

# Image Analysis of Grain and Chemical Composition of the Barley Plant as Predictors of Malting Quality in Mediterranean Environments

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## ABSTRACT

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We have explored the possibility of predicting the malting quality of barley grain, indicated by malt extract yield, by characteristics measured either on plants at anthesis or in mature dry grain by image analysis. To produce barley samples with varying levels of all the characteristics studied, we used grain from an experiment designed to study the influence of low-input husbandry practices on malting quality of barley by growing five malting genotypes at each of four environments (site × season) and with two different agronomic treatments (N fertilization and herbicide-mechanical

roguing of weeds). The results showed that nitrogen content in the plant at anthesis was a good predictor of grain protein content, this characteristic in turn being positively correlated with embryo size and grain volume, as estimated by image analysis, and negatively correlated with nonstructural carbohydrate content in the plant at anthesis. Extract yield was positively correlated with Kolbach index (ratio of soluble to total wort protein) and negatively correlated with wort viscosity and barley grain protein content. Thus, the only practical predictor of malt extract was grain protein content.

The possibility of predicting malting quality in a barley sample with the aid of related characteristics, other than the properties of the malt itself, has been of interest to barley breeders, grain producers, and merchants, who would like to discard undesirable genotypes or grain lots without the necessity of actually malting the grain. Early attempts to predict malting quality were made by the Grain Research Laboratory of Canada, where in the 1930s and 1940s, the so-called "prediction methods" were developed to assess the malting quality of a sample without actually malting it. These methods consisted of determining the potential extract or amylolytic activity of a previously digested barley flour with a solution of malt enzymes or with papain, respectively (Meredith et al 1962).

Similar studies were conducted in England by Bishop, who established the negative correlation between barley protein content and malt extract yield (Bishop 1930a,b, 1934; Bishop and Day 1933). Later, a correlation between certain electrophoretic bands of barley storage proteins (B fraction hordeins) and malting quality was reported (Baxter and Wainwright 1979). Additionally, Allison et al (1979) reported a negative correlation between malt extract yield and the energy required to mill a known weight of barley grain (milling energy), which was related to the hardness of its endosperm.

The search for techniques leading to an early assessment of malting quality without the necessity of malting has a paradigm in the near-infrared reflectance spectroscopy (NIRS) techniques that are used to estimate several characteristics, including protein and moisture content (Williams 1975),  $\beta$ -glucans (Allison et al 1978, Czuchajowska et al 1992, Szczodrak et al 1992), starch (Czuchajowska et al 1992), and malt extract yield (Henry 1985, Tragonrunga et al 1990). For breeding purposes, a further step has been to combine the predicting power of NIRS with the molecular mapping of quantitative trait loci (QTLs) linked to malting quality characteristics (Bezant et al 1997).

Recently, another promising nondestructive technique has emerged: image analysis of whole kernels. It is based on the digitalization of photographic images of grains and the subsequent estimation of characteristics that describe the morphology (size and shape) of grains (Chen et al 1989, Keefe 1992, Sapirstein 1995). This tech-

nique has been successfully used to screen wheat samples for flour-milling yield (Berman et al 1996) and also for evaluating the extent of modification of malt (Reinikainen et al 1996), although in the latter study, the work was with halved kernels stained with Calcofluor.

The accumulation of starch and protein in cereal grain are not synchronous processes. Generally, protein deposition starts earlier and ends before that of starch, both phenomena being controlled by different genetic factors (Jenner et al 1991). Environmental conditions during grain filling favorable for leaf senescence, such as high temperatures and drought, curtail starch accumulation, enriching the grain in protein. This has also been described for barley under Mediterranean environments in southern Spain (García del Moral et al 1997).

The nitrogen content of the barley plant at heading time has been studied, attempting to link it with the final grain yield and protein content. It has been reported that the protein present in the vegetative organs at the moment of maximum development (anthesis) is positively correlated with the final grain protein content (Austin et al 1977, García del Moral et al 1985). The rationale behind this correlation is that the main source of the grain protein in temperate cereals is protein in leaves, particularly the enzyme RuBisCo (Nair and Chatterjee 1990, Jenner et al 1991), whose hydrolysis is enhanced by high-temperature stress during grain filling.

The great importance of plant preanthesis reserves for grain yield in cereals in hot, dry areas has been widely recognized (Austin et al 1980, Lawlor et al 1981) and has also been shown for barley in the Mediterranean conditions of southern Spain (Ramos et al 1985). These preanthesis reserves are mainly nonstructural carbohydrates and contribute to the starch content of the grain when thermic and drought stresses are present during the grain filling period (McCaug and Clarke 1982, Savin et al 1997) as is usual in Mediterranean environments. When there is heat stress during grain filling, photosynthesis is curtailed and leaf proteins as well as soluble carbohydrates from stems are translocated to grains. This translocation could negatively affect malting quality through a decrease in extract yield because the accumulation of starch and protein in cereal grain are not synchronous processes; deposition of protein is well completed before starch synthesis ends. Therefore, a sudden stop to grain development imposed by drought and hot temperatures may favor the final increase of grain protein content.

