Use of near infrared spectroscopy for estimating levels of anthocyanin and DPPH-radical scavenging activity in corn

Tetsuo Sato,[a] Kentaro Eguchi[a] and Akira Sawai[b]

[a]*Analysis and Monitoring Office, National Agricultural Research Center for Kyushu Okinawa Region (KONARC), National Agriculture and Food Research Organization (NARO), Koshi-city, Kumamoto, Japan. E-mail: sato@affrc.go.jp*
[b]*Research Team for Year-round Grazing of Cattle, KONARC, NARO, Miyakonojo-city, Miyazaki, Japan*

Introduction

Corn (maize, *Zea mays*) has high nutritive value, and is one of the whole forage crops. The attentions of cattle breeders, farmers, and consumers are focused, not only on the nutritional constituents, but also on the physiologically functional elements in these crops. Some corn varieties and lines contain the antioxidant anthocyanin. Purple corn, which contains anthocyanin, might be useful to cattle health, and is also notified as a new industrial crop. In a corn breeding project, one of the aims is that of developing corn varieties with high levels of anthocyanin and DPPH(1,1-diphenyl-2-picrylhydrazyl)-radical scavenging activity.[1] However, the conventional methods for the analyses of anthocyanin and DPPH-radical scavenging activity are time-consuming, expensive, and very tedious. A simple method for their analyses is needed. In this study, we examined the feasibility of NIRS for estimating the levels of anthocyanin and DPPH-radical scavenging activity in different parts of the corn plant, including the cob, kernel, and leaf-stem.

Materials and methods

Samples

The corn samples were cultivated and harvested at the field of the Miyakonojo campus of KONARC in 2007 and 2008. Cob and leaf-stem were milled by a crushing mill through a screen ($\varphi = 2.0$ mm). Kernels were milled to a powder by a crushing mill.

Table 1. The fundamental statistics of the samples used.

		Calibration set		Prediction set	
Material	Constituent	Min – Max	Mean ± SD	Min – Max	Mean ± SD
Cob	Anthocyanin	0.04 – 68.17	9.26 ± 14.30	0.04 – 53.71	11.63 ± 13.76
	DPPH	0.97 – 371.95	56.54 ± 72.27	0.86 – 247.08	69.23 ± 65.81
Kernel	Anthocyanin	0.01 – 5.33	1.04 ± 1.14	0.03 – 4.37	0.94 ± 0.88
	DPPH	1.02 – 52.07	6.30 ± 6.27	0.00 – 16.42	5.55 ± 3.47
Leaf-Stem	Anthocyanin	0.09 – 34.13	9.34 ± 11.50	0.09 – 33.25	9.07 ± 11.49
	DPPH	8.47 – 152.17	51.72 ± 47.51	8.06 – 191.92	52.13 ± 53.14

Min: minimum, Max: maximum, *SD*: standard deviation.
Anthocyanin: The unit is μmol-cyanidin-3-*O*-glucoside equivalent gDW^{-1}.
DPPH: DPPH-radical scavenging activity. The unit is μmol-Trolox equivalent gDW^{-1}.

Chemical Analyses

The powdered samples were extracted with 4 mL of 1% trifluoracetic acid aqueous solution (v/v) for 24 h at room temperature. Six mL of water were added to the mixture, and it was filtered through a membrane filter (0.45 μm; Advantec Co. Ltd, Tokyo, Japan) prior to chemical analyses. The levels of anthocyanin and the DPPH-radical scavenging activity were measured at 520 nm by colorimetric methods using cyanindin-3-*O*-glucoside and Trolox (6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid) as respective standards.[2] The unit is μmol-cyanidin-3-*O*-glucoside equivalent gDW^{-1} for anthocyanin level, and μmol-Trolox equivalent gDW^{-1} for level of DPPH-radical scavenging activity, respectively. The fundamental statistics of these levels in the samples are described in Table 1.

The samples were divided into two sets: the calibration set and the prediction set.[3] The numbers of samples used were 81 (the calibration set) and 54 (the prediction set) for cob, 94 and 62 for kernel, and 51 and 33 for leaf-stem, respectively.

