Near infrared reflectance spectroscopy for selecting high amylomaize lines in breeding programmes

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Introduction

Maize crops have an essential role as food and feed for human and animal consumption and as a source of industrial products. Products from the maize kernel, especially corn starch, are the basis for nearly all industrial uses of maize.[1] The starch produced in nonmutant endosperms is composed of approximately 25% amylose [linear polymers of glucose linked by α-D(I → 4 glucosidic bounds] and 75% amylopectine [branched polymers of glucose primarily linked by α-D(I → 4) bounds as well as branches linked by α-D(I → 6 bounds].

The advent of routine genetic manipulation of plants in the past decade has made it possible, in theory at least, to increase yield and to provide novel raw materials through alteration of the pathway of starch synthesis.[2,3] In this context the endosperm mutants, *waxy* (*wx*) and *amylose extender* (*ae*), are two extreme hereditary elements of maize that alter the distribution of amylose and amylopectin in endosperm starch. The recessive *wx* allele, when homozygous, completely blocks amylose accumulation; this *wx* starch contains 0% amylose,[4] while the recessive *ae* allele increases the proportion of amylose in starch. Hybrids are now in production that have *ae* starches with 80% apparent amylose.[5]

Studies in this field have shown that wet milling of amylomaize is more difficult than yellow dent maize and its starch yield is lower. In addition, amylomaize starch granules comprise two distinct types, spherical and irregular and are smaller than normal starch granules.[6] Moreover, some of the granules do not lose all birefringence even after prolonged boiling.[7] The amylomaizes are used mainly for sizing of glass fibres prior to weaving, a component of gummed candies, preparation of a clear hot water dispersible, edible film for packaging foods, dyes and other soluble materials and coating paper to reduce water and fat absorption.[8] Amylomaize starch sales are small but are growing, especially as a "green chemistry" project for producing biodegradable materials as an alternative to raw materials derived from petrol chemistry.

As a consequence of the growing increase in the demand for maize with a high content of amylose, it seems useful to develop a more productive *ae* maize hybrid. In this context, our Institute is carrying out a research programme for selecting new maize materials with high levels of amylose in the kernel.

The amylose content of starch is commonly determined by one of the many variations of the classical reaction between amylose and iodine to form a blue complex, which is then measured either spectrophotometrically, potentiometrically, amperometrically or with colorimetric titration.[9–12] Other approaches to amylose measurement, such as size exclusion chromatography after fraction and enzymatic debranching,[9,13,14] are useful for fine-structure studies but are not adaptable to large sample numbers as required in breeding programmes. Amylose determination by near infrared (NIR) transmittance reflectance spectroscopy,[15,16] or differential scanning calorimetry, are more rapid but re-

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quire a reference method for calibration. In this paper we present the results obtained from a specific NIR calibration model developed for the prediction of amylose content in maize grain and ground kernels in breeding programmes for the development of superior amylomaize hybrids.

Material and methods

Plant materials

The experimental material used in this study is represented by six normal inbred lines and their high-amylose version. In addition, we selected 13 *ae* F5 lines, 1 commercial *ae* hybrid and 200 S4 *ae* lines. All samples were grown in 1998 at three different locations in Northern Italy. During harvesting, grain samples for each entry were dried in an oven until they reached a constant weight. The absorbance spectra (log l/*R*) of all samples were recorded in duplicate using a FOSS Elettric Model 6500 scanning monochromator with a range 400–250 nm. 200 grain samples, chosen by the SELECT algorithm,[17]were used to develop the NIR calibration for measuring the percentage of amylose in grains.

Chemical analyses

Amylose in starches was obtained from endosperms without pericarp and embryo tissues, according to the method used by Adkins and Greenwood[18] and estimated according to Knutson's method,[19] as a percentage of total starch. Starch was measured by the method used by Lorenzoni *et al.*[20] All analyses were carried out in duplicate.

