Uptake of humic substances by wheat plants: preferential accumulation in lipid fraction

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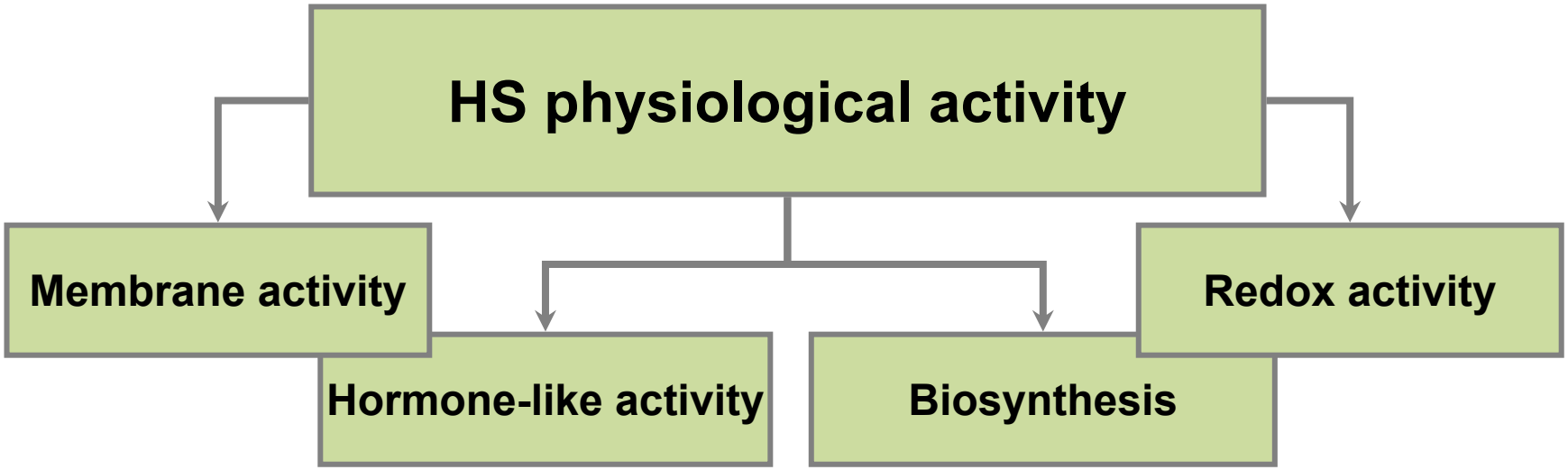
Probable mechanisms of HS physiological activity



- Do humic substances enter the plants?
- What is the distribution of HS in plants?

