JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Characterization of Maize Germplasm for the Chemical Composition of the Grain

NICOLA BERARDO,* GIANFRANCO MAZZINELLI, PAOLO VALOTI, PAOLO LAGANÀ, AND RITA REDAELLI

CRA—Maize Research Unit, via Stezzano 24, I-24126 Bergamo, Italy

The aim of this research was the evaluation of food grain quality-related traits in a collection of maize populations of different origins, currently used in the framework of breeding and genetic programs. A total of 1245 maize samples were scanned by near-infrared (NIR) spectroscopy to develop calibration equations to evaluate the content of crude protein, crude lipid, starch, and floatation area. The performances of the NIR equations developed in our study were assessed using the determination coefficient of cross-validation r^2 (ranging from 0.66 to 0.91) and the ratio of performance deviation (1.71−3.31) in flour starch and grain crude protein, respectively. Among the genotypes considered, 93 landraces belonging to the European Union Maize Landraces Core Collection (EUMLCC) were also analyzed for their content of lutein, zeaxanthin, and total carotenoids. Among the populations of the collection, several accessions, interesting from a nutritional point of view, were identified: VA25, VA158, VA282, VA284, VA285, VA567, VA572, VA814, VA950, VA1057, and VA1179. They showed protein and lipid contents ranging between 12.52 and 15.16% and 5.26 and 7.17%. The range of variation observed for antioxidants in the EUMLCC was quite large. Lutein varied between 1.03 and 21.00 mg kg^{-1} dm, zeaxanthin varied between 0.01 and 35.00 mg kg^{-1} dm, and total carotenoids ranged from 1.09 to 61.10 mg kg^{-1} dm. Recently, a single cross-hybrid was developed from the ITA0370005 population; this hybrid had a high carotenoids content and is currently being used by the Italian food industry.

KEYWORDS: Maize germplasm; NIR; EUMLCC; carotenoids

INTRODUCTION

The continuous and significant loss of genetic variability in most crops, observed in recent years, has stimulated a growing interest in the preservation of biodiversity. About 14 million species (*1*) and 1.1−6.6 billion populations (*2*) are estimated to compose the existing biodiversity of our planet, and about 27000 species are lost every year (*3*). After the United Nations Conference on Environment and Development held in Rio de Janeiro in 1992, the preservation of biodiversity in all species became a priority challenge for the new millennium.

In the 1950s, the diffusion of maize (*Zea mays* L.) hybrids, characterized by a superior yield performance, brought a progressive substitution of local populations in almost all of the European maize-growing countries. As a consequence, the genetic variability of the cultivated maize germplasm was reduced over the past 50 years, in terms of both number of alleles and genetic diversity between hybrids (*4*). The necessity to collect and maintain the traditional maize landraces emerged for the first time in past decades, as it became evident that some actions were to be taken to avoid a significant loss of the genetic variability existing in Europe for this species. In different countries, collections of populations (landraces, local varieties, and so on) were activated.

In this context, the CRA—Maize Research Unit began in the 1950s to collect many traditional maize populations grown in Italy before the introduction of hybrids and constituted a collection of about 1500 landraces, representing most of the genetic variability available in our country for this species. A part of these populations have been described in terms of plant morphology, ear characteristics, and some kernel chemical compounds (*5*, *6*). Recently, the genetic distances among 54 Italian landraces were also assessed at both the morphological and the molecular level (*7*). In addition to these landreaces, many traditional populations were also acquired from different countries and included in the germplasm collection.

Among these accessions, 93 landraces were selected to represent the European Union Maize Landraces Core Collection (EUMLCC), constituted through the research project RESGEN088 with the aim to preserve the genetic variability of maize in Europe. Information about the background of the research, the genotypes used, and the results obtained can be found at the Web site of the project (*8*).

Because maize is a relevant food source, the quantification of the grain constituents with a nutritional role is important for the best exploitation of the different genotypes. In this context,

* To whom correspondence should be addressed. Tel: 0039 035 313132. Fax: 0039 035 316054. E-mail: nicola.berardo@entecra.it.

10.1021/jf803688t CCC: $40.75
Published on Web 02/27/2009

Table 1. Laboratory Data of Maize Grain and Flour Samples[a]

traits	N	mean	range	SD	CV_{AS} (%)	SEL	CV_{AD} (%)
			Grain				
CP	381	10.61	6.31−14.81	1.64	15.46	0.08	0.79
CL	643	6.10	2.53−18.52	2.38	38.97	0.09	1.54
ST	180	65.18	58.55−73.09	2.99	4.59	0.21	0.32
FA	403	3146.45	2300.11−4332.97	411.36	13.07	20.49	0.65
			Flour				
CP	302	10.33	6.31−14.62	1.53	14.81	0.09	0.85
CL	302	5.13	2.53−8.30	1.25	24.33	0.07	1.40
ST	302	67.25	58.55−79.78	4.45	6.62	0.26	0.38

[a] CP, CL, ST in % dry matter basis.

