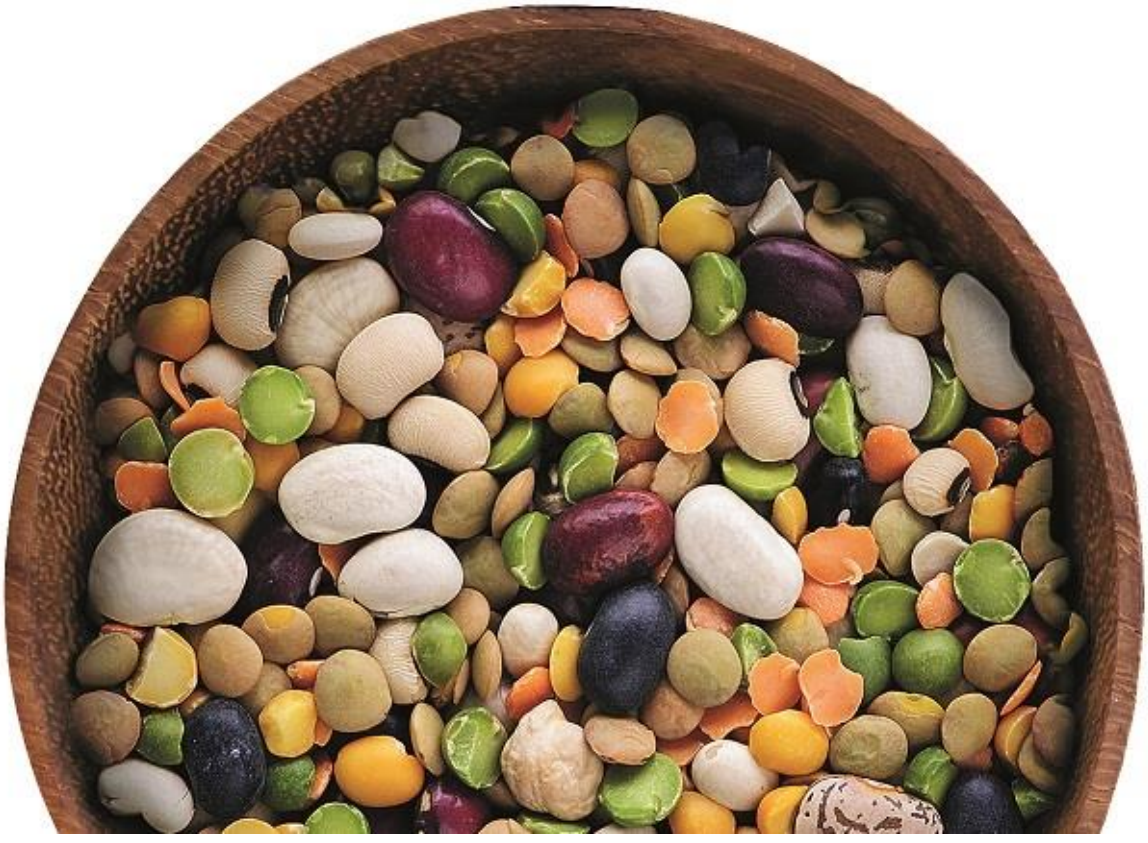
TRansition paths to sUstainable legume-based systems in Europe

Carbohydrate Assay 1: Sugars (Glucose, Sucrose, Fructose)

Date: September, 30th 2018



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Content

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1 Summary Information

1.1 Partner Summary

SOP Code	EU_TRUE_SOP_016
TRUE Partner Acronym	AUA
Primary Author	Ntatsi, Georgia (*ntatsi@aua.gr*)
Other Authors	Savvas, Dimitrios
Linked Reference and Hyperlink (if available)	Peter Geigenberger, Mohammad Hajirezaei, Michael Geiger, Uta Deiting, Uwe Sonnewald, Mark Stitt, 1998. Overexpression of pyrophosphatase leads to increased sucrose degradation and starch synthesis, increased activities of enzymes for sucrose-starch interconversions, and increased levels of nucleotides in growing potato tubers. Planta 205: 428-437 *https://doi.org/10.1007/s004250050340*
Associated files to use with the SOP [and function]	*Not applicable*

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1.2 SOP Summary

Title

Carbohydrate Assay 1: Sugars (Glucose, Sucrose, Fructose)

Brief description

Sugars are formed by the plant during photosynthesis. They can act as signalling molecules and regulators of gene expression and thus regulate plant growth (Eveland *et al.*, 2012). Soluble sugars are highly sensitive to environmental factor variations such as light, water and temperature which may lead to a significant reduction in the supply of soluble sugars to sink tissues due to a decreased efficiency of photosynthesis in source tissues. Sucrose and glucose either act as substrates for cellular respiration or as osmolytes to maintain cell homeostasis while fructose is related to secondary metabolites synthesis (Rosa *et al.*, 2009).