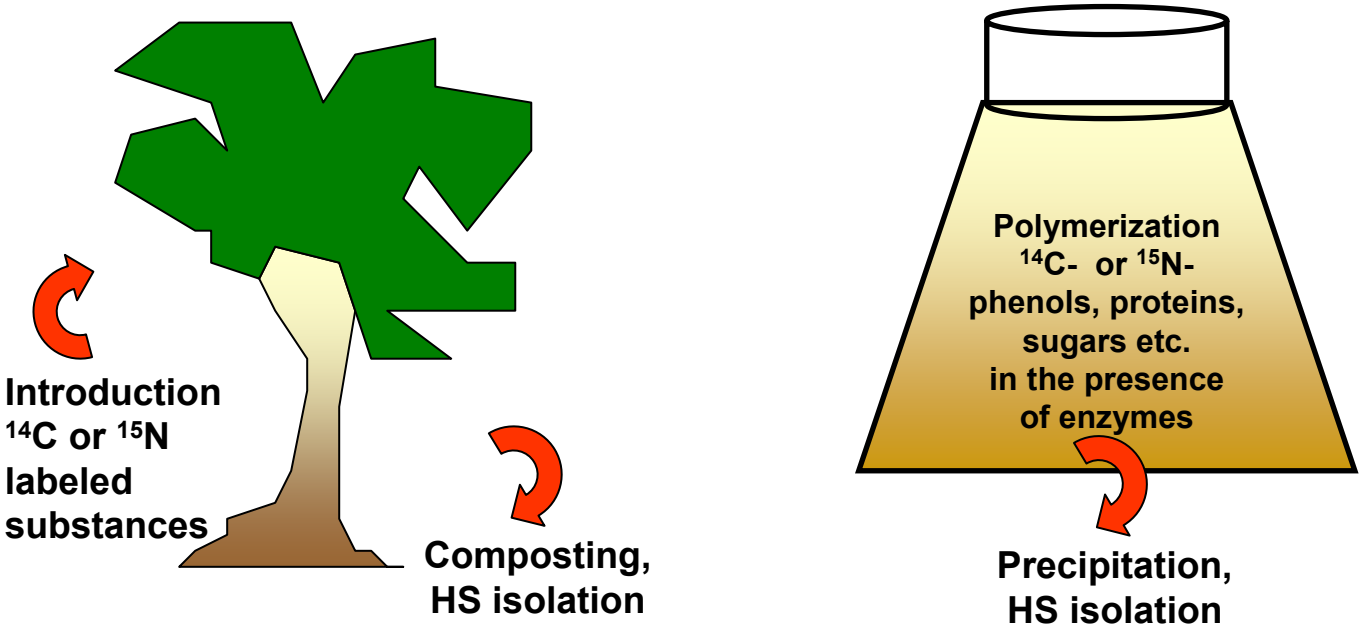
The goal

- to study uptake and distribution of HS in wheat seedlings

Preparation of labeled HS

Isolation from labeled substrate

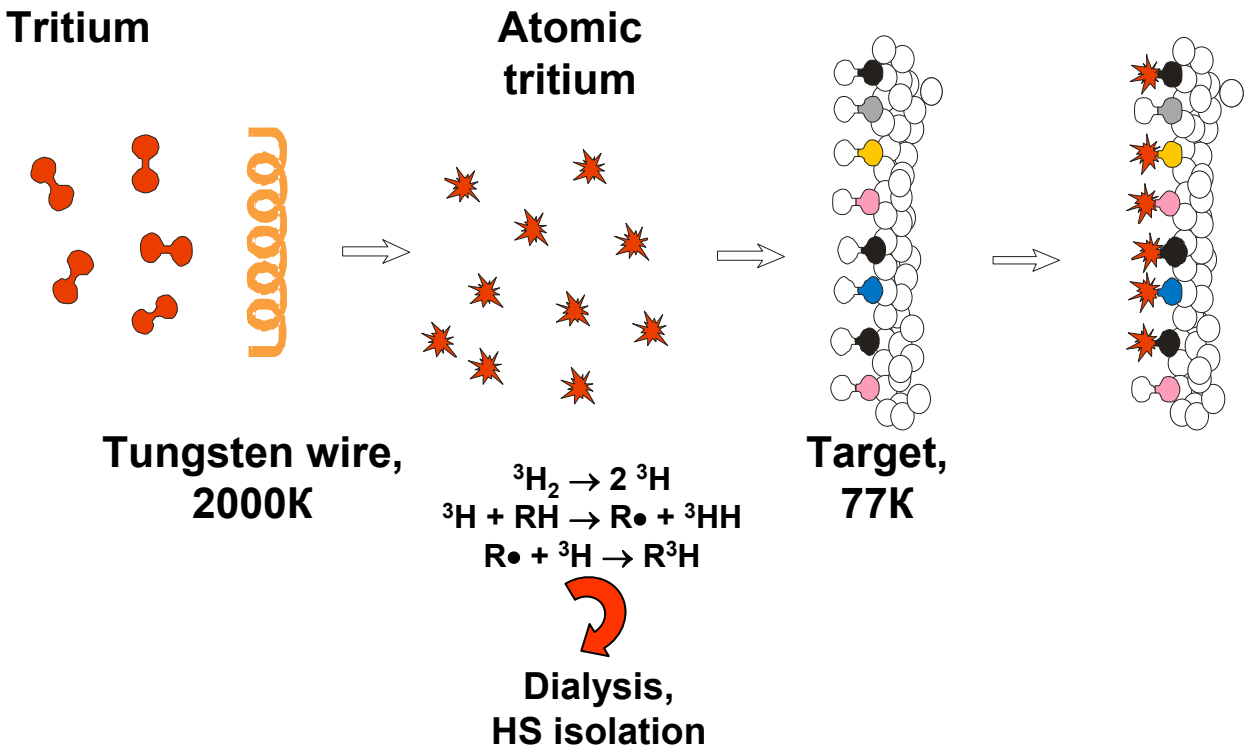
Synthesis from precursors



^{14}C or ^{15}N *analogues* of humic substances

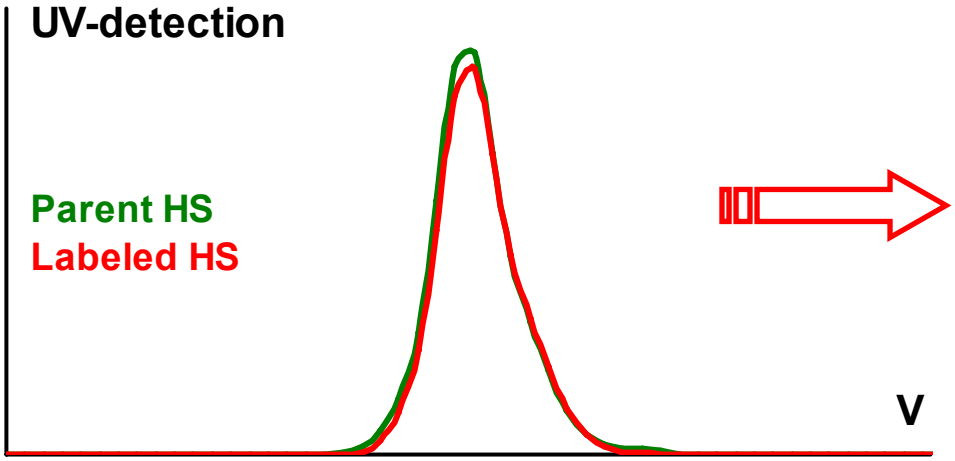
Preparation of ^{3}H-HS

Tritium thermal activation method

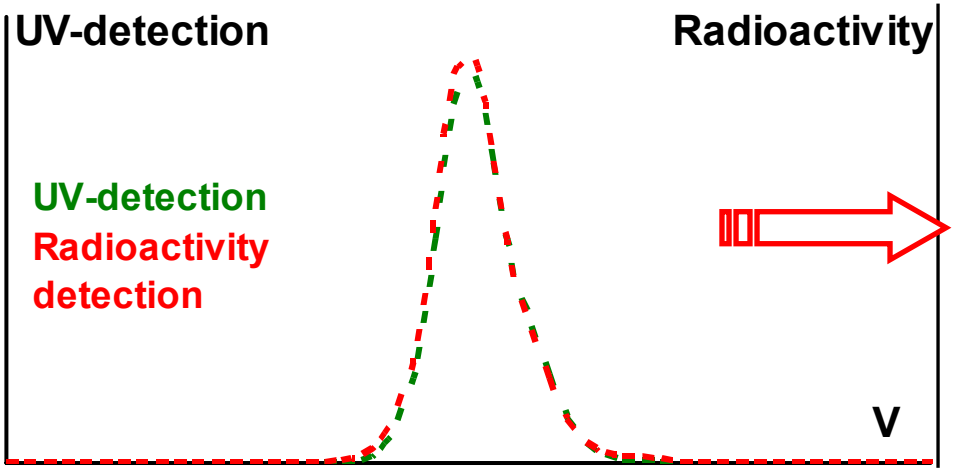


^{3}H humic substances

SEC analysis of ^{3}H-HS



