Application of Near-Infrared Diffuse Reflectance Spectroscopic Analysis for Estimating the Ratio of True Seed Weight to Fruit Weight in Sugar Beet Seed

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Abstract: The feasibility of near-infrared diffuse reflectance spectroscopic analysis (NIR analysis) for estimating the ratio of true seed weight to fruit weight (T/F) as well as water content was examined in sugar beet (Beta vulgaris L.) seeds of 61 F₁ hybrid lines and 4 standard cultivars. For the calibration, partial least squares (PLS) regression was carried out with second derivative spectra and the measured data using attached software (NSAS). For estimating T/F, calibration using 7 factors was the most valid with a correlation coefficient of calibration (R) of 0.943, standard error of calibration (SEC) of 1.26% and standard error of prediction (SEP) of 1.40%. By this calibration, sugar beet varieties could be classified into 4 levels according to the estimated T/F. For the estimation of the water content of sugar beet seed, calibration using 14 factors was optimal. The calibration was highly accurate since the R, SEC and SEP was 0.999, 0.23% and 0.27% respectively. Consequently, the true seed weight of sugar beet could be nondestructively and rapidly estimated by NIR analysis and weighing the air-dried seed. This technique should be useful in breeding selection for higher true seed weights, which would thereby improve the early growth of sugar beet varieties.

Key words: Near-infrared spectroscopy, Ratio of true seed weight to fruit weight, Sugar beet, Water content.

To improve the yield of drilled sugar beets, it is crucial to increase the early growth (Yoshida et al., 1983; Kurosawa and Saito, 1987; Yoshimura, 1996). In a previous paper, we suggested that there was a positive correlation between the early growth and the true seed weight (Mukasa and Ogata, 2001). The true seed is, however, tightly covered by the pericarp, making it very troublesome to examine its weight. For breeding large true seed lines, a simple and rapid determination of true seed weight is necessary.

Near-infrared (NIR) spectroscopy is widely applied to nondestructive and rapid determinations of food constituents (Ozaki, 1998). It has been adopted as an official method for determining the water and protein content of wheat in the United States and Canada. Also, NIR analysis has been examined to determine other parameters, such as acidity and hardness of plums (Onda et al., 1994), peroxidic value of edible oil (Hong et al., 1994) and classification of wheat flour (Chiba et al., 1995).

There are some studies on the determination of sucrose content in sugar beet root by NIR analysis. Murakami et al. (1993; 1994) improved the sensor part of NIR system and used it to determine the sucrose content of cut sugar beet root. Roggo et al. (2002) used thousands of brei samples and compared spectral data with sucrose content by different pre-treatment and regression methods in order to find a satisfactory model. However, NIR analysis has never been applied to determine true seed weight of sugar beet so far. In this report, we examined the feasibility of NIR analysis for estimating the ratio of true seed weight to fruit weight (T/F) in sugar beet seeds. In addition, a non-destructive method to measure seed water content was examined to determine the dry weight of true seeds.

Materials and Methods

1. Seed materials

Normally polished sugar beet seeds (botanically fruits) of 61 F₁ hybrid lines and 4 standard cultivars were used (Table 1) to provide wide range configurations. They were monogerms and supplied by the sugar beet breeding laboratory of National Agricultural Research Center for Hokkaido Region. The materials were divided at random into sample sets for calibration (33 varieties) and prediction (32 varieties). The same number of materials was polished further to change the T/F, and they were added to each sample set, i.e., 66 samples were used for calibration and 64 samples for prediction.

2. Near-infrared spectroscopy

A polyurethane sponge was attached to the cell of the near-infrared spectroscopic analyzer (NIR Systems 6500, FOSS) for scanning a small amount of sample.
The cell was filled with approximately 2 g of sugar beet seed and diffusive reflectance spectra were scanned in the wavelength range of 1100 nm to 2498 nm. The sample was refilled and scanned three times and the three spectra were averaged by attached software (NSAS). Air-dried seeds were used for the estimation of T/F.

To estimate the water content, we scanned three kinds of sugar beet seeds as above; (1) without any treatments (air-dried), (2) humidified in the constant temperature chamber at 25ºC and RH 95% for 24 hrs (humidified) and (3) dehumidified in the constant temperature chamber at 25 ºC and RH 30% for 24hrs (dehumidified).

3. Measurement of the ratio of true seed weight to fruit weight (T/F) and seed water content

Before NIR spectrum scanning, the weights of air-dried, humidified and dehumidified samples were measured. After all scanning, the samples were separated to true seeds and pericarps with tweezers and dried at 105 ºC for 24hrs. The T/F ratio was calculated on the basis of dry matter. Then, seed water contents were calculated from the weights of air-dried, humidified and dehumidified samples. The dry weight of the fruit was shown by the sum of true seed and pericarp dry weights.

