



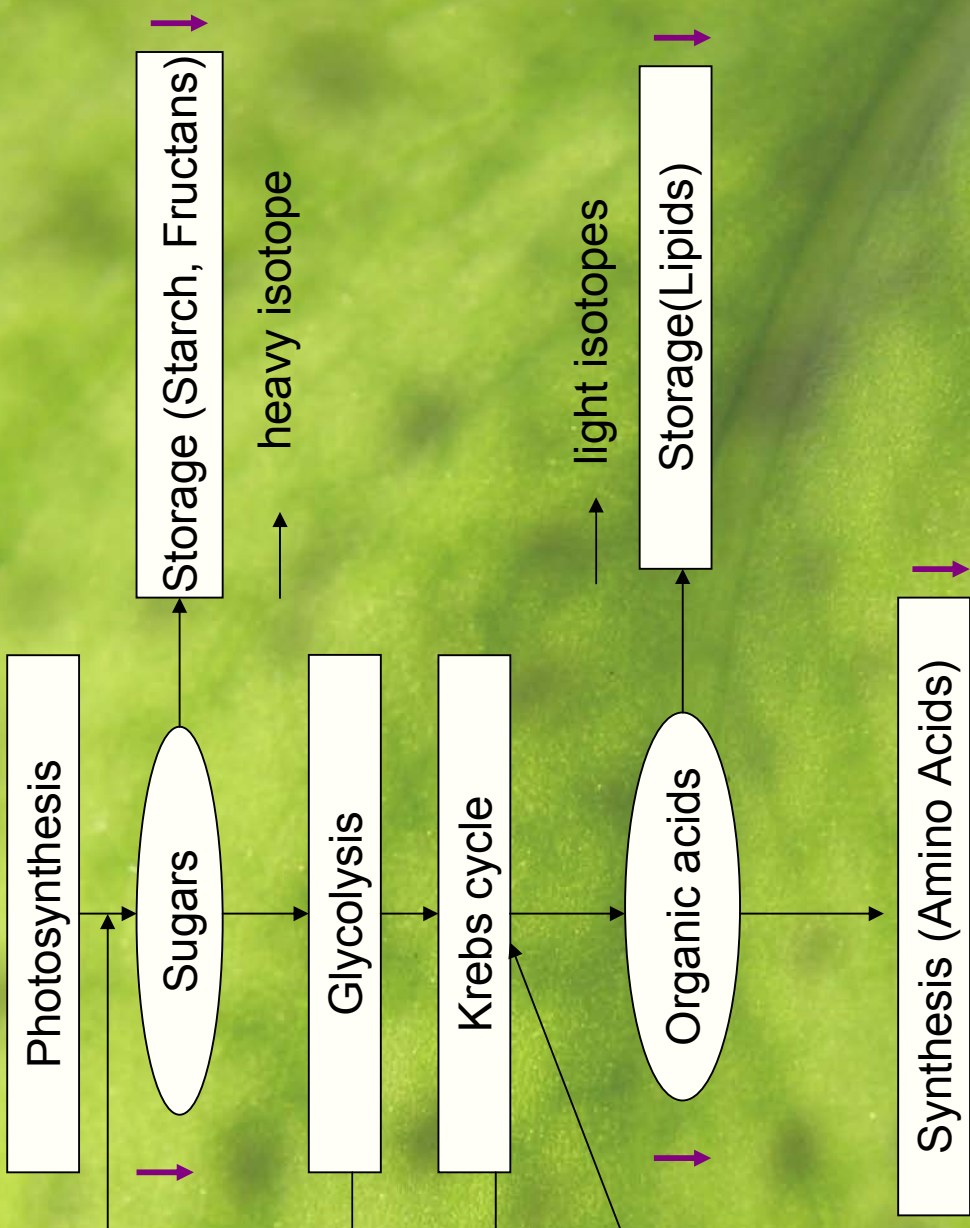
Compound-specific carbon isotope ratios of metabolites determined by LC/MS-IRMS

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Fractination on stomata
(alters through e.g. water stress)



heavy isotope

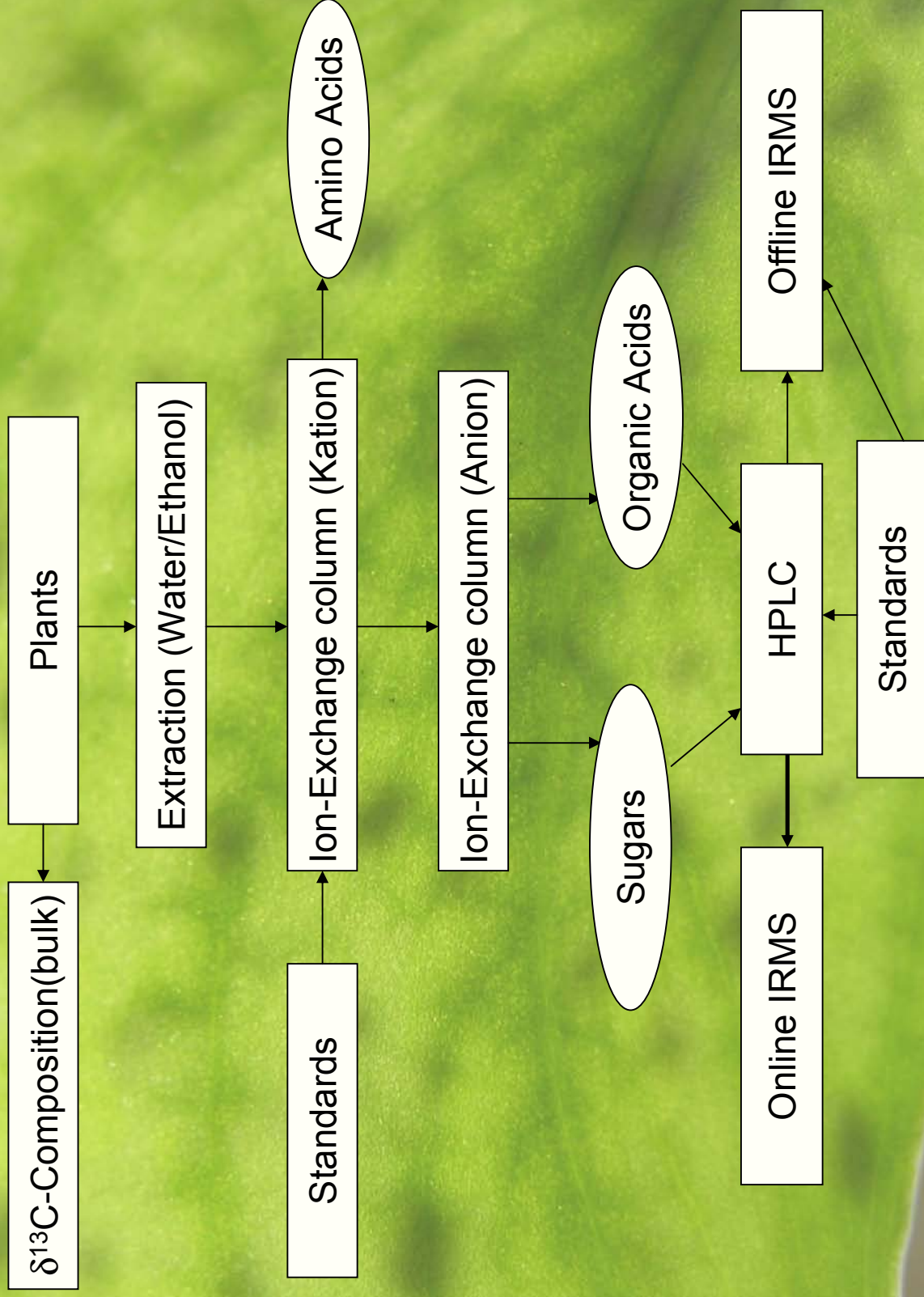
light isotopes

heavy CO₂ molecules,
anaplerotic reaction

Objectives

- To investigate plant metabolism
- To develop and evaluate the ^{13}C measurement of sugars and organic acids
 - to determine system limits
 - to compare online-offline values
 - to verify isolation procedure