Although only some of these methods have contributed substantially to the practical work of breeders, growers, merchants, and maltsters, efforts to discover new relationships with malt extract yield continue. In this article, we describe attempts to forecast the malting quality of barley grain: 1) from the chemical composition of the barley plant at heading and maturity, and 2) before malting, with the aid of image analysis of whole and halved dry grains.

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## MATERIALS AND METHODS

### Field Methods

To explore relationships between plant composition at anthesis and maturity, mature grain size and shape assessed through image analysis, and final malting quality, it was necessary to generate barley samples varying in these characteristics. The samples came from experiments designed to study the influence of low-input husbandry practices on malting quality of barley. Sources of variation in these experiments were: environment (sites and years), genotype (a set of near-isogenic mutant lines differing in plant type and life cycle length, induced in a malting cultivar widely grown in Spain, plus a control cultivar), and agronomic treatment (nitrogen fertilizer application, and weed control with herbicide versus mechanical weeding).

The growing sites were Valladolid ( $\approx 700$  m above sea level) and Lleida ( $\approx 200$  m above sea level), both with a Mediterranean climate, located 650 km apart in northern Spain. Valladolid is in the northern Plateau and Lleida is 100 km from the Mediterranean coast. The main difference in climate between the sites is temperature during winter, which is lower in Valladolid than in Lleida. Hence, Valladolid barley is sown in spring (February) and Lleida barley is sown in autumn (end of November). The experiments were performed at both sites over three years (1994, 1995, and 1996), but because of incomplete data, only the results from four environments (site  $\times$  season) are reported here: Lleida 95 and 96, and Valladolid 94 and 96 (L95, L96, V94, V96). The climatic conditions during grain development at both sites during the relevant years are presented in Fig. 1.

The genotypes are described in Table I. The three mutants B1, B2, and B3 were obtained from the spring malting cultivar Beka and have been characterized for malting quality and yield physiology (Molina-Cano 1982, Molina-Cano et al 1990, Romagosa et al 1993). Alexis is the high-quality, control cultivar used in the spring barley trials of the European Brewery Convention and is a very successful cultivar in Europe.

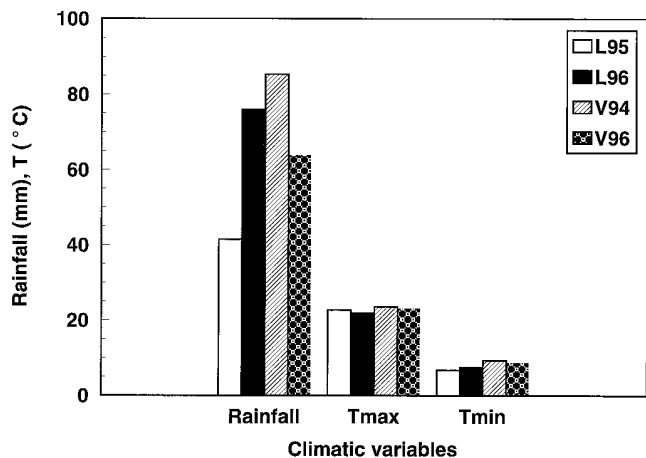


Fig. 1. Total rainfall (mm) and mean daily maximum and minimum temperatures during grain filling at four environments. L95 and L96 = Lleida 1995 and 1996; V94 and V96 = Valladolid 1994 and 1996.

TABLE I  
Genotype Description of Three Mutants (B1, B2, and B3) Obtained from Spring Malting Cultivar Beka and Alexis (Control Cultivar)

Genotype	Spike Density	Plant Height	Earliness at Heading	Leaf Area	Grain Filling Period
B1	Equal	Shorter	Earlier	Smaller	Equal
B2	Denser	Shorter	Equal	Smaller	Equal
B3	Denser	Equal	Earlier	Equal	Shorter
Alexis	Equal	Shorter	Later	Equal	Longer

The experiments at each environment were laid out as split-split plot designs with four replicates. The underlying principle of this design is that whole plots, to which levels of one factor are applied, are divided into subplots and then into sub-subplots, to which levels of the other two factors are applied; the main plots being the herbicide treatment and the subplots being the nitrogen treatment. The genotypes (sub-subplots) were distributed at random within each subplot. Sub-subplot size was  $8.0 \times 1.2$  m<sup>2</sup>, including six rows 20 cm apart.

In each sub-subplot, two 1-m row segments were marked at the tillering stage. Plants were sampled twice: the first at anthesis (when 50% of awns had emerged 2 cm from the sheath) and the second at maturity. Each sample was taken from one of the 1-m rows marked previously. All plants from each row segment were cut above ground and brought to the laboratory, where 20 tillers were taken at random from each sample. The samples taken at anthesis were dried in an oven at 60°C. The heads of the samples taken at maturity were detached from the stems and kept separate.

### Plant Analysis and Malting Quality

Nonstructural carbohydrates were determined on the plant samples taken at anthesis and at maturity (without spikes, in both cases) following the method of McCaig and Clarke (1982). Kjeldahl nitrogen and total protein content ( $N \times 6.25$ ) were also determined on these samples.