4. Calibration and prediction

All calculations were performed using the attached software (NSAS). Partial least squares (PLS) regression was performed with the second derivative (segment 12, gap 0) spectra of the samples for calibration and the measured values. In the algorithm of NSAS, the calibrations with 1~15 factors were automatically made by PLS. Those calibrations were evaluated by the samples for prediction, and the standard error of prediction (SEP) was calculated. Sixty-four samples were used for the prediction of T/F and 192 samples (64 samples x 3 pretreatments) for the prediction of seed water content.

Results and Discussion

1. Ratio of true seed weight to fruit weight (T/F) and seed water content

Table 2 shows the measured T/F ratios and water

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Table 1. F₁ hybrid lines and standard varieties used in the NIR analysis.

| Seed parent lines (2n) | Pollen parent lines | produced year |
|------------------------|-------------------|---------------|
| (183BR×232)            |                   | 1989          |
| (208BR×213BR)          |                   | 1993          |
| (172BR×213BR)          |                   | 1993          |
| (169×226BR)            |                   | 1993          |
| (195×226BR)            |                   | 1993          |
| (172BR×248)            |                   | 1994          |
| (208BR×220BR)          |                   | 1994          |
| (195×229BR)            |                   | 1995          |
| (195×230BR)            |                   | 1995          |
| (TK-80-2BR×249)        |                   | 1995          |
| (172BR×229BR)          |                   | 1995          |
| (172BR×183BR)          |                   | 1987,88       |
| (170×TK-80-2BR2)       |                   | 1988,89       |
| (183BR×219)            |                   | 1989          |

(2n): diploid, (4n): tetraploid.

Table 2. The ratio of true seed weight to fruit weight (T/F) and the water content in the samples of sugar beet seeds for calibration and prediction.

|                     | T/F | Water content of seed |                      |                      |                      |                      |
|---------------------|-----|-----------------------|---------------------|---------------------|---------------------|---------------------|
|                     | Cal.| Pre. | [1] Air-dried | Cal.| Pre. | [2] Humidified | Cal.| Pre. | [3] Dehumidified | Cal.| Pre. |
| N                   | 66  | 64 | 66 | 64 | 66 | 64 | 66 | 64 | 66 | 64 |
| Minimum             | 23.63 | 24.29 | 7.13 | 6.76 | 13.21 | 13.27 | 4.53 | 4.57 |
| Maximum             | 39.82 | 37.22 | 10.35 | 10.99 | 18.11 | 17.69 | 5.18 | 5.19 |
| Average             | 31.66 | 31.67 | 8.94 | 8.91 | 15.17 | 15.13 | 4.88 | 4.89 |
| Standard deviation  | 3.54 | 3.14 | 0.62 | 0.72 | 1.18 | 1.2 | 0.16 | 0.17 |
| Unit                | % | % | % | % | % | % | % | % |

Cal.: Calibration, Pre.: Prediction.
2. Calibration and prediction of T/F

Raw spectra data of the samples for calibration are shown in Fig. 1. The absorptions at 1450nm and 1930nm were probably caused by water and the absorptions around 2100nm and 2270nm might be attributed to cellulose that the pericarp contained. The spectral variations in the longer wavelength range were generally larger than those in the shorter wavelength range, so we suspected that the fruit size might affect the spectral shift (Iwamoto et al, 1994). To diminish the effect of the fruit size, we used the second derivative spectra for the calibration (Fig.2). In the second derivative spectra, the baseline shift of the spectra seemed to be corrected approximately.

In the algorithm of NSAS, calibrations using 1~15 factors were automatically performed by PLS and the correlation coefficient of calibration (R) and the standard error of calibration (SEC) were calculated. Then, these 15 calibrations were evaluated with the sample set for prediction, and the standard error of prediction (SEP) was calculated (Table 3). When the contents of air-dried, humidified and dehumidified samples for calibration and prediction. The range of T/F was 23.63%~39.82%, with a maximal difference of approximately 16 points. The range of the seed water content was 6.76%~10.99% in air-dried samples, 13.21%~18.11% in humidified samples and 4.53%~5.18% in dehumidified samples. The maximal difference was approximately 14 points in total. The sample sets for calibration and for prediction showed only small differences in terms of range, average values and standard deviation for both T/F and seed water content.
explanatory factors increased, R increased and SEC decreased, though SEP was saturated over 7 factors. Judging from SEP, the calibration using 7 factors was the most valid and optimal; R = 0.943 and SEC = 1.26 in this calibration. From the analysis of the first and the second loading of PLS factor, we estimated that the longer wavelength range over 1800nm mainly contributed to this calibration (data not shown).