Isolation of plant metabolites



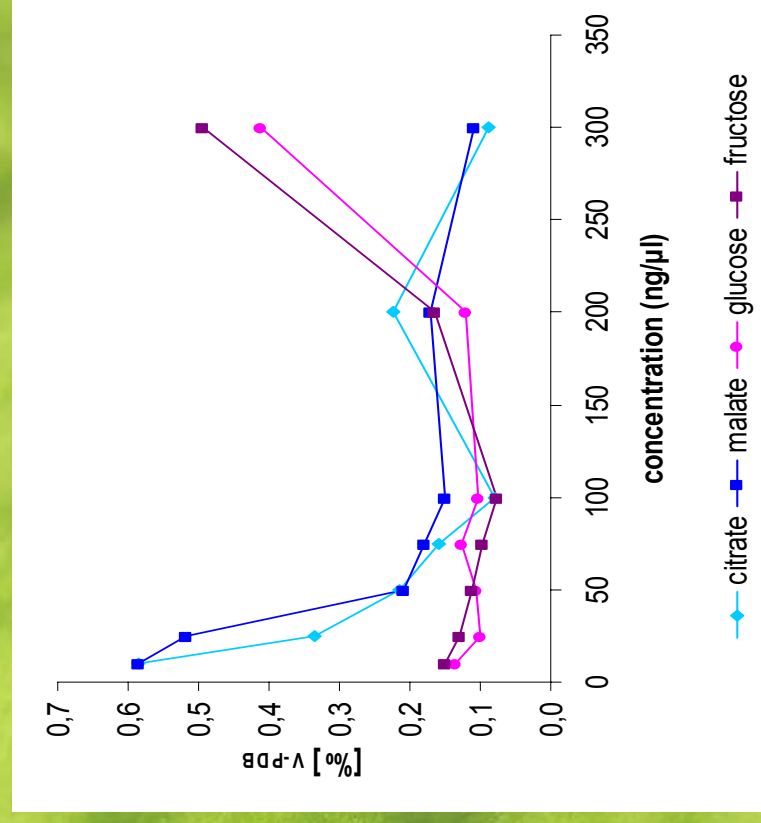
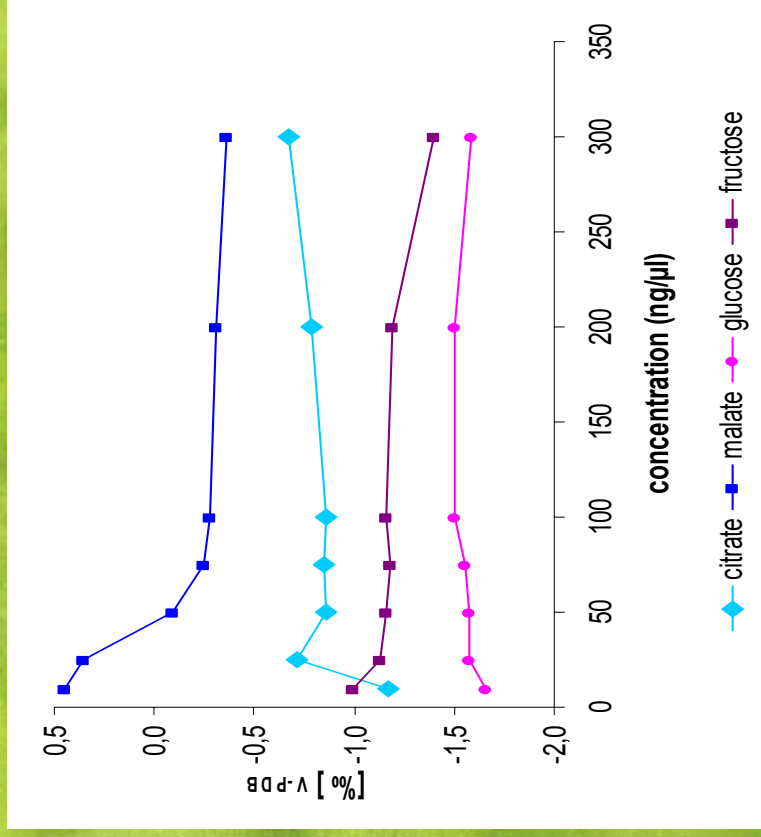
Operational parameters

- Linearity range?
- Oxidation capacity?
- Reaction time?

What determined precision and reproducibility?