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2 Protocol Steps

1st stage: Extraction

Leaves

- Keep the samples on ice
- Add 200 µL of ethanol 80% to 50 mg frozen powdered material
- Incubate for 20 min at 78 °C and 550 rpm shaking
- Centrifuge for 5 min at 14,000 rpm, 4 °C
- Transfer the supernatant in an eppendorf (keep on ice)
- Add 100 µL of ethanol 50% to the pellet
- Incubate for 20 min at 78 °C and 550 rpm shaking
- Centrifuge for 5min at 14,000rpm, 4 °C
- Transfer the supernatant in an eppendorf (keep on ice)

Roots

- Keep the samples on ice
- Add 400 µL of ethanol 80% to 50 mg frozen powdered material
- Incubate for 20 min at 78 °C and 550 rpm shaking
- Centrifuge for 5min at 14,000 rpm, 4 °C
- Transfer the supernatant in an eppendorf (keep on ice)
- Add 100 µL of ethanol 50% to the pellet
- Incubate for 20 min at 78 °C and 550 rpm shaking
- Centrifuge for 5min at 14,000 rpm, 4 °C
- Transfer the supernatant in an eppendorf (keep on ice)
- Add 100 µL of ethanol 50% to the pellet
- Incubate for 20 min at 78 °C and 550 rpm shaking
- Centrifuge for 5min at 14,000 rpm, 4 °C
- Transfer the supernatant in an eppendorf (keep on ice)

After the extraction, prepare a Master-Mix plate with 200 µL in each well (cell)→ Store at -80 °C

2nd stage: Glucose-Fructose-Sucrose Measurement

- Freshly prepare a master mix (for 100 samples)
  a. 20 mL (50 mM HEPES, pH = 7 + 5 mM $MgCl_2$ (store at 4 °C)
  b. 12 mg NADP
  c. 20 mg ATP (- 80 °C)
  d. 20 µL G6PDH (from yeast 127671)
- Dispense 200 µL of the master mix in a separate well for each sample to be measured
- Add sample (10 µL for leaves and 10 µL for roots)
- Mix plate on thermomixer (max 1,000 rpm)
- Use 3-4 wells for blank (only the Master Mix inside and the enzyme after the baseline)
- Place plate in a microplate reader (Anthos HtII) and read NADPH absorbance at 340 nm (against 405 nm as blank) until you get a baseline

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- Dissolve the enzyme and keep it on ice
  a. Hexokinase
     i. (From yeast, 1426362), centrifuge 50 µL and resuspend pellet in 300 µL HEPPES buffer
     ii. Add 2 µL of hexokinase to the master mix plate to determine glucose
     iii. Let reaction stabilise (about 20 min).

  b. PGI (Phosphoglucoisomerase)
     i. (From yeast, 128139), centrifuge 50 µL and resuspend pellet in 300 µL HEPPES buffer
     ii. Add 2 µL of PGI (phosphoglucoisomerase) to the master mix plate to determine Fructose
     iii. Let reaction stabilise (about 10 min)

  c. Invertase
     i. Dissolve 50 mg in 500 µL HEPES buffer
     ii. Add 4 µL of Invertase to the master mix plate to determine Sucrose
     iii. Let reaction stabilise (about 30 min)

Calculations

Sugars = (HL - BL) * ((E+L)/S) * (1/L) * DF * 1000

with Sugars = Glucose or Fructose or Sucrose (µmol mg^{-1} FW)
BS = Baseline
HL = Absorbance of each sugar
S = µL of supernatant
DF = Dilution Factor
L = mg of Leaves
E = µL of leaf extraction

To calculate the dilution factor (DF)

For example, if you extract 250 mg of tissue with 750 µL of extraction buffer, the dilution factor is 4 (250 µL + 750 µL) / 250 µL).

Additional References

Andrea L. Eveland, David P. Jackson 2012. Sugars, signalling, and plant development, Journal of Experimental Botany, Volume 63, Issue 9, 3367–3377. https://doi.org/10.1093/jxb/err379

Rosa, M., Prado, C., Podazza, G., Interdonato, R., González, J. A., Hilal, M., & Prado, F. E. (2009). Soluble sugars—Metabolism, sensing and abiotic stress: A complex network in the life of plants. Plant Signalling & Behaviour, 4(5), 388–393.

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3 Linked SOPs

SOP Code	SOP Function
EU_TRUE_SOP_017	Carbohydrate Assay 2: Starch

4 Disclaimer

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