Reactive oxygen species in leaves…
… and how to catch these

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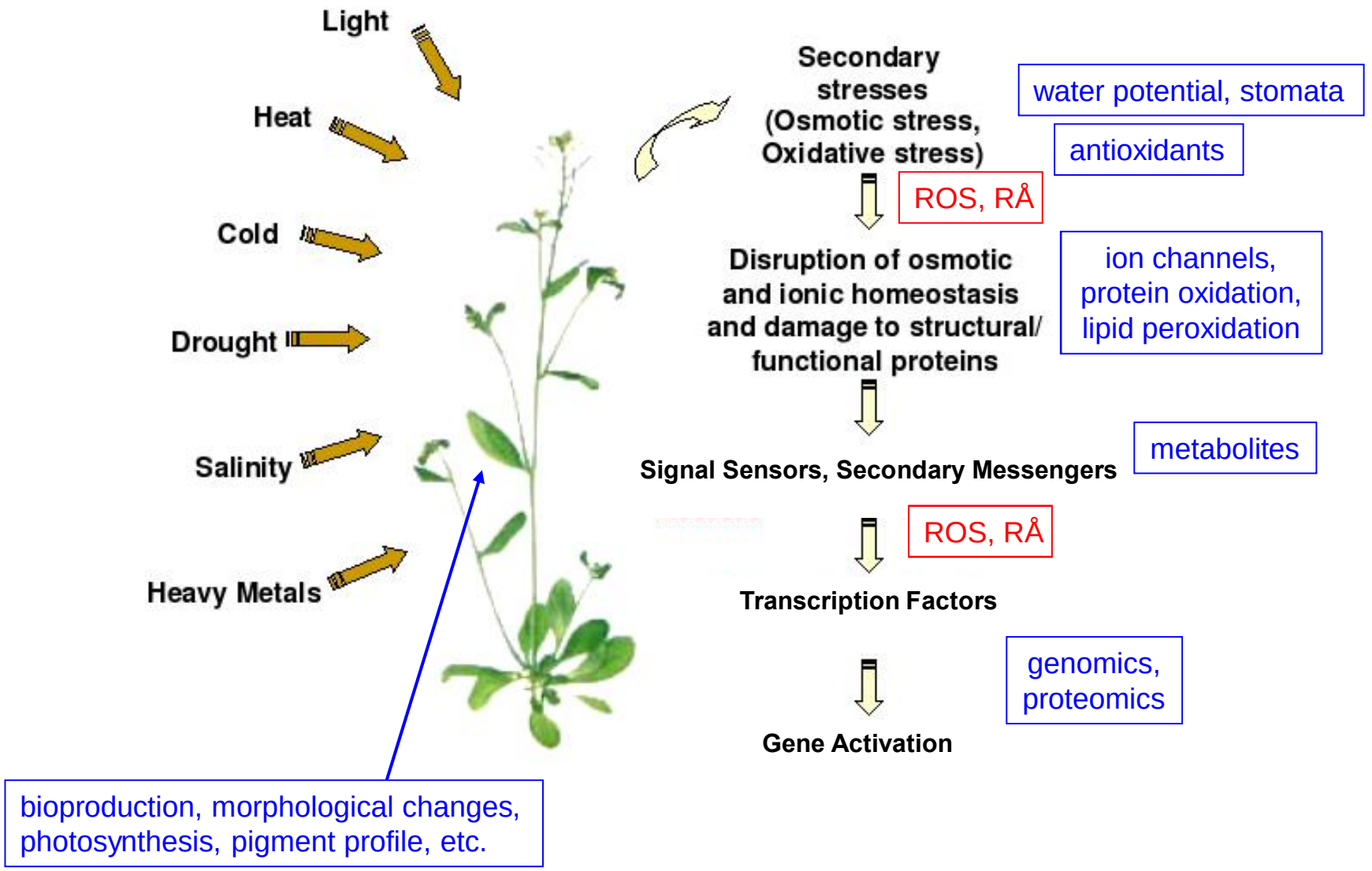
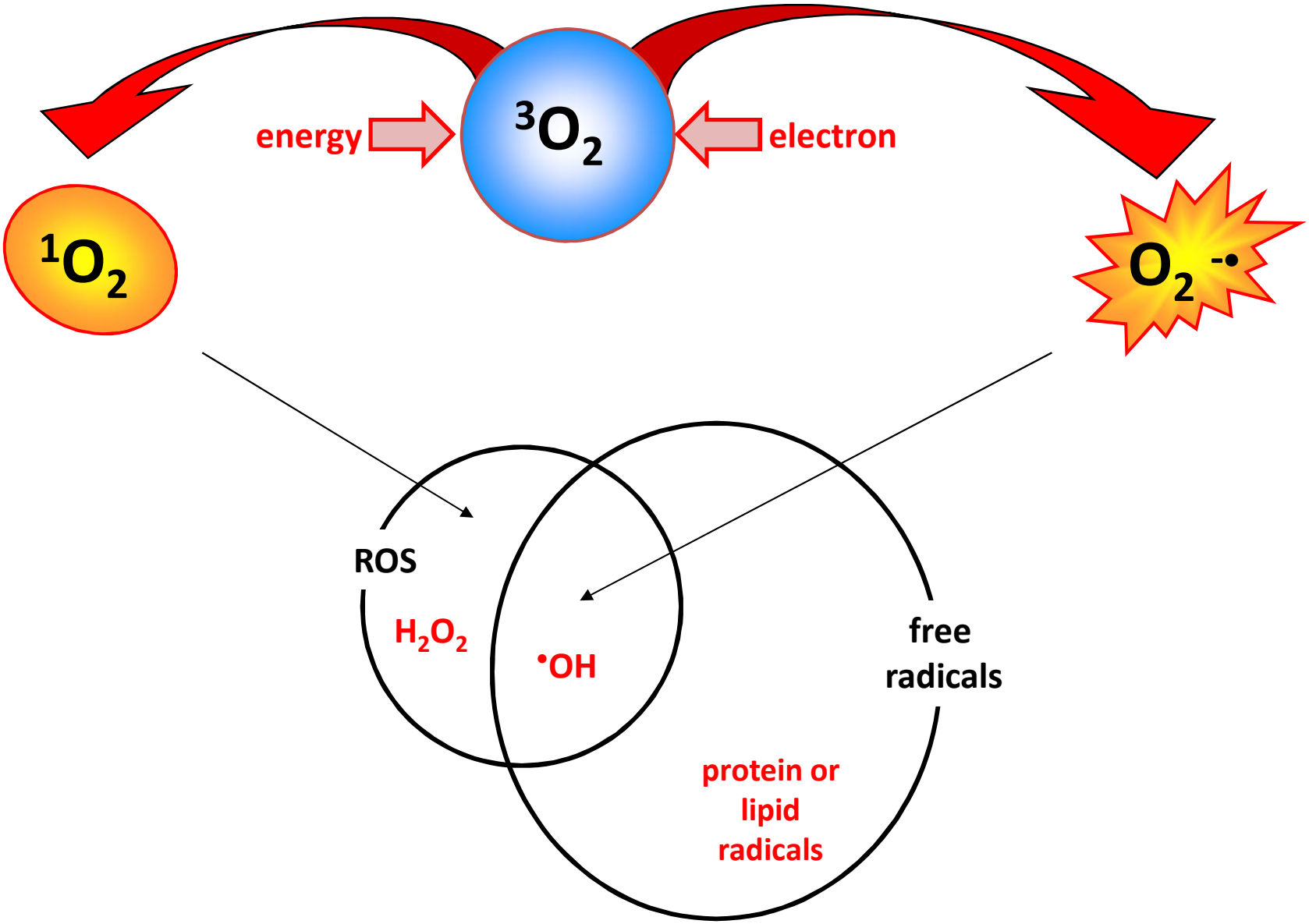
EPPN Summer School 2013