Table 2. NIRS Calibration Statistics for Maize Grain and Flour Traits[a]

traits	N	mean	SD	SEC	R^2	SECV	r^2	BIAS	RPD
				Grain					
CP	335	10.67	1.59	0.41	0.93	0.48	0.91	−0.29	3.31
CL	549	5.72	1.67	0.67	0.84	0.79	0.78	−0.48	2.11
ST	127	66.92	2.94	1.06	0.87	1.06	0.87	−0.64	2.77
FA	370	3125.66	392.47	147.68	0.86	174.94	0.80	−104.46	2.24
				Flour					
CP	259	10.42	1.47	0.43	0.91	0.51	0.89	−0.31	2.88
CL	182	4.95	0.89	0.35	0.84	0.40	0.80	−0.24	2.23
ST	164	67.57	2.60	1.49	0.67	1.52	0.66	−0.91	1.71

[a] N, number of samples used in the calibration; and r^2, determination coefficient of cross-validation.

Table 3. Mean, Range of Variation, and LSD for CP, CL, ST, and FA, As Determined in 996 Maize Accessions from Different Countries

	CP	CL	ST	FA
		Italy ($n = 547$)		
mean	11.48	5.00	65.68	3188.48
min	7.91	2.58	61.18	1933.14
max	15.42	7.74	69.67	4286.67
LSD ≤ 0.05	1.20	1.24	206	394.72
		Other Countries ($n = 449$)		
mean	11.36	5.10	65.88	3289.75
min	7.39	2.27	61.30	2094.92
max	15.12	7.67	70.07	4633.50
LSD ≤ 0.05	1.09	1.22	1.98	414.61

Table 4. Canonical Variable Means of Corn Grain Germplasm

	root 1	root 2
	Italian Populations	
floury	−0.259	−0.026
semivitreous	0.264	0.200
vitreous	0.662	−0.128
	Foreign Populations	
floury	0.170	0.004
semivitreous	−0.51	−0.174
vitreous	−0.600	0.315

the traditional germplasm represents a good source of genetic variability to explore and could help to identify the most suitable materials for the development of more nutritious foods. Near-infrared (NIR) spectroscopy is a well-established nondestructive screening method used in plant breeding and in the cereal industry for estimating a wide range of chemical components (*9*, *10*) and for the screening of a large number of samples. The reproducibility of the acquired spectra, the number of specific calibrations developed for the main components of plant tissues, and the high prediction performance make it the most efficient method to get a large number of chemical data in a reduced span of time.

The objectives of the present study were to (1) explore the variability existing for some chemical components of the grain in a large range of maize populations, including our available germplasm of local varieties and the EUMLCC, and (2) identify genotypes that could be interesting in terms of nutritional value.

MATERIALS AND METHODS

Maize Samples. Maize samples used in this study consisted of 1245 accessions from our germplasm collection, among which there were 633 traditional populations, collected in different Italian regions, and 519 accessions acquired from different countries (Albania, Austria, Canada, Czech Republic, Chile, China, Cyprus, Spain, Ethiopia, France, Germany, Japan, Morocco, Mexico, The Netherlands, Romania, Turkey, Hungary, the United States, and Russia); additionally, 93 populations represented the EUMLCC.

NIR Spectroscopy Analysis. All samples were submitted to NIR spectroscopy analysis, as both whole grains (about 20 g) and flours (4 g). Samples were scanned in the visible and NIR regions of the electromagnetic spectrum in reflectance (400−2500 nm) at 2 nm intervals using a scanning monochromator NIRS 6500 (NIRSystems). Spectral data were stored as a logarithm of the reciprocal of reflectance [log (1/*R*)]. The software for scanning, mathematical processing, and statistical analysis was supplied with the spectrophotometer by Infrasoft International (ISI, Port Matilda, PA).

A principal component analysis (PCA) was run, and the generalized Mahalanobis distances (*H*) were computed for each spectrum. All samples that had *H* values above 3 were considered as outliers. A maize subsample of the population was selected for laboratory analyses from grain spectra, based on neighborhood Mahalanobis distances (NH).

Technological and Chemical Analysis. The value of the floatation area (FA) was determined according to Ferrari (*11*), using a sample of 50 sound kernels. Kernels with a vitreous texture showed a value lower than 3100; the intermediate type values varied between 3101 and 3300; the values of the maize floury kernels were larger than 3301. For chemical analyses, the grain samples were milled using a Cyclotec Udy laboratory mill (Tecator-FossItalia-PD, Italy), with a 1 mm sieve. The protein content was determined according to the Dumas method (*12*). The lipid content was evaluated using the Soxtec System Tecator method. Carotenoids were extracted according to Berardo et al. (*13*) and evaluated by high-performance liquid chromatography according to the method described by Buratti et al. (*14*), with minor modifications. Briefly, 5 g of ground grain, brought to a constant humidity at 40 °C in a desiccator, was suspended in 3 mL of methanol, added by the internal standard, and extracted in the dark with about 50 mL of unstabilized tetrahydrofuran (THF). The sample was homogenized at a moderate speed for few minutes by using an Ultra-Turrax homogenizer (Janke & Kunkel, Staufen, Germany); the samples were kept refrigerated in an ice bath to avoid overheating and potential carotenoid damage. Following a 10 min centrifugation at 10000*g*, the supernatant was collected, and the residue was extracted again, until the solid was white. The pooled THF extracts were partitioned in petroleum ether; the extracts were kept refrigerated in an ice/solid NaCl mixture with stirring for 45 min to solidify the contained lipids. The ether was transferred in another amber-colored flask, and the solid lipid was re-extracted with petroleum ether by brief sonication of the melted lipid mass. The freezing−sonication step was repeated at least three times, or further, till an A_{450nm} <0.01 resulted for the ether supernatant. All solvents used were added by 0.1% BHT. The ethereal extracts were then concentrated under a nitrogen steam and transferred into 5 mL glass tubes. Each aliquot was finally dried under nitrogen flow and stored in the dark at −20 °C. All of the analyses were carried out in duplicate.