Labeled HS are identical to parent



Tritium is uniformly distributed in HS

Studied humic substances

- Coal humic acids
- Peat humic and fulvic acid
- Aquatic fulvic acid

Elemental analysis
Size-exclusion chromatography
^{13}C NMR analysis

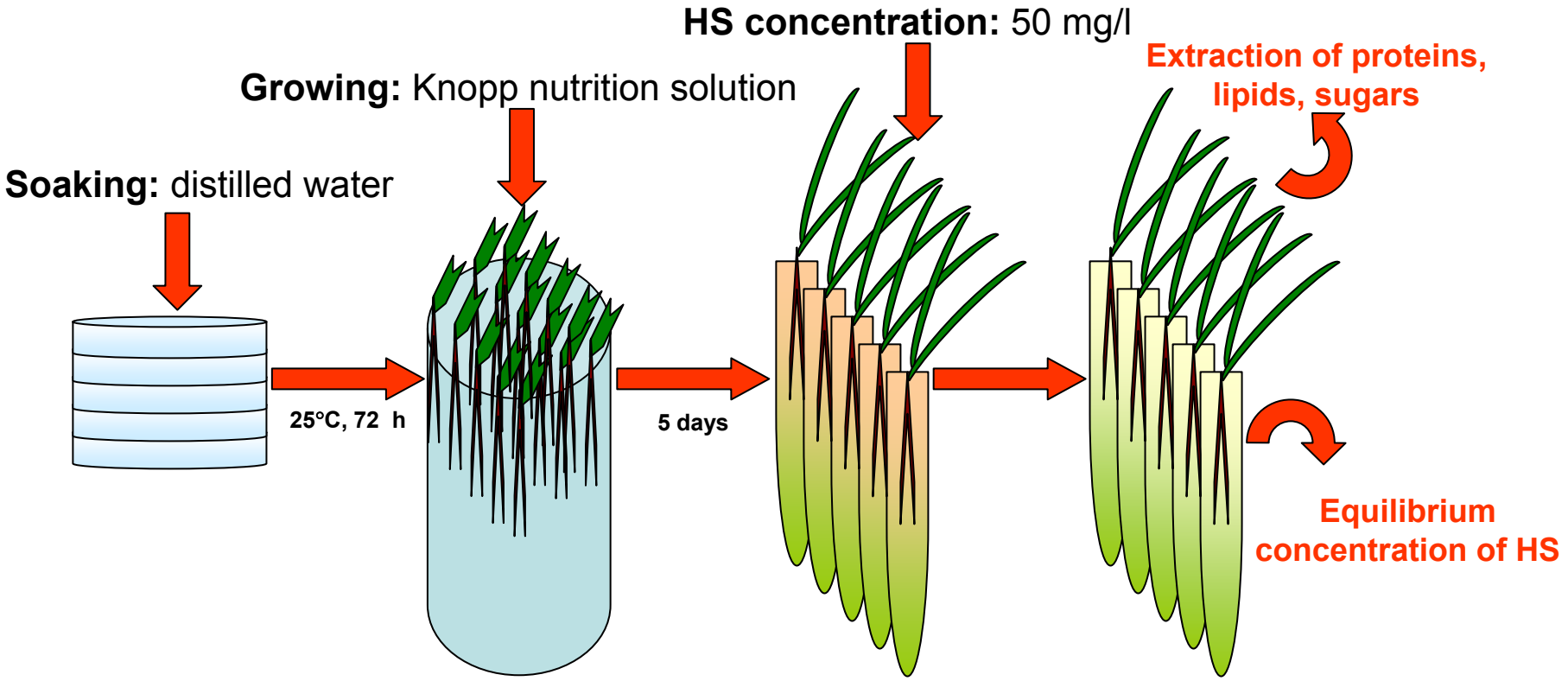
Labeling with tritium

Estimation of hydrophobicity (i.e. determination of K_{TW})

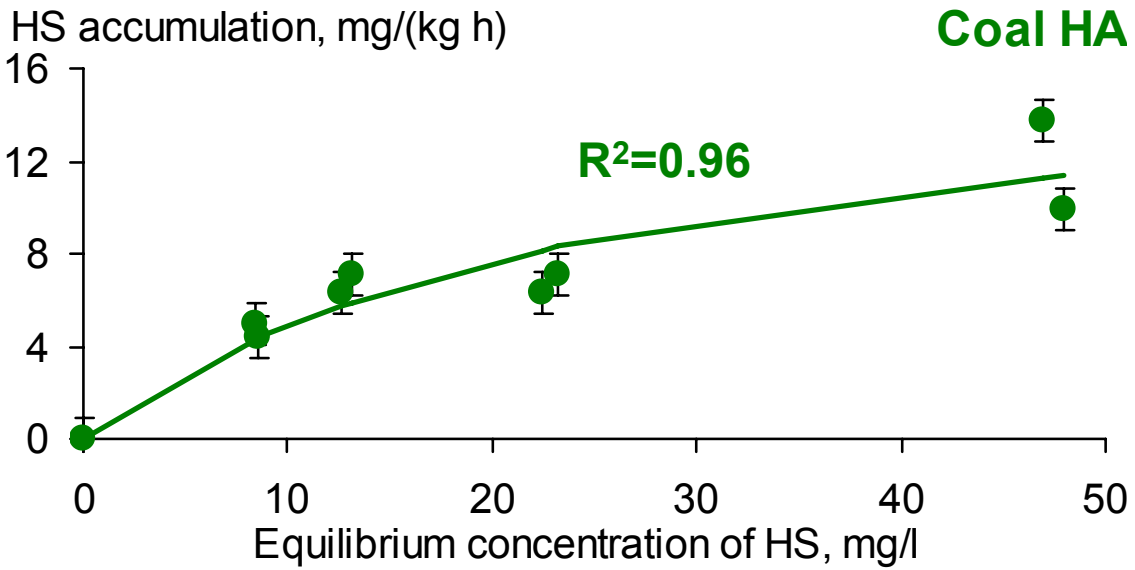
Bioassay experiments

Bioassay design

Target object*:* wheat *Triticum aestivum* L.