Outline

- How to prove that ROS are involved?
- The central role of ROS on stress responses
- What are ROS?
- Direct and indirect techniques
  - analytical methods
  - methods with potentials as imaging techniques

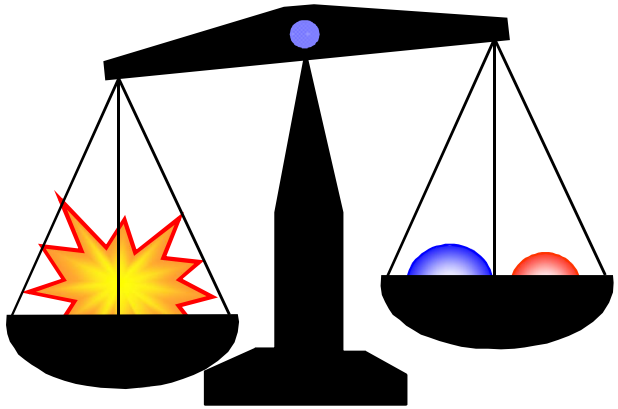
What to measure?



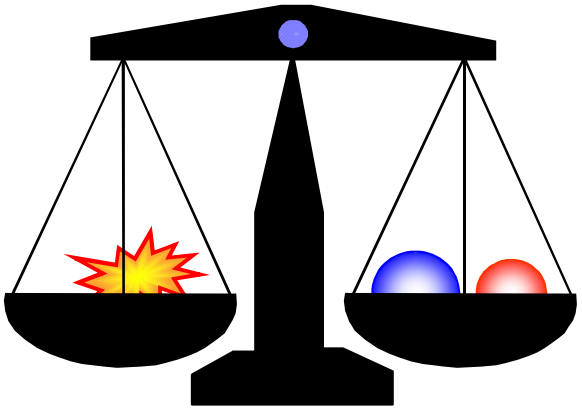

energy
$^3O_2$
electron
$^1O_2$
$O_2^{-\bullet}$
ROS
H_2O_2
$^\bullet OH$
free radicals
protein or lipid radicals

ROS – what do they do?

“bad ROS”
high yields,
uncontrolled production
effective detection

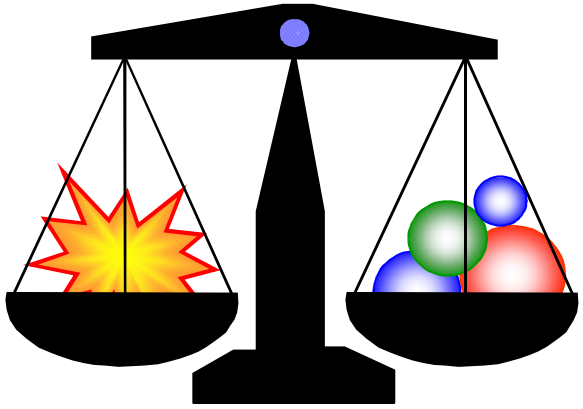


oxidative stress



ROS
(pro-oxidants)

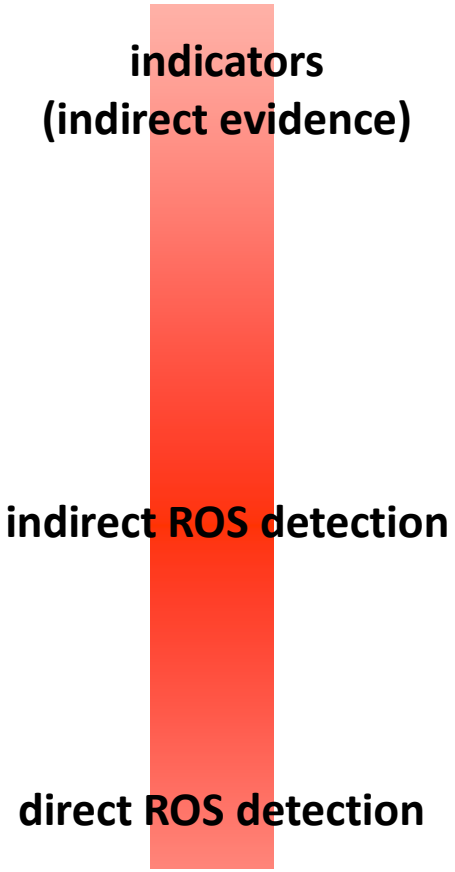
antioxidants



acclimation

“good ROS”, signals?
high yields, mostly neutralized
controlled production?
low detectable amounts

How to prove that ROS are involved? – Summary



is the effects of stress oxygen dependent?

does the antioxidant status change?

do plants with different antioxidants react differently?

do externally added ROS have the same effect?

„foot-printing" (detecting oxidized biomolecules)

reporter molecules: colorimetric, fluorescent or EPR active probes

H_2O_2 UV-absorption, $^1O_2$ photon emission

How to prove that ROS are involved? – References

Reviews:

Fischer, B.B., Hideg, É., Krieger-Liszkay, A. (2013) Production, detection and signaling of singlet oxygen in photosynthetic organisms. Antioxidants & Redox Signaling 18: 2145-2162.

