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Near infrared reflectance spectroscopy as a potential surrogate method for the analysis of $\Delta^{13}C$ in mature kernels of durum wheat

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Abstract. Carbon isotope discrimination ($\Delta^{13}C$) in grain is a potentially useful trait in breeding programs that aim to increase the yield of wheat and other cereals. Near infrared reflectance spectroscopy (NIRS) is used in routine assays to determine grain and flour quality. This study assesses the ability of NIRS to predict $\Delta^{13}C$ in mature kernels of durum wheat. Plants were grown in north-west Syria as this location provided 3 distinct Mediterranean trials that covered a wide range for $\Delta^{13}C$ values in grains (from about 12.9‰ to 17.6‰). We measured the spectral reflectance signature between 1100 and 2500 nm in samples from the same flour used in the conventional (i.e. mass spectrometry) determinations of $\Delta^{13}C$. By using principal components regression and partial least squares regression (PLSR), a model of the association between conventional laboratory analysis and these spectra was produced. Global regressions, which included samples from all 3 trials, and local models, which used samples from only one trial, were built and then validated with sample sets not included in calibration procedures. In global models, strong significant correlations ($P < 0.001$) were found between NIRS-predicted $\Delta^{13}C$ and measured $\Delta^{13}C$ values. PLSR gave r^2 values of 0.86 and 0.82 for calibration and validation sets, respectively. Although less strongly correlated, all local models selected for a subset of samples with significantly higher $\Delta^{13}C$ values. Local models also performed well when selecting samples from the other 2 trials. The advantages and possible limitations of NIRS are further discussed.

Additional keywords: NIRS, carbon isotope discrimination, PCR, PLSR, multivariate analysis, regression.

Introduction

Integrative physiological criteria are useful in breeding programs aimed at increasing yield in durum wheat and other cereals (Austin 1993; Araus 1996; Slafer *et al*. 1999). Among these criteria, several authors have reported using carbon isotope discrimination ($\Delta^{13}C$) of mature kernels (Acevedo 1993; Araus *et al*. 1998). In C_3 crops, such as wheat and other small grain cereals, $\Delta^{13}C$ may be useful in measuring indirect genetic variation of transpiration efficiency (TE), that is to say, the ratio of net assimilation to water transpired (Farquhar *et al*. 1982; Farquhar and Richards 1984; Hubick and Farquhar 1989; Condon *et al*. 1990). Moreover, when measured in plant dry mass, $\Delta^{13}C$ integrates TE over the period during which the dry mass is laid down. The consistent negative relationship between TE and $\Delta^{13}C$ across treatments, and the high broad-sense heritability of $\Delta^{13}C$, indicate that breeding programs could use this trait to modify TE, and hence increase the yield of water-limited C_3 crops (Condon *et al*. 1987; Condon and Richards 1992, 1993; Hall *et al*. 1994).

Owing to the cost of carbon isotope analyses, several surrogate techniques have emerged. Clark *et al*. (1995) proposed the use of near infrared reflectance spectroscopy (NIRS) for grasses; however, no further studies have yet been published for grasses or for other crops. In the laboratory, NIRS is currently used for quick, accurate, non-destructive, and highly repeatable assays for many biological materials. Such assays cover digestibility, content of nitrogen, energy, moisture, ash, crude fats, total reducing sugars, alkaloids, and a number of other plant compounds (see references in Finney *et al*. 1987; Shenk *et al*. 1992). NIRS can determine such miscellaneous compounds because of the vibrational and rotational energies associated with H bonds (Osborne and Fearn 1986).

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The quantity and quality of proteins, carbohydrates, lipids, and minerals in cereal grains depends on environmental and genotypic factors, which also affect $\Delta^{13}C$ values in kernels (see references in Finney *et al*. 1987; Araus *et al*. 1998; Stone and Savin 1999). The environmental effect is particularly evident for cereals grown under Mediterranean conditions, where drought during grain filling is the main constraint affecting $\Delta^{13}C$ and quality traits of grains (Araus *et al*. 1997*b*, 1999; Acevedo *et al*. 1999). Therefore, provided that the chemical composition of kernels covaries with $\Delta^{13}C$ because of the effect of common environmental and genotypical factors during the crop cycle, it may be possible to correlate the spectral reflectance signature in the near infra-red region with $\Delta^{13}C$ in kernels.