Kinetics of HS uptake by plants



Michaelis-Menten equation

$$V = \frac{V_{max}[C]}{K_m + [C]}$$

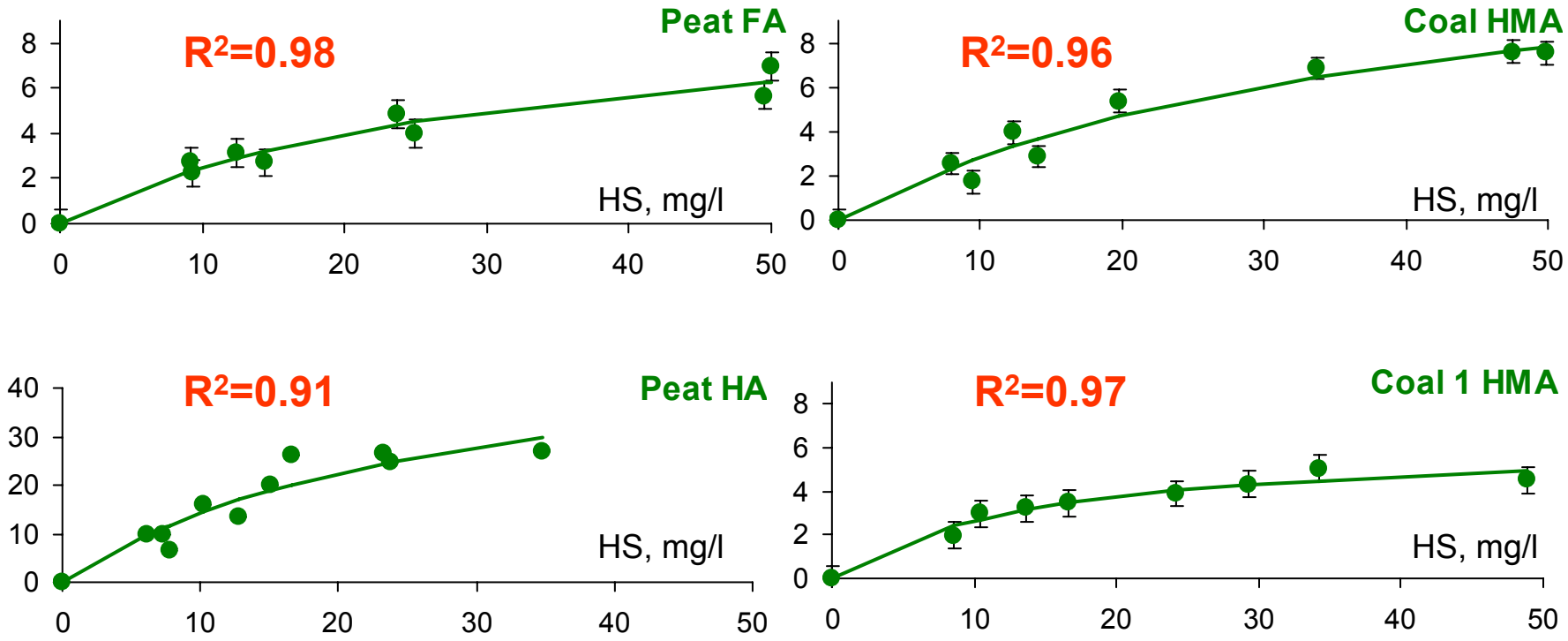
V is rate of nutrient uptake (amount/unit time)

[C] is nutrient concentration in the solution

V_{max} is the maximum rate of nutrient uptake due to saturation of the transporter mechanism