Development of NIR Prediction Equations. Calibrations were performed using a modified partial least-squares (MPLS) regression of WinISI. Calibration statistics included the following parameters: standard deviation of the population (SD), coefficient of determination (R^2) and regression slope, bias, standard error of calibration (SEC), and standard error of cross-validation (SECV). In the determination of SECV, 25% of the samples was used to validate a calibration model

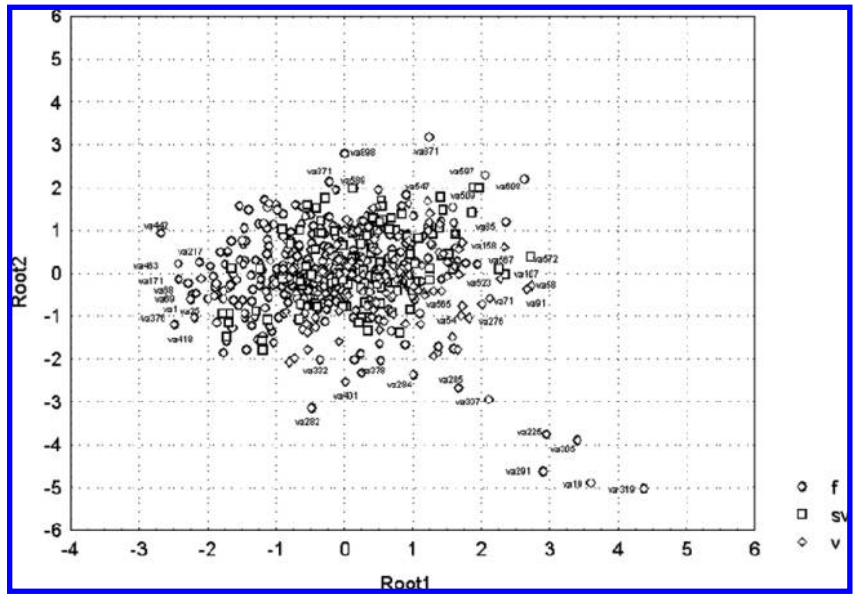

Figure 1. Scatterplot of canonical scores for root 1 and root 2 of Italian germplasm. Genotypes were classified as floury (○), semivitreous (□), and vitreous (◇).

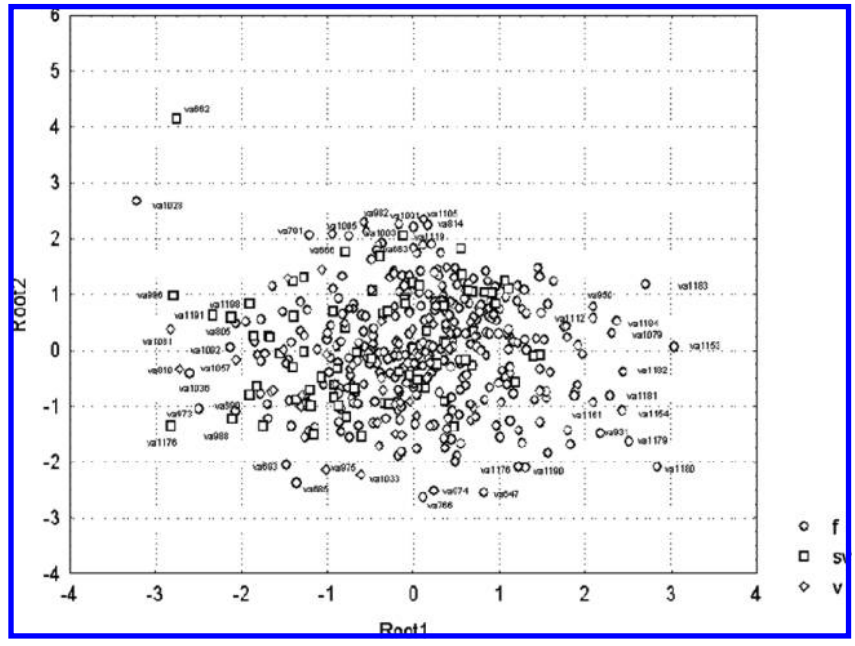

Figure 2. Scatterplot of canonical scores for root 1 and root 2 of foreign germplasm. Genotypes were classified as floury (○), semivitreous (□), and vitreous (◇).

developed with the remaining 75%. SECV was repeated four times, and the average was calculated. The ratio of performance deviation (RPD) was calculated as SD/SECV. Student's test (*t*) was used to identify *t* outlier samples.

Statistical Analyses. Analysis of variance (ANOVA) was performed using AGROBASE GENERATION II version 18.2.1 (Agronomix Software, Inc., Winnipeg, Manitoba, Canada). Canonical and discriminant analyses and unweighted pair-group arithmetic method (UPGMA) were performed using the appropriate options of STATISTICA (StatSoft Inc., Tulsa, OK).

RESULTS AND DISCUSSION

In the present study, we used a maize germplasm collection, representing the genetic diversity of cultivated maize, to predict the food quality of the grain by NIR.