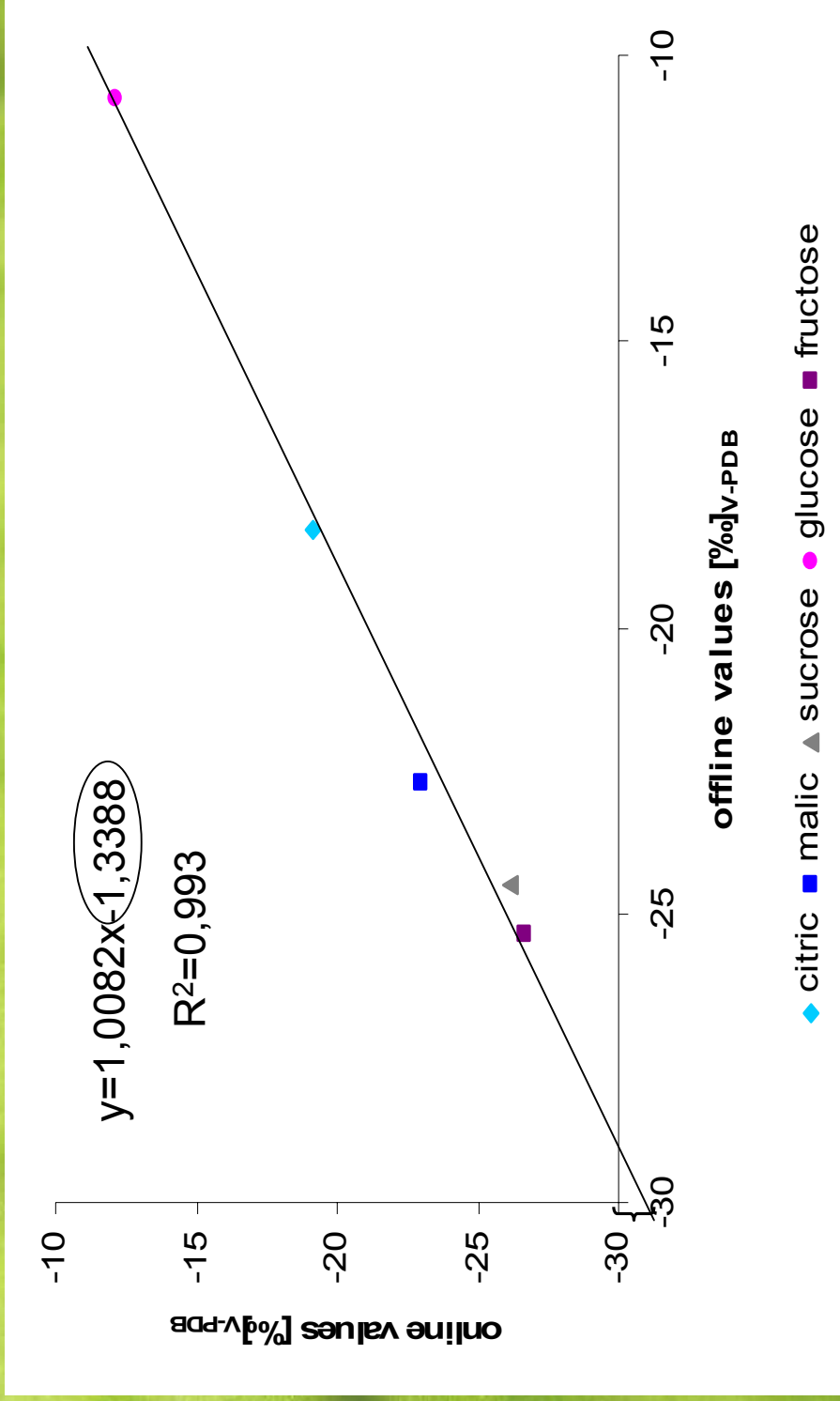
Δ online-offline

St Dev



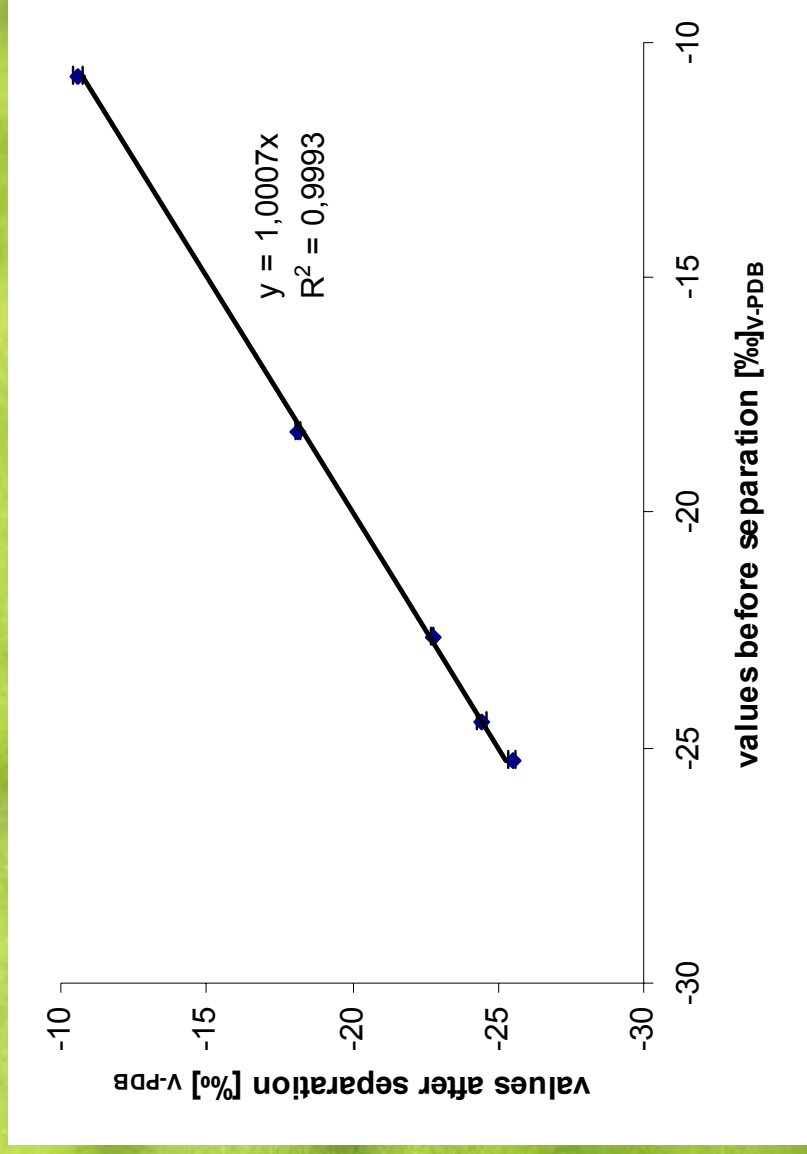
- Optimal amount 50-100 ng/ μ l
- Oxidation level best between 15-30 V for organic acids and 19-30 V for sugars
- Flow rate is optimal by 400 μ l/min for acids and 500 μ l/min for sugars

