Protein and Lysine Content, Grain Yield, and Other Technological Traits in Durum Wheat under Mediterranean Conditions

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INTRODUCTION

Durum wheat is one of the cereals most grown in the Mediterranean basin. It is cultivated essentially for pasta making, including couscous and bulgur, although in the Mediterranean countries, a large part of the harvest is devoted to bread preparation (1). This makes the quality breeding and evaluation a main trend in the research area of durum wheat.

Different environmental variables such as climate, soil, and agronomic practices exert a strong influence on different technological quality parameters of wheat. This effect is particularly marked in Mediterranean environments, where the climate, characterized by increasing water deficit and thermal stress during grain filling, may cause large fluctuations, not only of grain yield, but also of grain quality traits, mainly protein content (2). Wheat quality is influenced by genotype, environmental factors, and the interaction between genotype and environment (3), but the relative magnitude of environmental, genetic, and G × E effects on quality is unclear (4).

Protein content, which is one of the most important traits in quality evaluation and breeding of durum wheat, is known to be influenced mainly by climatic parameters, cultivar, nitrogen fertilizer rate, time of nitrogen application, residual soil nitrogen, and available moisture during grain filling (5). Recently, Daniel and Tribol (6) reported that the percentage of proteins in the flour increased with the increase of temperature and nitrogen supply, whereas the quantity of proteins per grain was affected negatively by high temperatures and affected positively by N fertilization. Many studies have focused on the relationship between protein content and grain yield. Campbell et al. (7) demonstrated that highest protein was obtained under conditions promoting lowest yields in spring wheat, which leads to the inverse relationship between protein content and grain yield. Similar results had been obtained by García del Moral et al. (8) in triticale and Novaro et al. (9) in durum wheat. In the same context, Beninati and Busch (10) reported that the development of high-protein, high-yielding cultivars is believed to be hindered by the predominantly negative correlation of the two traits, which ranged in their study from relatively low (−0.25) to high (−0.77) associations for spring and winter wheat.

Cereal proteins are low in lysine, one of the essential amino acids that cannot be synthesized by humans and must therefore be obtained entirely from dietary sources. Thus, lysine content constitutes an important feature for defining the nutritive value of flour obtained from cereals. There are no works in the literature dealing with the relationship between lysine and protein content in durum wheat, as far as we are aware. In bread wheat, early works in 1960s demonstrated a negative correlation between these two traits (11, 12).

The objectives of this work were (i) to determine the magnitude of variation present in a set of durum wheat cultivars and the degree of additive and multiplicative effects on the traits studied, (ii) to investigate the relationship between grain yield and some quality parameters under Mediterranean conditions, with emphasis on protein content, and (iii) to study the relationships between lysine and protein content.

MATERIALS AND METHODS

Three experimental field trials were conducted during the 1997–1998 season in a representative area of the cereal-growing region of southern Spain, a Mediterranean-type growing region of southern Spain.
Ochı´char, and Jerez, respectively. Rainfall amounts were 549.8 mm, 458.2 mm, and 609.7 mm at CIFA (Vertisol Uderts soil) under rainfed conditions. Total amounts were measured on a 250-g sample and expressed as kg hL1.

RESULTS AND DISCUSSION

Analyses of Variance. The analyses of variance (Table 1) showed a large effect of sites and cultivars and a relative low magnitude of the interaction. Within the main factors, sites and cultivars had a considerable effect on all the characters with the exceptions of protein per kernel in the case of sites, and lysine content in the case of cultivars, but these effects were not significant. The most important interaction effects were found on TKW, vitreousness, and protein content per kernel.

The results clearly show the high influence of site on all characters. In this regard, it is useful to underline that in previous works Peterson et al. (4), Pecetti and Annichiarico (17), and Novarro et al. (9) found similar results in durum wheat. In fact, Peterson et al. (4) encountered a large effect of environment on many traits but effect of genotype was more determinant for SDS test. For protein content, the environmental effect overcomes genotype by environment (G × E) interaction effects. These results are confirmed in our study and were also found in other works (18, 19, 20). A small G × E interaction reveals the lack of multiplicative effects in determining the considered character that appeared essentially influenced by additive effects of the environment and genotype. Novarro et al., (9), and as also shown in our results (Table 1), revealed that for characteristics such as grain yield and protein content, highly influenced by additive environmental effects, a large variability over environments must be expected. On the contrary, for traits such as SDS test and TKW, for which the genotype effect is important, it is easier to identify the interest of a cultivar over sites.

Lysine content (expressed in % of protein) was mainly influenced by environmental growing conditions, as shown in the analysis of variance, and this might be due to its dependency on protein level in the grain, which is also under environmental control.