Laboratory Analyses. Laboratory analyses of maize samples were carried out for crude protein (CP), crude lipid (CL), starch (ST), and FA. For each trait, the number of samples analyzed (*N*), the mean values, the range of variation, the SD, the coefficient of variation among samples (CV_{AS}), and the standard error of laboratory (SEL) are shown in **Table 1**. The repeatability was excellent for all biochemical parameters determined in the grain flour, and the coefficients of variation among duplicates (CV_{AD}) varied from 0.38 to 1.40%. For the physical parameter determined in the grain samples, that is, FA, CV_{AD} was also good (0.65%), whereas CL had the highest CV_{AD} (1.54%) and ST had the lowest value (0.32%).

Calibration from Whole Grain and Flour Spectra. The quality of standard analyses is a prerequisite for developing NIR calibration. The NIR absorbance for various generic functional groups (*15*, *16*) may be correlated to the major grain and flour constituents. The use of whole grains to

Table 5. List of the 77 Accessions Belonging to the EUMLCC Analyzed in This Study and Their Contents of Lutein, Zeaxanthin, Total Carotenoids (mg kg^{-1} dm), CP, and CL (% dm)

sample no.	accession code	lutein	zeaxanthin	total carotenoids	CP	CL
1	DEU1460010	1.87	0.87	2.82	11.67	5.11
2	DEU1460013	4.00	1.76	5.96	12.50	4.25
3	DEU1460026	2.85	1.19	4.18	14.84	5.43
4	DEU1460158	5.67	2.27	9.30	13.92	5.43
5	DEU1460239	5.63	2.00	9.06	10.25	4.81
6	DEU1460312	5.66	1.61	7.54	11.86	5.05
7	ESP0070127	6.27	5.59	12.45	10.93	6.71
8	ESP0070217	8.98	11.90	25.40	11.24	6.07
9	ESP0070339	15.70	10.85	32.05	11.62	4.90
10	ESP0070441	1.50	1.64	3.21	12.17	5.08
11	ESP0070784	6.37	7.54	18.25	12.44	5.22
12	ESP0070892	6.26	10.40	21.10	11.13	5.36
13	ESP0090025	4.29	3.62	9.03	12.67	5.70
14	ESP0090032	3.00	6.25	11.80	12.66	4.97
15	ESP0090033	7.24	12.30	24.15	12.19	5.67
16	ESP0090067	4.72	4.30	11.20	13.37	4.91
17	ESP0090205	9.48	4.32	18.10	10.56	5.99
18	ESP0090214	16.15	6.16	27.20	11.37	5.03
19	ESP0090300	13.00	3.08	17.50	13.26	4.74
20	ESP0090315	1.17	0.49	2.11	12.74	6.27
21	ESP0090343	6.70	4.04	14.95	13.10	5.10
22	ESP11973C03	13.95	9.75	25.85	10.79	6.12
23	ESP11981040	1.03	0.01	1.09	11.80	4.96
24	ESP11981047	3.18	1.37	4.70	11.53	4.95
25	ESP11982012	3.83	7.31	13.90	12.09	5.04
26	ESP11982019	9.47	7.23	20.40	13.30	5.40
27	ESP11982031	4.44	4.30	10.50	10.89	5.68
28	ESP11985022	4.11	1.72	6.03	11.14	5.20
29	FRA0410006	6.20	2.11	9.32	11.76	4.27
30	FRA0410010	3.99	0.13	4.50	11.74	4.96
31	FRA0410015	4.17	1.45	5.83	12.65	4.60
32	FRA0410022	10.03	4.27	15.95	12.04	5.21
33	FRA0410023	9.63	11.10	26.65	13.45	4.67
34	FRA0410031	9.86	1.87	12.75	12.72	4.95
35	FRA0410090	13.75	22.50	46.40	11.23	5.19
36	FRA0410194	4.79	4.91	12.95	11.58	4.88
37	FRA0410619	4.29	6.13	12.00	12.09	4.62
38	FRA0410625	8.72	17.10	31.00	12.34	4.56
39	FRA0410969	12.10	11.00	27.80	13.06	4.45
40	GRC0010012	3.77	5.84	10.60	11.64	4.95
41	GRC0010016	11.45	5.63	19.65	13.14	4.69
42	GRC0010017	7.07	14.40	27.60	12.26	5.37
43	GRC0010051	5.12	7.00	14.65	11.31	5.51
44	GRC0010084	5.10	4.97	12.95	11.00	5.61
45	GRC0010160	2.99	4.63	7.76	12.21	5.82
46	GRC0010165	5.10	5.57	13.15	12.61	5.68
47	GRC0010179	5.27	1.52	7.32	13.89	5.31
48	ITA0370005	21.00	28.20	61.10	11.99	4.58
49	ITA0370058	3.91	6.35	12.35	12.48	5.56
50	ITA0370071	5.28	8.02	15.90	10.98	6.04
51	ITA0370088	6.89	7.94	16.75	10.56	6.13
52	ITA0370143	3.73	5.58	10.30	13.32	4.62
53	ITA0370152	6.40	8.74	18.55	13.09	5.46
54	ITA0370154	8.51	35.00	51.50	12.83	5.50
55	ITA0370171	6.90	5.48	14.90	10.82	5.07
56	ITA0370185	12.60	27.65	52.60	11.24	5.12
57	ITA0370195	5.19	7.22	16.15	12.43	5.03
58	ITA0370336	9.05	15.65	31.05	10.92	4.87
59	ITA0370373	6.52	14.45	28.75	12.36	4.78
60	ITA0370466	4.23	2.54	7.91	12.99	5.01
61	ITA0370475	2.16	2.33	4.60	12.40	5.49
62	ITA0370477	2.56	3.01	2.69	11.45	5.03
63	ITA0370488	4.48	6.99	14.10	12.44	4.93
64	PRT00100019	6.59	5.02	12.75	13.01	4.96
65	PRT00100049	6.26	5.73	14.40	12.32	5.31
66	PRT00100088	2.52	0.01	2.65	13.39	5.56
67	PRT00100120	4.30	2.31	6.83	12.09	4.44
68	PRT00100186	4.90	2.19	7.33	12.98	5.30
69	PRT00100291	1.48	0.01	1.55	12.39	5.81
70	PRT00100530	9.97	12.75	28.80	11.62	5.94
71	PRT00100569	14.30	20.90	46.45	11.25	5.87
72	PRT00100813	10.95	2.39	14.55	13.39	5.62
73	PRT00100815	4.12	2.35	6.67	11.39	5.41
74	PRT00100828	3.44	1.28	4.89	11.05	5.27
75	PRT00100867	3.42	0.93	4.67	13.16	5.32
76	PRT00100916	15.85	9.51	29.65	13.61	4.75
77	PRT00101526	5.10	2.94	8.30	12.59	5.62
mean		6.65	6.75	16.11	12.17	5.23
LSD0.05		0.49	0.83	0.86	0.41	0.34

