

Image Analysis of Whole Grains: A Noninvasive Method to Predict Semolina Yield in Durum Wheat

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ABSTRACT

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Durum wheat grain samples ($n = 327$) harvested during 1998 and 1999 in eight different Italian agroclimatic areas and representative of the main Italian cultivars were analyzed. Image analysis of whole grains allowed five size and shape descriptors (length of minor and major axes, perimeter, area, and ellipsoidal volume) to be measured on 100 grains for

each sample. By multiple regression analysis, shape measures more valid to explain semolina yield were identified. By combining these measures with test weight or 1,000-seed weight, equations useful to predict semolina yield were developed.

Image analysis is a noninvasive technique suitable to analyze grain quality attributes to obtain reliable predictions of quality in breeding programs because the whole-grain samples are left intact to plant after testing (Keef 1992; Sapirstein 1995).

Image analysis systems have been developed on a variety of applications in agriculture. Storlie et al (1989) devised a method for estimating leaf area and plant weight in corn and soybean; Draper and Travis (1984) used image analysis for characterizing size and shape of plants; many researchers studied cereal products to describe textural appearance of bread crumb (Bertrand et al 1992; Sapirstein et al 1994; Zayas 1993; Zghal et al 1999); Symons et al (1996) developed an objective instrumental method for counting specks in semolina, whereas Bacci et al (1995) studied an automatic system for the evaluation of durum wheat grain alterations such as yellow berry and shriveling. Moreover, image analysis techniques have been applied both to kernel classification and discrimination (Zayas et al 1985, 1986, 1994; Sapirstein et al 1987; Thomson and Pomeranz 1991; Zayas and Steele 1996; Sapirstein and Kohler 1999) and to differentiation among wheat-grain samples according to grain morphology or other quality attributes that relate to milling quality (Marshall et al 1986; Zayas et al 1986; Symons and Fulcher 1988a,b; Draper and Keef 1989; Neuman et al 1989; Sapirstein 1995; Wrigley and Morris 1995; Troccoli and Di Fonzo 1999).

Berman et al (1996) applied image analysis of whole-grain samples to predict milling quality in selecting for this character in bread wheat while preserving intact seed for sowing; they found four parameters (area, length of minor and major axes, and ellipsoidal volume) plus test weight to be related to flour yield.

On the basis of these results on bread wheat, the present work was performed to 1) determine in durum wheat the relationships among kernel shape and size measures obtained by image analysis, 1,000-seed weight, test weight, and semolina yield; 2) establish the relative importance of these variables in determining semolina yield; and 3) define equations useful to predict semolina yield at different stages of breeding programs.

MATERIALS AND METHODS

Samples

Grain samples ($n = 327$) of durum wheat cultivars included in a national network of performance testing trials spread over several sites in Italy during 1997–98 and 1998–99 were analyzed. The culti-

vars were representative of all genotypes currently grown in Italy of both Italian and foreign origin. Many cultivars and several locations were considered: 20 cultivars and 48 locations for the first growing season and 22 and 46, respectively, for the second. Due to the environmental diversity of Italy, locations were grouped into eight different agroclimatic areas: Po Valley, Adriatic Coast, Tyrrhenian Coast, North-Central Apennines, Central-South Apennines, Ionian Coast, Sicily, and Sardinia. For each cultivar, composite samples were performed by mixing grains from locations belonging to the same agroclimatic area (Lukow and McVetty 1991; Mariani et al 1995).

Image Analysis

A specific macro (a succession of image analysis procedures) was developed on an image analysis system which included a video camera (JVC TK-C 1380), a 5,000 K light, and KS 400 software (Zeiss). It was used to study the durum wheat kernel shape and dimensions. Images were captured for each sample as duplicate sets of 50 grains randomly chosen and arranged to minimize contact among the grains (Berman et al 1996). For each sample, different measures useful for describing kernel shape, such as area, perimeter, length of minor and major axes, and ellipsoidal volume, were determined. As described by Berman et al (1996), on the assumption that a grain is an ellipsoid with circular cross section, the ellipsoidal volume was computed applying the formula $\pi \cdot \text{AMAX} \cdot \text{AMIN}^2 / 6$, where AMAX and AMIN are major and minor axes lengths, respectively.