Grain samples were screened over a 2.5-mm sieve and micro-malted according to the protocol of Molina-Cano et al (1991). The malt samples were analyzed for extract yield, total and soluble protein, Kolbach index, and viscosity according to the official methods (EBC 1987).

### Image Analysis of Grains

*External morphometry.* Twenty-five dry grains per replicate were photographed before being digitized with an Olympus-ST binocular microscope equipped with a video camera. The area, perimeter, and major and minor axes lengths of each grain were calculated with the aid of the image analysis program (Visilog v. 4, Noesis Vision Inc., Quebec, Canada). Variables calculated were: Sphericity =  $(\text{perimeter})^2/4 \times \pi \times \text{area}$ . The approximation of grain shape to the sphere (1.0) is: Volume =  $4/3 \times \pi \times 1/2(\text{major axis}) \times 1/2(\text{minor axis})^2$ .

*Internal morphometry.* To quantify the relative proportion (percentage) of each of the main components of the grain including husks (lemma, palea, testa, and pericarp), aleurone layer, embryo,

TABLE II  
Recorded Characteristics

Type <sup>a</sup>	Description
1	Plant protein content at anthesis (%)
1	Plant nonstructural carbohydrate content at anthesis (%)
1	Plant protein content at maturity (%)
1	Plant nonstructural carbohydrate content at maturity (%)
1	Barley grain protein content (%)
2	Area (mm <sup>2</sup> )
2	Perimeter (mm <sup>2</sup> )
2	Length of the major axis (mm)
2	Length of the minor axis (mm)
2	Sphericity
2	Volume (mm <sup>3</sup> )
3	Relative area occupied by the husks (%)
3	Relative area occupied by the embryo (%)
3	Relative area occupied by the aleurone layer (%)
3	Relative area occupied by the endosperm (%)
4	Malt extract yield (%)
4	Malt Kolbach index
4	Wort viscosity (cSt)
5	Grain yield (g/m <sup>2</sup> )

<sup>a</sup> 1 = Plant and grain chemical composition; 2 = image analysis from the dorsal view of the intact grains; 3 = image analysis from cut and stained grains; 4 = malting quality; 5 = agronomic.

and endosperm, the 25 grains were fixed in a mixture of 5% pure acetic acid, 5% neutral formic aldehyde of 40% and 90% ethanol of 70%. A representative subsample of five grains was taken and cut with a freezing microtome on a plane approximately at right angles to the ventral furrow (dorsiventral) plane. To highlight the endosperm, the sectioned grains were stained with diluted Lugol (5% I<sub>2</sub> and 10% IK in distilled water) to show starch before being photographed and digitized. The husks, aleurone, embryo, and endosperm were then easily distinguished after staining.

### Statistical Analysis

Statistical analyses included analysis of variance (ANOVA) and principal component analysis (PCA) calculated on a correlation matrix (SAS Institute, Cary, NC). The PCA calculations were first performed on the matrix made up with all 19 characteristics studied (Table II), but later excluded all the correlated characteristics used in subsequent calculations (lengths of the axes, area, perimeter) used to calculate sphericity and volume and, later on, excluded some other characteristics with very small loadings on the three first axes (plant carbohydrate and protein at maturity). Hence, the final matrix was made up of 12 characteristics (Table III). An additional characteristic (grain yield, YLD) was included only for reference and was not included in the final PCA calculation because its loadings (level of importance) on the first three axes were negligible. The PCA graphs were drawn with SigmaPlot for Windows (Jandel Scientific, San Rafael, CA).

## RESULTS

### Climatic Differences

Although Valladolid and Lleida have markedly different climates, if we consider the grain-filling period at both sites for the relevant years (April-May at Lleida and May-June at Valladolid), as shown in Fig. 1, the differences in mean daily maximum and minimum temperatures averaged monthly are quite small. There are some differences, however, in rainfall, which was lowest in L95. In this year, rainfall in autumn was also very low in Lleida, and it was necessary to irrigate after sowing. Later, two irrigations each of ≈40 mm were applied. These dry conditions resulted in a low plant population and a grain yield half the normal (Table III).

A major difference between both sites is the consistently low temperature in Valladolid during winter, making it obligatory for spring cultivars to be sown in early spring (February), while at Lleida, with milder winter temperatures, spring barley is sown in autumn (November). Harvest took place at middle June in Lleida and at the end of June in Valladolid. Therefore, the barley life cycle is very different at the two sites (7 months at Lleida, 4.5 months at Valladolid).

### Relative Importance of Different Sources of Variation on the Measured Characteristics

Although not of main concern in this study, we summarized the relative influence of the different sources of variation (environments, genotypes, nitrogen fertilization, and herbicide treatment) on plant composition, image analysis characteristics, and malting quality parameters measured.

Overall, the factor accounting for most of the variance was the environment, the effect of which was highly significant for all the characteristics measured. The factor of second importance was nitrogen fertilization, followed by the genotype. Herbicide (herbicide treatment vs. mechanical roguing of weeds) had almost no influence on any of the characteristics. Generally, the most important interaction was that of environment by nitrogen fertilization.