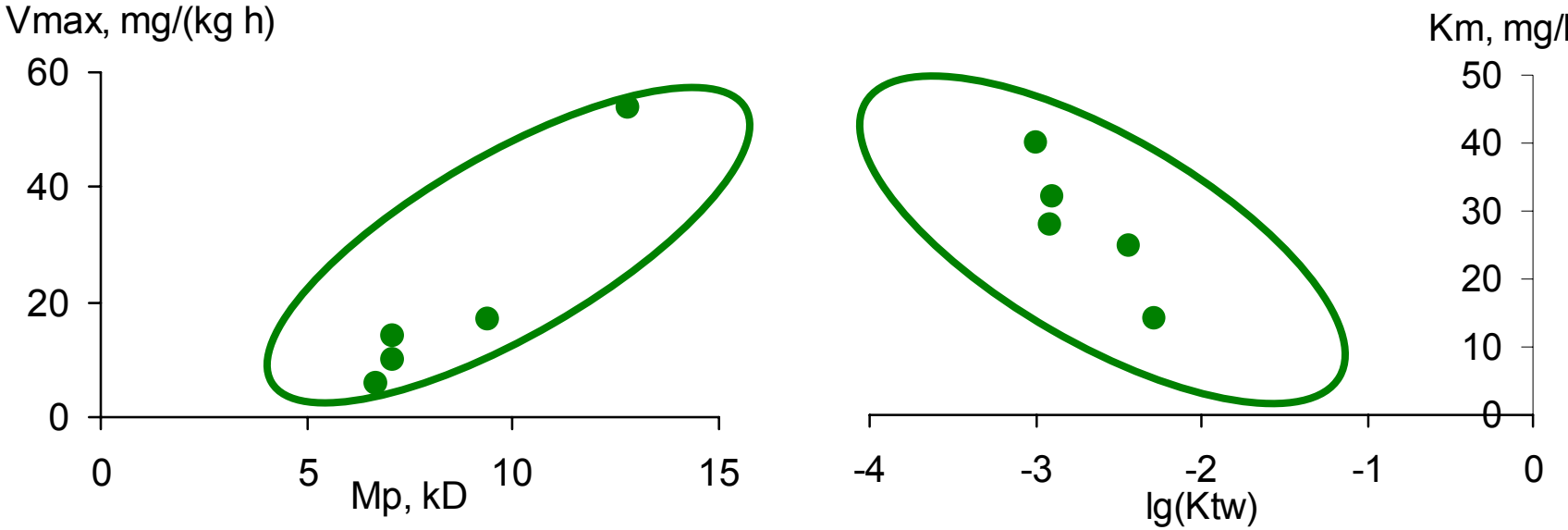
K_m is the Michaelis constant (in units of concentration)

Kinetics of HS uptake by plants



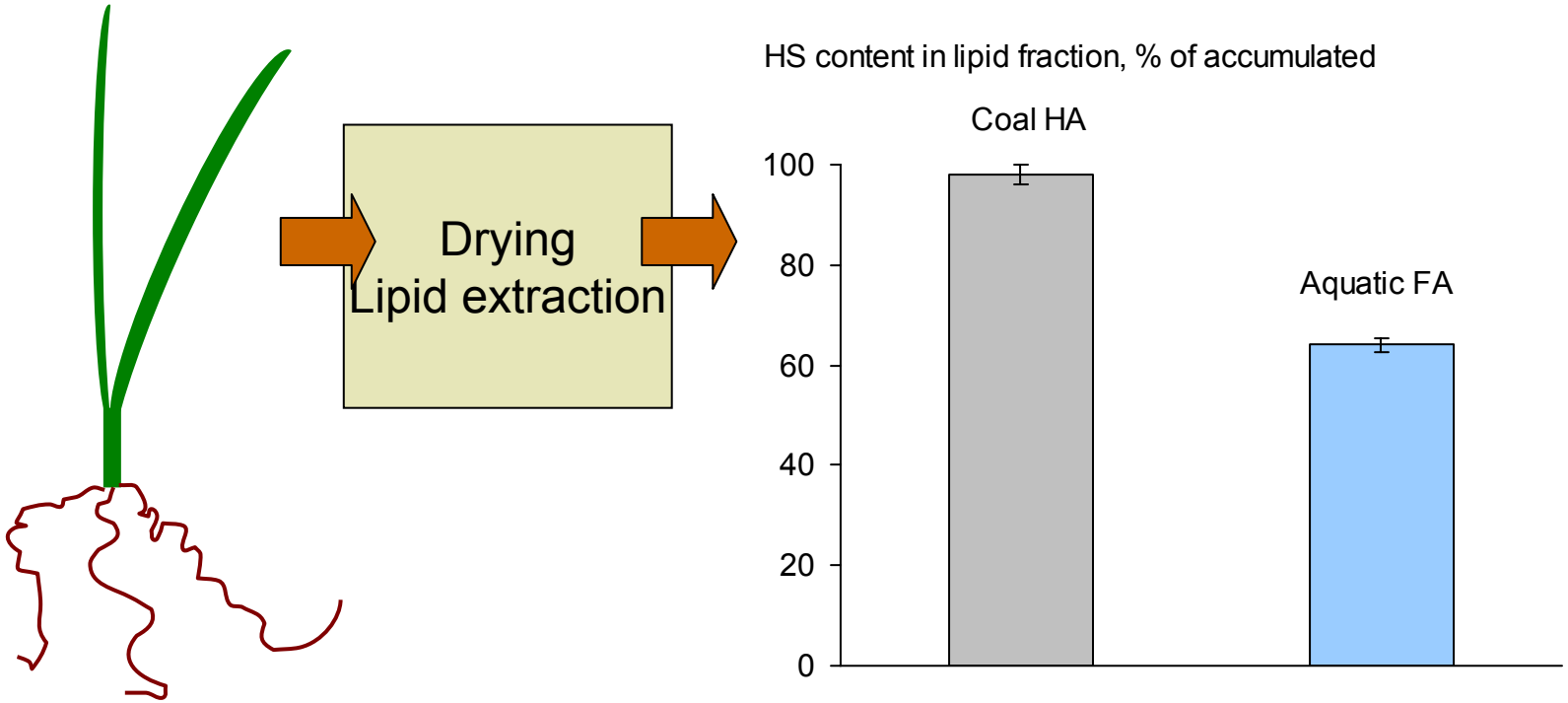
- Kinetics of HS uptake by plants can be described using Michaelis-Menten equation
- Uptake of HS by plants is hypothesized to be carrier-mediated (transporter-limited)