Technological Tests

Test weight, 1,000-seed weight, and semolina yield were considered. Test weight was measured on a sample of 250 g and expressed as kg/hL; 1,000-seed weight was determined as the mean of two samples of 15 g each; and semolina was obtained on a sample of 3.5 kg by laboratory-scale milling on a Buhler MLU 202 test mill with three breaking and three sizing passages. The milling equipment (mill and purifier) was adjusted to obtain semolina within 0.9% ash content, according to Italian law. The semolina yield was the percentage of semolina on a total product basis.

Statistical Analysis

Simple correlations between all variables were computed. Factor analysis was performed using the software package SPSS (Norusis 1996) on the correlation matrix to evaluate simultaneously all the variables and relationships. Principal component analysis was used for factor extraction; the number of factors needed to adequately describe the data was determined on the basis of eigenvalues, and percentage of total variance accounted for different factors. Eigenvalues >1.0 were chosen, and a plot of the eigenvalues (scree plot) was also used to determine the number of factors to be considered. The varimax method was chosen for orthogonal factor rotation to minimize the number of variables with high loadings on a factor

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and to enhance the interpretability of the factors. The rotation redistributes the variance of the extracted factors; therefore, eigenvalues and percentage of variance accounted for by each factor were calculated again. The association among variables and factors measured by factor loadings can be graphically represented by plots in which orthogonal axes are the factors, taken two at a time, and coordinates of variables are the factor loadings.

Analysis of variance (ANOVA) for multiple regression of the five shape measures and test weight or, alternatively, 1,000-seed weight, versus semolina yield was performed by SPSS backward elimination method for controlling the removal of independent variables from the regression model. Coefficients of multiple correlation were used to evaluate the suitability of the chosen regressions, and standard partial regression coefficients were computed to estimate the relative worth of the independent variables involved (Steel and Torrie 1981). The comparison of the standard partial regression coefficients indicates the relative importance of the independent variables; to simplify this comparison, the relative worth values were expressed as percentages of their total. Moreover the *t* statistics for partial regression coefficients were used to provide a further clue regarding the relative importance of each independent variable. Then the collinearity statistic, expressed as tolerance, was studied to reveal possible strong correlations among the independent variables.

On the basis of the relative worth of standard partial regression coefficients, *t* value significance, and results of collinearity diagnostic, the independent variables useful to predict semolina yield were chosen and multiple regression analysis was performed again.

Finally, the predictive equations for semolina yield, including alternatively test weight or 1,000-seed weight, were computed. Moreover, to control the relationship between the semolina yield observed values and the expected ones, the percentage of observed values lying within, above, and below the confidential limits of the expected values was calculated.

RESULTS AND DISCUSSION

Mean and standard deviation of all the variables measured on 327 samples are reported in Table I; the variability values for test weight, 1,000-seed weight, and semolina yield are those usually found for durum wheat in Italy. Simple correlations (Table II) for all possible pairs of variables produced significant results, as also

TABLE I

Mean Value and Standard Deviation (SD) of Variables Considered

Variables	Code	Mean	SD
Minor axis length, mm	AMIN	3.23	0.28
Major axis length, mm	AMAX	7.45	0.37
Perimeter, mm	PERIM	18.25	0.80
Area, mm ²	AREA	17.81	1.86
Ellipsoidal volume, mm ³	VOL	41.99	6.53
Test weight, kg/hL	TW	79.46	3.17
1,000-seed weight, g	SEEDW	42.62	5.29
Semolina yield, %	SEM	65.64	4.05