Online values vs. Offline values

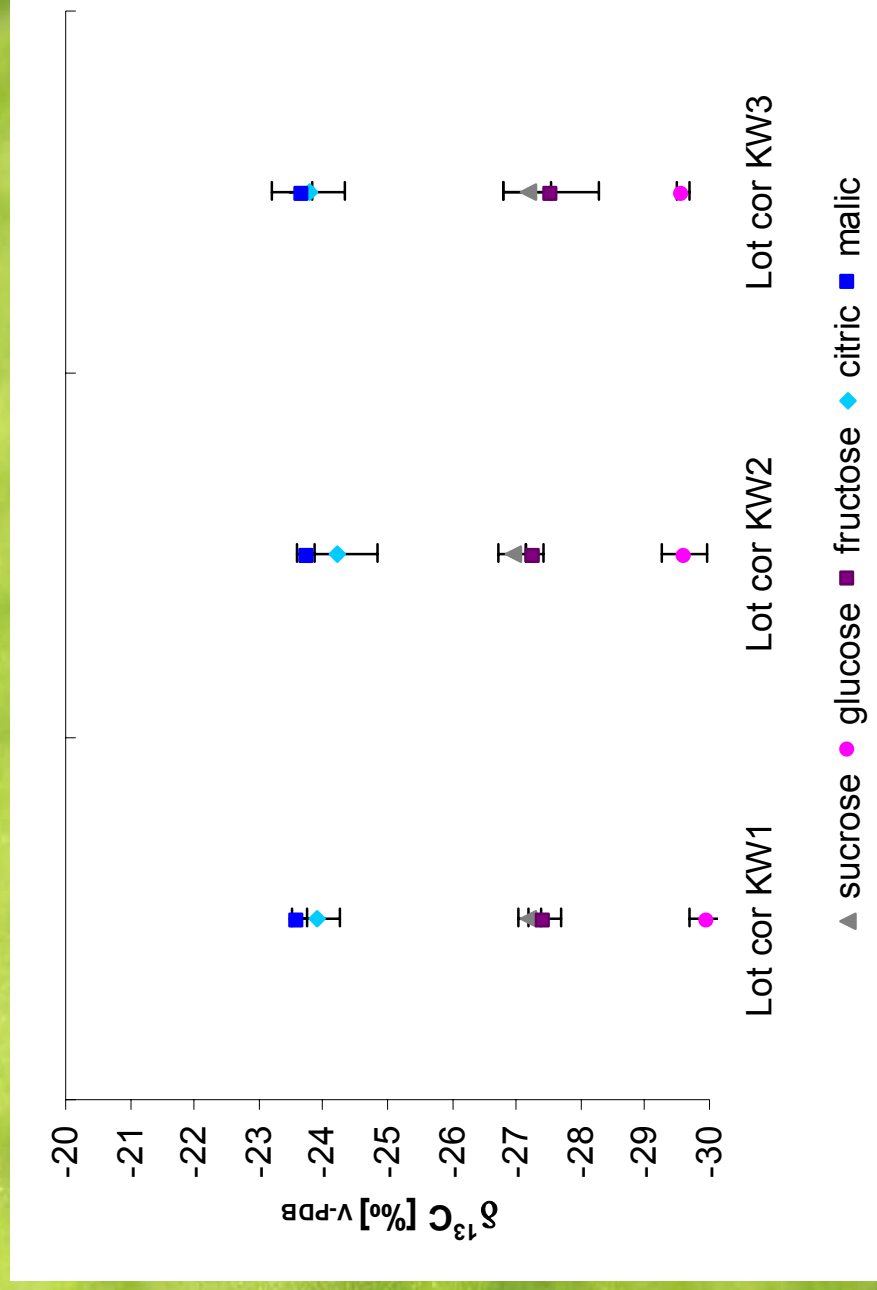


Evaluation of separation procedure

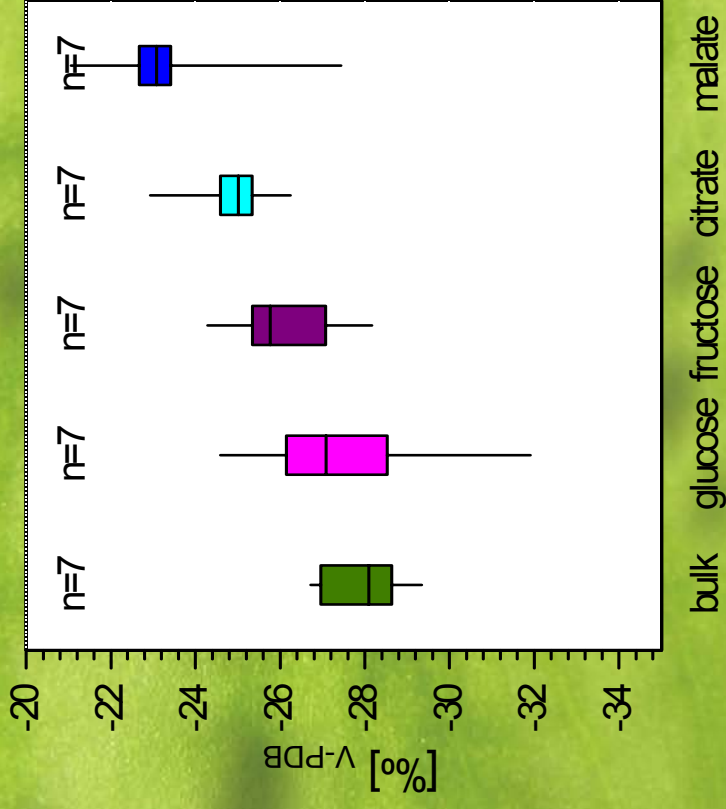
- $n=5$,
- Analytical mean
st dev for
sugars= 0,16‰;
for acids=0,08‰;
- Reproducibility of
the independent
samples
- st dev 0,20 ‰ for
sugars
and 0,09‰ for
organic acids