HS uptake vs. HS properties



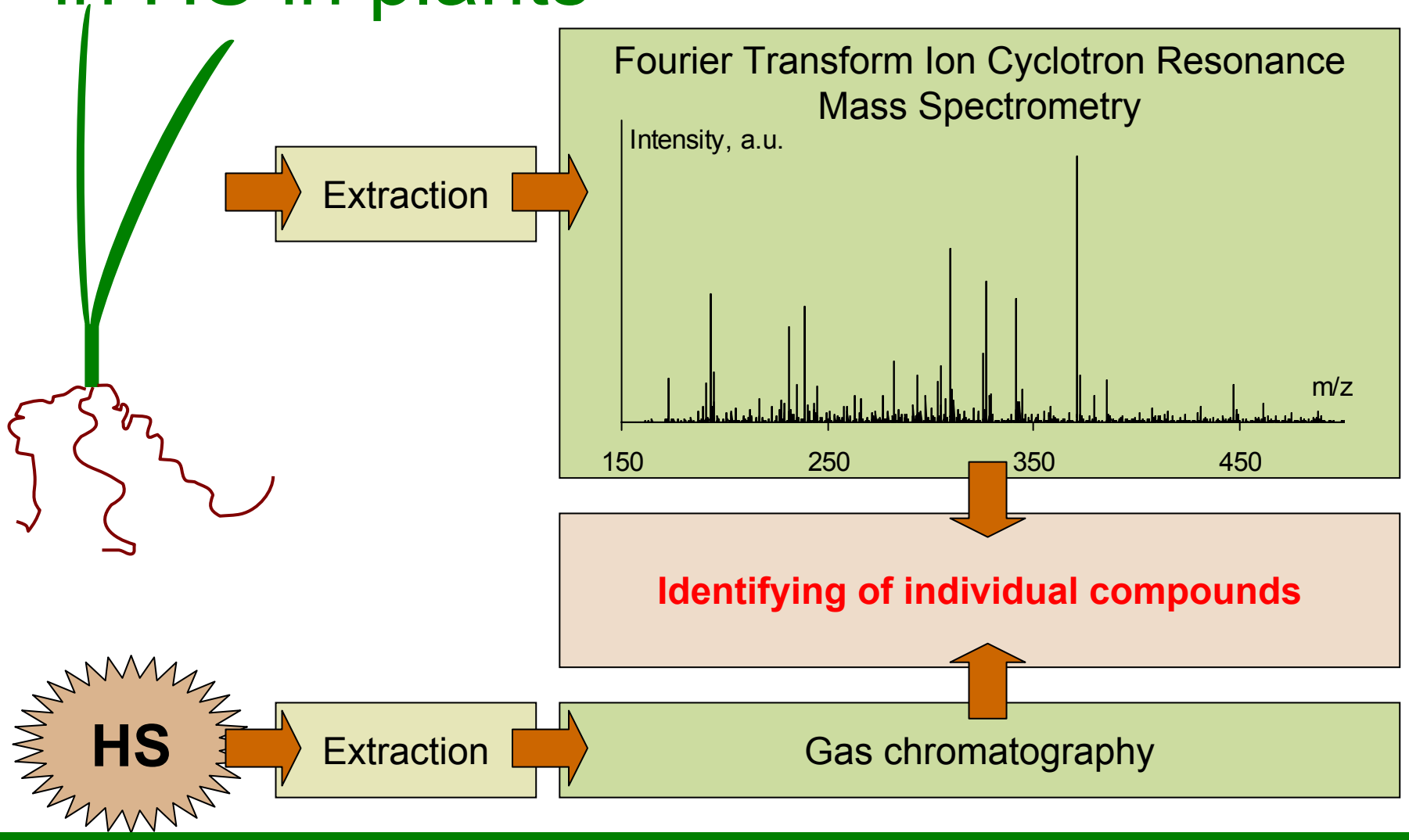
- The higher hydrophobicity, the less K_m
- The higher molecular weight of HS, the higher V_{max}