develop calibration equations has been studied for other cereals (*9*, *10*), in the scope of eliminating the tedious grinding step and developing a nondestructive measurement method.

The regression coefficients of vis–NIR equations of whole grain and flour showed several distinct peaks where a functional group absorbed in the NIR region. Vis–NIR bands that have been assigned to chemical functional groups, present in whole grain and flour, could be used to interpret the specific vis–NIR absorption bands. The functional group bands for grain and flour were assigned according to the literature (*15*−*20*).

The dominant wavelengths for protein fell on 1196, 1250, 1484−1516, 1750−1800, and 2130−2180 nm, corresponding to the second overtone C−H CH_3 groups stretching mode, third overtone N−H stretching in the symmetrical mode, the first overtone N−H stretching mode, third overtone C−O C−N stretching and N−H bending modes, second and third overtone N−H bending, and stretching C−H C═O stretching combination band modes. The main regression coefficients obtained for lipids fell on wavelengths 1240, 1580, 1716−1724, 2076−2092, 2140, 2308, and 2380 nm, corresponding to the second overtone C−H stretching mode, third overtone −C═C− conjugated chains stretching mode, first overtone C−H and C−O stretching, a first overtone O−H combination band, first overtone C−H stretching and C−O combination band modes, second overtone CH bending, and third overtone C−H stretching and C−C combination bands, respectively. The dominant absorption bands attributed to ST fell on wavelengths 1460, 1620−1680, 2280−2284, and 2326−2334 nm, corresponding to the first overtone OH stretching mode, second overtone C−H stretching mode, C−H stretching and CH_2 deformation band modes, and C−H CH_2 deformation combination band modes, respectively. The main FA equation coefficients were detected at 896, 1048, 1620−1680, and 1820 nm corresponding to the third overtone CH stretching mode, second overtone NH stretching mode, first overtone CH stretching hardness, first and second overtone OH, and C−O stretching and combination band modes, respectively; all of these groups could be ascribed to ST or cellulose and hardness.

We obtained acceptable reproducibility of NIR spectra acquisition on both whole grain and flour. The performances of the NIR equations developed in our study for CP, CL, ST, and FA were evaluated using SECV, BIAS, and RPD, which are the most significant statistics for a rapid appraisal of calibration performance (*21*). The calibration performance was good for CP, particularly in the grain (**Table 2**): R^2 was 0.93, and RPD was 3.31, whereas the regression line between actual and predicted CP values had a slope of 1.00 (data not shown) with a bias of −0.29. The corresponding values for CP in the flour were slightly lower ($R^2 = 0.91$ and RPD = 2.88), whereas the bias value was similar (−0.31), indicating a good firmness of the calibration performance. A quite similar result was observed for CL calibrations, with a close R^2 value (0.84), and RPD values of 2.11 and 2.23 for whole grain and flour calibration, respectively. On the basis of RPD value, the calibration performance for ST was better when using the whole grain (2.77) than the flour (1.71), whereas the bias had an opposite trend (−0.64 and −0.24 for grain and flour, respectively). The FA calibration was quite good, showing a high R^2 value (0.86) and a RPD value over 2.

While these equations remain to be validated using independent samples, they can already have an application in breeding and technology programs to compare varieties. The quality and potential of the NIR equations here presented are due both to