Near infrared spectroscopic measurements

A SpectraStar 2400 (Unity Scientific, USA) was used to measure the NIR reflectance spectra in the wavelength range from 1200 to 2400 nm at 1 nm intervals. Samples were packed in a standard cup on a standard drawer for NIR measurements.

Statistical analysis

The SensoLogic (Sensologic GmbH, Germany) was used on SpectraStar 2400 spectral data with chemical data for multiple linear regression (MLR) analysis, and was also used for PLSR/PCR (partial least squares regression/ principal component regression) analysis. The conditions to obtain the derivatives for the pretreatment of NIR spectra were as follows: gap 10, segment 10 for the first

Table 2. Calibration and prediction statistics using MLR.

Calibration				Prediction			
Material	Constituent	Pretreatment	Selected wavelengths (nm)	*r*	*SEP*	Bias	*RPD*
Cob	Anthocyanin	Log 1/*R*	1579, 1588, 1647, 1655, 1664, 1673	0.90	5.92	0.46	2.32
	DPPH	Log 1/*R*	1641, 1651, 1659	0.95	21.03	–3.63	3.12
Kernel	Anthocyanin	Log 1/*R*	1342, 1642, 1652, 1661, 2344	0.84	0.52	0.14	1.69
	DPPH	Log 1/*R*	1205, 1639, 1647	0.60	3.20	0.35	1.08
Leaf-stem	Anthocyanin	D2	1285, 1585, 1651, 1698, 2274	0.99	1.61	0.29	7.14
	DPPH	D22	1335, 1374, 1404, 1655, 2330	0.95	16.72	–0.88	3.18

r = coefficient of correlation between chemical and NIR spectroscopy methods.
SEP = standard error of prediction.
Anthocyanin units are µmol-cyanidin-3-*O*-glucoside equivalent gDW^{-1}.
DPPH:DPPH-radical scavenging activity: units are µmol-trolox equivalent gDW^{-1}.

derivative (abbreviated as d1), gap 10, segment 10 for the second derivative (d2), and gap 5, segment 5 for another second derivative (d22). First, the data analyses were carried out on the calibration sets. Then, the performance of the calibration equations were evaluated using the prediction set.

Results and discussion

Statistical analysis by MLR

Table 2 describes the results of the statistical analysis by MLR analysis.

Figure 1 also shows these results. As for cob and leaf-stem analysis, based on the values of r and RPD, the levels of anthocyanin and the DPPH-radical scavenging activity were successfully estimated. The calibration models were adequate for rough breeding selection. On the other hand, for kernel analysis, because of the low *RPD* values, the levels of anthocyanin and the DPPH-radical scavenging activity were not well-estimated, possibly because the range of the levels was small.

Statistical analysis by PLSR/PCR

Table 3 describes the results of the statistical analysis by PLSR/PCR.

In this case, the scattering graphs (scatter plots, not shown) were similar to those of Figure 1, i.e. the results obtained by MLR analysis. As for cob and leaf-stem analysis, based on the values of *r* and *RPD*, the levels of anthocyanin and the DPPH-radical scavenging activity were also

