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Quantification of Oil Content in Intact Sugar Beet Seed by Near-Infrared Spectroscopy

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Abstract: Sugar beet seed oil reserves play an important role in successful germination and seedling development. The purpose of this study was to establish a non-destructive near-infrared (NIR) methodology with good predictive accuracy to quantify stored seed oil in sugar beet seed. Reflectance NIR spectra were acquired from viable monogerm seeds. Calibration equations were developed using partial least squares. The optimized calibration model reached a Pearson correlation of 0.946; an independent prediction test reached a correlation of 0.919 and a Root Mean Square Error of Prediction of 0.388. The possible role of the outer pericarp in the prediction of oil content was additionally considered. The results indicate that the model is suitable for a rapid and accurate determination of the oil content in both polished and unpolished sugar beet seeds. This NIR application might help to understand the role of seed energy reservoirs in sugar beet germination and further plant growth.

Keywords: near-infrared spectroscopy; oil content; seed reserves; sugar beet

1. Introduction

Sugar beet (*Beta vulgaris* L. subsp. *vulgaris* var. *altissima*) is an important agronomic crop worldwide because, together with sugarcane, it is the world's main source of sucrose and an increasingly important source of bioethanol. The crop yield is highly influenced by seed germination and seedling vigour. Moreover, the quality of germination is highly dependent on the primary energy sources gathered in the seed.

The sugar beet embryo stores oil, protein, and starch as initial energy reservoirs. Before the germinating embryo is able to initiate photosynthesis, it relies solely on these stored reserves for energy and carbon. The content of seed oils, also known as triacylglycerols, has been positively correlated to germination earliness. In fact, about 80% of plant species rely almost exclusively on stored seed oil for germination and plant establishment [1,2]. In a sugar beet seed, oil seems to be the main energy supply required for seed vigour and successful seedling growth, especially under stressful environmental conditions [3–6]. The concept of seed vigour comprises a combination of successful and rapid germination with an additional stress tolerance towards diverse environments. Seed vigour is consequently a very complex trait influenced by multiple elements. However, some important factors that affect the earliness of germination and later plant fitness have been identified including an efficient mobilization of seed storage reserves during germination, the content of oil in seed, and the specific fatty acid composition of that oil [2,7–9].

This study concentrates on the quantification of oils stored in seed through near-infrared (NIR) spectroscopy technology coupled to chemometrics. The NIR technique has been used already in the early 1960s to detect the moisture content in seeds [10]. The charm of this methodology relies greatly on its uncomplicated sample presentation; it is a fast (a single measurement takes only a few seconds), non-destructive and reagent-free methodology, with levels of precision comparable to the primary reference methods and easily adaptable to industrial measurements [11,12]. Over the years, the NIR applications in agriculture have slowly, but steadily, been spreading and are nowadays well established in the seed sector for determining protein, moisture, and oil content [13–18]. Most of the NIR research in seed has focused on agronomically important crops, mainly cereals and oilseeds, while in the sugar beet sector NIR research has prioritized until now the quantification of yield-related traits and traits affecting sugar quality [12,19,20].

What is usually referred to as sugar beet seed is botanically actually a fruit. However, for sake of brevity and functionality, these dispersal units are traditionally called seeds. The technically true seed in sugar beet remains confined by a thick and compact coat of sclerenchyma cells originating from the fruit, known as pericarp [8]. Partial removal of the outer layer of the pericarp, also called soft pericarp, is a commonly used procedure to achieve uniform seed ready to be planted. The so-called polished seeds are those with most of the soft or outer pericarp removed, while unpolished seeds refer to those with an intact pericarp. The role of the outer pericarp in the present NIR application was also considered.

NIR applications focusing on sugar beet seed have not been released so far. This study wants to set a first step towards a better understanding of the role of the seed energy sources in this crop. The development of a non-destructive method that can be widely used in the sugar beet sector could greatly enhance future research in this field.

2. Materials and Methods

Monogerm sugar beet (*Beta vulgaris vulgaris altissima*) seed-lots from the breeding company Strube Research GmbH and Co KG were measured every 2 nm in the spectral range 850 to 1650 nm with the diode array PSS 1720 NIR spectrometer (256 pixel detector) under reflection (Polytec GmbH, Waldbronn, Germany). One measurement encompassed a seed batch of 50 g representative of a given seed lot. The 50 g seed aliquots were filled in 1 cm deep plates and were measured during four seconds under rotation. During these four seconds, 125 single NIR measurements were implemented and automatically averaged into a single NIR spectrum corresponding to a given seed sample.

Polished seed samples were available from six different years (2011, 2013, 2014, 2015, 2016, and 2017) and comprised 186 hybrids and 101 inbred lines. NIR spectra from unpolished seed samples comprising 62 hybrids from 2011 were also collected.