TABLE II
Simple Correlation Coefficients Between Variables^a

	AMIN	AMAX	PERIM	AREA	VOL	TW	SEEDW
AMIN							
AMAX	0.358**						
PERIM	0.601**	0.839**					
AREA	0.746**	0.692**	0.937**				
VOL	0.783**	0.612**	0.880**	0.983**			
TW	0.328**	0.141*	0.291**	0.368**	0.347**		
SEEDW	0.675**	0.505**	0.728**	0.826**	0.831**	0.478**	
SEM	0.426**	0.253**	0.414**	0.500**	0.515**	0.450**	0.525**

^a AMIN = minor axis length, AMAX = major axis length, PERIM = perimeter, VOL = volume, TW = test weight, SEEDW = 1,000-seed weight, and SEM = semolina yield; * and ** indicate *P* = 0.05 and 0.01, respectively.

was reported by Marshall et al (1986), Matsuo and Dexter (1980), Ghaderi et al (1971), and Schuler et al (1994). Particularly high is the association between volume and area, area and perimeter, perimeter and major axis, and 1,000-seed weight with volume and area.

Factor analysis was used to evaluate simultaneously all the variables and their relationships. The strength of the linear association among the variables, evaluated by Kaiser-Meyer-Olkin test (=0.8), confirms the sampling adequacy to be correctly analyzed by factor analysis. The results of principal components analysis and the scree plot allowed two factors to be identified, explaining 79.1% of total variance (Fig. 1). The loadings of the two factors after orthogonal rotation and the communalities for each variable are reported in Table III, which also shows the new distribution of the total variance for each factor. The first factor appears linked with perimeter, area, major axis, and ellipsoidal volume; the second factor is associated with test weight and semolina yield, instead of seed weight and minor axis load on both factors (Table III and Fig. 2).

TABLE III
Varimax Rotated Factor Matrix^a

Code ^b	Factor		Communality (%)
	1	2	
PERIM	0.94		94
AREA	0.90		96
AMAX	0.87		75
VOL	0.86		92
SEEDW	0.69	0.57	80
AMIN	0.62	0.49	62
TW		0.84	71
SEM		0.75	63
Eigenvalue	4.12	2.22	
Variance	51.4	27.7	

^a Factor loadings on each of the two factors identified and communalities for each variable. Loadings <0.5 in absolute value are omitted. Below the matrix, the variance explained by each factor (eigenvalue) after rotation and the relative percentage on the total variance are displayed.

^b PERIM = perimeter, AMAX = major axis length, VOL = volume, SEEDW = 1,000-seed weight, AMIN = minor axis length, TW = test weight, SEM = semolina yield.

TABLE IV
Analysis of Variance for Multiple Regression of Five Shape Measures plus Test Weight (TW) or 1,000-Seed Weight (SEEDW) vs. Semolina Yield

Variation Source ^a	df	TW		SEEDW	
		MS	<i>F</i>	MS	<i>F</i>
Regression	6	317.14	29.44**	270.53	23.23**
Residual	320	10.77		11.64	
Total	326				
<i>R</i>		0.596**		0.551**	
Adjusted <i>R</i> ²		34.4%		29%	

^a Multiple correlation coefficient (*R*) and adjusted *R*². MS = mean square and ** indicates *P* = 0.01.

ANOVA for multiple regression of the five shape measures and test weight, or 1,000-seed weight on semolina yield (Table IV) shows the regression mean square to be highly significant when tested on residual variance; the significance of multiple correlation coefficients confirms the combined effect of the six independent variables (predictors) in determining semolina yield. The percentage of variance explained by multiple correlation actually is 34 and 29%, respectively, when test weight or 1,000-seed weight is considered.