HS in lipid fraction of plants

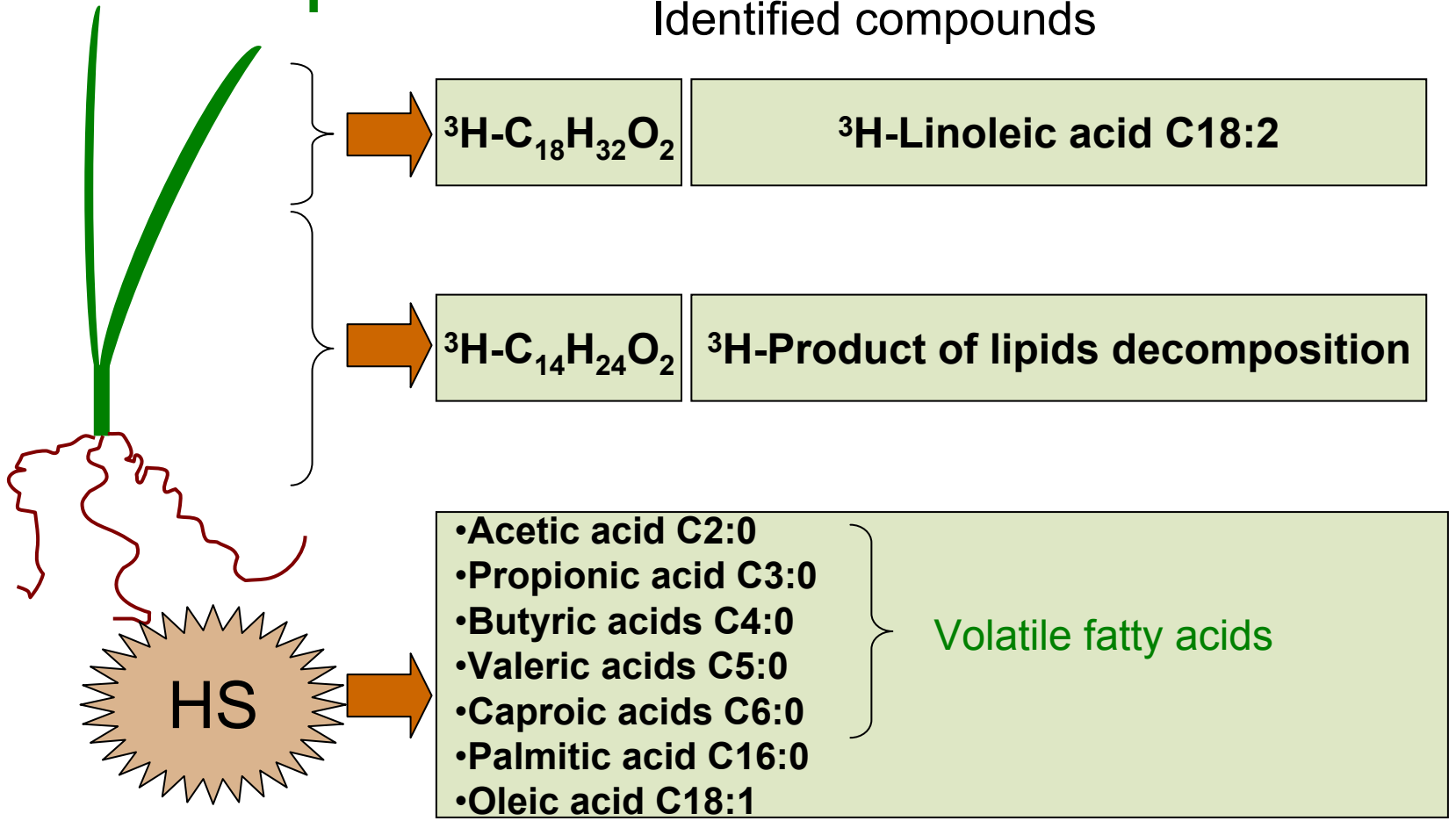


- Mainly hydrophobic fragments of HS are accumulated by plants?