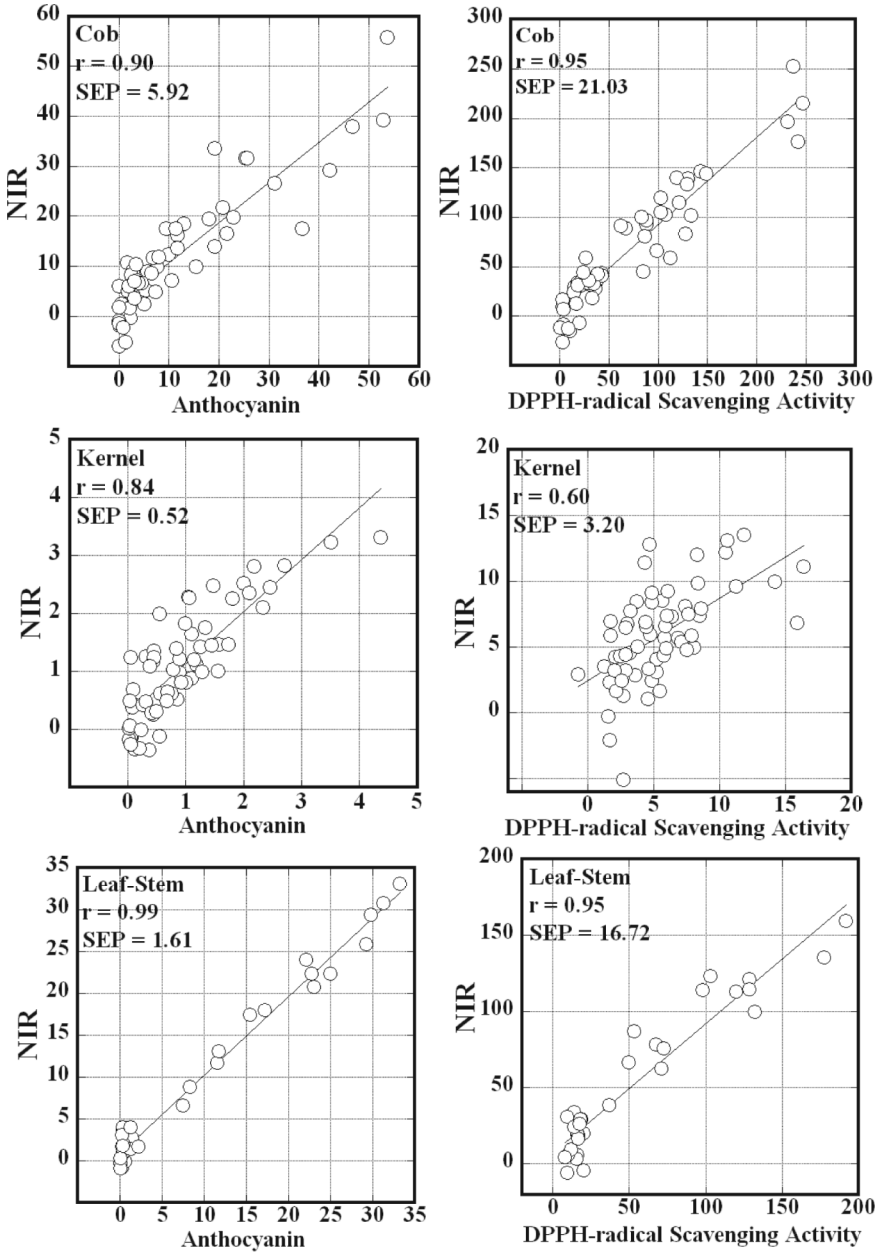

Figure 1. Scattering graphs of analytical results by MLR analysis.

Table 3. Calibration and prediction statistics using PLSR/PCR.

Material	Constituent	Treatment	Method	*N.* factors	*r*	*SEP*	Bias	*RPD*
Cob	Anthocyanin	D22	PLSR	6	0.88	6.74	0.51	2.04
	DPPH	D1	PCR	13	0.93	24.6	0.00	2.49
Kernel	Anthocyanin	D22	PLSR	7	0.75	0.59	0.13	1.49
	DPPH	D1	PLSR	5	0.64	3.12	0.53	1.11
Leaf-stem	Anthocyanin	D2	PLSR	5	0.98	2.44	0.65	4.71
	DPPH	D1	PLSR	4	0.94	18.8	−2.55	2.82

r = coefficient of correlation between chemical and NIRS methods.
SEP = standard error of prediction.
Anthocyanin units are μmol-cyanidin-3-*O*-glucoside equivalent gDW^{-1}.
DPPH:DPPH-radical scavenging activity: units are μmol-trolox equivalent gDW^{-1}.
N. Factors = number of PLSR or PCR factors.

successfully estimated, with usefulness adequate for rough breeding selection. On the other hand, for kernel analysis, the low *RPD* values again indicated that the levels of anthocyanin and the DPPH-radical scavenging activity were not estimated very well.

We also measured the NIR spectra using the InfraAlyzer 500 (Bran+Luebbe Co., Germany). The spectra were then analyzed using IDAS (Bran+Luebbe) and the Unscrambler (version 9.6, Camo Co., Norway), with similar results.[3] Based on these results, NIR spectroscopy is considered to be useful for estimating the levels of anthocyanin and DPPH-radical scavenging activity in cobs and leaf-stems of corn for rough breeding selection simply, easily, and rapidly.

References

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