Seed colour assessment in rapeseed (*Brassica napus* L.) by near-infrared reflectance spectroscopy

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ABSTRACT

Selection for seed colour in *Brassica* oil crop breeding depends on the availability of an adequate method for measurement. In order to overcome the inaccuracy of visual scoring, seed colour was determined by using a digital optical-picture analysis system generating brightness values as follows: yellow (140-255), brown (70-139) and black (45-69). In a second step, these brightness values were used for the development of a near-infrared reflectance spectroscopy (NIRS) calibration. A total number of 8,870 inbred lines segregating for seed colour, harvested in 1998 and 1999 from the breeding nurseries, were scanned with a NIRS system. 360 samples covering the whole range of seed colour brightness values were randomly selected to perform a calibration procedure. Four calibration algorithms were developed on basis of following wave-length ranges of the instrument: 400-770 nm (visible light, VIS), 400-1,098 nm, 1,100-2,498 nm (near-infrared spectral region, NIR) and 400-2,498 nm. The calibration algorithms were independently validated with 280 samples taken from the same large inbred line collection described above. External validation was successfully performed using sets of 400 samples derived from the breeding nurseries harvested 2000 and from the genetically different Yellow2 population, which had grown in a different environment and year (2001). Since assessment of colour is normally performed in the visible range of light, the standard errors of calibration (SEC) and the standard errors of performance (SEP) for the calibrations including the visible range of light were smaller than those found for the specific NIR range.

Key words: *Brassica napus* - seed colour - digital optical-picture analysis - NIRS

INTRODUCTION

Yellow seed colour is an important objective in breeding of winter oilseed rape (*Brassica napus*). The improvement of a range of seed traits - such as oil content as well as protein and crude fibre content of the meal - is associated with yellow seed colour due to a thinner seed coat as compared to black seeds (Friedt et al. 2002, Baetzel et al. 2003). The determination of seed colour has been based often on visual assessment. However, this procedure is not useful when thousands of samples have to be screened or when an accurate quantification is needed. Therefore, the objective was to achieve a fast and accurate determination of seed colour by using a digital optical-picture system. Subsequently, these brightness values were used for the calibration of near-infrared reflectance spectroscopy (NIRS) system, which is the method of choice for non-destructive, high-speed and multi-compositional analysis of *Brassica* oilseeds (Daun et al. 1994, Van Deynze and Pauls 1994, Velasco et al. 1996, 1999, Font et al. 2003).

MATERIAL AND METHODS

Inbred lines were derived from crosses between different dark-seeded *B. napus* lines and yellow-seeded *B. napus* genotypes (*YE1*, *YE2*) as described in more detail elsewhere (Baetzel et al. 1999, 2003). In addition to visual scoring with a scale 1 to 9, in which 1 represented bright yellow, 5 brown and 9 black seeds, the seed colour was assessed by using a digital optical-picture analysis system. For this purpose, the seed material was photographed with a digital camera, equipped with a charge-coupled device (CCD) sensor, and the images were processed by a personal computer-based programme (GTA Sensorik, Neubrandenburg, Germany) generating brightness values as follows: yellow (140-255), brown (70-139) and black (45-69). The seed colour value of an individual sample comprises the mean value of all seeds, as the system counts the seeds and analyses the frequency of seeds of each of the ranges of

brightness values, respectively. The imaging method was used as reference measurement of seed colour in order to calibrate the NIRS instrument.

A total of 8,870 inbred lines segregating for seed colour and harvested in 1998 and 1999 from our breeding nurseries were screened with a NIRS monochromator instrument (NIRS system model 6500, Foss NIRSystems, Hamburg, Germany) covering the spectrum range of 400 to 2,500 nm. Intact rapeseed samples (ca. 3,0 g) were placed in a NIRS standard ring cup with 38 mm inner diameter, the spectra of each sample were scanned twice. 360 samples were randomly selected to develop calibration algorithms. Data transformation on the spectral information were carried out with WinISI II Near-infrared software (version 1.04, Infrasoft International, LLC, Port Matilda, PA, USA) using the 'modified partial least squares' (MPLS) regression method (Shenk and Westerhaus 1991, 1995) as well as 'standard normal variate' (SNV) for correction of scatter effects and the 'detrend' mathematical procedure (Barnes et al. 1989) as specific adjustments to compute the calibration equations. The derivation treatment '1,4,4,1' was applied, where the 1st digit is the number of the derivative, the 2nd is the gap over which the derivative is calculated, the 3rd is the number of data points in a running average or smoothing, and the 4th is the 2nd smoothing (Shenk and Westerhaus 1995). Four different ranges of wavelength were tested (400-770 nm, 400-1,098 nm, 1,100-2,498 nm and 400-2,498 nm) to develop separate calibration algorithms with the same mathematical procedures. After the prediction equations were developed we used various *B. napus* material for validating the calibration algorithms (Table 1).