Identifying of individual compounds in HS in plants

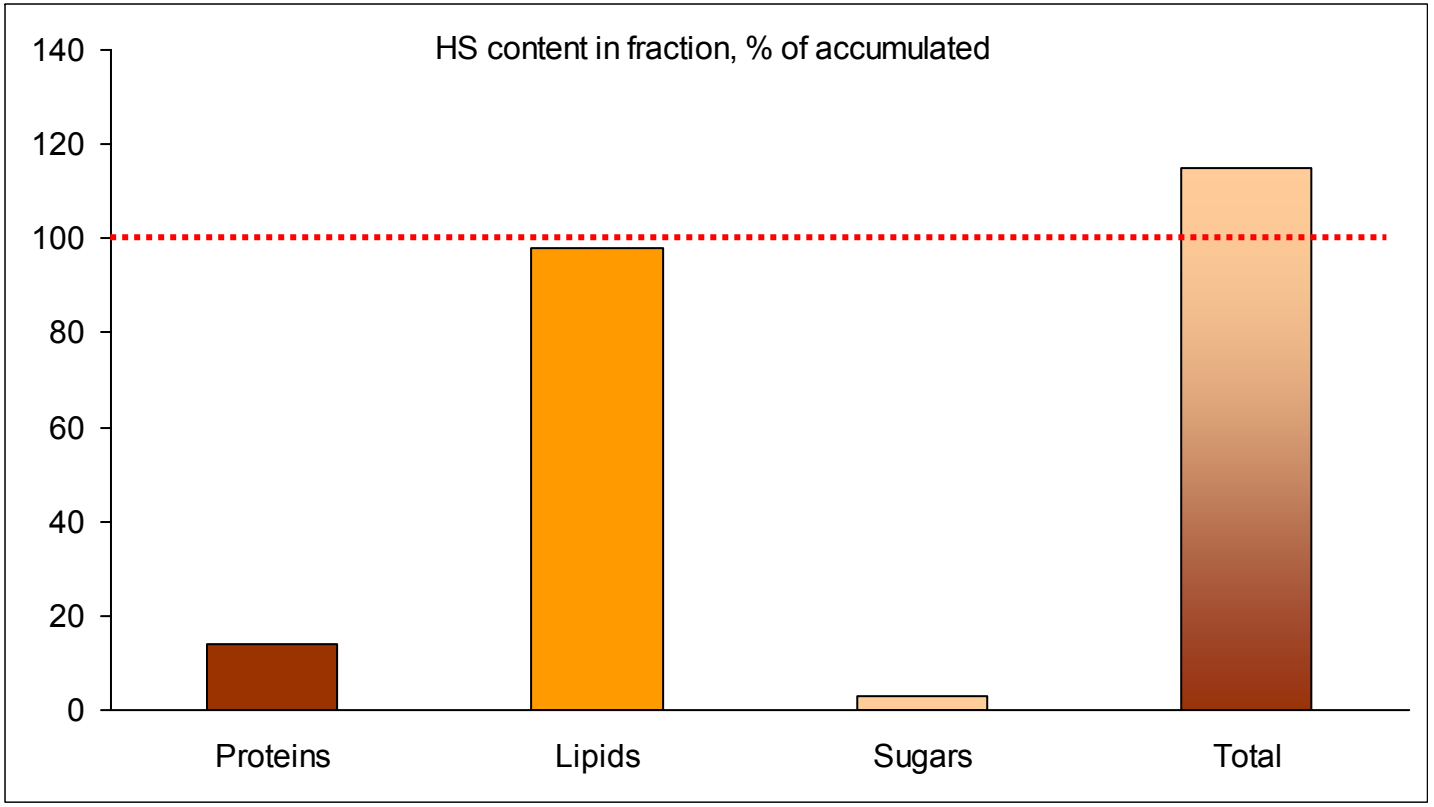


Identifying of individual compounds in HS in plants



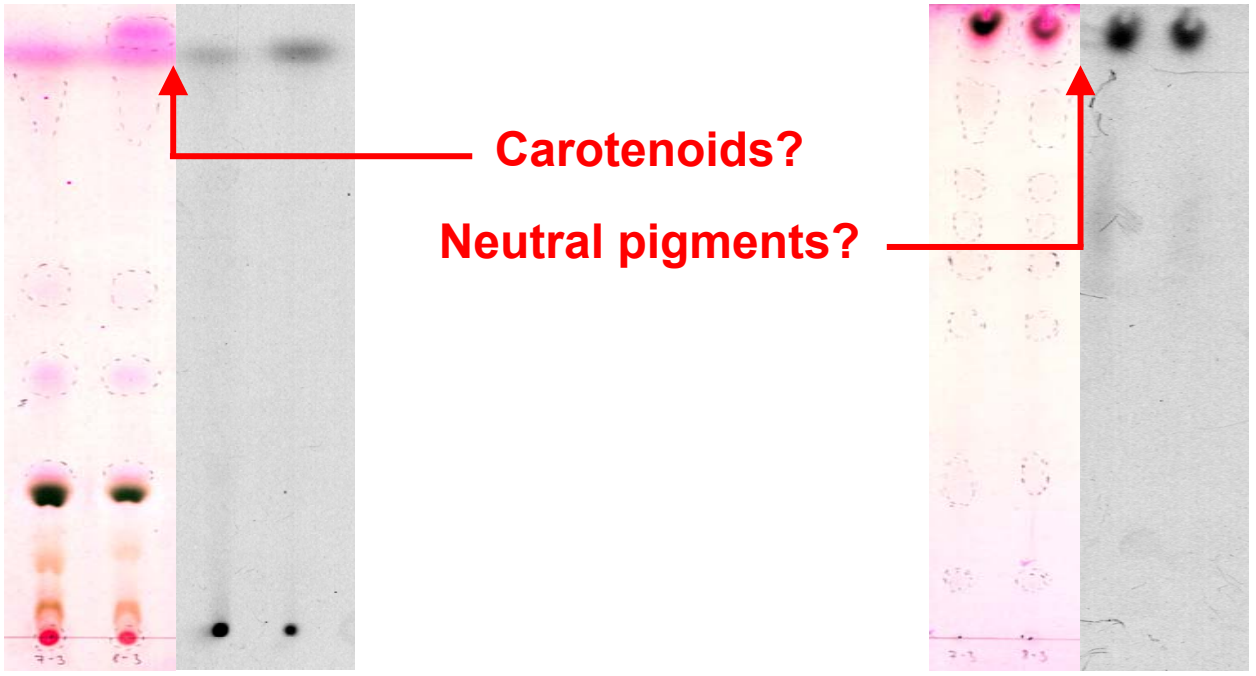
Ø HS can be involved in lipid metabolism of plants

Uptake of HS by plants: further issues to solve



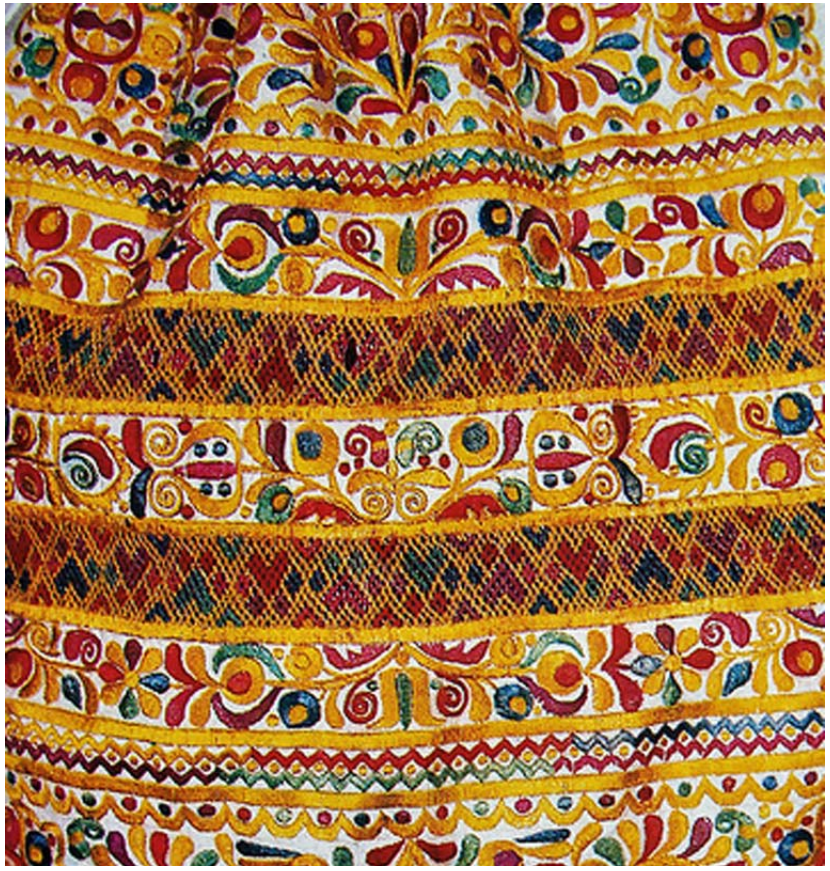
Uptake of HS by plants: further issues to solve

Thin layer chromatography and tritium autoradiography of lipid fraction



Uptake of HS by plants: further issues to solve

- Sum of HS determined in proteins, lipids, and sugars exceeded 100% of HS accumulated by plants
- Lipids are a heterogeneous mixture of different compounds
- Further study of distribution of HS in plants is needed



Thanks for your attention!

Ďakujem!