Hideg, É., Ayaydin, F. (2013) Fluorescent ROS Probes in Imaging Leaves. In: Plant Image Analysis: Fundamentals and Applications (Gupta, S. D. and Ibaraki, Y. Eds.) CRC Press, Boca Raton USA, in press. ISBN 978-1466583016

Sass, L., Majer, P., Hideg, É. (2012) Leaf Hue Measurements: A High-Throughput Screening of Chlorophyll Content. In: High-Throughput Phenotyping in Plants: Methods and Protocols Methods in Molecular Biology Vol. 918 (Normanly, J., ed.) Springer Science + Business Media LLC, Ch.6. pp. 61-69. ISBN 978-1-61779-994-5

Hideg, É., Kálai T, Hideg K. (2011) Direct detection of free radicals and reactive oxygen species in thylakoids. In: Methods in Molecular Biology Vol. 684 (Carpentier, R., ed.), Humana Press Inc., Ch.16, pp. 187-200. ISBN 978-1-60761-924-6

Hideg, É., Vass,I. Kálai,T, and Hideg,K. (2000) Singlet oxygen detection with sterically hindered amine derivatives in plants under light stress. Methods in Enzymolology 319, 77-85.

Hideg, É. (2008) A comparative study of fluorescent singlet oxygen probes in plant leaves. Central European Journal of Biology, 3, 273-284

Research papers mentioned in the talk:

Kálai. T, Hideg, É., Ayaydin, F., Hideg, K. (2013) Synthesis and potential use of 1,8-naphthalimide type 1O2 sensor molecules. Photochemical and Photobiological Sciences, 12, 432-438.

Majer, P., Hideg, É. (2012) Developmental stage is an important factor that determines the antioxidant responses of young and old grapevine leaves under UV irradiation in a green-house. Plant Physiology and Biochemistry 50, 15-23.

Majer, P., Sass, P., Horváth, V.G., Hideg, É. (2010) Leaf hue measurements offer a fast, high throughput initial screening of photosynthesis in leaves. Journal of Plant Physiology, 167, 74-76.

Šnyrychová, I., Ayaydin, F., Hideg, É. (2009) Detecting hydrogen peroxide in leaves in vivo – a comparison of methods. Physiologia Plantarum 135, 1-18.

Hideg, É., Kós, B.P. Schreiber, U. (2008) Imaging of NPQ and ROS formation in tobacco leaves: heat inactivation of the water-water cycle prevents down-regulation of PS II. Plant Cell Physiology, 42, 1879-1886.

Hideg, É., Rosenqvist, E., Váradi, Gy., Bornman, J., Vincze, É. (2006) A comparison of UV-B induced stress responses in three barley cultivars. Functional Plant Biology 33, 77-90.

Hideg, É., Mano,J., Ohno,Ch. and Asada,K. (1997) Increased levels of monodehydroascorbate radical in UV-B irradiated broad bean leaves. Plant Cell Physiology 38, 684-690.

How to prove that ROS are involved? – examples

indicators
(indirect evidence)

indirect ROS detection

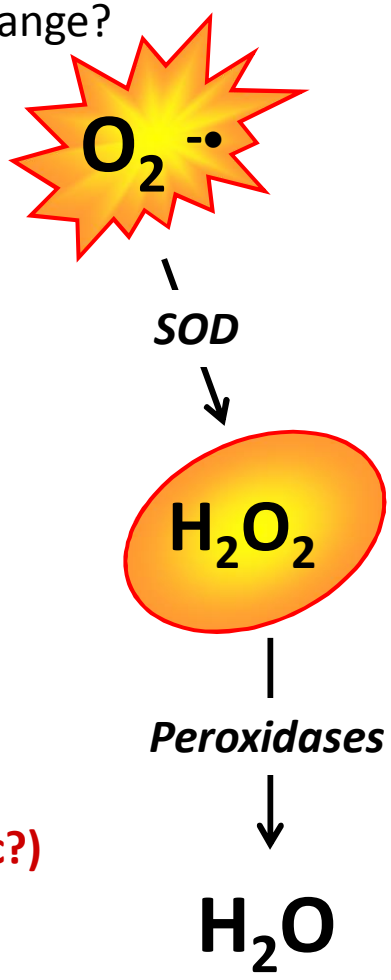
direct ROS detection

is the effect of stress oxygen dependent?

does the antioxidant status change?

enzymes
Å **mRNA, protein, activity?**

non-enzymatic antioxidants
Å **amounts, redox status?**
(ascorbate, GSH/GSSG)
Å **chemical identity, action?**
(total capacities vs. specific?)