However, the chemical basis of the relationship of $\Delta^{13}C$ with the near infra-red spectrum is unknown, so we must use empirical calibration techniques prior to NIRS computing. The reason for this is that such calibration methods do not require previous knowledge of the compounds involved. For empirical calibration modern multivariate techniques, such as principal components regression (PCR) and partial least squares regression (PLSR), are the most recommended. The principles behind these methods have been described in-depth in the literature (Beebe and Kowalski 1987; Martens and Naes 1989). This paper studies the feasibility of NIRS to evaluate the differences in $\Delta^{13}C$ of mature durum wheat kernels. We propose the use of NIRS as an indirect selection tool during early generations of a plant-breeding program designed to increase yield.

Materials and methods

Plant material and growth conditions

Durum wheat (*Triticum turgidum* L. var. durum) genotypes from the Durum Core Collection at the International Centre for Agricultural Research in the Dry Areas (ICARDA) were cultivated in 1995 under rain-fed conditions in Breda (BR) and Tel Hadya (THR), 2 sites in north-west Syria. These trials showed consistent differences in rainfall and evapotranspirative demand. A third trial was planted at Tel Hadya under support irrigation (THI). Growth conditions are detailed elsewhere (Araus *et al*. 1997*a*, 1998). The conditions of the distinct trials, as well as the large set of genotypes assayed, allowed us to obtain a wide range of variations in $\Delta^{13}C$ (Araus *et al*. 1997*a*, 1998). Thus, Araus *et al*. (1998) reported significant differences in the genotypes within the 3 trials, even after removing the effect of days to heading. Kernels from about half the plots harvested in their study were later used for NIRS and $\Delta^{13}C$ determinations, but working with a different set of genotypes for each trial because of the lack of enough sample for NIRS analysis.

Carbon isotope analysis

Samples of mature kernels were ground and then oven-dried at 60°C for 48 h. The $^{13}C/^{12}C$ ratios were determined by mass spectrometric analysis as reported elsewhere (Araus *et al*. 1997*a*, 1998). Results were expressed as $\delta^{13}C$ (Farquhar et al. 1989), where $\delta^{13}C$ (‰) = $[(R_{sample}/R_{standard}) - 1] \times 1000$, and R is the $^{13}C/^{12}C$ ratio. A secondary standard calibrated against Peedee belemnite carbonate was used for comparison. Sample sizes of 5–10 mg were used. Replicate samples differed by less than 0.10‰. $\Delta^{13}C$ was then calculated from δ_a and δ_p, where a and p refer to air and plant, respectively (Farquhar *et al*. 1989): $\Delta^{13}C = (\delta_a - \delta_p)/(1+\delta_p)$. On the Peedee belemnite scale, free atmospheric CO_2 has a current deviation, δ_a, of approximately −8.0‰ (Farquhar *et al*. 1989).

Near infrared reflectance determinations

A subset of samples from the same oven-dried and ground kernels used for $\Delta^{13}C$ determinations was analysed using a NIRSystems spectrophotometer (Foss-NIRSystems Europe, Raamsdonksveer, Holland) equipped with a fibber-optic probe for quantitative analysis. Reflectance spectra were obtained from 315 samples, performing 32 triplicate scans per sample at 2-nm intervals from 1100 nm to 2500 nm. An average spectrum was then computed from the data. The spectrophotometer was controlled by its bundled software (NSAS version 3.20).

Construction of global models

Spectra were imported from the NSAS file into the program Unscrambler version 6.0 (CAMO Ltd, New Market, UK), which included the PCR and PLSR algorithms used in the building of models. We divided sampled spectra from each of the trials into 2 independent sets: the first was used for calibration purposes, while the second was used to evaluate the predictive ability of the model. Samples were selected to obtain a calibration set with regular increments for $\Delta^{13}C$ (i.e. flat calibration). The number of samples, $\Delta^{13}C$ range, mean, and standard deviation for each set are shown in Table 1. First, global models were built to help select the regression algorithm. Using the full calibration sets, global models were performed with PCR and PLSR. To correct baseline shifts, which are often related with particle size, spectra were mean-centered, but not scaled, using the SNV algorithm (Barnes *et al*. 1989). The number of factors used in each model was determined by cross-validation (Wold 1978) and the root mean standard error of prediction (RMSEP) was calculated for calibration samples to assist in model selection as follows:

$$\mathrm{RMSEP} = \sqrt{\frac{\sum (Y_{ref} - Y_{nirs})^2}{(N-1)}}$$

Table 1. Main descriptive statistics for $\Delta^{13}C$ (‰) for the sample sets used in the models