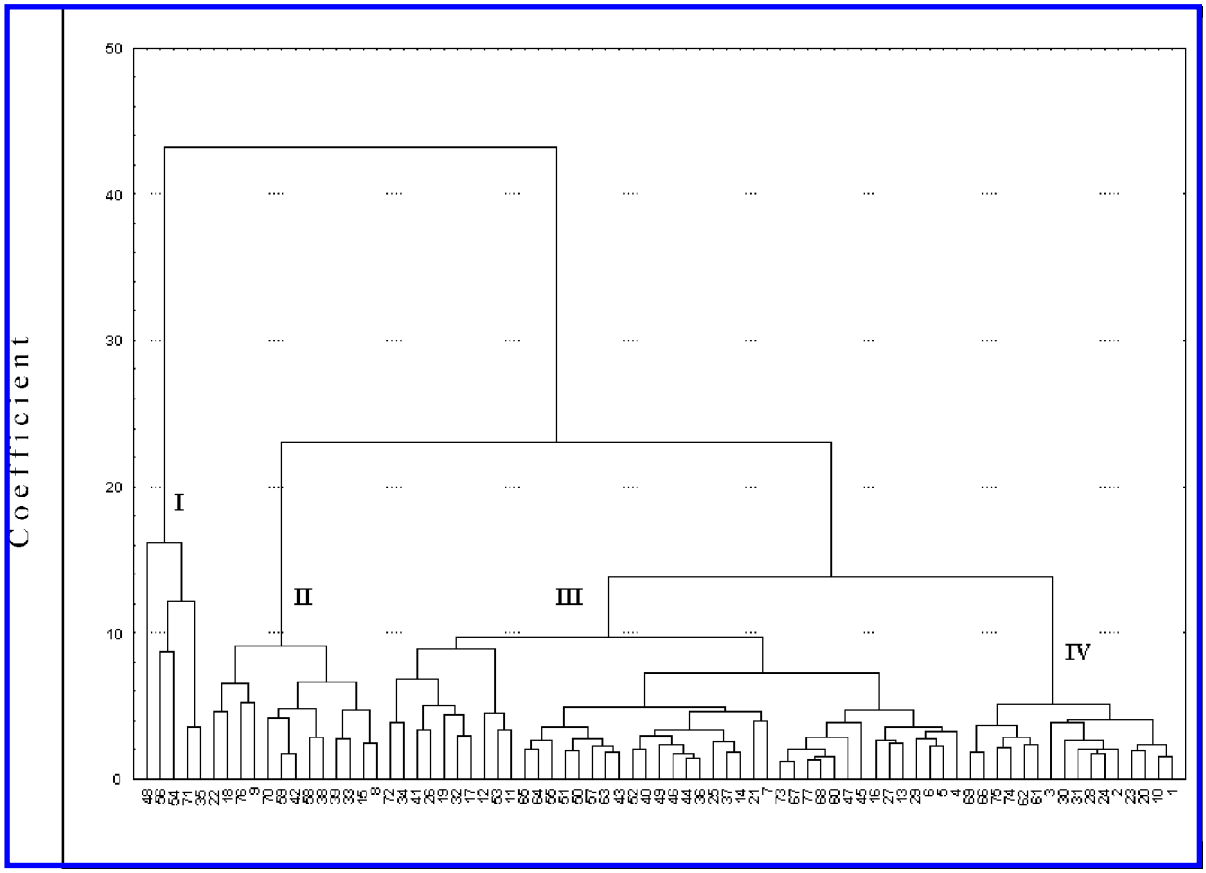

Figure 3. UPGMA clustering of 77 EUMLCC based on qualitative traits.

the use of a collection of accessions that covers a broad variability of consumers' usages and the accuracy of the developed models.

Statistical Analysis of Maize Germplasm. By means of NIR prediction equations, we obtained a set of chemical data regarding 996 maize populations from both Italy and foreign countries. Each group was analyzed separately by ANOVA, using a one-factor model with two replications; the mean values, the range of variation, and the least significant difference (LSD) ≤ 0.05 values for each trait in the two groups are reported in **Table 3**. In general, the CP content varied between 7.39 and 15.42%, the CL content varied between 2.27 and 7.74%, while the ST content ranged from 61.18 to 70.07%. This is in agreement with the data available in the literature for proximate analysis (*22*, *23*). The means of the two groups of accessions were compared statistically using Student's *t* test and were found to be significantly different for all traits (data not shown).

To obtain a more informative description of these materials, discriminant analysis was carried out. As a first step, the genotypes were classified as floury, semivitreous, and vitreous on the basis of the texture characteristic of the endosperm, as expressed by the value of the FA. The two sets (Italian and foreign populations) were then analyzed using two specific canonical variables from discriminant analysis, root 1 and root 2, that resulted mostly effective in separating floury genotypes from the others in both sets of populations, as shown by the comparison of their mean values (**Table 4**). Root 1 and root 2 can be visualized in the plots of individual scores from the two data sets (**Figures 1** and **2**). In the plot of the Italian populations (**Figure 1**), a group of 28 floury accessions, which showed a large range of variation in the value of the FA (3315.07−4013.27), were distinguished from the others. Among them, some were characterized by high CP and CL contents: VA25 (14.24 and 6.03%, respectively), VA282 (14.38 and 6.75%), VA284 (15.16 and 6.22%), and VA285 (14.83 and 7.17%). Six semivitreous accessions were also distinguished, two of which had results high in CP and CL: VA567 (12.53 and 6.26%) and VA572 (12.52 and 5.76%). Finally, among the six distinguished vitreous populations, VA158 was considered the most interesting for its CP and CL contents (14.43 and 5.93%).

In the plot of the foreign populations (**Figure 2**), most of the distinguished accessions (47 populations) had a floury texture. Among them, three genotypes of different origins (Romania, the United States, and Cyprus) were identified, with a high CP or CL content: VA814 (14.57 and 5.57%), VA950 (13.17 and 5.75%), and VA1179 (13.59 and 5.26%). Also a vitreous population, VA1057, was found interesting for its CP and CL contents (14.10 and 6.25%).