Mean Comparison Among Sites. The site mean values of the recorded traits are shown in Table 2. The irrigated site (CIFA) favored a good grain yield, higher TKW, and better lysine content. Technological traits such as TW, semolina color, SDS test, and vitreousness were better expressed in Jerez (rainfed site) probably, because of soil fertility and adequate distribution of rains.

Protein content showed a high percentage in samples from the lowest-yielding site (Ochıchar), whereas in samples from a site with favorable conditions (CIFA), where high grain yield was obtained, low protein contents were recorded. Our results are in accordance with those obtained by Campbell et al. (7). Differences

<table>
<thead>
<tr>
<th>source of variance</th>
<th>grain yield (x 106)</th>
<th>TKW</th>
<th>TW</th>
<th>semolina color</th>
<th>SDS</th>
<th>vitreousness</th>
<th>grain protein</th>
<th>lysine content</th>
</tr>
</thead>
<tbody>
<tr>
<td>site</td>
<td>2</td>
<td>55.8***</td>
<td>583.6***</td>
<td>84.9***</td>
<td>8.59***</td>
<td>10.1***</td>
<td>84.2***</td>
<td>82.3***</td>
</tr>
<tr>
<td>cultivar</td>
<td>9</td>
<td>1.5***</td>
<td>83.1***</td>
<td>21.5***</td>
<td>3.00***</td>
<td>9.1***</td>
<td>88.2***</td>
<td>7.9***</td>
</tr>
<tr>
<td>site × cultivar</td>
<td>18</td>
<td>0.8***</td>
<td>50.3***</td>
<td>1.8***</td>
<td>0.31***</td>
<td>0.5***</td>
<td>53.7***</td>
<td>4.1***</td>
</tr>
<tr>
<td>blocks (site)</td>
<td>9</td>
<td>1.2***</td>
<td>18.0***</td>
<td>2.8***</td>
<td>0.07***</td>
<td>0.8***</td>
<td>6.8***</td>
<td>2.8***</td>
</tr>
<tr>
<td>error</td>
<td>81</td>
<td>0.2</td>
<td>12.4</td>
<td>1.3</td>
<td>0.03</td>
<td>0.2</td>
<td>3.0</td>
<td>1.0</td>
</tr>
</tbody>
</table>

TKW = thousand kernel weight; TW = test weight; SDS = sedimentation volume. *, **, and *** indicate significant at 0.05, 0.01, and 0.001 levels of probability, respectively.

* TKW = thousand kernel weight; TW = test weight; SDS = sedimentation volume. * , ** , and *** indicate significant at 0.05, 0.01, and 0.001 levels of probability, respectively.
Table 2. Mean Site Values of Different Traits Studied in Durum Wheat Grown at Three Sites of Southern Spain during the 1997–1998 Growing Season

<table>
<thead>
<tr>
<th></th>
<th>grain yield (kg ha⁻¹)</th>
<th>TKW (g)</th>
<th>TW (kg hL⁻¹)</th>
<th>semolina color b values</th>
<th>SDS (ml)</th>
<th>vitreousness (%)</th>
<th>grain protein (mg kernel⁻¹)</th>
<th>lysine content (g/100 g of protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CIFA</td>
<td>4523 a</td>
<td>49.9 a</td>
<td>81.1 c</td>
<td>14.7 c</td>
<td>5.4 b</td>
<td>91.0 c</td>
<td>6.4 a</td>
<td>2.5 a</td>
</tr>
<tr>
<td>Ochichar</td>
<td>2162 c</td>
<td>42.4 c</td>
<td>81.8 b</td>
<td>15.1 b</td>
<td>4.8 c</td>
<td>92.2 b</td>
<td>6.7 a</td>
<td>1.9 c</td>
</tr>
<tr>
<td>Jerez</td>
<td>3292 b</td>
<td>44.7 b</td>
<td>83.9 a</td>
<td>15.7 a</td>
<td>5.8 a</td>
<td>93.9 a</td>
<td>6.4 a</td>
<td>2.2 b</td>
</tr>
<tr>
<td>LSD</td>
<td>184 1.6</td>
<td>0.5</td>
<td>0.2</td>
<td>0.8</td>
<td>0.5</td>
<td>0.3</td>
<td>0.1</td>
<td></td>
</tr>
</tbody>
</table>

* TKW = thousand kernel weight; TW = test weight; SDS = sedimentation volume. Values with the same letter do not differ significantly at the 0.05 probability level using the least significant difference test (LSD).

