained area of the four variants of Arabidopsis, tomato, and pepper was assessed with the ImageJ software (n=6).

Oxidative stress-protective potential of SF against damaging effect of PQ on *A. thaliana* rosette leaves assessed with four different methods

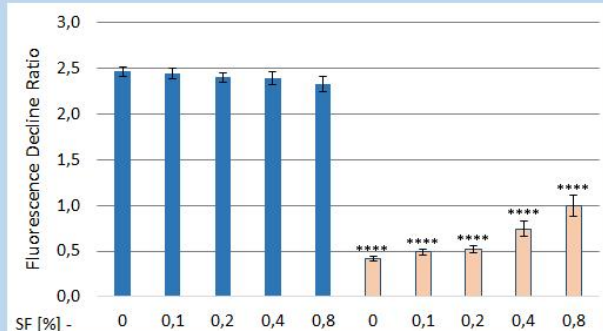
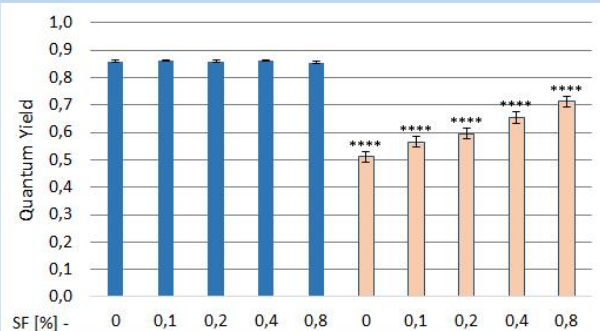
A)



A) Phenotype:

Plants, pre-treated with increasing concentrations of SF, followed by PQ treatment. (Best examples shown).

B)



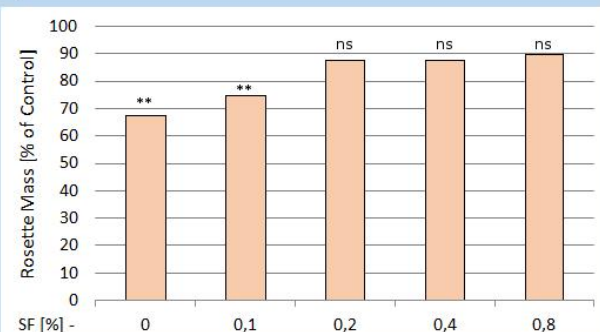
B) PAM fluorimetry:

Quantum Yield and Fluorescence Decline Ratio were determined in-situ. Lower values indicate higher degree of stress.

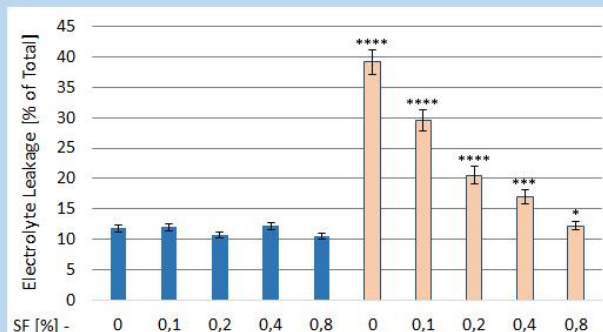
C) Mass of rosette leaves:

Mass increases with application of even higher concentrations of SF, reaching plateau after 0.2% at about 87-89% of control (Mock-treated).

C)



D)



D) Electrolyte leakage:

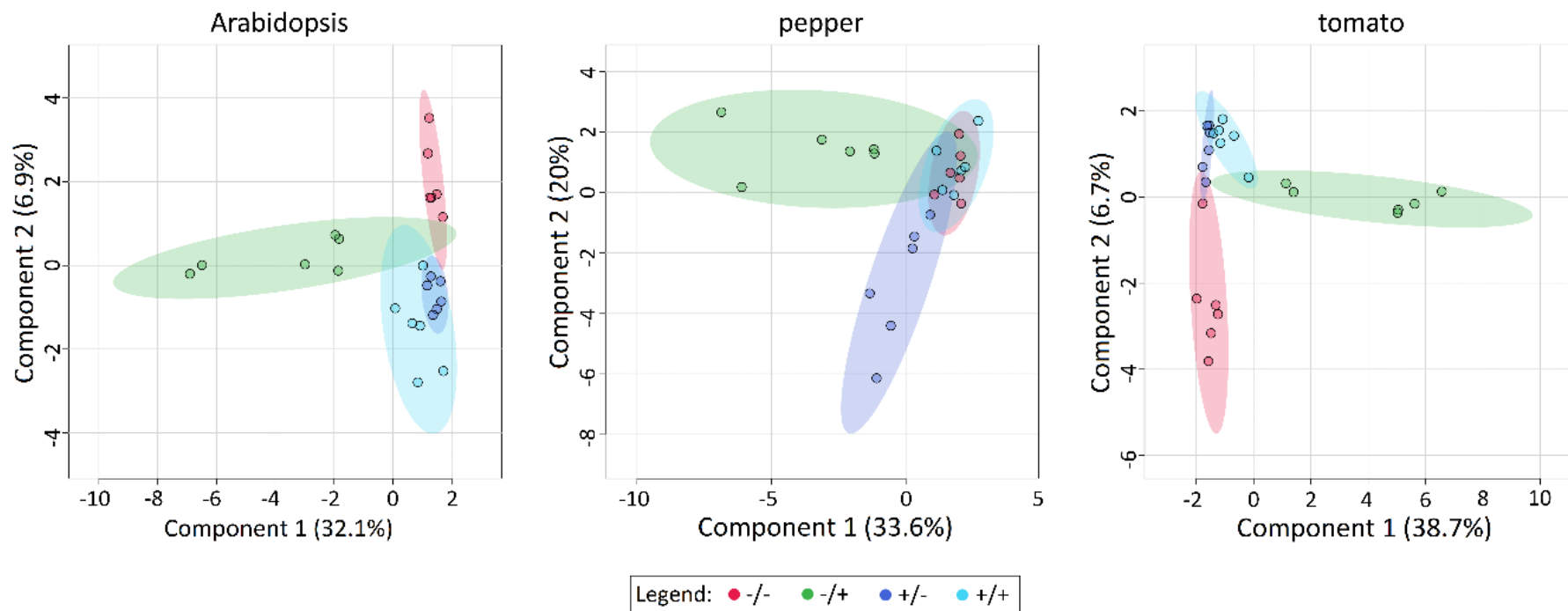
EL was determined on detached whole rosettes. Higher values correspond with higher degree of damaged and dead cells.

Legend: ■ - Mock, ■ - Paraquat

15 μ M PQ, SE, Student T-test

2D sPLSDA Scores plots for 89 metabolites of Arabidopsis, pepper and tomato

Distinct metabolomic profiles



Sparse Partial Least Squares Discriminant Analysis (sPLSDA): supervised technique, each sample's group is defined in advance for more efficient separation.

The -SF/+PQ treatment (light green clusters) groups separately from all the others, which show much more similar distribution and partial or complete overlapping (n=6). AT – *Arabidopsis thaliana*, CA – *Capsicum annuum*, SL – *Solanum lycopersicum*.



Follows:

- Electrolyte leakage assay
- Root length measurement
- Surface area measurement