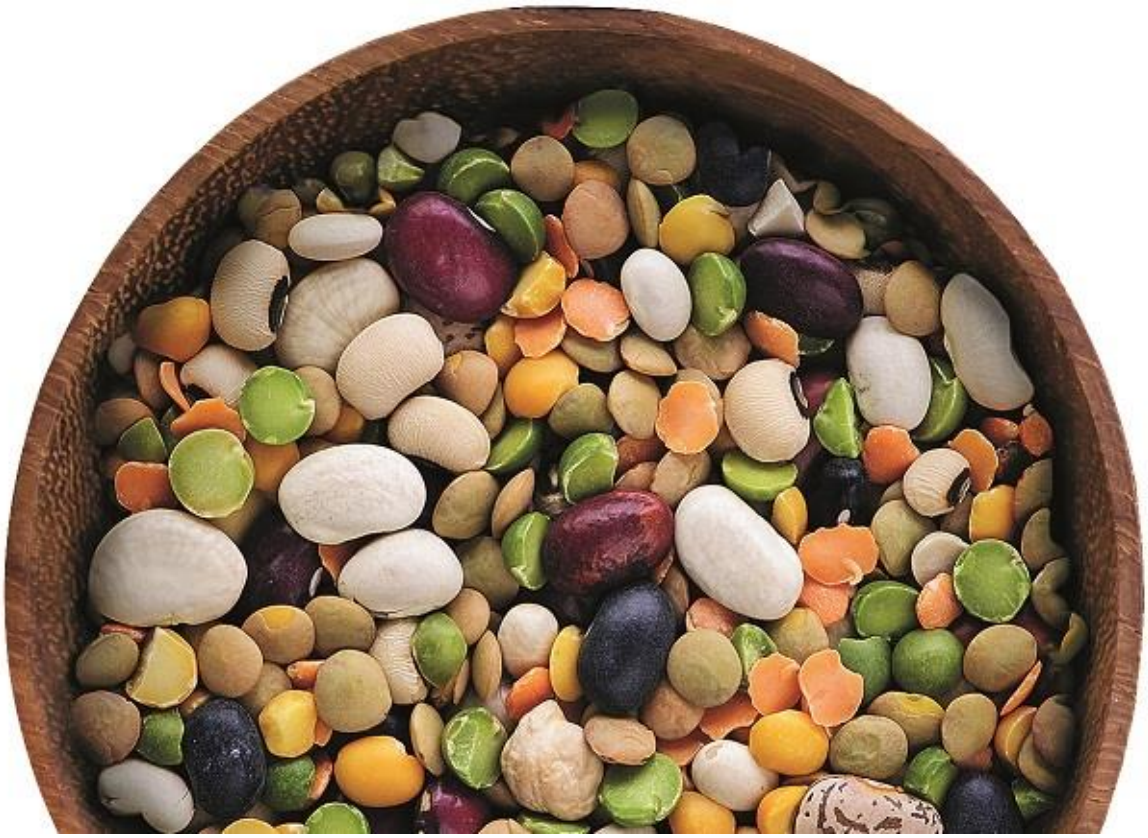
TRansition paths to sUstainable
legume-based systems in Europe

Oxidation parameters 1: MDA

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_020
TRUE Partner Acronym	AUA
Primary Author	Ntatsi, Georgia (*ntatsi@aua.gr*)
Other Authors	Savvas, Dimitrios
Linked Reference and Hyperlink (if available)	D. Mark Hodges, John M. DeLong, Charles F. Forney, Robert K. Prange 1999 Improving the thiobarbituric acid-reactive-substances assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds Planta 207: 604-611 *https://link.springer.com/article/10.1007/s004250050524* Yong He, Zhujun Zhu, Jing Yang, Xiaolei Ni, Biao Zhu 2009 Grafting increases the salt tolerance of tomato by improvement of photosynthesis and enhancement of antioxidant enzymes activity Environmental and Experimental Botany 66: 270–278 *https://www.sciencedirect.com/science/article/pii/S0098847209000343*
Associated files to use with the SOP [and function]	*Not applicable*

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1.2 SOP Summary

Title

Oxidation parameters 1: Malondialdehyde (MDA)

Brief description

The occurrence of malondialdehyde (MDA), a secondary end-product of the oxidation of polyunsaturated fatty acids, is considered a useful index of general lipid peroxidation. Malondialdehyde is formed through auto-oxidation and enzymatic degradation of polyunsaturated fatty acids in cells.