Reproducibility of metabolite extraction from plants

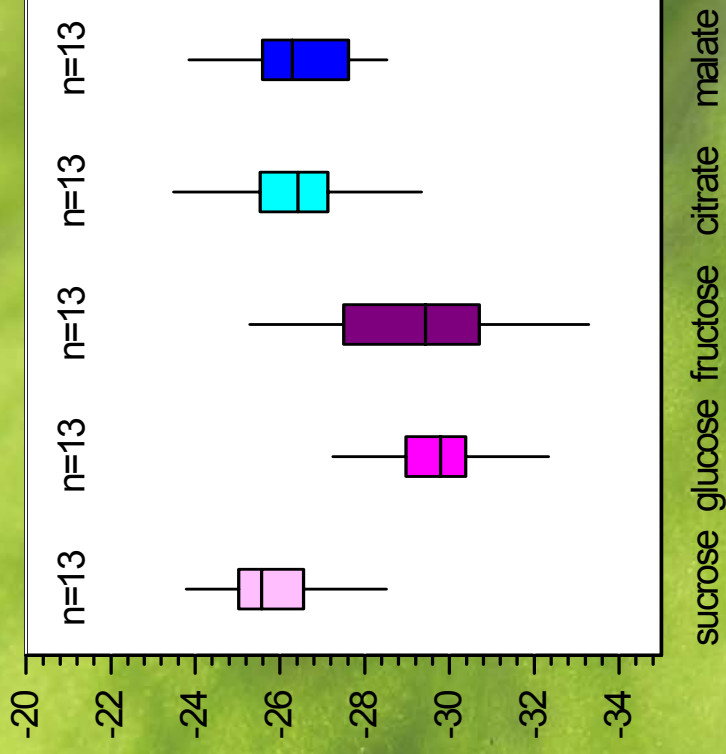


$\delta^{13}\text{C}$ -Composition of sugars and organic acids in plants



bulk glucose fructose citrate malate

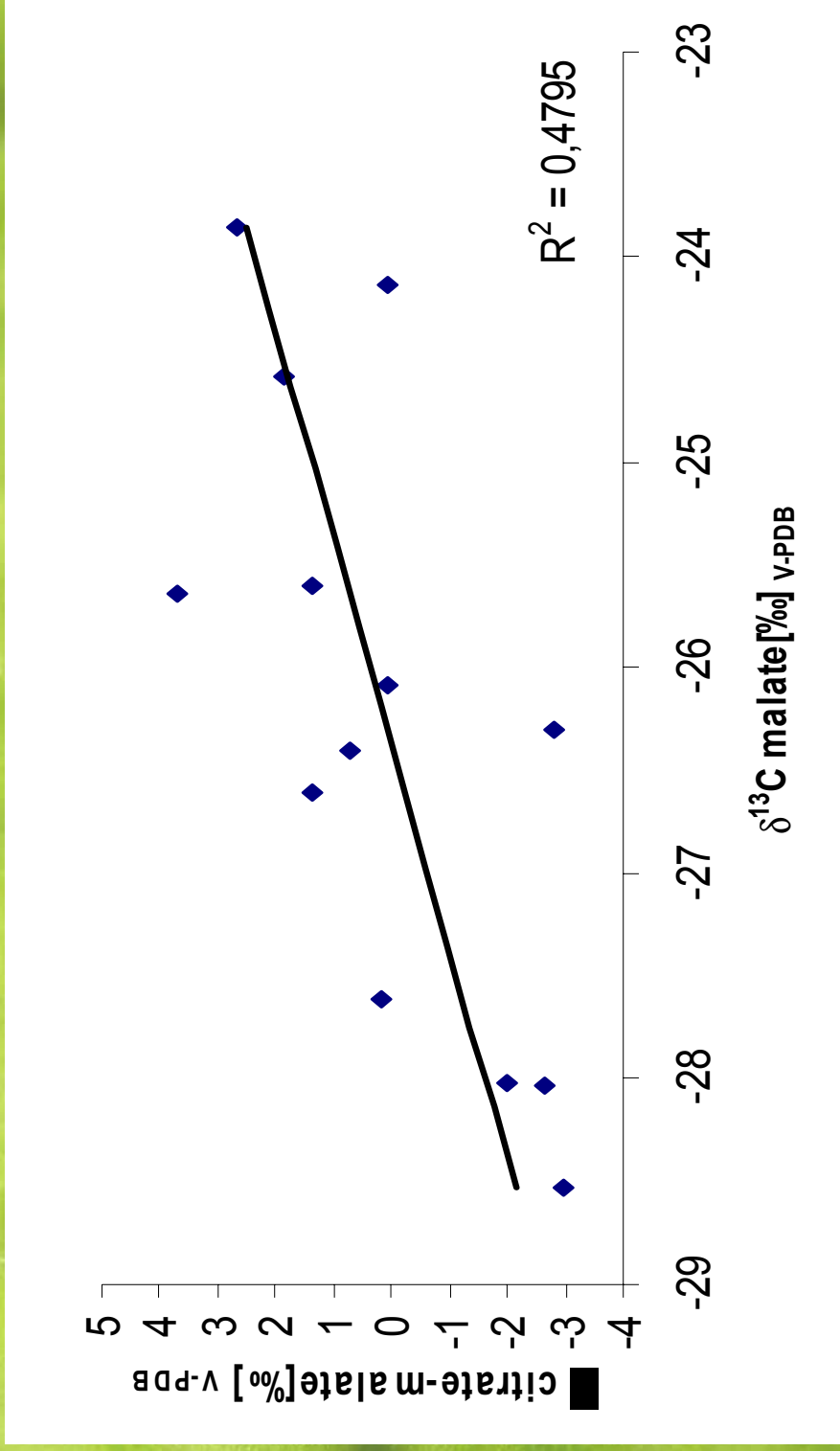
September 2003



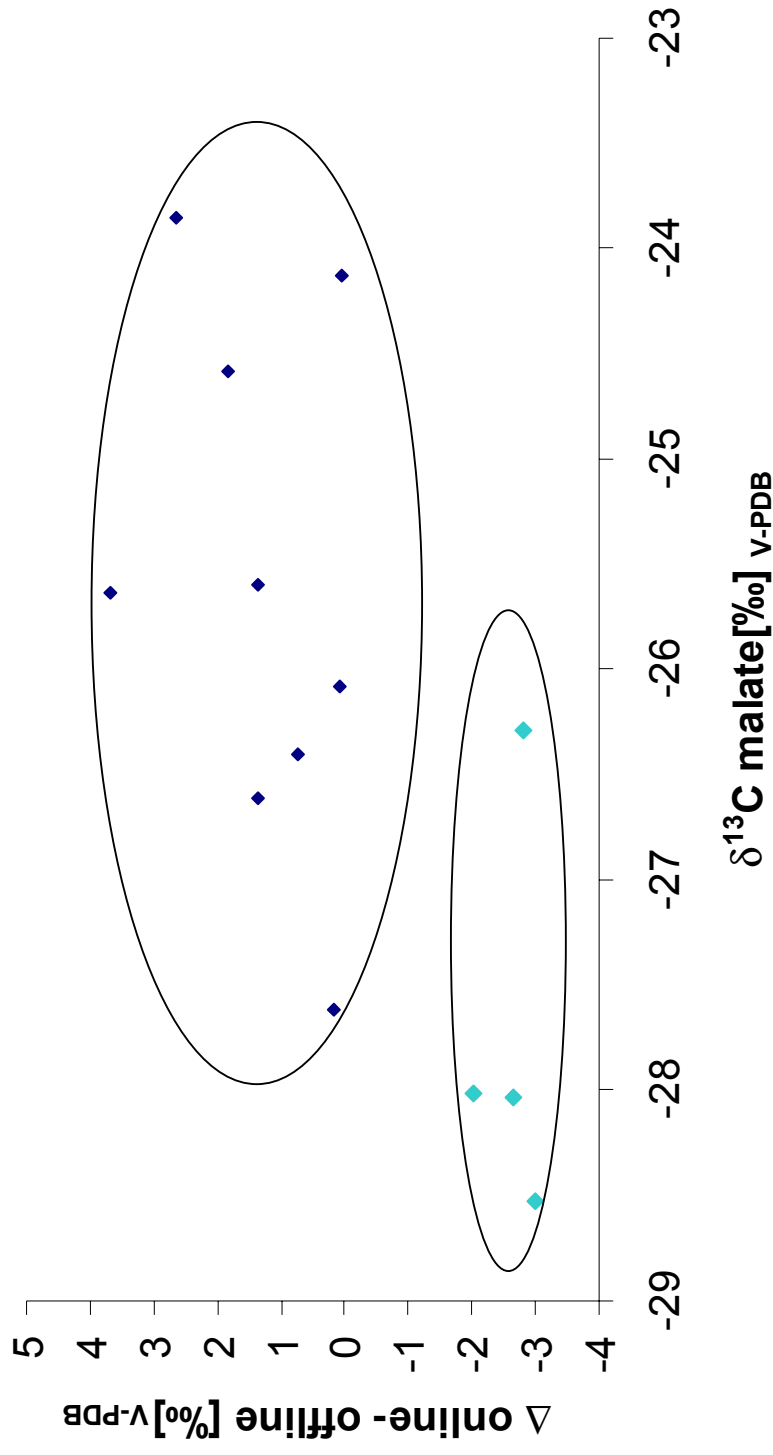
sucrose glucose fructose citrate malate