Qualitative Characterization of the EUMLCC. The characterization of the EUMLCC for a complete valorization of its genetic resources was a main step in the RESGEN 088 project. A wide, interesting variability was described in these accessions for resistance to insects, in both early and late populations (*24*, *25*) and for forage digestibility (*26*). In the present study, the existent variability for the content of carotenoid components, CP, and lipid in the populations of the EUMLCC was explored; in **Table 5**, the mean values and LSDs ($p \leq 0.05$) for each trait are reported. The ranges of variation observed for carotenoids (1.03−21.00 mg kg^{-1} for lutein, 0.01−35.00 mg kg^{-1} for zeaxanthin, and 1.09−61.10 mg kg^{-1} for total carotenoids) were larger than those observed in previous reports (*27*, *28*) and indicated the presence of an interesting variability among the maize accessions for the ability to accumulate these compounds. Lutein and zeaxanthin represented both 41% of total carotenoids, according to the figures reported in previous studies (*29*). When the contents of total carotenoids were averaged across the

landraces from each country, Italy and France showed the highest values, 22.45 and 18.65 mg kg^{-1}, respectively (data not shown). The variability observed for CP content (10.25−14.84%) and CL content (4.25−6.71%), on the other hand, was less effective in discriminating the landraces, also on a geographical basis. A positive, significant correlation ($p \leq 0.01$) was found among the carotenoid components, which, on the other hand, were not correlated with protein and lipid contents (data not shown).

Cluster analysis was performed to reveal association between landraces. Genetic similarity was calculated from chemical data by UPGMA cluster analysis based on Euclidian distance coefficients, with similarity coefficients. Cluster analysis divided the 77 landraces into four distinctive groups (**Figure 3**). The content of total carotenoids was the discriminating trait among the groups: A linear decreasing gradient was noted from sample 48 (61.10 mg kg^{-1}) to sample 1 (2.82 mg kg^{-1}). The clusters obtained with the chemical data did not appear clearly related to the geographic origin of the landraces analyzed; in fact, the populations belonging to different national germplasms were distributed uniformly across all of the groups, with the exception of the six German populations, which were all found in group IV. In fact, the moderate variability of the European germplasm due to the recent introduction of the crop in Europe and the numerous exchanges of cultivated populations among countries do not make it possible to distinguish different European maize races based on variability or origin (*30*). Five populations (samples 48, 56, 54, 71, and 35), which showed the highest content in carotenoids, were clustered together in group I, apart from all of the other genotypes. It is interesting to note that landraces ITA0370005 and PRT00100569 (samples 48 and 71, respectively), besides having a high content in carotenoids, also showed a good level of resistance to corn borer infestation (*24*). The identification of favorable chemical and physiological characteristics in landraces could be useful for the exploitation of these traditional genotypes, for example, by the introduction of the desired traits in elite germplasm. Recently, a maize hybrid was developed from population ITA0370005, with a high carotenoids content, and is currently being used by an Italian beer industry.

In conclusion, the screening of the Italian and foreign germplasm in our collection revealed the presence of a wide genetic variability for some antioxidant components. The other traits analyzed also showed a good range of variation. From the results of these analyses, some genotypes were identified that can be considered interesting in breeding programs. As an example, the characteristics of Italian landraces Nostrano dell'Isola and Marano (high contents of protein, lipid, and carotenoids and a vitreous texture) were recently introduced into high-yielding, modern genotypes, which are now being exploited for the production of more nutritious maize-based foods.

LITERATURE CITED

(1) Myers, N.; Mittermeier, R. A.; Mittermeier, C. G.; Da Fonseca, G. A. B.; Kent, J. Biodiversity hotspots for conservation priorities. *Nature* **2000**, *403*, 853–858.

(2) Hughes, J.; Daily, G. C.; Ehrlich, P. R. Population diversity: its extent and extinction. *Science* **1997**, *278*, 689–692.

(3) Wilson, E. O. *The Diversity of Life*; Belknap Press: Cambridge, MA, 1992; p 423.

(4) Reif, J. C.; Hamrit, S.; Heckenberger, M.; Schipprack, W.; Maurer, H. P.; Bohn, M.; Melchinger, A. E. Trends in genetic diversity among European maize cultivars and their parental components during the past 50 years. *Theor. Appl. Genet.* **2005**, *111*, 838–845.

(5) Brandolini, A.; Brandolini, A. Classification of Italian maize (*Zea mays* L.) germplasm. *Plant Genet. Res. Newsletter* **2001**, *126*, 1–11.

(6) Lucchin, M.; Barcaccia, G.; Parrini, P. Characterization of a flint maize (*Zea mays* convar. *mays*) Italian landrace: I. Morpho-phenological and agronomic traits. *Genet. Resour. Crop Evol.* **2003**, *50*, 315–327.

(7) Hartings, H.; Berardo, N.; Mazzinelli, G.; Valoti, P.; Verderio, A.; Motto, M. Assessment of genetic diversity and relationships among maize (*Zea mays* L.) Italian landraces by morphological traits and AFLP profiling. *Theor. Appl. Genet.*, **2008**, *117*, 831–842.

(8) http://www.montpellier.inra.fr/gap/resgen88.

(9) Orman, B. A.; Schumann, R. A., Jr. Comparison of near-infrared spectroscopy calibration methods for the prediction of protein, oil, and starch in maize grain. *J. Agric. Food Chem.* **1991**, *39*, 883–886.