Table 3. Coefficients of Correlation among Traits of Durum Wheat Grown in Three Sites of Southern Spain during the 1997–1998 Growing Season (n = 30)

<table>
<thead>
<tr>
<th></th>
<th>TKW</th>
<th>TW</th>
<th>semolina color</th>
<th>SDS</th>
<th>vitreousness</th>
<th>grain protein</th>
<th>lysine content</th>
</tr>
</thead>
<tbody>
<tr>
<td>grain yield</td>
<td>0.50**</td>
<td>0.01</td>
<td>−0.14</td>
<td>0.16</td>
<td>−0.72***</td>
<td>−0.27</td>
<td>0.60***</td>
</tr>
<tr>
<td>TKW</td>
<td>−0.33</td>
<td>0.38**</td>
<td>−0.10</td>
<td>−0.20</td>
<td>−0.65***</td>
<td>0.28</td>
<td>0.59***</td>
</tr>
<tr>
<td>TW</td>
<td>0.66***</td>
<td>−0.05</td>
<td>0.48**</td>
<td>0.15</td>
<td>−0.10</td>
<td>−0.16</td>
<td>−0.09</td>
</tr>
<tr>
<td>semolina color</td>
<td>0.09</td>
<td>0.46**</td>
<td>0.07</td>
<td>−0.20</td>
<td>−0.41*</td>
<td>0.24</td>
<td>0.53**</td>
</tr>
<tr>
<td>SDS</td>
<td>0.13</td>
<td>0.46**</td>
<td>0.07</td>
<td>−0.20</td>
<td>−0.41*</td>
<td>0.24</td>
<td>0.53**</td>
</tr>
<tr>
<td>vitreousness</td>
<td>−0.10</td>
<td>−0.24</td>
<td>−0.24</td>
<td>0.53**</td>
<td>−0.86***</td>
<td>−0.46*</td>
<td>−0.86***</td>
</tr>
<tr>
<td>grain protein (%)</td>
<td>−0.10</td>
<td>−0.24</td>
<td>−0.24</td>
<td>0.53**</td>
<td>−0.86***</td>
<td>−0.46*</td>
<td>−0.86***</td>
</tr>
<tr>
<td>grain protein (mg kernel⁻¹)</td>
<td>−0.10</td>
<td>−0.24</td>
<td>−0.24</td>
<td>0.53**</td>
<td>−0.86***</td>
<td>−0.46*</td>
<td>−0.86***</td>
</tr>
</tbody>
</table>

*a TKW = thousand kernel weight; TW = test weight; SDS = sedimentation volume. *, **, and *** indicate significant at 0.05, 0.01, and 0.001 levels of probability, respectively.

between sites in the quantity of protein accumulated per kernel were statistically nonsignificant and appear to be mainly under genotypic dependency as has been shown in Table 1. Lysine content exhibited higher values under the irrigated conditions of CIFA, and the lowest percentages were obtained in the rainfed site of Ochichar.

Correlations Among Characteristics. Table 3 shows the correlation matrix calculated over cultivar means in each site (i.e., n = 30). Protein content was negatively correlated with grain yield (r = −0.72***), TKW (r = −0.65***), and lysine content (r = −0.86***). The inverse relationship between protein content and grain yield was found in numerous works in bread wheat underlined the negative correlation between TKW and grain protein percentage (30). Protein content was not a consequence of protein per kernel, TW, and vitreousness. Novaro et al. (9) stated that the increase in yellow-berry percentage (100 − vitreousness) determines a significant decrease of test weight (TW) and then negatively influences the semolina yield. Porceddu (1) noted that vitreousness is correlated to semolina color, and industries continue to prefer durum wheat low in nonvitreous grain. The positive correlation between grain protein percentage and protein per kernel was probably due to a decrease in kernel weight demonstrated with the inverse relationship between TKW and grain protein percentage (Table 3). These results contradict those of Motzo et al. (23) who encountered no correlation between kernel weight and grain protein and thus concluded that the positive relation between grain protein percentage and protein content per kernel was not a consequence of decrease in kernel weight.

Environment Characterization Based on the Studied Traits. Analyses of variance showed that most of the variability present in our results could be attributed to differences between sites. To detect the combination of characters that best explained the existing variability, principal component analysis (PCA) was carried out on the correlation matrix based on the 9 traits shown in Figure 1. The first three PCA axes accounted for 77.3% of total variance: 36.2, 27.2, and 13.9 for axes 1, 2, and 3, respectively.

Toward the positive direction of axis 1 (Figure 1a), there was a joint increase of grain yield, TKW, and lysine content. Toward its negative direction, there was an increase in protein percentage. On axis 2, a positive relationship appeared between TW, pigment content, and vitreousness in the negative side, whereas on its positive direction, only protein per kernel appeared. On axis 3 (Figure 1b), there was a clear separation of SDS volume, which interacted negatively with a group of traits in the other side of the axis formed by protein per kernel, TW, and vitreousness.