How to prove that ROS are involved? – examples

indicators
(indirect evidence)

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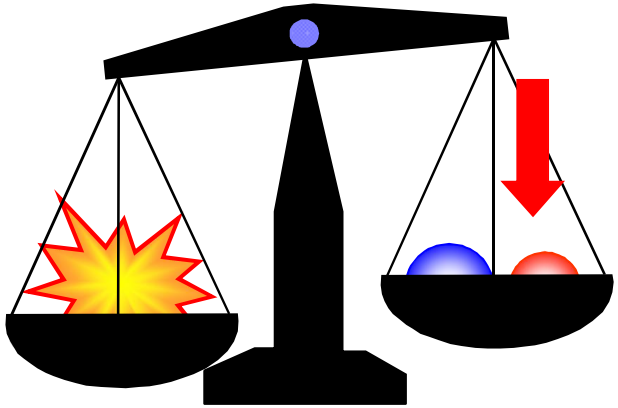
direct ROS detection

is the effects of stress oxygen dependent?

does the antioxidant status change?

do plants with different antioxidants react differently?

- **same species, different cultivars**
- **same species grown under different conditions**
- **transgenic plants**



Are antioxidant reinforced plants more stress tolerant?

How to prove that ROS are involved? – examples

indicators
(indirect evidence)

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direct ROS detection

is the effects of stress oxygen dependent?

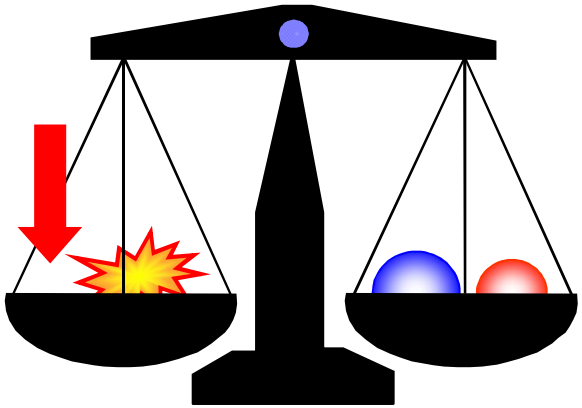
does the antioxidant status change?

do plants with different antioxidants react differently?

do externally added ROS have the same effect?

ROS elicitors:

- **superoxide sources (e.g. methyl-viologen)**
- **singlet oxygen photosensitizers**



How to prove that ROS are involved? – examples

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„foot-printing" (detecting oxidized biomolecules)

- **protein oxidation (e.g. carbonyl detection)**
- **lipid peroxidation products (e.g. TBARS)**
- **DNA damage (e.g. comet assay)**

How to prove that ROS are involved? – examples

indicators
(indirect evidence)