April 2005

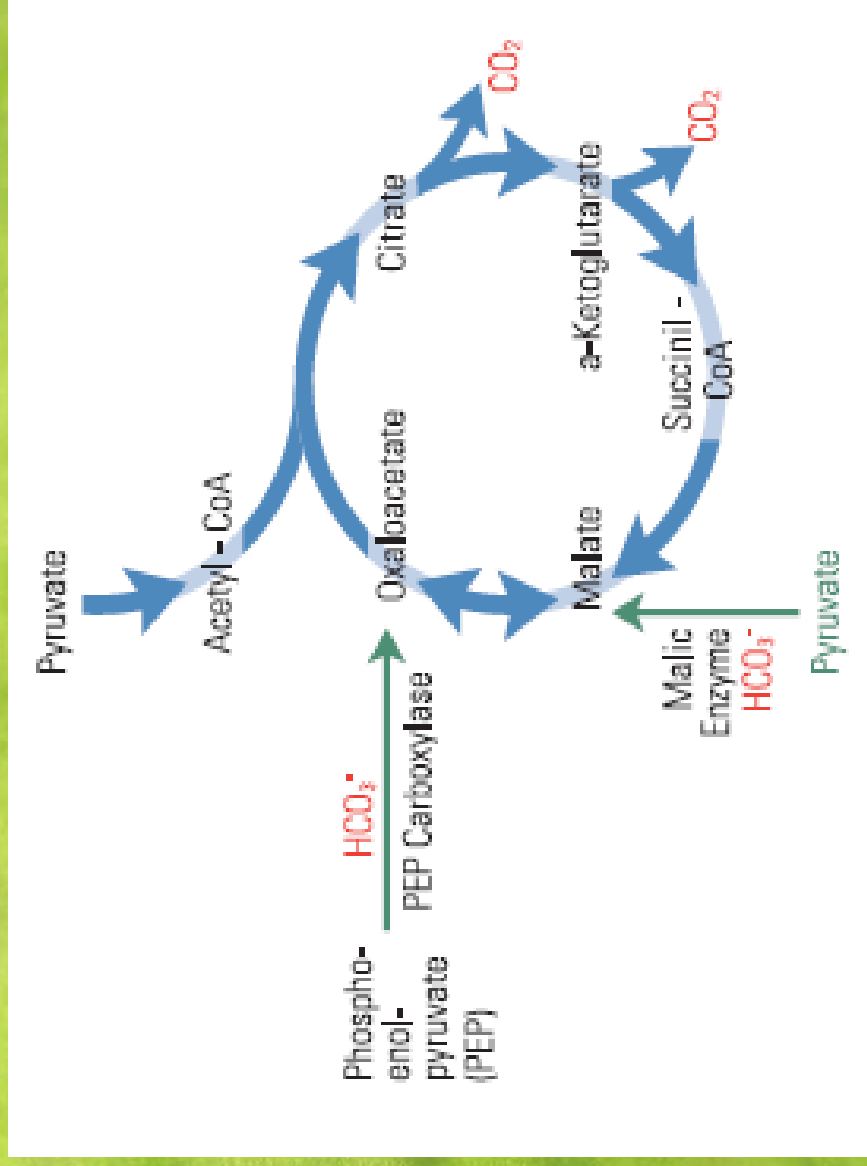
Is it an anaplerotic reaction fixation?



Is it an anaerobic reaction fixation?



Anaplerotic reaction for Krebs cycle



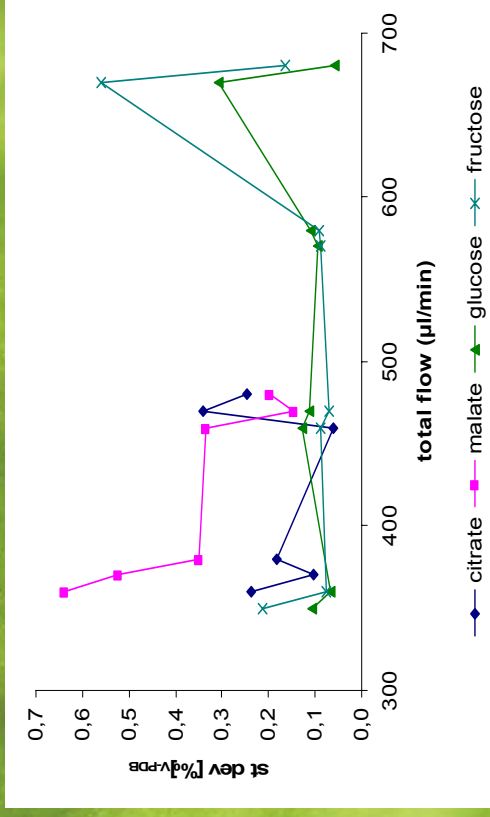
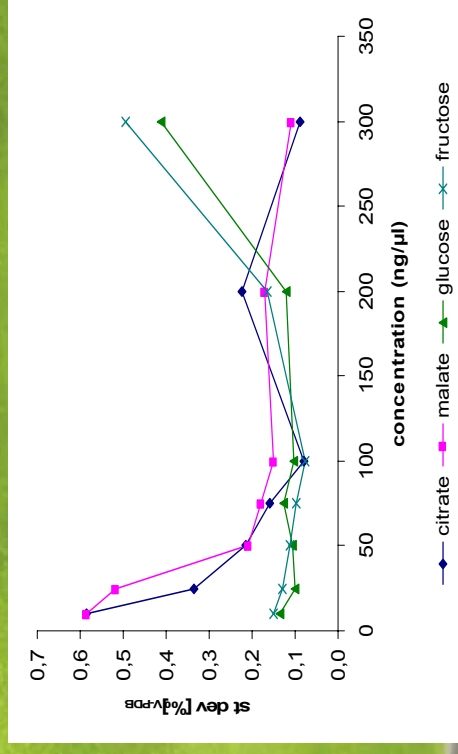
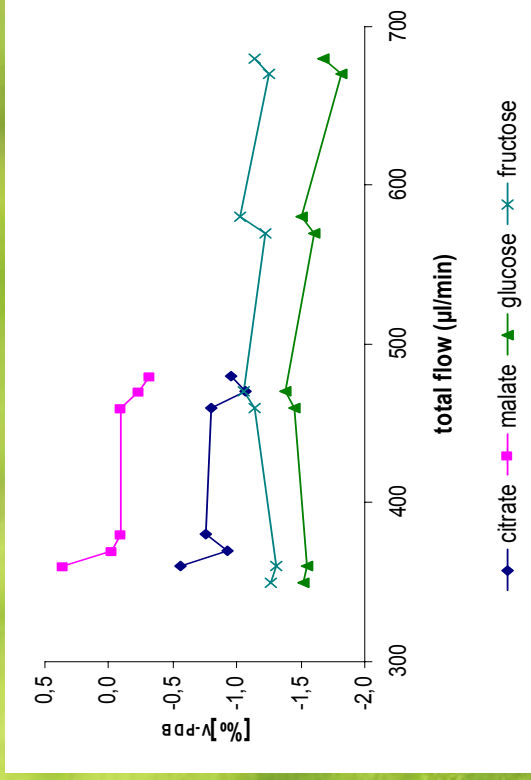
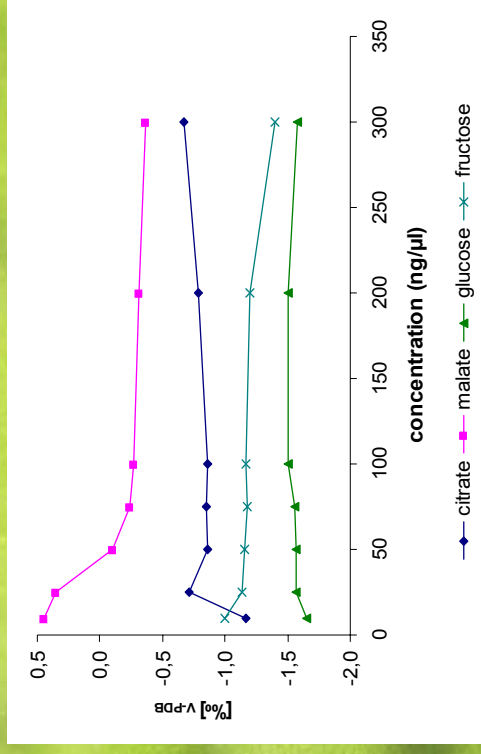
Summary