The points plotted on the plane determined by the first two axes (Figure 1a) are grouped in clusters related to sites. Axis 1 discriminates between sites according to its moisture regimen whereas axis 2 separated, at a lesser extent and in its negative direction, between Granada (CIFA and Ochichar) and Jerez trials. Grain yield, TKW, and lysine content were higher in the irrigated site CIFA. Ochichar favored better protein percentages. In addition to acceptable values of grain yield and protein percentage, Jerez favored a good expression of other quality parameters such as TW, pigment content, and vitreousness. In general, rainfed sites of our study better stimulated the genetic potential of cultivars in terms of producing grains with good quality. This finding agrees with similar work by Borghì et al. (2) who concluded that the Mediterranean climate causes a large fluctuation in grain yield, but offers the opportunity for production of high-quality wheats.

Similarly to Figure 1a, points plotted on the plane determined by axes 1 and 3 are grouped in clusters related to sites (Figure 1b). Cultivars in each site appear to be distributed along axis 3, which lead to the conclusion that this axis mainly discriminates between cultivars. The variation between cultivars was mostly explained by traits that were under genetic determination, such as protein per kernel, TW, vitreousness, and...
SDS volume. These results confirmed those obtained by the analyses of variance that were previously mentioned.

**Relationship between Protein Content and Grain Yield.** Regression of cultivar mean values in each site for protein content on grain yield (Figure 2) shows a high negative correlation between these two traits. This inverse relationship between grain yield and grain protein concentration is reported widely in the literature and is frequently confirmed in studies of grain yield and protein. As discussed by Simmonds (24), this relationship is curiously consistent, but no simple physiological interpretation is available.

Dilution of protein by non-nitrogen compounds in the grain seemed to be the primary cause for the negative association between grain yield and protein content (21). Penning de Vries et al. (25) have estimated that, in theory, 1 g of glucose produced by photosynthesis can be used by the crop to produce 0.83 g of carbohydrates or 0.40 g of protein when NO\textsubscript{3} is the N source. This implies that an increase in protein content uses more photosynthate, thus decreasing available photosynthate...
for carbohydrate synthesis and leading to relative decreases in grain yield. Because nitrogen is the most predominant constituent of proteins, nitrogen accumulation and redistribution during the plant life cycle is of great interest in order to understand the protein—grain yield relationship. Cox et al. (26) demonstrated that total grain nitrogen was influenced both by translocation and current assimilation of nitrogen, whereas its concentration was influenced by grain yield and the dilution effect of carbohydrate deposition through photosynthesis.

Conditions that promote leaf senescence during grain growth, such as drought or higher temperatures, favor protein deposition over starch accumulation in the grain because the production and/or translocation of carbohydrates to the grain is more sensitive to adverse growing conditions that is protein production (7, 27). In the same way, under conditions that shorten the duration of grain filling period, starch deposition appears to be more affected than protein accumulation (8, 28). This is also confirmed by the climatic conditions of our experiments (data not shown), so that the rainfed trial (Ochíchar) was the warmest and driest site during grain filling period, followed by Jerez trial, and finally the irrigated trial (CIFA).

Although the grain protein percentage was negatively related to grain yield and varied with growing conditions, the protein per kernel appeared unchangeable over the sites (Table 2), thus confirming the suggestions made by several authors (7, 8, 27, 28, 29) that protein synthesis in the grain is less affected by environmental factors than is starch synthesis, which could determine variation in grain protein percentage.

**Relationship between Lysine and Protein Content.** Under the conditions of our study, a highly negative correlation \( r = -0.864^{***} \) was encountered between lysine content (as expressed in % of protein) and protein content (Figure 3), suggesting that the percentage of lysine increases as the protein level decreases. There are no literature references on the nature of this relationship in durum wheat as far as we are aware. Nevertheless, in bread wheat an inverse relationship was found in the early works by Lawrence et al. (11) and McDermott and Pace (12). As discussed by Lawrence et al. (11), this negative correlation might be accounted for by changes in the proportion of individual proteins with different lysine contents as the total protein content changes; by changes in the lysine content of the proteins themselves; or by changes in the proportions of free lysine present. Moreover, the same authors stated that the proportion of endosperm proteins tends to be lower in wheats of low total protein, and that the endosperm proteins of low-protein wheat tend to have a higher concentration of lysine.

It is worthy to comment here on the positive relationship found in our work between lysine content and grain yield (Table 3), which means that an increase in grain yield will be accompanied with a decrease in protein quantity, but an increase in the nutritional quality of these proteins. However, improvement of lysine content while maintaining or increasing protein content in durum wheat appears to be hampered by the negative correlation between both characters.

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**LITERATURE CITED**