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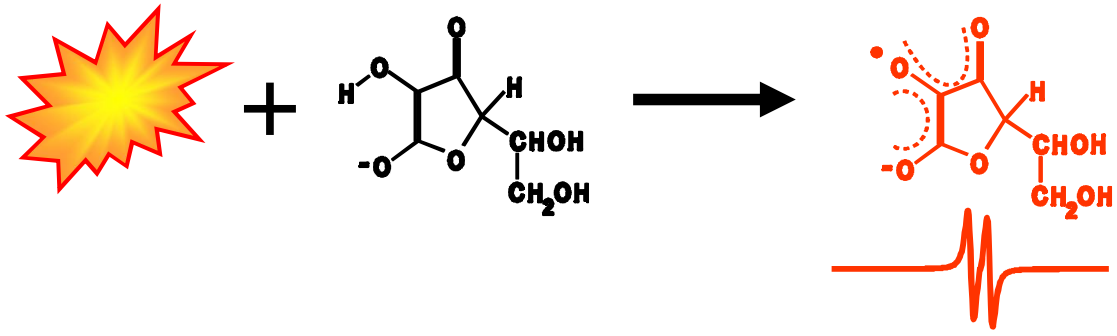
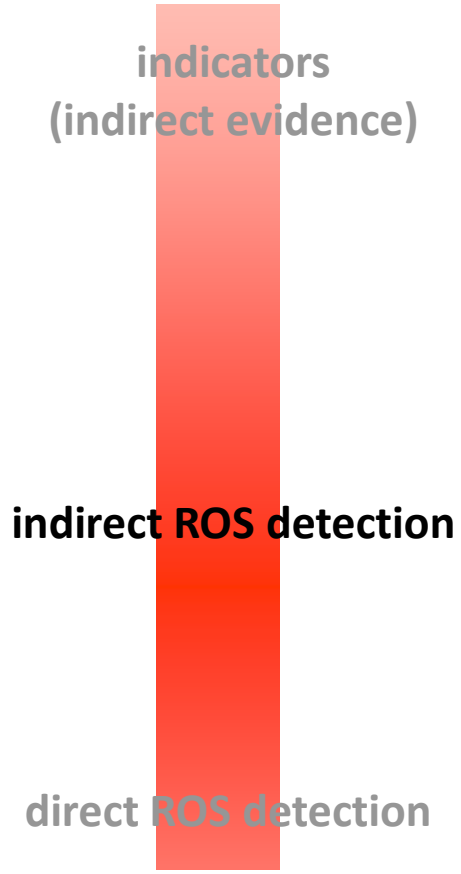
problem:
spin traps and adducts are unstable in leaves!!
one possible solution:
ascorbate as "built-in" spin trap

reporter molecules: colorimetric, fluorescent or **EPR active probes**



How to prove that ROS are involved? – examples



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„foot-printing" (detecting oxidized biomolecules)

reporter molecules: **colorimetric**, fluorescent or EPR active probes

Molecules to change colour by ROS oxidation:

- **DAB for H_2O_2**
- **NBT for superoxide radicals**

How to prove that ROS are involved? – examples

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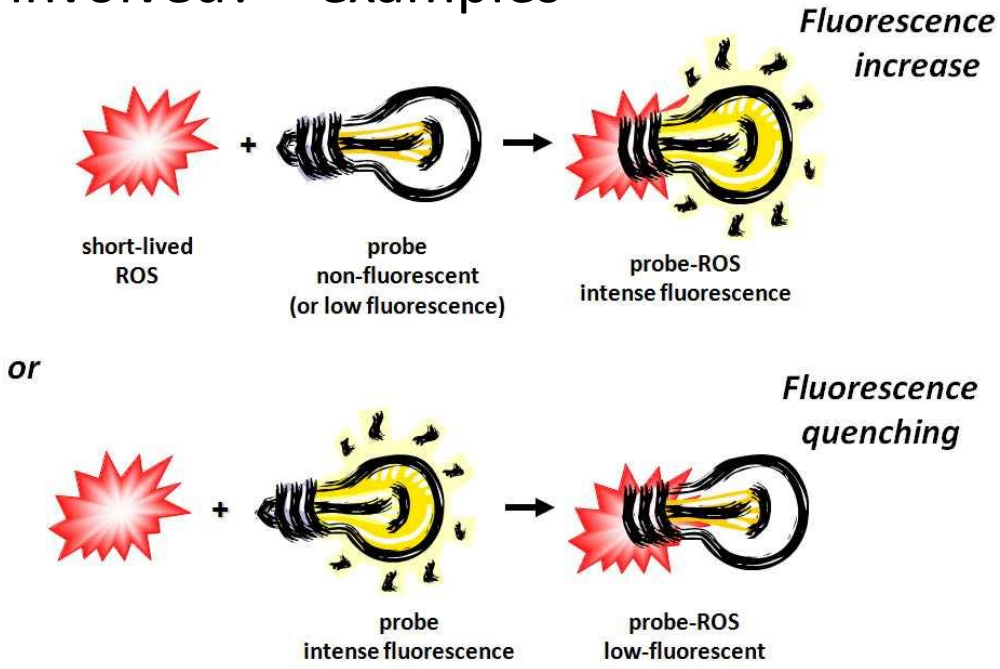

reporter molecules: colorimetric, **fluorescent** or EPR active probes

Molecules to change fluorescence by ROS oxidation:

- **Amplex Red for H_2O_2**
- **……**
- **……**

How to prove that ROS are involved? – examples

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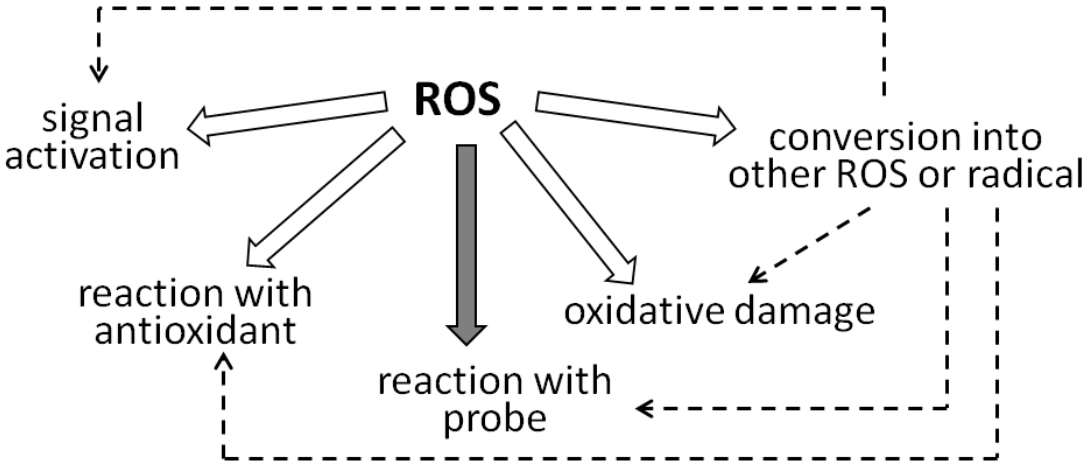

Hideg & Ayaydin 2013

Good for imaging, BUT use with care:

- ***ROS selectivity? (check yourself!)***
- ***artefacts? (light or redox sensitivity?)***
  (sample autofluorescence?)
- ***localization in sample? (how to put in?)***
  (check subcellular localization, confocal imaging)
- ***probes always compete for ROS***

How to prove that ROS are involved? – examples

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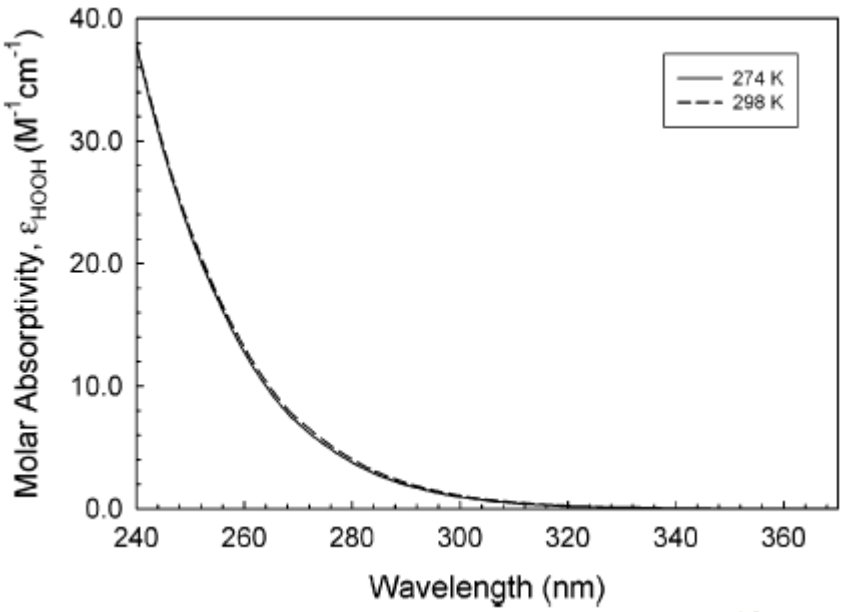