The correlation between the values estimated by NIR and the measured values is shown in Fig. 3. The correlation coefficient of prediction (r) was 0.903 and SEP was 1.40%. We did not consider this calibration to be very accurate since SEP was not very small. On the other hand, the measured values of T/F ranged from 23.63% to 39.82% in the samples and the maximal difference was approximately 16 points (Table 2). We concluded that this calibration could roughly classify the sugar beet varieties into 4~5 levels based on estimated T/F.

3. Calibration and prediction of seed water content

As in the case of T/F, the algorithm of NSAS automatically performed the calibrations by PLS using 1~15 factors. Then, these 15 calibrations were evaluated with the sample set for prediction, and the standard error of prediction (SEP) was calculated (Table 4). The calibration was highly accurate even if the calibration had used only one factor. The larger the number of factors, the larger the R and the smaller the SEC. The decrease of SEP with increasing number of factors was saturated over 14 factors; therefore we concluded that 14 factors were optimal for this calibration.

The correlation between the values estimated by NIR and the measured values is shown in Fig. 4. The correlation coefficient of prediction (r) was 0.998 and SEP was 0.27%. We considered this calibration is quite accurate since r was substantially high and SEP was small. Near-infrared spectroscopy has been used to determine the water content of wheat in the United States and Canada; clearly, the water content of the sugar beet seed can be measured on the same principle.

In conclusion, T/F could be roughly estimated and the seed water content could be precisely estimated by NIR analysis without the destruction of the samples. The dry weight of seeds (botanically fruit) could be determined from the air-dried weight and the seed water content estimated by NIR analysis. Then, the true seed weight could be roughly determined from the dry weight of seed and the T/F estimated by NIR analysis. Breeding selection using true seed weight

Table 3. Calibration and prediction of the ratio of true seed weight to fruit weight in sugar beet seed.

| Factor | R ¹ | SEC ² | SEP ³ | Bias |
|--------|-----|-------|-------|------|
| 1      | 0.405 | 3.27  | 3.17  | -0.123 |
| 2      | 0.540 | 3.04  | 2.86  | 0.161  |
| 3      | 0.787 | 2.24  | 2.22  | 0.230  |
| 4      | 0.870 | 1.81  | 2.06  | 0.073  |
| 5      | 0.886 | 1.72  | 2.15  | -0.025 |
| 6      | 0.934 | 1.33  | 1.61  | -0.268 |
| 7      | 0.943 | 1.26  | 1.40  | -0.151 |
| 8      | 0.954 | 1.14  | 1.43  | -0.168 |
| 9      | 0.962 | 1.05  | 1.56  | -0.306 |
| 10     | 0.970 | 0.94  | 1.44  | -0.245 |
| 11     | 0.978 | 0.81  | 1.45  | -0.086 |
| 12     | 0.980 | 0.78  | 1.45  | -0.100 |
| 13     | 0.982 | 0.75  | 1.43  | -0.081 |
| 14     | 0.983 | 0.74  | 1.44  | -0.080 |
| 15     | 0.985 | 0.70  | 1.43  | -0.050 |

¹: R: Correlation coefficient of calibration,
²: SEC: Standard error of calibration,
³: SEP: Standard error of prediction.
could be simply and rapidly carried out by NIR analysis, and could well improve the early growth of sugar beets in drilling cultivation. Although we did not discuss in detail which substances caused the diffusive reflection in this experiment, it was revealed that NIR analysis would be useful for the practical estimation of true seed weight.

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* In Japanese with English summary
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Table 4. Calibration and prediction of the water content in sugar beet seed.

| Factor | R$^1$ | SEC$^2$ | SEP$^3$ | Bias
|-------|-------|--------|--------|-----|
| 1     | 0.973 | 1.01   | 0.98   | 0.017 |
| 2     | 0.994 | 0.49   | 0.47   | -0.012 |
| 3     | 0.995 | 0.44   | 0.44   | -0.003 |
| 4     | 0.996 | 0.40   | 0.43   | 0.010 |
| 5     | 0.996 | 0.38   | 0.42   | 0.000 |
| 6     | 0.997 | 0.34   | 0.36   | -0.001 |
| 7     | 0.997 | 0.32   | 0.34   | -0.008 |
| 8     | 0.998 | 0.29   | 0.34   | 0.010 |
| 9     | 0.998 | 0.28   | 0.32   | 0.005 |
| 10    | 0.998 | 0.26   | 0.30   | -0.001 |
| 11    | 0.999 | 0.24   | 0.29   | 0.013 |
| 12    | 0.999 | 0.24   | 0.28   | 0.011 |
| 13    | 0.999 | 0.24   | 0.28   | 0.008 |
| 14    | 0.999 | 0.23   | 0.27   | 0.009 |
| 15    | 0.999 | 0.23   | 0.28   | 0.014 |

1) R: Correlation coefficient of calibration.
2) SEC: Standard error of calibration.
3) SEP: Standard error of prediction.

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Fig. 4. Prediction of seed water content by NIR analysis.