N, number of samples; All_c, calibration sample set of the global model; All_v, validation sample set of the global model; BR_c, THR_c, THI_c, calibration sample sets of the local models for Breda, Tel Hadya Rainfed, and Tel Hadya under support Irrigation, respectively; BR_v, THR_v, THI_v, validation sample sets for each of the local models

Sample set	N	Range (‰)	Mean ± s.d. (‰)
		Calibration	
All_c	135	12.91–17.67	15.41 ± 1.25
BR_c	42	12.91–14.83	13.92 ± 0.54
THR_c	38	14.03–16.21	15.34 ± 0.47
THI_c	55	15.24–17.67	16.58 ± 0.63
		Validation	
All_v	180	12.92–17.60	15.55 ± 1.26
BR	55	12.92–14.81	13.97 ± 0.50
THR_v	39	14.80–16.06	15.36 ± 0.32
THI_v	86	15.27–17.60	16.66 ± 0.49

Table 2. NIRS calibration and validation statistics of the models for $\Delta^{13}C$ (‰) determination using the combined samples from the three environments (i.e. global models)

AL1, global model using PCR-based calibration; AL2, global model using PLSR-based calibration; All_c, calibration sample set of the global model; All_v, validation sample set of the global model; N, number of samples; r^2, determination coefficient; RMSEP, root mean standard error of prediction

Model	Sample set	Method	N	Factors	r^2	RMSEP (‰)	Intercept (‰)	Slope
				Calibration				
AL1	All_c	PCR	135	14	0.82***	0.53	2.75	0.82
AL2	All_c	PLSR	135	9	0.86***	0.46	2.10	0.86
				Validation				
AL1	All_v	PCR	180	14	0.82***	0.55	1.84	0.88
AL2	All_v	PLSR	180	9	0.82***	0.55	1.48	0.90

***$P < 0.001$.

where Y_{ref} and Y_{nirs} are the $\Delta^{13}C$ values of each sample either determined by mass spectrometry (reference) or predicted by NIRS, respectively, and N is the number of samples. In addition, the coefficient of determination (r^2), the intercept, and the slope of the linear regression between the predicted and measured values were used to select the best-fit model. To evaluate the performance of the selected model, RMSEP, r^2, and the parameters of the regression line were also calculated for the validation set.

Construction of local models

Three local models were performed using PLSR to predict the $\Delta^{13}C$ values of kernels grown in each of the 3 trials (BR, THR, and THI). We calculated the RMSEP, r^2, intercept, and slope as described above. Moreover, we tested the performance of the models by contrasting with samples from the other 2 trials. To do this, the whole sample set from each trial was used for validation, as no sample from a given set was included in the calibration procedures of the other sets. As a reference point for local regressions, we also assessed the predictive ability of the PLSR global model independently within the 3 growth conditions.

Results

Performance of $\Delta^{13}C$ global models

Table 2 shows the main calibration and validation statistics for the global models of the combined values of $\Delta^{13}C$ determination in the 3 trials. Figure 1 shows the regression lines for the NIRS-predicted $\Delta^{13}C$ values versus measured values for the best global model. PLSR showed a lower RMSEP (0.46‰) and a higher determination coefficient ($r^2 = 0.86$) than PCR (RMSEP = 0.53‰ and $r^2 = 0.82$). Moreover, PCR required 14 factors to describe 82.2% of $\Delta^{13}C$ variability in the calibration set, whereas PLSR described 86.4% using only 9 factors. In addition, both validation and calibration regression lines for PLSR showed a lower intercept and higher slope, and therefore better fit the measured values than PCR (see Table 2).

Performance of $\Delta^{13}C$ local models

The calibration and validation results for the local models are summarised in Table 3. These models were built using only PLSR. All models showed highly significant correlations ($P < 0.001$) in calibration sets. When compared with the calibration performance of the PLSR global model (see AL2 in Table 2), the RMSEP was clearly lower in BR and THR (0.30‰ and 0.20‰, respectively), whereas r^2 and the slope were just slightly lower (both values of 0.71 for BR and 0.83 for THR). In contrast, the THI model showed the lowest r^2 and slope (0.35 and 0.37, respectively) for calibration, and even the RMSEP (0.51‰) was higher than that obtained in