There were a few exceptions to these general trends: neither wort viscosity nor grain sphericity were influenced by nitrogen fertilization, and the only factor influencing aleurone size was the environment (not genotype or nitrogen fertilization).

Table III shows the mean values of environments, genotypes, and nitrogen fertilization as well as standard errors. It is noteworthy that at L95 (environment 1), where the low number of spikes per square meter resulted in a grain yield half of normal, grain and endosperm size were large. In addition, the grain had high protein content and produced malt with low extract yield. This was the result of a greater availability of nitrogen for each developing grain. Also note that in this environment, the amount of nonstructural carbohydrates in the tillers at anthesis was as high as it was at maturity (data not presented), indicating little translocation of these carbohydrates to the ears, presumably because of the ample supply from current photosynthesis during grain filling.

Nitrogen fertilization (40 kg/ha) resulted in grain of greater volume, husk content, embryo, and aleurone size, and protein content. This grain was produced on plants with higher protein and lower nonstructural carbohydrate content at anthesis. This grain, in turn, produced malt with lower extract yield and Kolbach index. Here again, large grain size was correlated with high protein content and lower extract yield.

### Relationships Between Plant Composition at Anthesis, Grain Size and Shape, and Malting Quality

PCA was conducted first on the 19 characteristics listed in Table II, but the almost negligible influence and redundancy of seven of those characteristics led to a PCA on a correlation matrix (Table IV) based only on the 12 characteristics listed in Table III, excluding grain yield.

The first three PCA axes in Figs. 2 and 3 accounted for 64% of total variance: 25, 22, and 17% for axes 1, 2, and 3, respectively.

**TABLE III**  
Mean Values for Treatments and Standard Error (SE) of 12 Characteristics Used for Principal Component Analysis and Grain Yield

Variable <sup>a</sup>	Environment					Genotype					N Fertilization			
	1 (L95)	2 (L96)	3 (V94)	4 (V96)	SE	Beka	BI	B2	B3	Alexis	SE	0 kg/ha	40 kg/ha	SE
EXT	78.07	80.44	79.56	77.85	0.18	79.25	78.84	78.21	79.13	79.46	0.20	79.36	78.60	0.13
KOL	41.40	45.00	43.27	32.48	0.56	42.35	40.36	39.46	40.26	40.25	0.63	41.30	39.77	0.40
VIS	1.47	1.45	1.49	1.52	0.01	1.49	1.47	1.52	1.48	1.46	0.01	1.49	1.48	0.01
SPH	1.49	1.53	1.53	1.56	0.01	1.53	1.52	1.48	1.51	1.60	0.01	1.53	1.53	0.01
VOL	77.29	52.76	57.79	45.27	0.75	57.46	57.91	55.80	58.69	61.54	0.84	57.52	59.04	0.53
HUS	11.63	12.79	12.50	13.46	0.17	12.89	11.98	13.00	12.65	12.44	0.19	12.19	13.00	0.12
EMB	8.39	8.31	8.25	8.03	0.09	8.29	8.24	8.27	8.46	7.96	0.10	7.50	8.99	0.06
END	78.46	77.32	77.39	76.88	0.16	77.16	78.23	77.10	77.14	77.92	0.18	78.72	76.30	0.11
ALE	1.53	1.59	1.88	1.64	0.08	1.64	1.56	1.66	1.75	1.69	0.09	1.61	1.70	0.06
PAN	12.68	11.09	15.02	8.80	0.20	11.83	12.83	11.85	11.05	11.92	0.22	11.05	12.75	0.14
CAN	194.97	147.94	122.65	216.30	6.71	167.55	188.91	160.17	171.04	164.66	7.50	191.40	149.53	4.75
GRP	14.18	13.34	14.33	12.87	0.17	13.90	13.91	13.78	13.78	13.02	0.19	13.38	13.98	0.12
YLD	216	544	588	513	12.27	452	445	458	472	498	13.72	453	476	8.68

<sup>a</sup> EXT (malt extract yield, %); KOL (wort Kolbach index); VIS (wort viscosity, cSt); SPH (mature barley grain sphericity); VOL (mature barley grain volume, mm<sup>3</sup>); HUS (mature barley grain husks, %); EMB (mature barley grain embryo, %); END (mature barley grain endosperm, %); ALE (mature barley grain aleurone, %); PAN (barley plant protein at anthesis, mg/g); CAN (barley plant soluble carbohydrates at anthesis, mg/g); GRP (mature barley grain total protein, %); YLD (barley grain yield, g/m<sup>2</sup>) included for reference.

Because we can explain most of the variation by considering these three axes, we used the projections of the data points on two different planes, those determined by the axes 1 and 2 and 1 and 3 (Figs. 2 and 3). Each figure is composed of A and B graphs. The A graphs (left of each figure) represent the eigenvectors of the characteristics that most influence each axis. The length of the projection of each of them on each principal component axis (PC1 to PC3) measures the weight (loading or eigenvalue) of its influence on that axis, whereas the cosine of the angle between any two vectors is inversely proportional to their correlation. For instance, in Fig. 2A mature barley grain embryo relative size (EMB %) has a positive weight on axis 2 (PC2) of 0.47, while mature barley grain endosperm relative size (END %) has a negative weight on the same axis of 0.55. This means that EMB and END relative sizes make a significant contribution to axis 2 and are negatively correlated (the vectors form an angle between 90° and 180°, a negative cosine).