Lipid peroxidation was determined following the method of He *et al.*, 2009 and Hodges *et al.*, 1998. Malondialdehyde (MDA) was extracted together with 5 mL of 5% (w/v) trichloroacetic acid at approximately 0°C. The thiobarbituric acid test was used to assay the MDA content in leaves according to the method of Hodges *et al.* (1999). The concentration of thiobarbituric acid reactive substances (TBARS) was calculated as MDA equivalents using the extinction coefficient of 155 mmol mol^{-1} cm^{-1} for malondialdehyde and expressed on a dry weight (DW) basis. Lipid peroxidation was determined according to the method of He *et al.*, 2009 and Hodges *et al.*, 1998.

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2 Protocol Steps

- 0.1 g fresh tissue homogenised in 1 mL of 0.1% (w/v) trichloroacetic acid (TCA).
- The homogenate was centrifuged at 10,000 χ *g* (max rpm) for 10 min at 4 °C.
- The reaction mixture in a total volume of 1 mL containing:
  - ✓ in the **+TBA solution:** 0.5 mL of extracts, 0.5 mL of 0.65% (w/v) TBA (2-thiobarbituric acid) made in 20% TCA) (heat the reaction mixture of TBA and TCA);
  - ✓ and in the **- TBA solution:** 0.5 mL of extracts, 0.5 mL of 20% TCA)
- The reaction mixture was heated at 95 °C for 30 min and then stopped by quickly placing it in an ice-bath for 5-10 min.
- After centrifugation at 10,000 χ *g* for 10 min, the absorbance of the supernatant at 440 (+TBA), 532 (+TBA, -TBA) and 600 nm (+TBA, -TBA) was read.
- After subtracting the non-specific absorbance at 600 nm of a solution containing plant extract incubated without TBA from an identical solution containing TBA, the TBA-MDA concentration was determined by its extinction coefficient of 157 mM^{-1} cm^{-1}.

Malondialdehyde equivalents were calculated in the following manner. First calculate:

A = [(Abs532 + TBA) - (Abs600 + TBA) - (Abs 532 - TBA- Abs600 - TBA)]

B = [(Abs440 + TBA - Abs600 + TBA) 0.0571]

with Abs532 + TBA: Absorbance of the +TBA solution at 532 nm
Abs600 + TBA: Absorbance of the +TBA solution at 600 nm
Abs 532 - TBA: Absorbance of the - TBA solution at 532 nm
Abs600 - TBA: Absorbance of the - TBA solution at 600 nm
Abs440 + TBA: Absorbance of the +TBA solution at 440 nm
Abs600 + TBA: Absorbance of the +TBA solution at 600 nm

Then with A and B, calculate

MDA equivalents (nmol. mL^{-1}) = (A-B) / 0,157

MDA equivalents (nmol. mL^{-1} g^{-1}) ={(A-B) / 0,157) (1/g of sample)}

We calculate 1 mL → 0.1g (We use 100 mg diluted in 1 mL of TCA, TBA) so 10mL → 1 g. That means that we need to multiple the MDA equivalents (nmol. mL^{-1} g^{-1}) ={((A-B)/0.157) (1/g of sample)} with 10.

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- MDA equivalents (nmol. ml^{-1} g^{-1}) ={((A-B)/0.157) (1/g of sample)} *10

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3 Linked SOPs

SOP Code	SOP Function
EU_TRUE_SOP_022	Oxidation parameters 2: H_2O_2
EU_TRUE_SOP_023	Oxidation parameters 3: Electrolyte leakage

4 Disclaimer

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