To assess the usefulness of each predictor, the significance of the *t* values and the relative worth of the standard partial regression coefficients were used (Table V). When multiple regression included the test weight, the *t* statistic reveals the importance of ellipsoidal volume and test weight, but when the relative worth values are considered, area also appears to be a useful predictor. Instead, when

1,000-seed weight is considered, the *t* statistic is highly significant for seed weight, but the relative worth reveals importance also for area, volume, and perimeter (Table V).

The SPSS backward elimination method was applied with these results to select the most important predictor variables. This method automatically stops the removal of the variables at the last three variables: AREA, volume (VOL), and test weight (TW) when TW is considered; perimeter (PERIM), VOL, and seed weight (SEEDW) when seed weight is included. Analyzing the results of collinearity (Table VI), AREA and VOL were strongly correlated, with tolerance values close to 0; only TW appeared completely independent. These results were confirmed by factor analysis (Table III); TW loads on factor 2, whereas AREA and VOL are related to factor 1 and lie on the same position (Fig. 2). To avoid the collinearity between AREA and VOL and considering the factor loadings (Table III and Fig. 2), AREA was eliminated, assuming ellipsoidal volume to be a

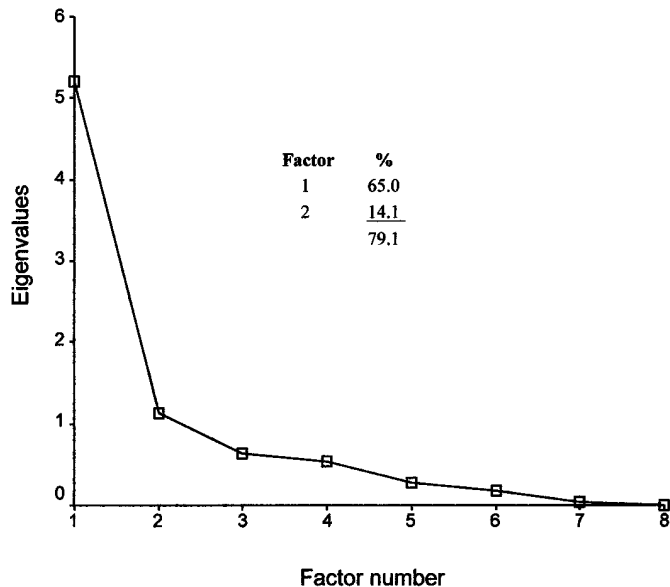


Fig. 1. Principal component analysis: scree plot and percentage of total variance accounted for by each factor.

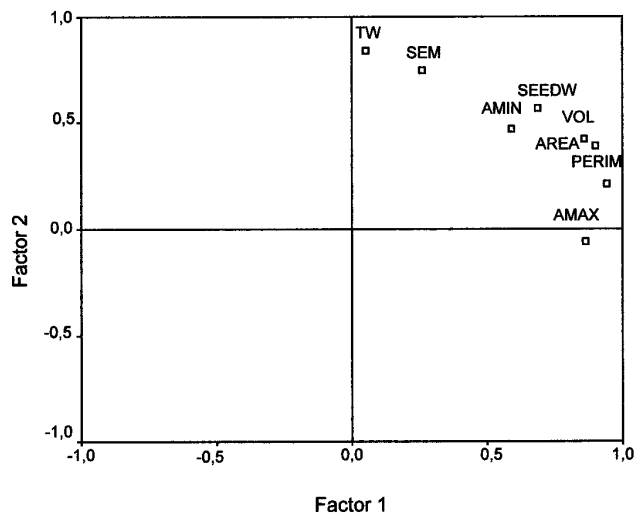


Fig. 2. Factor analysis distribution of the eight variables on the plot defined by factors 1 and 2.