The B graphs of Figs. 2 and 3 represent the projection on the planes 1–2 and 1–3 of the 80 points representing the means of the 80 different treatment combinations (environment, genotype, nitrogen application, and herbicide). For our purposes, there are 80 samples of malting barley of varying grain size, shape, composition, and malting quality that are grouped using the axes. Given that the main source of variation was environment, the 80 points are grouped into the four environments studied.

*Axis 1 measures the balance between plant composition and grain protein and grain size (volume).* Toward its positive direction, there is a joint increase of barley plant protein at anthesis (PAN), barley grain total protein (GRP), barley grain volume (VOL), and Kolbach index, or ratio soluble to total protein in the wort (KOL). Plant protein content at anthesis and final grain protein content are strongly correlated, and both are correlated with VOL and KOL. Toward its negative direction, although with lower weights, there is the increase in barley plant nonstructural carbohydrates at anthesis (CAN) and barley grain husk size (HUS). It should be noted that there is a highly significant negative correlation between CAN and PAN. Therefore, when the barley plant has a high protein and low carbohydrate content at anthesis, there will probably be a parallel rise in grain protein content and VOL and

KOL of the malt. Thus, when we go toward the positive direction on axis 1, there is an increase in PAN, GRP, VOL, and KOL.

*Axis 2 measures grain structure (image analysis) as influenced by plant composition at anthesis.* END is negatively correlated with HUS and EMB and positively correlated with CAN. Therefore, the higher the carbohydrate content in the plant at anthesis, the bigger the relative size of the future endosperm and the smaller the husk and embryo. Thus, when we go toward the negative direction on axis 2, the size of the endosperm increases, due to the greater supply of nonstructural carbohydrates from the plant at anthesis, and the relative sizes of embryo and husk decrease.

*Axis 3 measures malt extract yield and protein degradation during malting.* This axis is dominated by malt extract, because malt extract yield (EXT) has almost twice the weight on it than the remaining characteristics. EXT is positively correlated with KOL and negatively correlated, although less strongly, with GRP. And, although KOL and GRP were positively associated on axis 1, here the association between EXT and KOL is much greater, indicating the positive contribution to extract yield of the part of total protein solubilized during mashing (KOL). Although this association may subsequently induce severe quality losses due to higher beer turbidity and shorter shelf life, here it actually increases the quantity of solutes contributed to wort by malt (extract yield). Therefore, in the positive direction of axis 3 there is a joint increase of EXT and KOL.

In Figs. 2B and 3B we have represented the 80 original mean observations on the planes 1–2 and 1–3, respectively. On each graph, the points belonging environments 1–4 are circled (L95, L96, V94, and V96, respectively). Each point has also a plus (+) or minus (–) sign, denoting with or without nitrogen fertilization. Fig. 2B shows that the grain structure (axis 2), estimated by image analysis, does not differentiate between environments but clearly separates the grain populations into those with or without nitrogen, increasing the size of the endosperm, as opposed to husks and embryo, when there is no application of nitrogen to the crop (negative direction of the axis). This relationship holds both within and between environments. Nitrogen application and environmental effects were acting additively as far as the characteristics on axes 1 and 2 are concerned.

TABLE IV  
Correlation Coefficients and Probability Levels for 12 Characteristics Used for Principal Component Analysis Calculations<sup>a,b</sup>

	EXT	KOL	VIS	SPH	VOL	HUS	EMB	END	ALE	PAN	CAN
KOL	<b>0.68771</b> (0.0001)										
VIS	<b>-0.36606</b> (0.0008)	<b>-0.4999</b> (0.0001)									
SPH	0.1878 (0.0953)	-0.06101 (0.5909)	-0.13624 (0.2282)								
VOL	-0.1857 (0.0991)	<b>0.28827</b> (0.0095)	<b>-0.21759</b> (0.0525)	<b>-0.28428</b> (0.0106)							
HUS	-0.10528 (0.3527)	<b>-0.33334</b> (0.0025)	<b>0.28706</b> (0.0098)	0.04603 (0.6851)	<b>-0.49307</b> (0.0001)						
EMB	-0.16066 (0.1546)	0.00182 (0.9872)	-0.10128 (0.3714)	-0.08107 (0.4747)	0.12396 (0.2733)	<b>0.23805</b> (0.0335)					
END	0.13455 (0.2341)	0.19517 (0.0827)	-0.15442 (0.1714)	-0.02736 (0.8096)	<b>0.29076</b> (0.0089)	<b>-0.78227</b> (0.0001)	<b>-0.7555</b> (0.0001)				
ALE	0.11008 (0.331)	0.13785 (0.2227)	0.10854 (0.3379)	0.18244 (0.1053)	-0.12034 (0.2877)	-0.12978 (0.2512)	0.14459 (0.2007)	-0.20696 (0.0655)			
PAN	0.06355 (0.5755)	<b>0.43446</b> (0.0001)	-0.08079 (0.4762)	-0.16973 (0.1323)	<b>0.39771</b> (0.0003)	<b>-0.21678</b> (0.0534)	<b>0.31538</b> (0.0044)	-0.06799 (0.549)	0.18533 (0.0998)		
CAN	-0.19512 (0.0828)	<b>-0.34458</b> (0.0017)	-0.02595 (0.8193)	-0.01394 (0.9023)	0.01936 (0.8647)	-0.09733 (0.3904)	<b>-0.3232</b> (0.0035)	<b>0.29557</b> (0.0078)	<b>-0.24472</b> (0.0287)	<b>-0.6895</b> (0.0001)	
GRP	<b>-0.31746</b> (0.0041)	0.14022 (0.2148)	-0.00752 (0.9472)	<b>-0.34487</b> (0.0017)	<b>0.44913</b> (0.0001)	-0.16077 (0.1543)	<b>0.32454</b> (0.0033)	-0.07176 (0.5271)	-0.01825 (0.8724)	<b>0.58342</b> (0.0001)	<b>-0.31572</b> (0.0043)