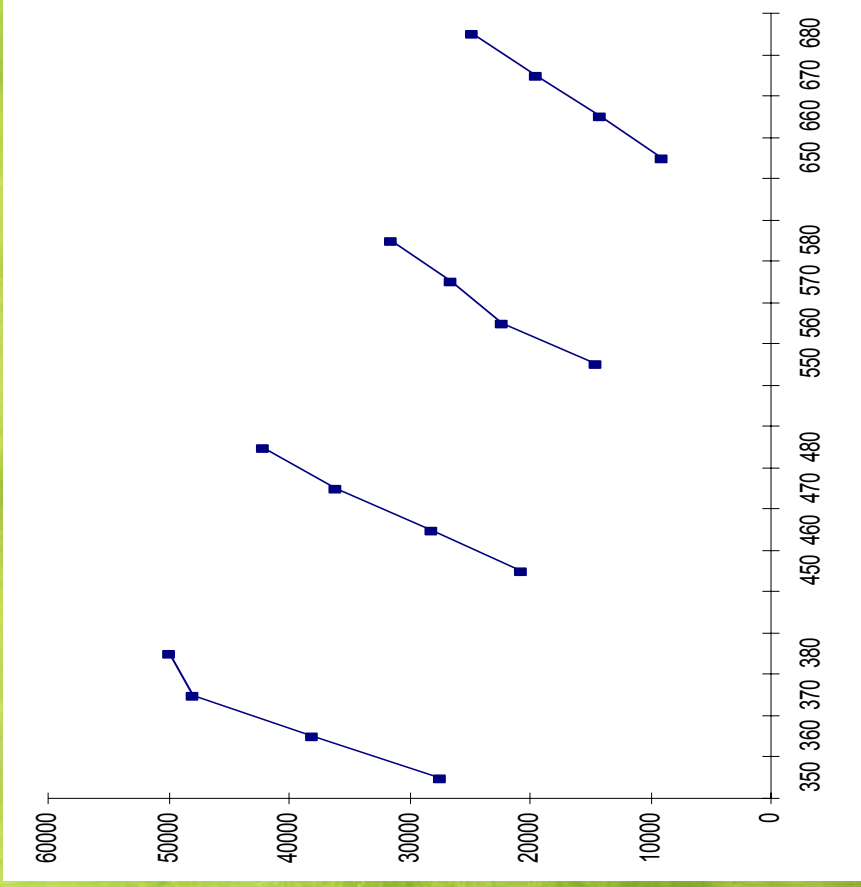
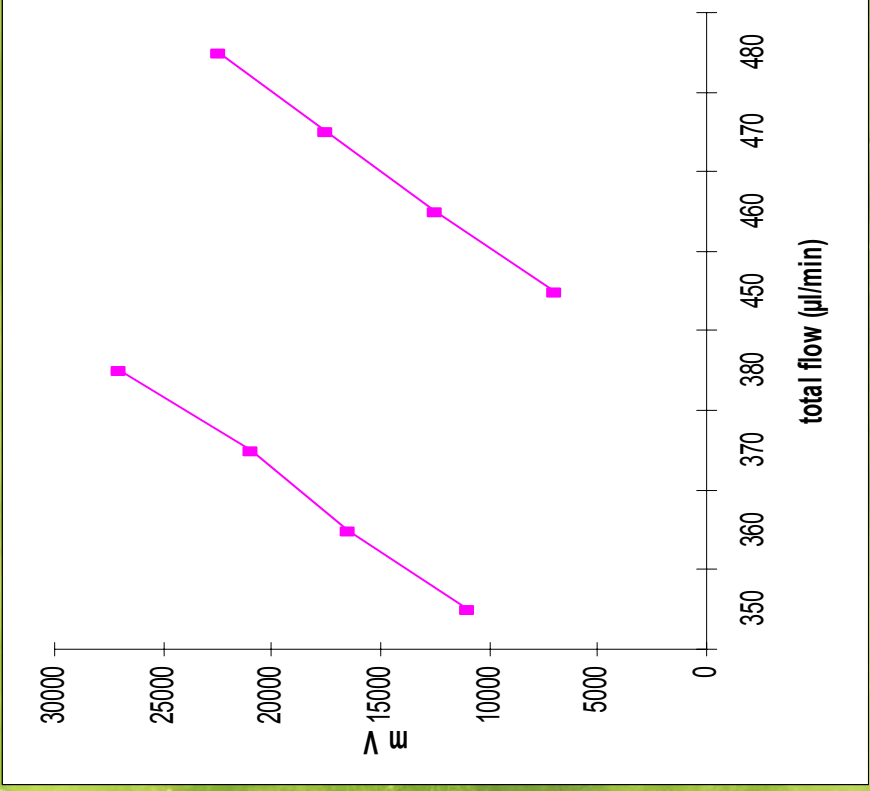
- LC/MS system was evaluated for measuring
- Standard running protocol was set up
- Seasonal differences in plants were found

Thank you for your attention



What determined precision and reproducibility?



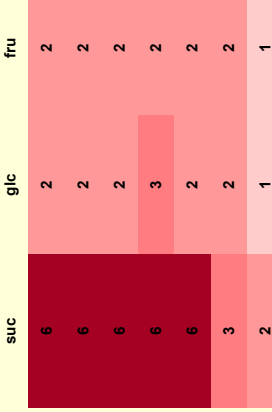


species

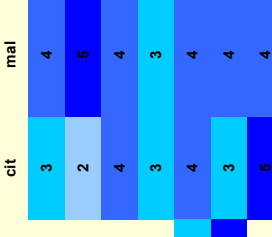
Cyn cit
Tri fla
Phi pra
Fes sub
Fes pra
Alo pra
Bro ere

Gräser

concentration



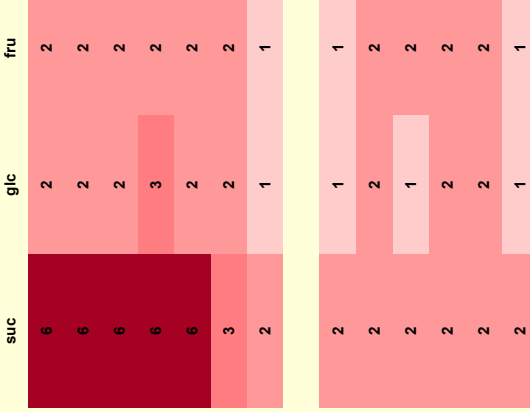
$\delta^{13}\text{C}$ -values



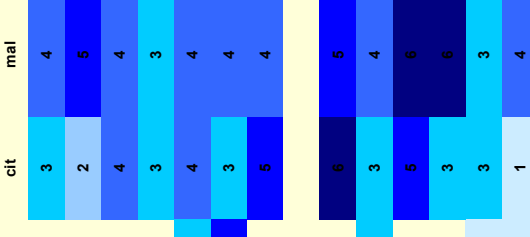
Lat pra
Lot cor
Tri pra
Tri dub
Ono vic
Med var