TABLE V
Partial Regression Coefficients (b), *t* Values, Standard Partial Regression Coefficients (b'), and Relative Worth

Variables ^a	TW				SEEDW			
	b	<i>t</i>	b'	Worth (%)	b	<i>t</i>	b'	Worth (%)
AMIN	-0.27	-0.25	-0.02	1.1	-0.04	-0.04	-0.003	0.3
AMAX	0.14	0.13	0.01	0.8	-0.46	-0.43	-0.04	4.2
PERIM	-0.22	-0.19	-0.04	2.6	-0.94	-0.79	-0.19	18.8
AREA	-0.89	-0.87	-0.41	24.6	0.51	0.50	0.23	23.3
VOL	0.53	2.49*	0.85	51.3	0.14	0.66	0.23	22.6
TW	0.41	6.31**	0.32	19.4				
SEEDW					0.23	3.58**	0.31	30.8

^a AMIN = minor axis length, AMAX = major axis length, PERIM = perimeter, and VOL = volume, TW = test weight, SEEDW = 1,000-seed weight, * and ** indicate P = 0.05 and 0.01, respectively.

TABLE VI
Collinearity: Tolerance Values for 6, 3, and 2 Predictor Variables of Semolina Yield

Variables ^a	TW			SEEDW		
	6	3	2	6	3	2
AMIN	0.350			0.350		
AMAX	0.220			0.221		
PERIM	0.038			0.039	0.226	
AREA	0.009	0.033		0.010		
VOL	0.017	0.033	0.879	0.019	0.149	0.310
TW	0.774	0.858	0.879			
SEEDW				0.299	0.310	0.310

^a AMIN = minor axis length, AMAX = major axis length, PERIM = perimeter, and VOL = volume, TW = test weight, SEEDW = 1,000-seed weight.

more effective measure to describe kernel shape; the new collinearity values calculated for VOL and TW showed that these two variables were not correlated. For SEEDW, the three predictors chosen were highly correlated (Table VI) and the elimination of PERIM improves the tolerance values for VOL and SEEDW, but the results show them still associated. Factor analysis (Table III and Fig. 2) also gives evidence of this result; in fact, SEEDW is loaded equally on both factors and is partially linked to shape measures loading essentially on factor 1; therefore, the collinearity between these two parameters, VOL and SEEDW, is unavoidable.

Berman et al (1996), studying bread wheat, found that four kernel shape parameters (area, minor and major axes lengths, and ellipsoidal volume) plus test weight were the best predictors of milling quality. They also reported "the prediction equation does not necessarily serve to indicate what aspects of grain shape should provide better milling quality because the component measures are highly positively correlated". We also found kernel shape measures to be highly correlated but, on the basis of factor analysis results, *t* statistics, relative worth, and collinearity diagnostics, we state that ellipsoidal volume only, among shape measures, is effective in predicting semolina yield. It must be noted the ellipsoidal volume is calculated using the length of major and minor axes that are the only measures among those obtained by image analysis used in this work. Finally, ANOVA for multiple regressions considering volume, test weight or, alternatively, seed weight as independent variables and semolina yield as the dependent variable was calculated again (Table VII), and the standard partial regression coefficients and relative worth were computed to estimate the relative importance of the independent variables involved (Table VIII). Considering jointly the results of Tables VII and VIII, it can be noted that R^2 values do not change compared with those measured on all the variables (Table IV), whereas the significance of the *t* test for the two independent variables selected, as well as worth, is enhanced, thereby establishing ellipsoidal volume, test weight, or seed weight to be the most efficient predictors for semolina yield.

Next, two equations for predicting semolina yield were calculated using ellipsoidal volume and seed weight or test weight alternatively, so as to be applied in early generation or during advanced stages of breeding programs (Table IX). The comparison of actual and predicted semolina yields (Table X) allows the goodness-of-fit of the proposed equations to be established. The equations overestimated only 28% of observed values, whereas 72% of predicted values provided reliable results. Values of the semolina yield, predicted on

TABLE VII
Analysis of Variance for Multiple Regression
of Two Shape Measures Chosen Plus Test Weight (TW)
or 1,000-Seed Weight (SEEDW) vs. Semolina Yield

Variation Source ^a	df	TW		SEEDW	
		MS	F	MS	F
Regression	2	932.84	86.75**	791.77	68.11**
Residual	324	10.75		11.62	
Total	326				
R		0.591**		0.544**	
Adjusted R ²		35%		29%	

^a Multiple correlation coefficient (*R*) and adjusted R^2 ; MS = mean square; ** indicates $P = 0.01$.

