



HAL
open science

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

Véronique Solé-Jamault, Aude Le Goff, Sophie Rolland, Nathalie Nesi

► **To cite this version:**

Véronique Solé-Jamault, Aude Le Goff, Sophie Rolland, Nathalie Nesi. Towards a reproducible and high-throughput workflow to quantify globulins and napins, the two major seed storage proteins in oilseed rape. IRC 2019, Jun 2019, Berlin, Germany. <hal-03236082>

HAL Id: hal-03236082

<https://hal.inrae.fr/hal-03236082v1>

Submitted on 26 May 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



HAL Authorization

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

Véronique Solé-Jamault¹, Aude Le Goff¹, Sophie Rolland², Nathalie Nesi²

¹ UR1268 BIA, INRA, F-44316 Nantes, France

² UMR1349 IGEPP, INRA-Agrocampus Ouest-Université Rennes1, F-35650 Le Rheu, France

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.

UR 1268 **bia** Biopolymères Interactions Assemblages
IGEPP Institut de Génétique, Environnement et Protection des Plantes
 This work was supported by:



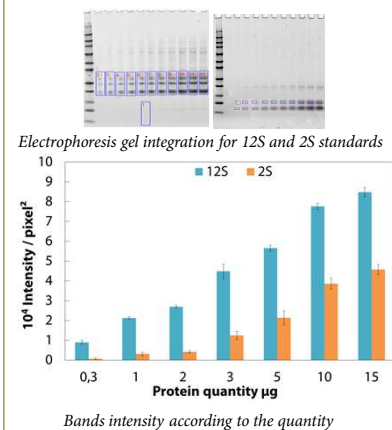
Objective Establish a simple, high-throughput method to assess the 12S and 2S contents in oilseed rape

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 Mutigaugue Software (Fujifilm).



- ⊕ Fast, no need to defatting
- ⊕ Coomassie staining differs from 12S and 2S

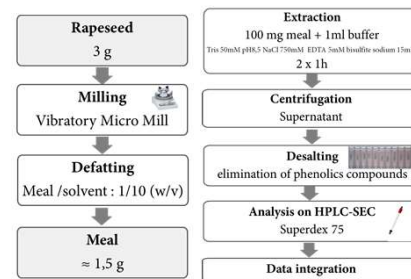
⇒ Coomassie blue interact with Arginine

	Uniprot	Arg content
2S	P80208	7
12S	P33524	27

⇒ Staining kinetics, sensitivity

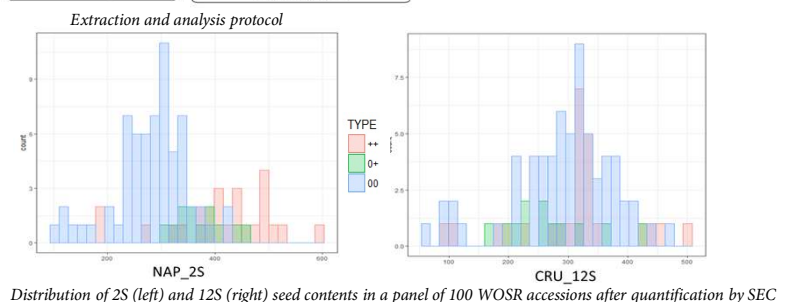
12S and 2S contents estimated by Size Exclusion Chromatography

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.



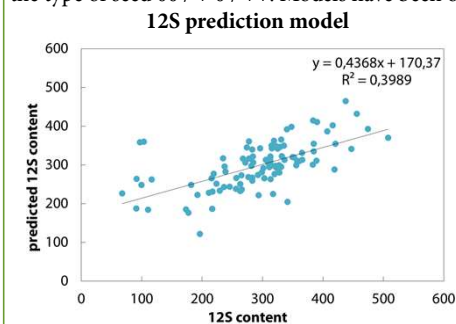
- ⊕ Robust
- ⊕ Very long, 8 samples in triplicates / day

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



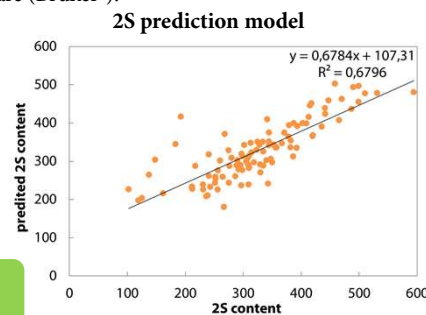
Near InfraRed Spectroscopy

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®).



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

- ⊕ Fast, non destructive
- ⊕ To be improved



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

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).