Seed samples were sent for oil content determination to the Institute for Feed Analysis (LUFA Nord-West) in Oldenburg (Germany). Oil was extracted from 287 polished and 62 unpolished seed samples using light petroleum according to the regulation (EC) No 152/2009 Annex III, H. The same seed aliquots that had been measured with NIR were used for oil determination.

The volume of the outer pericarp in the unpolished seed samples was measured with a computer tomography imaging system internally developed by the company Strube D&S GmbH (Söllingen, Germany).

A Partial Least Squares Regression (PLSR) was used to develop calibration models. As a first step and in order to obtain a representative and at the same time independent validation set, an algorithm was applied into the available 287 polished samples to select one in every five samples (58 samples in total). The remaining 229 samples formed the calibration set. Internal full cross-validation was used to evaluate the efficiency of the different calibration models and the external independent validation set was used to test additionally their prediction aptitude. The 62 unpolished samples were used for a further independent prediction test of the calibration model constructed with the polished samples.

Several spectrometric data pre-treatments consisting of combinations of absorbance, first derivative, second derivative, standard normal variate, smoothing and normalization were tested. To the resulting chemometric models, the following criteria were applied in order to choose the optimal calibration design: low Root Mean Square Error of Cross-Validation (RMSECV), low bias, low Standard Error of Prediction (SEP) and Root Mean Square Error of Prediction (RMSEP), and high Pearson correlation coefficient (*R*) in calibration and prediction between lab-measured and NIR-estimated oil values. The Ratio of Prediction to Deviation (RPD) was calculated as the ratio between the standard deviation of the validation set to the RMSEP. All chemometrical analyses were performed with the SensoLogic GmbH software package (Norderstedt, Germany).

3. Results

The oil content in the 287 analyzed polished seed samples ranged from 2.02% to 7.93% from the original seed weight. The average oil content was 5.8% with a standard deviation of 0.92. The calibration model with the best performance for the prediction of oil content in sugar beet seed reached a Pearson correlation coefficient of $R = 0.946$ (Figure 1) and an RPD of 3.02. To enhance the calibration performance, a spectrometric data pre-treatment consisting of a second derivative followed by a standard normal variate transformation was found to be optimal. The prediction of the independent validation set reached $R = 0.919$. Both correlations in calibration and prediction were found to be highly significant. The prediction showed a bias of −0.052, indicating a good concordance between the predicted NIR values and the laboratory reference data. The low bias between both data sets was subsequently reflected in the SEP and RMSEP parameters since the SEP squared is roughly equal to the RMSEP squared minus the bias square. Both parameters SEP and RMSEP were equal to 0.388.

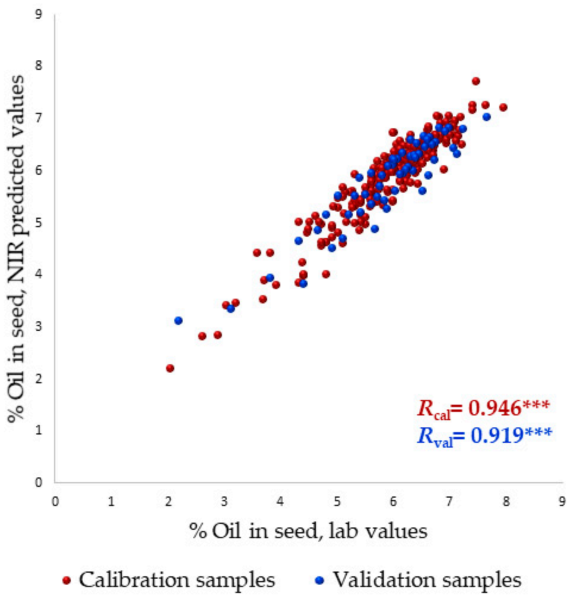

Figure 1. NIR calibration model for oil quantification in polished sugar beet seed. Calibration ($n = 229$) and validation samples ($n = 58$) are represented. Correlation coefficients (*R*) for both data sets are also indicated. *** Significantly different at $p < 0.001$.

To assess the possible effect of the thickness of the outer pericarp layer in the oil content prediction, the calibration model developed with polished seed was applied to unpolished samples (Figure 2). The oil content in the 62 available unpolished seed samples ranged from 4.01% to 5.92% from the original seed weight. The volume of the intact outer pericarp of these samples ranged from 6.17 to 13.10 mm^3. The prediction of this independent unpolished validation set reached $R = 0.868$ with a bias of 0.023. In addition, SEP and RMSEP reached values of 0.220 and 0.219, respectively. Considered altogether, these parameters reveal a good adjustment between the NIR-predicted and laboratory oil values. To further discern if the total volume of the outer pericarp had any effect upon the prediction error, the differences between reference and predicted values were tested for a normal distribution using the Skewness and Kurtosis tests, respectively. Both tests were highly significant (significantly different at $p < 0.001$), indicating that the error in the prediction is independent of the pericarp volume.