H_2O_2 UV-absorption, $^1O_2$ photon emission

How to prove that ROS are involved? – examples

indicators
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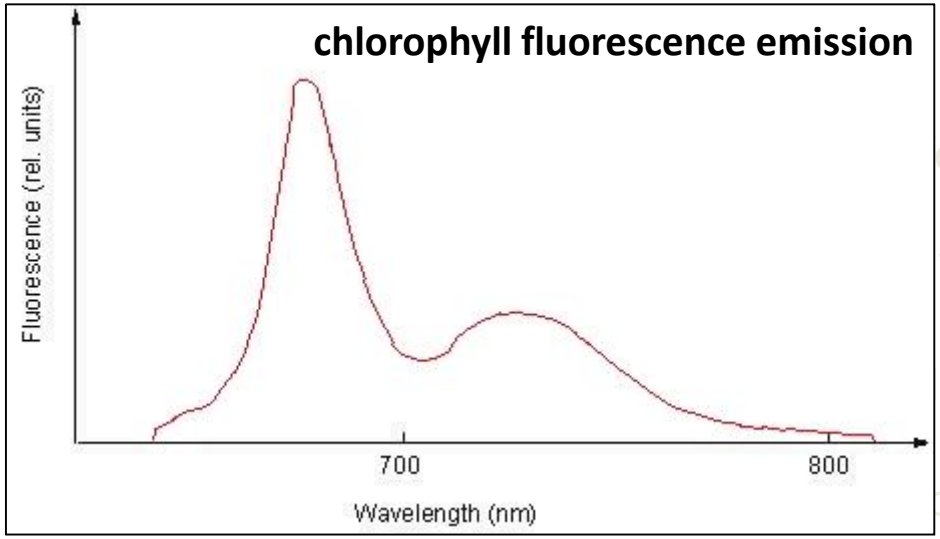


H_2O_2 UV-absorption, $^1O_2$ photon emission

$O_2(^1\Sigma_g^+) \rightarrow O_2(^3\Sigma_g^G) + h\nu$ (762 nm)

$2\ O_2(^1\Delta_g^+) \rightarrow 2\ O_2(^3\Sigma_g^G) + h\nu$ (634 nm)

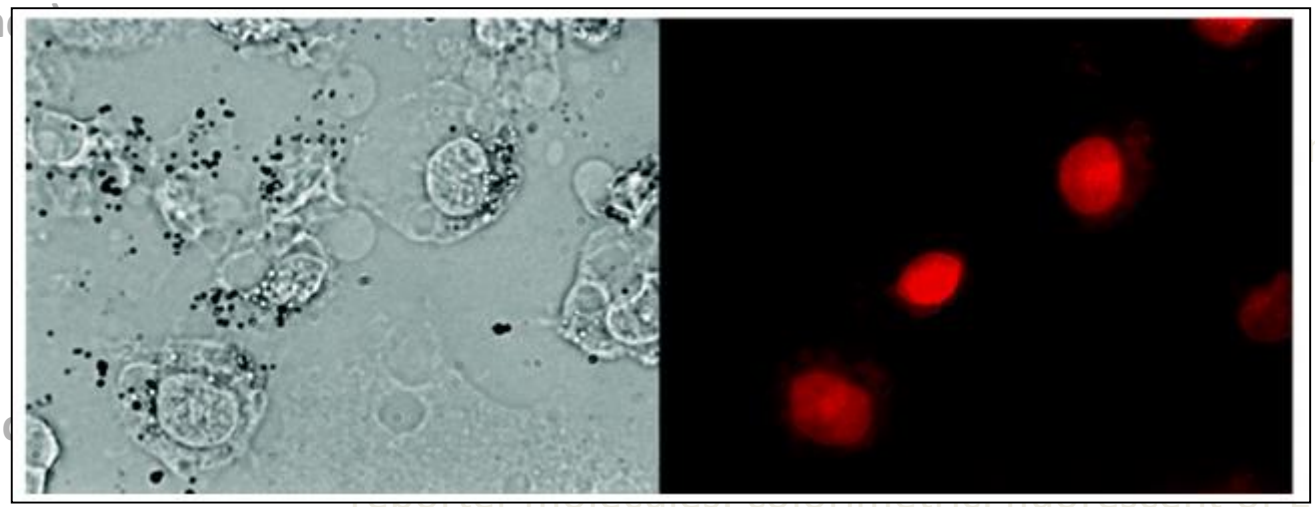
How to prove that ROS are involved? – examples

indicators
(indirect eviden

indirect ROS dete

direct ROS detection

plant cells? Å **chlorophyll phosphorescence**
Å **$^1O_2$ quenchers and antioxidants**



$^1O_2$ IR photoemission from photosensitizer in HeLa cells
Hartz, Lambert & Ogilby 2007 Photochem. Photobiol. Sci., 6, 1106-1116

H_2O_2 UV-absorption, $^1O_2$ IR photon emission

$O_2(^1\Delta_g^+) \rightarrow O_2(^3\Sigma_g^G) + h\nu$ (1269 nm)