Leguminosen

concentration



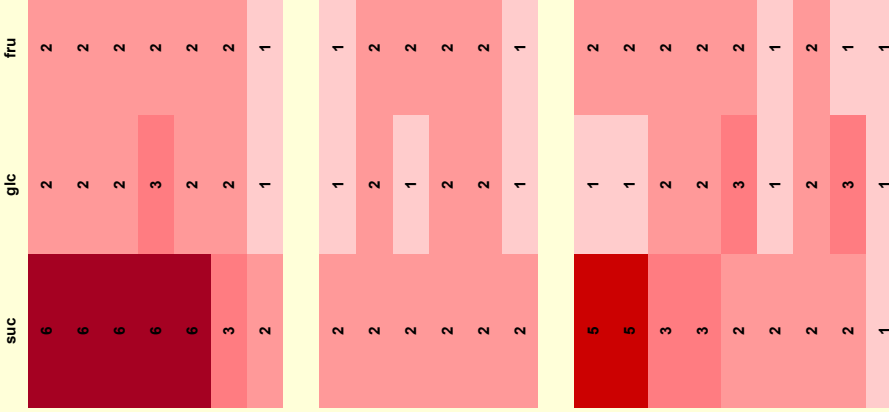
$\delta^{13}\text{C}$ -values



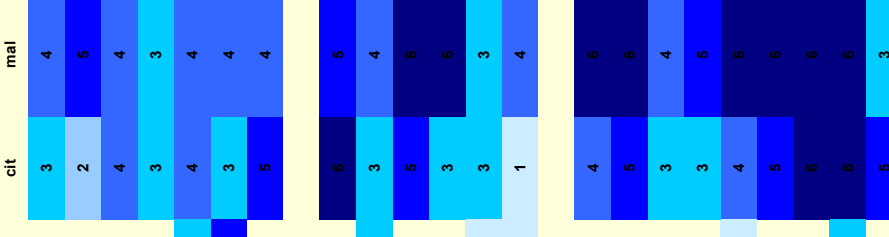
Leo his
Leo aut
Tar off
Pru vul
Pru lu
Pri ver
Pla med
Ver cha
Bel per

Kleine Kräuter

concentration



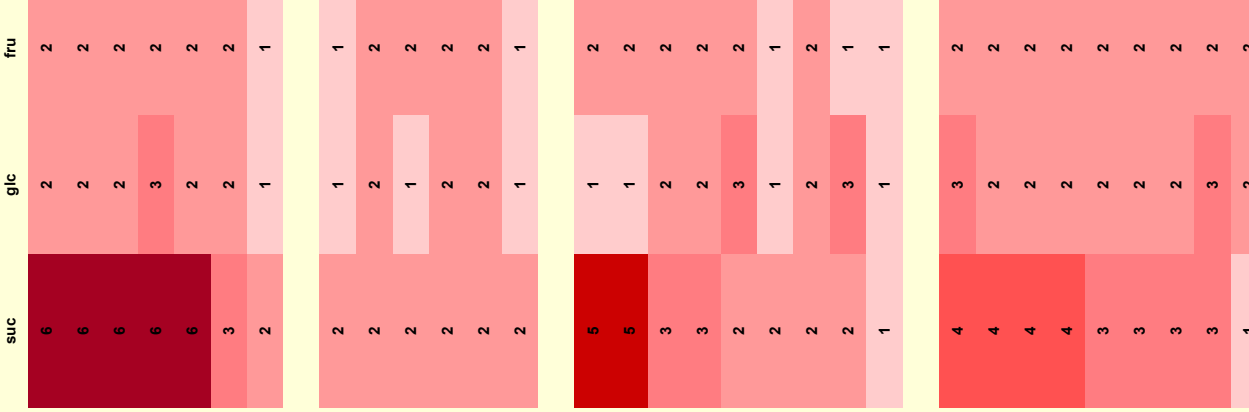
$\delta^{13}\text{C}$ -values



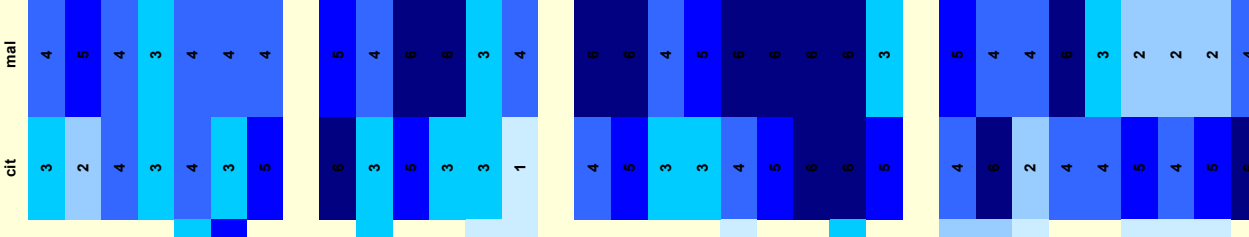
Ran acr
San off
Drau
Ger pra
Cen jac
Leu vul
Ach mill
Kna arv

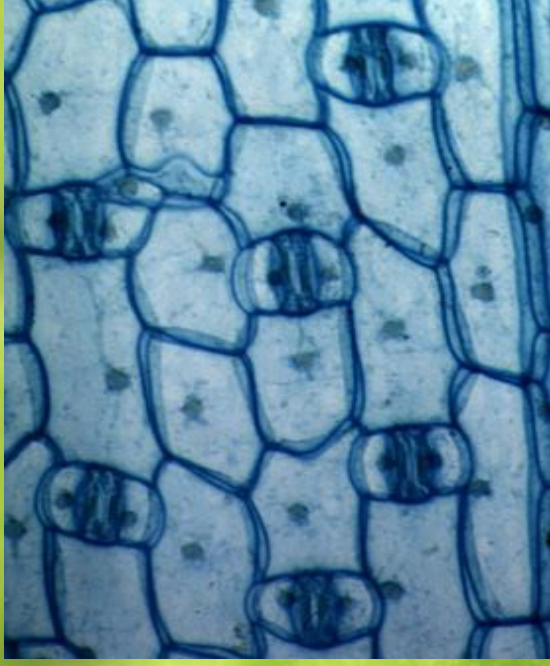
Große Kräuter

concentration

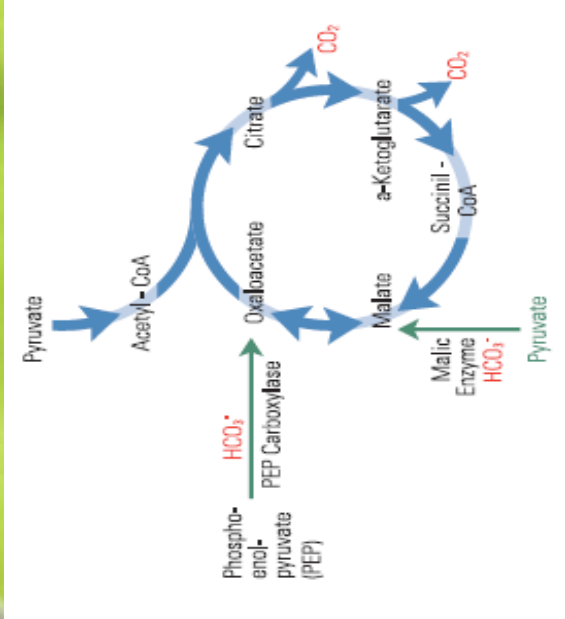


$\delta^{13}\text{C}$ -values





Stomata conductance



Anaplerotic reaction