TABLE VIII
Partial Regression Coefficients (*b*), *t* Values, Standard Partial Regression Coefficients (*b'*), and Relative Worth
for Two Independent Variables Chosen

Variables ^a	TW				SEEDW			
	<i>b</i>	<i>t</i>	<i>b'</i>	Worth (%)	<i>b</i>	<i>t</i>	<i>b'</i>	Worth (%)
VOL	0.25	8.53**	0.41	57	0.16	3.03**	0.25	44
TW	0.39	6.44**	0.31	43				
SEEDW					0.24	3.76**	0.32	56

^a VOL = volume, TW = test weight, SEEDW = 1,000-seed weight; ** indicates $P = 0.01$

the basis of the equations defined, and the 95% confidence limits were computed using given values of ellipsoidal volume and test weight or 1,000-seed weight as x_1 and x_2 (Table XI). To obtain an increase of 1 unit for semolina yield, the test weight must increase by 2 units and volume by 1.5 units when the first equation is applied; for the second equation, to have the same increase, volume and 1,000-seed weight must be increased by 2.5 units.

CONCLUSIONS

Image analysis, a noninvasive method, allows grain morphology parameters to be measured on a small quantity of seed that is left intact for sowing after testing.

This investigation allowed two equations to be determined for predicting semolina yield by using image analysis measures.

Several points must be emphasized. Among the different measures captured by image analysis, only major and minor axes lengths are essential because they allow ellipsoidal volume to be calculated. Ellipsoidal volume is the best predictor, jointly with test weight or 1,000-seed weight, in determining semolina yield. The variables chosen for predictive equations can be fruitfully used in selecting new lines. In early selection, semolina yield can be determined by 1,000-seed weight joined with ellipsoidal volume, whereas the equation with test weight can be used only in advanced stages of selection; more seed is required for evaluating this variable. Predictive equations can be considered of general use because our samples derived from two years and many genotypes and sites belonging to different Italian agroclimatic areas.

TABLE IX
Predictive Equation for Semolina Yield (SEM) Including Test Weight
(TW) or 1,000-Seed Weight (SEEDW)

Y	=	a	+	(<i>b</i> ₁ · <i>x</i> ₁)	+	(<i>b</i> ₂ · <i>x</i> ₂)
SEM	=	23.76	+	0.25 VOL	+	0.39 TW
SEM	=	48.77	+	0.16 VOL	+	0.24 SEEDW

TABLE X
Semolina Yield (SEM) Observed Values (%)
Within, Above, and Below Confidence Limits of Expected Values^a

SEM	VOL + TW (%)	VOL + SEEDW (%)
Within	42a	41c
Above	30b	31d
Below	28	28

^a VOL = volume, TW = test weight, SEEDW = 1,000-seed weight; a + b = 72%; c + d = 72%; TW vs. SEEDW, $r = 0.478^{**}$, $P = 0.01$.

TABLE XI
Expected Values of Semolina Yield (SEM) for Given Values
of Ellipsoidal Volume (VOL) and Test Weight (TW)
or 1,000-Seed Weight (SEEDW)

SEM (%)	VOL (mm ³)	TW (kg/hL)	VOL (mm ³)	SEEDW (g)
+1.0	+1.5	+2.0	+2.5	+2.5
62 ± 1.1	37.5	73.0	32.5	33.5
65 ± 0.6	42.0	79.0	40.0	41.0
68 ± 1.1	46.5	85.0	47.5	48.5

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