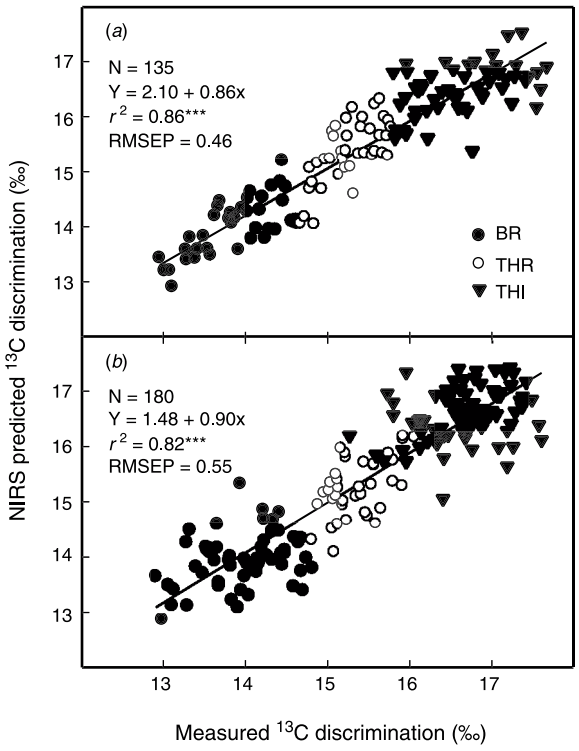

Fig. 1. Relationships between carbon isotope discrimination ($\Delta^{13}C$) predicted by NIRS and $\Delta^{13}C$ measured by mass spectrometry for (*a*) calibration and (*b*) validation procedures of the PLSR global model (AL2). N, number of samples; r^2, determination coefficient; RMSEP, root mean standard error of prediction; BR, THR, THI, samples from Breda, Tel Hadya rainfed, and Tel Hadya under support irrigation, respectively.

Table 3. NIRS calibration and validation statistics for determining $\Delta^{13}C$ (‰) within each of the three environments using either global or local models

BR, THR, THI, local models for Breda, Tel Hadya rain-fed, and Tel Hadya under support irrigation using PLSR-based calibration; AL2, global model using PLSR-based calibration; BR_c, THR_c, THI_c, calibration sample sets for each of the local models; BR_v, THR_v, THI_v, validation sample sets for each of the local models; N, number of samples; r^2, determination coefficient; RMSEP, root mean standard error of prediction; Diff, difference between the average value of $\Delta^{13}C$ (‰) for the two extreme quarters of samples ranked according the NIRS-predicted value (significance level of the *t*-test for the mean is also indicated)

Model	Sample set	N	Factors	r^2	RMSEP (‰)	Intercept (‰)	Slope	Diff ± s.e. (‰)
				Calibration				
BR	BR_c	42	6	0.71***	0.30	4.06	0.71	
THR	THR_c	38	7	0.83***	0.20	2.58	0.83	
THI	THI_c	55	3	0.35***	0.51	10.50	0.37	
				Validation				
AL2	BR_v	55	9	0.11*	0.57	9.47	0.32	0.45 ± 0.19*
AL2	THR_v	39	9	0.29***	0.45	2.06	0.86	0.34 ± 0.14*
AL2	THI_v	86	9	0.09**	0.57	11.77	0.29	0.39 ± 0.15*
BR	BR_v	55	6	0.16**	0.51	9.09	0.35	0.54 ± 0.17**
THR	THR_v	39	7	0.17*	0.42	8.07	0.46	0.37 ± 0.12**
THI	THI_v	86	3	0.12**	0.49	12.56	0.24	0.41 ± 0.15**

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Table 4. NIRS validation statistics for local models predicting $\Delta^{13}C$ (‰) of samples grown in environments other than that in which they were calibrated

BR_{v+c}, THR_{v+c}, THI_{v+c}, validation sample sets resulting from the combination of the former calibration and validation set of samples of the local models for Breda, Tel Hadya rain-fed, and Tel Hadya under support irrigation, respectively; N, number of samples; r^2, determination coefficient; RMSEP, root mean standard error of prediction; Diff, difference between the average value of $\Delta^{13}C$ (‰) for the two extreme quarters of samples ranked according the NIRS-predicted value (significance level of the *t*-test for the mean is also indicated)