(10) Williams, P. C. Observations on the use, in prediction of functionality in cereals, of weights derived during development of partial least squares regression. *JNIRS* **1996**, *4*, 175–187.

(11) Ferrari, E. La qualità molitoria del mais. Risultati di prove sperimentali. *Molini d'Italia* **1995**, *1*, 45–55.

(12) Kirsten, W. J. Rapid, automatic, high capacity Dumas determination of nitrogen. *Microchem. J.* **1982**, *28*, 529–547.

(13) Berardo, N.; Brenna, O. V.; Amato, A.; Valoti, P.; Pisacane, V.; Motto, M. Carotenoids concentration among maize genotypes measured by near infrared reflectance spectroscopy (NIRS). *Innovative Food Sci. Emerging Technol.* **2004**, *5*, 393–398.

(14) Buratti, S.; Pellegrini, N.; Brenna, O. V.; Mannino, S. Rapid electrochemical method for the evaluation of the antioxidant power of some lipophilic food extracts. *J. Agric. Food Chem.* **2001**, *49*, 5136–5141.

(15) Shenk, J. S.; Workman, J. J., Jr.; Westerhaus, M. O. Application of NIR spectroscopy to agricultural products. In *Handbook of Near-Infrared Analysis*; Burns, D. A., Ciurciak, E. W., Eds.; M. Dekker, Inc.: New York, Basel, Hong Kong, 1992; pp 383−431.

(16) Murray, I.; Williams, P. C. Chemical principles of near infrared technology. In *Near Infrared Technology in the Agricultural and Food Industries*; Williams, P. C., Norris, K., Eds.; AACC: St. Paul, MN, 1987; pp 17−34.

(17) Williams, P. C.; Norris, K. H. Qualitative applications of near-infrared reflectance spectroscopy. In *Near Infrared Technology in the Agricultural and Food Industries*; Williams, P. C., Norris, K., Eds.; AACC: St. Paul, MN, 1987; pp 241−246.

(18) Paulsen, M. R.; Watson, S. A., Singh, M. Measurement and maintenance of corn quality. In *Corn: Chemistry and Technology*, 2nd ed.; White, P. J., Johnson, L. A., Eds.; AACC: St. Paul, MN, 2003; pp 159−219.

(19) Pomeranz, Y.; Martin, C. R.; Taylor, D. D.; Lai, F. S. Corn hardness determination. *Cereal Chem.* **1984**, *61*, 147–150.

(20) Pomeranz, Y.; Czuchajowska, Z.; Lai, F. S. Comparison of methods for determination of hardness and breakage susceptibility of commercially dried corn. *Cereal Chem.* **1986**, *63*, 39–43.

(21) Williams, P. C. Implementation of near-infrared technology. In *Near-Infrared Technology in the Agricultural and Food Industries*, 2nd ed.; Williams, P. C., Norris, K. H., Eds.; American Association of Cereal Chemistry: St. Paul, MN, 2001; pp 145−169.

(22) www.cropcopmposition.org.

(23) Reynolds, T. L.; Nemeth, M. A.; Glenn, K. C.; Ridley, W. P.; Astwood, J. D. Natural variability of metabolites in maize grain: Differences due to genetic background. *J. Agric. Food Chem.* **2005**, *53*, 10061–10067.

(24) Malvar, R. A.; Butron, A.; Alvarez, A.; Ordas, B.; Soengas, P.; Revilla, P.; Ordas, A. Evaluation of the European Union Maize Landrace Core Collection for resistance to *Sesamia nonagrioides* (Lepidoptera: Noctuidae) and *Ostrinia nubilalis* (Lepidoptera Crambidae). *J. Econ. Entomol.* **2004**, *97* (2), 628–634.

(25) Malvar, R. A.; Butron, A.; Alvarez, A.; Padilla, G.; Cartea, M. E.; Revilla, P.; Ordas, A. Yield performance of the European Union Maize Landraces Core Collection under multiple corn borer infestations. *Crop Prot.* **2007**, *26* (5), 775–781.

(26) Brichette Mieg, I.; Moreno-Gonzales, J.; Lopez, A. Variability of European maize landraces for forage digestibility using near infrared reflectance spectroscopy (NIRS). *Maydica* **2001**, *46*, 245–252.

(27) Chander, S.; Meng, Y.; Zhang, Y.; Jianbing, Y.; Li, J. Comparison of nutritional traits variability in selected eighty-seven inbreds from Chinese maize (*Zea mays* L.) germplasm. *J. Agric. Food Chem*. **2008**, *56*, 6506–6511.

(28) Egesel, C. O.; Wong, J. C.; Lambert, R. J.; Rocheford, T. R. Combining ability of maize inbreds for carotenoids and tocopherols. *Crop Sci*. **2003**, *43*, 818–823.

(29) Ortiz-Monasterio, J. I.; Palacio-Rojas, N.; Meng, E.; Pixley, K.; Trethowan, R.; Pena, R. J. Enhancing the mineral and vitamin content of wheat and maize through plant breeding. *J. Cereal Sci*. **2007**, *46*, 293–307.

(30) Revilla, P.; Soengas, P.; Cartea, M. E.; Malvar, R. A.; Ordas, A. History and isozyme variability of European maize populations. *Maydica* **2003**, *48*, 141–152.

Received for review July 18, 2008. Revised manuscript received February 4, 2009. Accepted February 5, 2009. This research was funded by the Italian Ministry of Agriculture, project Risorse Genetiche vegetali/FAO.

JF803688T