Towards a reproducible and high-throughput workflow to quantify globulins and napins, the two major seed storage proteins in oilseed rape

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This work was supported by:

Seed storage proteins (SSP) in oilseed rape consist of 12S globulins (cruciferins) and 2S albumins (napins) that stand for around 70% of total seed proteins. Since napins contain more sulfur residues than cruciferins, they display a higher value for food or feed usages. Recent results demonstrated the potentialities of genetics to improve the 12S/2S ratio. However, the current methods to assess the SSP balance are still time consuming and difficult to handle, thus preventing the phenotyping of large sets of accessions.

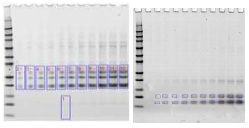
Objective Establish a simple, high-throughput method to assess the 12S and 2S contents in oilseed rape

Plant material 100 accessions of winter oilseed rape :

24	++	high erucic acid (EA) and glucosinolates (GSL)
12	0+	low EA and high GSL
64	00	low EA and GSL

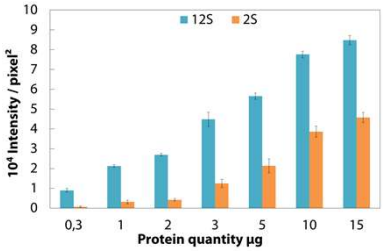
Electrophoresis quantification

Increasing amounts of 12S and 2S standards or seed extracts were separated onto electrophoresis gel and stained with Coomassie Blue. Gels were scanned and the level of SSP was determined by image analysis using Mutigauge Software (Fujifilm).



Electrophoresis gel integration for 12S and 2S standards

☺ Fast, no need to defatting
☹ Coomassie staining differs from 12S and 2S



Bands intensity according to the quantity

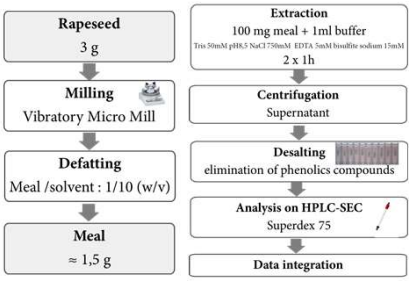
⇨ Coomassie blue interact with Arginine

	Uniprot	Arg content
2S	P80208	7
12S	P33524	27

⇨ Staining kinetics , sensitivity

12S and 2S contents estimated by Size Exclusion Chromatography

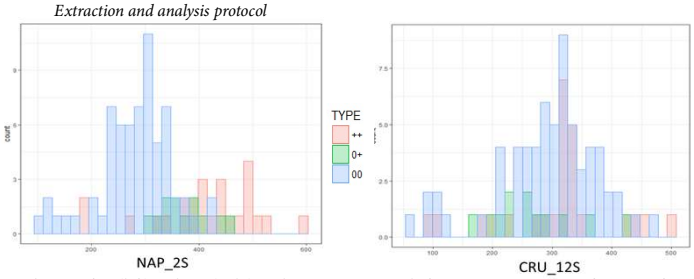
SSP were extracted from milled and defatted seeds. Pigments were removed by desalting chromatography. Samples were injected onto size exclusion chromatography (SEC - S75 Increase, GE Healthcare) equipped with a refractometer detection. After calibration, SSP contents was deduced from area response. Highly reproducible results were obtained. Therefore SEC was retained as the reference method.



Extraction and analysis protocol

☺ Robust
☹ Very long, 8 samples in triplicates / day

Bérot et al., J. Chromatogr. B, 818, 2005, 35-42
Defaix C. , et al. Food Chemistry, 287, 2019, 151-159.

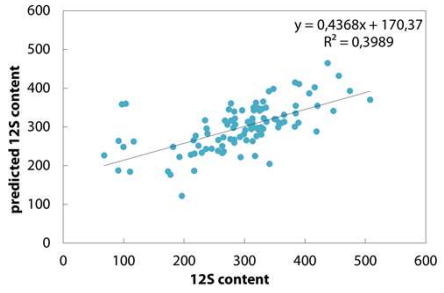


Distribution of 2S (left) and 12S (right) seed contents in a panel of 100 WOSR accessions after quantification by SEC

Near InfraRed Spectroscopy

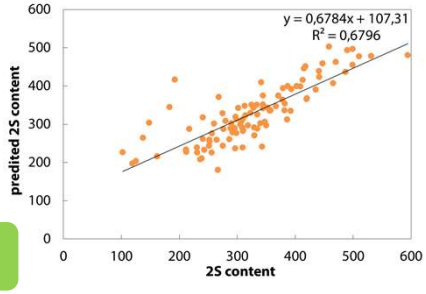
Spectrum acquisition was performed using a FT-NIR spectrometer (MPA, FT-NIR multi-function analyzer, (Bruker Optics, Ettlingen, Germany)). Seeds were placed in a rotating quartz cup with a diameter of 51 mm (Bruker, reference IN311-S). The spectra were collected in reflectance mode from 4,000 to 10,000 cm^{-1} with an optical resolution of 16 cm^{-1} and averaged 64 scans. A principal component analysis was carried out on these spectra in order to confirm that there is no grouping according to the type of seed 00 / + 0 / ++. Models have been optimized using the OPUS 8.1 software (Bruker®).

12S prediction model



Data treatment	1st derivative Normalization
Factor numbers	5
RMSECV	63.7
RPD	1.3
Prediction error < 10 %	46 samples /100

2S prediction model



Data treatment	2nd derivative
Factor numbers	6
RMSECV	58
RPD	1.52
Prediction error < 10 %	60 samples /100

☺ Fast, non destructive
☹ To be improved

Conclusion:

Encouraging results for 2S quantification with NIRS. The current calibrations can be used to roughly sort out the accessions upon 12S/2S content but need further improvements (e.g., wider calibration sets).

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