Model	Sample set	N	Factors	r^2	RMSEP (‰)	Intercept (‰)	Slope	Diff ± s.e. (‰)
BR	THR_{v+c}	77	6	0.15**	0.44	9.80	0.36	0.40 ± 0.11***
BR	THI_{v+c}	141	6	0.26***	2.71	5.90	0.48	0.80 ± 0.12***
THR	BR_{v+c}	97	7	0.32***	1.16	3.29	0.69	0.71 ± 0.14***
THR	THI_{v+c}	141	7	0.19***	2.80	7.89	0.36	0.63 ± 0.12***
THI	BR_{v+c}	97	3	0.23***	3.29	11.52	0.41	0.72 ± 0.13***
THI	THR_{v+c}	77	3	0.09**	2.36	13.80	0.25	0.33 ± 0.12*

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

the PLSR global model (see Table 2). Therefore, although there is a relative loss of statistical performance (lower r^2 and slope) in local models when compared with the global model AL2, calibration RMSEP was, in general, reduced compared with the results obtained with AL2 (Table 2). Regarding validation performance, although the r^2 values were generally low, all correlations were still significant ($P < 0.05$). Moreover, the validation results within each environment showed that local models (for either BR, THR, or THI) seemed to better fit the measured data than the best global model (AL2 in Table 3). Thus, RMSEP values were always lower for local models than for global ones, whereas r^2 was higher in BR and THI, but lower in THR.

To further evaluate the performance of local models in selecting $\Delta^{13}C$, a comparison of 2 subsets of samples from each trial was carried out. Each subset comprised one-quarter of the validation set, and contained the highest and lowest NIRS-predicted $\Delta^{13}C$ values (Table 3). Differences between the predicted highest and lowest quarters were consistently significant, with absolute differences between means ranging from 0.34‰ to 0.54‰ (Table 3). Compared with the global regression model, local models slightly increased the difference between quarters, but the level of significance was higher ($P < 0.05$ and $P < 0.01$ for global and local models, respectively) due to a reduction of the dispersion within the selected subsets.

In addition, we examined the validation of the performance of local models using samples from distinct trials; the results are shown in Table 4. When compared with results obtained with their own validation sets, although RMSEPs were considerably higher for most of the trials (except for THR predicted by the BR model), correlations

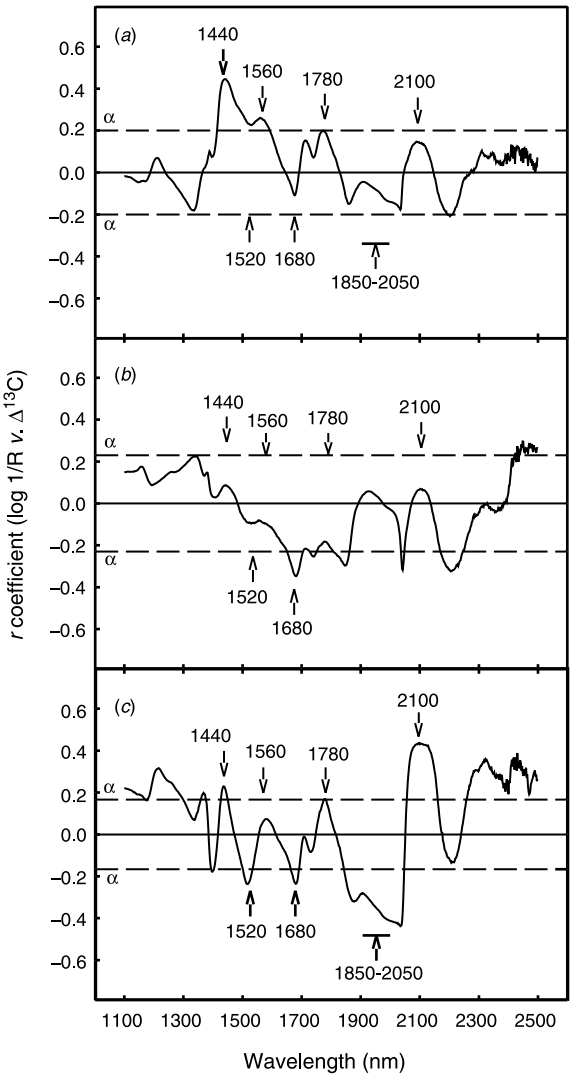

Fig. 2. Correlation coefficients (r) between carbon isotope discrimination ($\Delta^{13}C$) and each of the wavelengths in the near infrared region of the spectrum for the samples of the 3 environments assayed: (*a*) Breda, (*b*) Tel Hadya rain-fed, and (*c*) Tel Hadya under support irrigation. Some relevant peaks have been marked. For more details see the **Discussion**. α, threshold value for significant correlations ($P < 0.05$).

were generally higher and more significant ($P < 0.01$) (see Table 3). In addition, absolute differences between opposite quarters of validating samples were greatly increased for BR and THI (up to 0.72‰ and 0.80‰, respectively), whereas THR remained in the same range (from 0.33‰ to 0.40‰).