Near infrared analysis

For each entry, approximately 2 g of ground grain or 20 g of whole kernels were sampled and packed into a black aluminium cup containing a rectangular quartz window. All samples were irradiated with NIR monochromatic light and the diffuse reflectance collected by means of lead sulphide detectors in a Foss NIRSystems 6500 scanning monochromator. All spectral data were recorded as log 1/*R* where *R* = reflectance, in the wavelength range 400–2498 nm at every 2 nm. The mathematical transformation (1, 5, 5) of the spectral data was carried out before derivation of the regression models. The first number indicates the derivative used, the second is the length of the segment expressed as data points and the last shows the length of the smoothing segment. A modified partial least squared (MPLS) regression technique was used to develop the calibration equations. The equation selected as the best for each chemical fraction was obtained using the following criteria:

1. the lowest standard error of cross-validation; this was obtained by dividing the data into sets of four and predicting each fourth value by calibrations developed from the other three values. Samples with large residuals were omitted and cross-validation was performed again;
2. the wavelengths that correspond most closely with the particular chemical fraction

Results and discussion

A total of 400 NIR spectra were collected from grain and ground endosperm samples for both normal and amylomaize. Figure 1 shows an example of the grain and ground endosperm samples in the main NIR region where the differences in spectral shape are more evident and are assigned to starch and amylose overtones. A total of 105–118 for whole grain and 126–184 for ground samples, respectively, were used to develop the equations of calibration and cross-validation. Table 1 gives the statistical parameters related to the best calibration equations obtained for each type of material analysed. The amylose percentage in starch granules of maize varies widely in amylose concentration (17–94%), covering almost all the variations usually observed in normal and amylomaize endosperm types. For both types of grain material, the coefficient of determination (R^2) in the equation developed

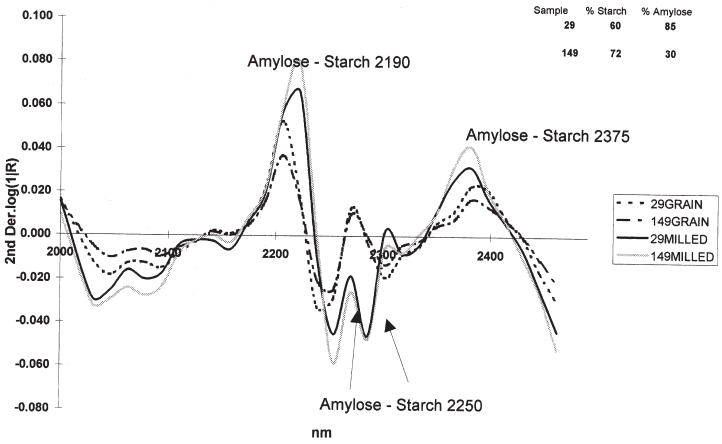

Figure 1. The greatest non water-reflectance difference region spectra of two grains (29GRAIN and 149GRAIN) and their counterpart milled (29MILL and 149MILL) samples with different percentages of starch in the kernel and amylose in starch granules.

for determining amylose percentages was very high, although, for ground material, the coefficient was slightly higher (0.95 vs 0.88, whereas a low standard error of calibration (*SEC*) was observed (3.13 and 4.73) for both estimations, confirming an excellent fit of the parameters used in the calibration equations. The cross-validation statistics for amylose percentages shows a similar trend, although a lower value for grain than for ground grain was noted for the second.

An NIR calibration was also developed for the starch content of two types of sample, whole and ground grains. The range of variation of the starch content was narrower in comparison to amylose content, varying from 53% to 81%. As a consequence of this variation, the R^2 value for this parameter was lower in respect of amylose content, with values ranging from 0.86 for grain to 0.84 for ground grains of maize. However, the *SEC* for starch percentage was appreciably lower for both ground and intact grains with comparable values (2.01 vs 2.09).

These results show that the potential for NIR techniques detecting amylose percentages in maize kernels and other cereals[15,16] is encouraging. The ability to measure percentages of amylose in maize inbreds or hybrids during the selection programmes and evalute kernels for industrial applications in the starch industry, both quickly and accurately, is an important goal.

Table 1. Statistics of the calibration equation of best fit and cross-validation for starch and amylose percentages in grain and ground maize samples including *SEC* and *SECV*.

Parameters	*n* sample	Range	Mean	*SEC*	R^2	*SECV*	r^2
Amylose (grain)	118	17–94	58.78	3.13	0.95	5.19	0.87
Starch (grain)	105	58–81	64.33	2.09	0.86	3.18	0.64
Amylose (ground)	184	17–94	60.78	4.73	0.88	6.99	0.73
Starch (ground)	126	58–81	65.62	2.01	0.84	2.49	0.75

Conclusion

The determination of amylose percentages in starch granules of maize grain by using NIR methods can be accomplished with precision comparable to the conventional colorimetric method. The MPLS models have routinely demonstrated standard errors less than 5% of amylose percentage content in starch granules of maize kernels. Another important aspect is the possibility of analysing grain without milling; the best results were obtained when we used whole grain rather than ground material, thus reducing labour costs for preparing the samples for analyses.

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