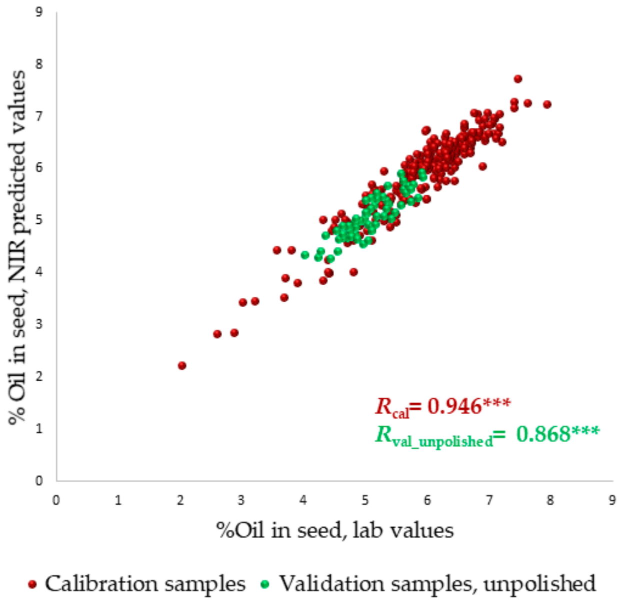

Figure 2. Prediction of unpolished seed samples using a calibration model developed with polished seed. Calibration (n = 229) and validation samples (n = 62) are represented. Correlation coefficients (R) for both data sets are also indicated. *** Significantly different at: $p < 0.001$.

4. Discussion

Oil content is a highly variable trait among seeds from different plant species, fluctuating from 1% in banana (*Musa paradisiaca* L.) to 76% in cocoplum (*Chrysobalanus icaco* L.) [21]. In sugar beet, the oil content in the seed has been previously described between 6.3 and 5.6% from the original seed weight [22,23]. In accordance with these studies, the average oil content is 5.8% across all examined seed samples.

Whilst among plant species the variation is high, within one species the oil content in seed seems to be rather a stable trait, more affected by genetic control than by environmental factors. The heritability of the seed oil content in plants has been repeatedly described to exceed 50% [9,24–27]. This seed oil reservoir seems to influence, in a great measure, germination behavior and seedling development. Seeds that germinate earlier and grow faster could, under equal environmental conditions, have a competitive advantage. A successful germination in sugar beet plays a crucial role in determining future plant fitness and consequently, the amount of stored seed oils might have an impact on plant yield. The oil content in sugar beet seed could be under breeding selection dragged along with yield traits selection. A better understanding of the significance of these oil reserves might be therefore meaningful for current sugar beet breeding programs and ultimately for the sugar industry.

Non-destructive successful NIR applications to measure oil in seed have been published so far for several oilseed crops [11,28–31]. Those cultivars are grown primarily for their rich seed oil content, which ranges from about 20% of the seed weight for soybeans to over 40% for sunflower and rapeseed. In contrast, sugar beet presents an average of approximately 6% of seed oil. The present study demonstrates that an accurate quantification of seed oil with NIR is possible even when the oil content in the seed is much lower than in oil crops.

The goodness of fit in the prediction is supported by our found RPD of 3. The RPD parameter helps to evaluate the efficiency of a NIR application, by pointing at possible deviations of the NIR prediction from the reference data. In a work by Williams [32], an RPD value of 3.0 was classified as good and the resulting application recommended for quality control analyses.

The process of polishing or removal of the outer pericarp layer is routinely applied to sugar beet seed because it is known to increase germination success [33]. Oil quantification of unpolished seed might be advantageous to assess seed quality before processing the seed any further, resulting in saving time and resources. Interestingly, the high correlation found for unpolished seed between predicted and reference oil values (R = 0.868) indicates that the outer pericarp layer does not seem to affect the oil prediction. A bit more specifically and as shown by the normality tests, the volume of the outer pericarp is not related to the prediction error, implying that even at its highest volume (13.10 mm^3 for the samples available in this study), the thickness of this layer is not interfering with NIR wavelength penetration and with the subsequent prediction of oil content.

More data are still needed to unravel the influence of the seed metabolic reserves on germination, seed vigour and the following robustness of seedlings and adult plants. Nevertheless, our present study shows that a simple and accurate determination of oil in a non-oilseed crop is feasible. The non-destructivity of the method is especially important in seed studies because seed composition can be analyzed before germination and, thus, it can be directly related to seedling development. In consequence, NIR applications in seeds might potentiate new ways to improve sugar beet seed quality while assisting further progress in achieving increased crop yield.

Author Contributions: R.M.-A. developed the NIR application and prepared figures and the original draft. M.G.R.-L. selected seed samples, ran the statistical tests and coordinated the reference analysis. A.S. conceptualized the study and supervised all steps of the research. All three authors contributed to data curation and editing of the manuscript.

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Conflicts of Interest: The authors declare no conflicts of interest.

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