Discussion

An insight into the chemical basis of NIRS models to assess $\Delta^{13}C$

To explain the chemical basis of NIRS in $\Delta^{13}C$ evaluation, correlation coefficients (r) between measured $\Delta^{13}C$ values and each of the wavelengths assayed in the near infrared region of the spectrum were plotted for each of the 3 environments (Fig. 2). Even when there were differences in the relative importance of the bands between trials, the wavelengths of these peaks generally coincided. Among the most significant correlations, we found some peaks that, according to the literature (Goddu and Delker 1960; Osborne and Fearn 1986; Shenk *et al*. 1992), corresponded to spectral signatures of starch (1440 nm and 2100 nm), bands from peptide bonds (1980 nm), and amine groups (1520 nm and 1680 nm). In addition, wavelengths associated with the starch to gluten ratio (1560 nm and 1780 nm) and water content (1850–2040 nm) were also detected. Some of these bands have been included in calibrations to determine the moisture and relative amounts of proteins and starch in wheat flour and baked products (Osborne 1992). Therefore, this suggests that the amounts of starch, protein, and water in ground kernels may be the main parameters related (indirectly) with $\Delta^{13}C$ that are quantified by NIRS.

Performance of NIRS approach in estimating $\Delta^{13}C$

Both PCR and PLSR techniques used in constructing models involve the decomposition of a spectrum matrix with k wavelengths into a number of factors, thus allowing the dimensional reduction to address a given problem with no appreciable loss of relevant information. PCR and PLSR differ mainly in the procedure of factor determination. While PCR determines the factors that best describe the variability in the spectrum matrix (principal components), the PLSR factors are not optimal for estimating this matrix, but are rotated to simultaneously describe the variable to regress. As the main sources of variation in NIRS spectrum seem to be quite different from those directly associated with $\Delta^{13}C$, one could expect to obtain better performances with PLSR than with PCR models (Beebe and Kowalski 1987; Martens and Naes 1989). Indeed, even though the validation performance of the 2 models was quite similar, the low number of components used by PLSR implies that this model is more robust. Therefore, PLSR seemed to be the most recommended for this approach.

Although the RMSEP was about 5 times greater than the difference between replicates in $\Delta^{13}C$ mass spectrometry determinations (0.10‰), our models provide a broad pattern of the parameter studied, thus showing a relationship between NIRS information and $\Delta^{13}C$ values in wheat kernels. Generally, we observed that performance was increased in models calibrated for each site. The major constraint of this approach lies in its empirical nature, which makes it necessary to carry out a previous calibration, preferably in the same growing conditions of the samples that we wish to evaluate. However, as the RMSEP was much lower whenever the range of values predicted was within the calibration range (for example, the prediction of THR samples by BR and AL2 models was better than that of the

THI model), a global model with a wide range, such as AL2, might also be used to select high $\Delta^{13}C$ values in distinct environments. We should bear in mind that the strong correlations of the global models across the 3 trials were mainly due to environmental differences in $\Delta^{13}C$, not genotypical, so we should consider the performance within each trial to get insight into their potential ability to select genotypes (Table 3). However, due to the lack of continuity of genotypes across the 3 environments, we cannot separate the relative contributions of genotypic and environmental factors to these NIRS-predicted $\Delta^{13}C$ values. On the other hand, we have shown that a model calibrated in given growing conditions does not fit the absolute values of $\Delta^{13}C$ for an environment other than that in which it was calibrated, but it can still select a group of samples with higher mean $\Delta^{13}C$. This result suggests that the variations in the chemical composition of grains considered by local models must be quite similar in the 3 environments assayed, therefore implying the effect of a common environmental factor.