<sup>a</sup> EXT (malt extract yield, %); KOL (wort Kolbach index); VIS (wort viscosity, cSt); SPH (mature barley grain sphericity); VOL (mature barley grain volume, mm<sup>3</sup>); HUS (mature barley grain husks, %); EMB (mature barley grain embryo, %); END (mature barley grain endosperm, %); ALE (mature barley grain aleurone, %); PAN (barley plant protein at anthesis, mg/g); CAN (barley plant soluble carbohydrates at anthesis, mg/g); GRP (mature barley grain total protein, %).

<sup>b</sup> Probability levels are in parentheses below each correlation. Correlations in bold type are significant at  $P < 0.05$ .

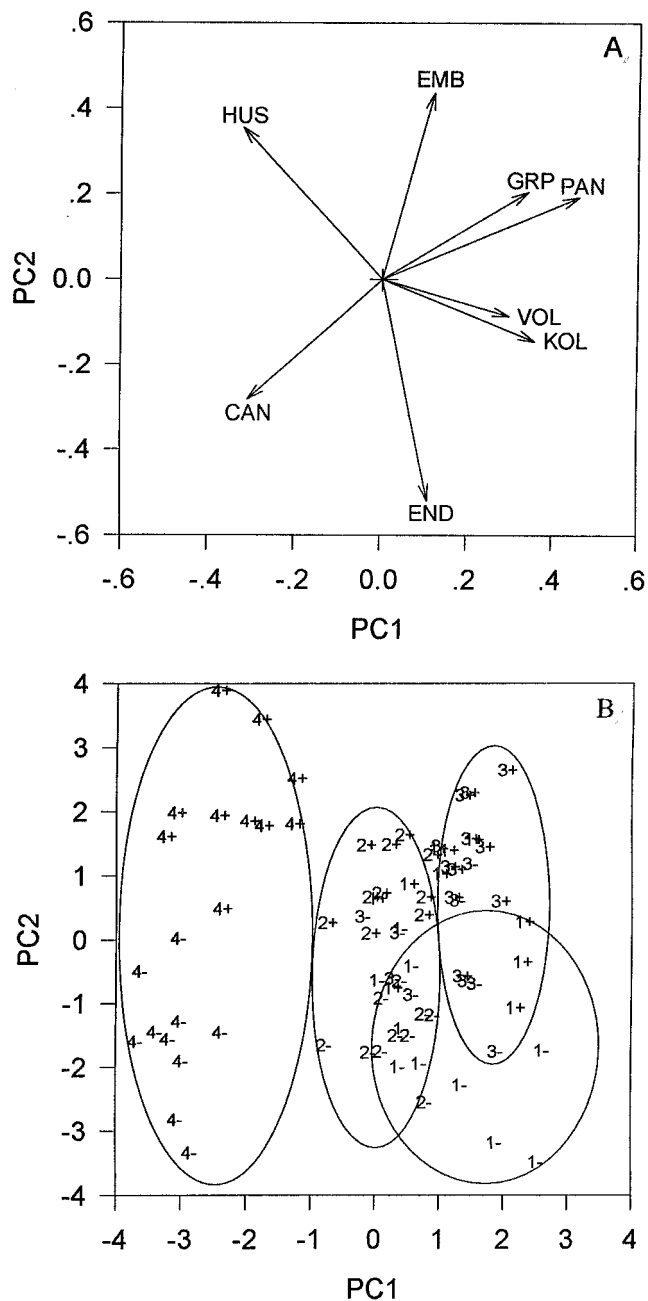
Conversely, on axis 1 there is a discrimination between environments that is related to plant carbohydrate and protein reserves at anthesis and to grain protein content and size, in so far as there is a gradient of PAN, CAN, GRP, VOL, and KOL from V96 (4) toward V94 (3), with L96 (2) and L95 (1) being intermediate. This gradient is also evident from the data in Table III.

Therefore, the distribution of the points on plane 1–2 validates the association of characteristics suggested by the axis loadings. The predictive power of the association of characteristics is confirmed by the point groupings.