Table 1. Different *B. napus* breeding materials segregating for seed colour (cf. Baetzel et al. 1999, 2003) used for validating the developed calibration equations

Material	Number	Year	Genotype	Origin
ZGWR99	280	1998/1999	Breeding lines (based on *YE1*)	Rauischholzhausen
ZGWR00	400	2000	Breeding lines (based on *YE1*)	Rauischholzhausen
Yellow2	1,205	2001	F_3 inbred population (based on *YE2*)	KWS-Saat, Einbeck

RESULTS and DISCUSSION

Near-infrared (NIR) reflectance analyzers are routinely used in quality breeding of rapeseed (Daun et al. 1994). Based on brightness values determined by a digital optical-picture analysis system as reference method the aim of the present study was to utilise a NIR analyzer for measurement of seed colour in order to facilitate *B. napus* breeding programmes. Table 2 shows the calculated statistics for the developed calibration equations and the independent validation of these calibration functions with a set of 280 breeding lines (ZGWR99), other than the 360 samples of the calibration set but randomly chosen out of the same sample stock (Table 1). As indicated by the coefficients of determination (R^2) all calibration equations for seed colour showed a close relationship between NIRS values and brightness values obtained by reference measurement.

Table 2. Cross-validation and independent validation statistics of developed NIRS calibration equations for estimating *B. napus* seed colour using a validation set of 280 genotypes (ZGWR99)

		Calibration				Independent validation			
Calibration	**[nm]**	**SECV**	**SEC**	**R^2**	**#**	**SEP (c)**	**Bias**	**R^2**	**Slope**
Farb770.eqa	400-770	0.346	0.33	0.969	46	0.293	-0.002	0.975	1.025
Farbgesa.eqa	400-1,098	0.322	0.315	0.972	86	0.294	-0.081	0.976	1.084
Farbgesb.eqa	1,100-2,498	0.512	0.459	0.942	173	0.492	-0.083	0.929	1.040
Farbges.eqa	400-2,498	0.318	0.304	0.974	259	0.297	-0.050	0.975	1.045

SECV = standard error of cross-validation, SEC = standard error of calibration, R^2 = coefficient of determination, # = number of wavelength, SEP (c) = standard error of performance corrected for bias, Bias = systematic error.

The equations including the visible (VIS) spectrum range of light (400-770 nm) showed the best correlation between estimated and reference values of the validation set. As measuring of colour is normally only possible in the visible region of light, the standard error of calibration (SEC) and the standard error of performance (SEP and SEP (c) corrected for bias) for the calibrations including the VIS spectrum are lower as those for the near-infrared (NIR) spectrum region (1,100-2,498 nm) of light. As shown in Table 2 it is also possible to measure seed colour in the NIR spectrum with satisfactory precision. In this case we suppose a high correlation between a main compound of the seed coat and seed colour, probably a fraction of crude fibre (cf. Archibald and Kays 2000, Font et al. 2003).
In order to test the reliability of the developed calibration equations by external validation, we took the two material groups, ZGWR00 and Yellow2 (Table 1), which were not used for the calibration and first validation procedure (Table 3). Again we observed that for all calibration equations including the VIS range of light the NIRS predictions were characterised by a low bias or systematic error and a high coefficient of determination. The values for the second material group (Yellow2) were slightly higher than in the other external validation set (ZGWR00), especially all bias values were significant. This is not surprising as this population segregating for seed colour does not belong to the basic material used for calibration equation development.

Table 3. External validation statistics of the developed NIRS calibration equations for estimating seed colour in rapeseed

	External validation with ZGWR00					External validation with Yellow2				
[nm]	SEP	SEP (c)	Bias	R^2	Slope	SEP	SEP (c)	Bias	R^2	Slope
400-770	0.452	0.451*	0.037	0.94	1.184	0.715	0.518*	0.492*	0.80	1.103
400-1,098	0.390	0.374	-0.114	0.96	1.176	0.579	0.514*	0.266*	0.82	1.152
1,100-2,498	1.254	0.679*	1.054*	0.87	0.785	2.110	0.650	2.007*	0.72	0.819
400-2,498	0.386	0.332	0.198*	0.96	1.109	0.821	0.504*	0.648*	0.82	1.086

SEP = standard error of performance, SEP (c) = standard error of performance corrected for bias, Bias = systematic error, R^2 = coefficient of determination.

CONCLUSION

The present study has shown that NIR spectrophotometers are suitable instruments for prediction of seed colour in rapeseed. It is concluded that it is necessary to include the visible range of light (400-770 nm) in the spectrum region of measurement to achieve accurate seed colour assessment (cf. Van Deynze and Pauls 1994, Velasco et al. 1996). The existing calibration equations should be further improved by inclusion of additional rapeseed breeding material. Since reduction of crude fibre content is actually the more interesting objective rather than yellow seededness as such, the breeding material segregating for seed colour will be used to extend multi-compositional NIRS analysis also for calibration of acid detergent fibre (ADF) content of intact seeds in order to avoid laborious extraction and destruction of valuable rapeseed material (cf. Font et al. 2003).

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