Water availability is considered the main environmental factor affecting $\Delta^{13}C$, especially under Mediterranean growing conditions (Acevedo 1993; Araus *et al*. 1997*b*, 1999). In addition, water availability is reported to dramatically affect many characteristics associated with grain quality, all of which have a distinct chemical nature (Smika and Greb 1973; Brocklehurst *et al*. 1978; Campbell *et al*. 1981; Rao *et al*. 1993; Garcia del Moral *et al*. 1995; Savin and Molina-Cano 2000). Therefore, the strong performance of the validation regression in the global models is consistent with the strong effect of environmental factors on $\Delta^{13}C$ and other chemical properties of the grain. In contrast, the lower performance of local models (and that of the global model when predicting differences between plots within a trial) relies on the miscellaneous nature of the factors (either environmental or genetic) responsible for the differences in grain quality and $\Delta^{13}C$ across genotypes. For example, although genotypical differences in $\Delta^{13}C$ depend in part on plant water status (i.e. the amount of water each genotype can use), constitutive (i.e. in absence of stress) differences in transpiration efficiency may also be involved (Farqhuar and Richards 1984; Acevedo 1993; Araus *et al*. 1997*c*, 1998). In addition, genotypical differences in grain quality are affected by water availability; however such differences also depend on constitutive characteristics which do not necessarily affect $\Delta^{13}C$ in kernels. Moreover, the poorer performance of local models for $\Delta^{13}C$ evaluation through NIRS may be also due to the indirect relationship between grain quality and $\Delta^{13}C$. Thus, the use of NIRS to assess the $\Delta^{13}C$ value from the grain composition involves, by definition, an additional step (the extrapolation between grain composition and $\Delta^{13}C$), which introduces a source of error.

The range of values and the number of samples used in each kind of model can also explain the distinct performance of global and local models. Thus, the results for $\Delta^{13}C$ prediction by NIRS using global models (RMSEP = 0.55‰, r^2 = 0.82) are not far, in terms of statistical performance, from the well-established NIRS procedures for ash, protein, or starch content determinations (Osborne and Fearn 1986; Osborne 1992; Shenk *et al*. 1992). Moreover, they are in agreement with those obtained by Clark *et al*. (1995) for NIRS evaluation of $\Delta^{13}C$ in grasses, where RMSEP were between 0.35‰ and 0.59‰ and r^2 ranged from 0.75 to 0.93. These authors included a wide range of variation for $\Delta^{13}C$ (up to 4.9‰) in each sample set, and worked with values ranging from 17.1‰ to 24.8‰. In our study, total variation for this parameter over the 3 environments was around 4.8‰ (from about 12.9‰ to 17.7‰). Therefore, our global model, which worked with a range of values close to those in the work of Clark *et al*. (1995), gave similar results. In contrast, local models showed a considerably higher RMSEP and lower r^2 than those found in the previous study. This could be explained by the lower range of $\Delta^{13}C$ values used by our local models, along with the few samples available for the construction of representative calibration and validation sets. Indeed, the sample sets assayed here included local variations in $\Delta^{13}C$ between 1.3‰ and 2.3‰.

Nevertheless, despite these constraints, NIRS can select for either high or low values of $\Delta^{13}C$ in all 3 environments assayed. In addition, this selective ability is not limited to the modelled environment, thus reducing the need to use independent calibration procedures to compensate for environmental variations due to spatial or temporal changes (i.e. different sites or seasons). From our results, NIRS appears to be potentially useful in breeding programs whenever an integrative estimation of TE is required. Depending on the target environment, breeders could select for either high or low NIRS-predicted $\Delta^{13}C$. Well-watered plants usually show positive correlations between $\Delta^{13}C$ and yield, whereas in extreme water-limited environments negative correlations have been reported (Acevedo 1993; Richards 1996; Voltas *et al*. 1999). Indeed, within the trials assayed in our work, where $\Delta^{13}C$ was positively correlated with yield, differences in $\Delta^{13}C$ between NIRS-selected extreme subsets may account for a grain yield increment of about 30% (Araus *et al*. 1998). Moreover, NIRS evaluations of $\Delta^{13}C$ could be combined, without added cost, with the grain quality evaluations that are performed routinely with NIRS in breeding programs (see Osborne 1992; Hollamby and Bayraktar 1996). The main advantage of this potential method is that NIRS measurements can be performed on a whole grain basis, therefore allowing at the same time evaluation of grain quality and estimation of the yield performance in water-limiting environments without any loss of sample. This would be of special interest during the first generations of a breeding program, when the amount of sample available for each genotype is usually low. However, further work is required in order to determine the genotypic component of NIRS-predicted $\Delta^{13}C$ values,

which must be well known before the adoption of this method in breeding programs.

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