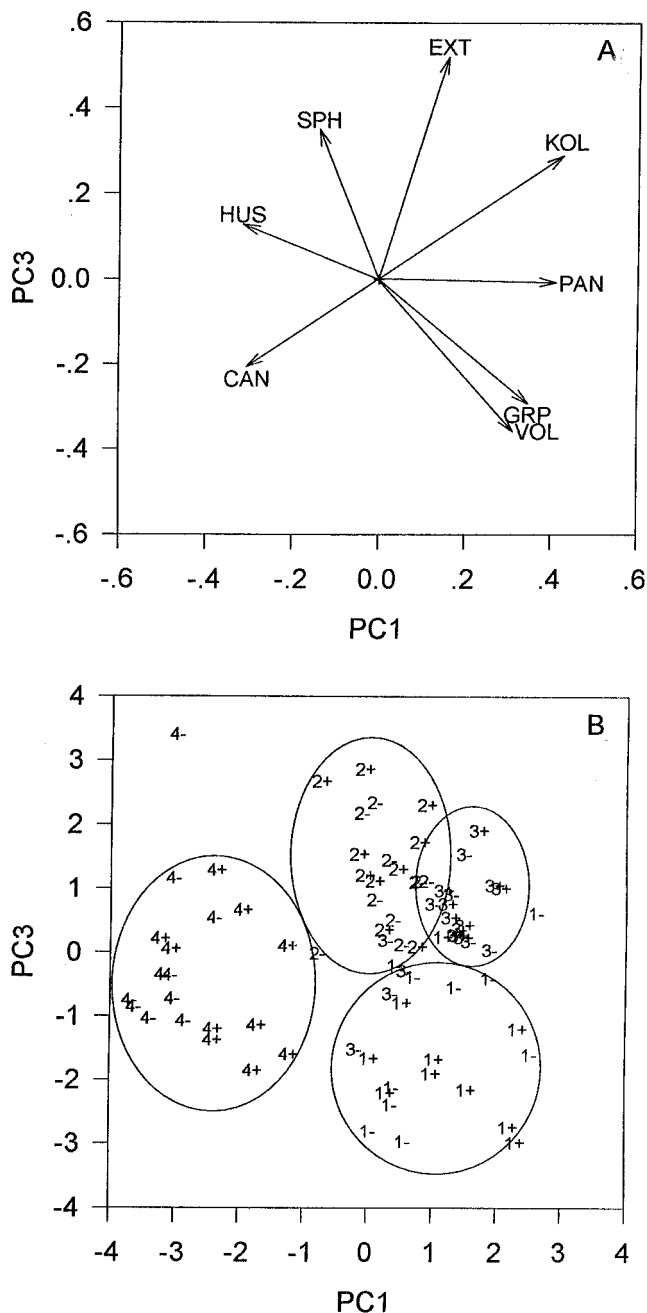
In Fig. 3B, where axis 3 is related to an increase in both EXT and KOL toward its positive direction, it can be seen that the grouping

of the environments following the positive direction on axis 3 shows a gradient from low (1 and 4) to high extract yield (2 and 3). The clustering of the four environments for KOL parallels that explained for EXT. The groupings following the positive direction of axis 1 show the increase in PAN and GRP from environment 4 to 3, with an intermediate situation for environments 1 and 2.

The clustering of points related to axis 1 also validates the meaning of this axis. There is an increase in PAN from environment 4 to 3, with environments 1 and 2 located in between. The same is true for GRP.



**Fig. 2.** Principal component analysis (PCA) projections on axes 1 and 2, accounting for 46.8% of total variance. **A.** Eigenvectors of characters that most influence each axis; length of projection on PC1 and PC2 indicates the weight (loading or eigenvalue) of its influence on that axis, whereas cosine of the angle between any two vectors is inversely proportional to their correlation. Variable definitions given in Table III. **B.** Projection on plane 1–2 of 80 points representing 80 means of different treatment combinations (environment, genotype, nitrogen [ $\pm$ ], and herbicide). Environments 1–4: Lleida 95, Lleida 96, Valladolid 94, Valladolid 96.



**Fig. 3.** Principal component analysis (PCA) projections on axes 1 and 3 accounting for 41.8% of total variance. **A.** Eigenvectors of characters that most influence each axis; length of projection on PC1 and PC3 indicates the weight (loading or eigenvalue) of its influence on that axis, whereas cosine of the angle between any two vectors is inversely proportional to their correlation. Variable definitions given in Table III. **B.** Projection on plane 1–3 of 80 points representing 80 means of different treatment combinations (environment, genotype, nitrogen [ $\pm$ ], and herbicide). Environments 1–4: Lleida 95, Lleida 96, Valladolid 94, Valladolid 96.

## DISCUSSION

### Barley Protein Content and Malt Extract Yield

The discovery of the negative correlation between barley protein content and malt extract yield (Bishop 1930a,b, 1934; Bishop and Day 1933) made this characteristic the most useful for the prediction of malting quality when choosing among grain lots intended for malting. Since then, the widespread use of this negative correlation by the industry has led to an uncritical acceptance that may overlook possible dangers. A valid single conversion factor for all the environments and cultivars is improbable. For example, for the same cultivars grown in Spain and in Scotland (Molina-Cano et al 1995), the Spanish lots with 2% more total protein yielded more extract than their Scottish counterparts, probably due to different hordein and  $\beta$ -glucan contents and a more complete degradation of proteins and  $\beta$ -glucans during malting caused by a superior enzymatic activity in the Spanish lots. Further research has demonstrated the existence of different patterns of synthesis of hordeins and  $\beta$ -glucans in barley grain in Spain and Scotland (Swanston et al 1997). In studies investigating this phenomenon, analyzing malting results from all over Europe (J. L. Molina-Cano, *unpublished*) we have found different patterns of extract development, depending on climate.

In the results presented here, GRP and EXT, though significantly negatively correlated (Table IV), are not as closely related as other characteristics belonging to the same type in Table II (e.g., GRP with PAN and EXT with KOL). However, GRP was the characteristic measured at the plant and whole grain level (Type I in Table II) most closely linked to EXT.

A different picture emerges for the correlations between GRP and EXT within environments (data not presented), suggesting that the relationship depends on the growing conditions. Although the correlation between EXT and GRP is negative and significant in three out of the four environments, the actual values vary substantially:  $-0.69$  (L95),  $-0.82$  (L96),  $-0.94$  (V96), and nonsignificant in V94. Furthermore, even in environments where GRP and EXT are negatively correlated, the other characteristics linked to EXT are different. Thus, KOL is positively correlated with EXT in three out of the four environments, but EXT and GRP are correlated in only two. Unfortunately, KOL cannot be used to predict EXT; they are determined at the same time. KOL is also dependent on malt processing protocols.

In spite of these limitations, the use of the negative correlation between extract and protein remains useful, provided the limitations are taken into account. Given the correlations presented in Table IV, EXT may be predicted by first predicting GRP and assuming that the higher the GRP, the lower the EXT of the future malt.

For plant characteristics linked to grain protein content, Table IV shows that GRP is significantly correlated with two plant characteristics at anthesis: positively with PAN and negatively with CAN. The more plant protein and the less plant nonstructural carbohydrates at anthesis, the higher the grain protein content at harvest. Thus, we can use PAN (which is easier to determine than CAN) as a predictor of GRP. Therefore, the analysis of plant protein content at anthesis, will permit us to forecast the future grain protein content. This, in turn, will allow us to reject those barley production fields with high potential grain protein content and will help us to concentrate on fields that may give grain acceptable for malting. Some of these fields will produce grain that will have to be rejected for malting at the elevator, but the frequency of these rejected batches will undoubtedly be lower than before. This is important when malting barley is produced under contract.

### Predicting Malting Quality with Image Analysis

Although Berman et al (1996), successfully used this technique to screen wheat samples for flour-milling yield, we did not find any size-shape characteristic able to predict EXT. However, malting quality is actually the overall expression of a complex aggregate

of characteristics, dependent on: 1) grain physical composition, 2) grain enzymatic potential, and 3) malting conditions (mainly steeping and germination). The study of Berman et al (1996) was of a different nature, because they were predicting flour-milling yield from characteristics at the same level (i.e., those related to grain structure), whereas EXT depends on enzymatic as well as grain structural properties.

Among the grain size and shape characteristics determined by image analysis, Table IV shows that two characteristics, VOL and EMB, were positively and significantly correlated with GRP, whereas SPH (grain sphericity) was negatively correlated with GRP. Hence, the bigger the grains and embryos, the higher the protein content, and the less spheric the grain shape, the lower the GRP (the larger the sphericity value, the less spheric the grain). These correlations are possibly a consequence of the environments and genotypes used in this study because the usual belief is that VOL is negatively correlated with grain protein content. It is, however, interesting to note the positive significant correlation between EMB and GRP, while END shows no correlation with grain protein content. This suggests that, under our experimental conditions and with the genotypes we compared, the embryo was contributing significantly to increase the total grain protein content.

As discussed above, we may assume that grain protein content will generally be used to predict malt extract yield. The hordein composition (the main fraction of barley protein) is a factor, together with other endosperm characteristics such as  $\beta$ -glucan composition, that may disturb the linearity of the correlation of EXT to GRP (Molina-Cano et al 1995) and could be taken into account when attempting to improve the prediction of EXT.

## CONCLUSIONS

The factor accounting for most of the variance in plant composition at anthesis, grain size and shape, and malting quality, was the environment, its effect was highly significant for all the parameters measured. Application of nitrogen was the second most important factor. Nitrogen fertilizer decreased endosperm and increased husk and embryo sizes. Large grain size (volume) was associated with high protein content and low extract yield. Barley plants with a high protein content and low nonstructural carbohydrate content at anthesis produced grain with a high protein content and size (volume) and Kolbach index of the derived malt. Malt extract yield and Kolbach index were strongly positively correlated.

None of the characteristics studied were able to predict malt extract yield better than barley grain protein content. The protein content of the barley grain can be predicted by analyzing plant protein content at anthesis. Of the genotypes and environments studied, the bigger the grains and embryos, the higher the grain protein content; the less spheric the grain shape, the lower the protein content of the barley grain.

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