Variations in yield and gluten proteins in durum wheat varieties under late-season foliar versus soil application of nitrogen fertilizer in a northern Mediterranean environment

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Variations in yield and gluten proteins in durum wheat varieties under late-season foliar *vs.* soil application of nitrogen fertilizer in a northern Mediterranean environment

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Running title: Effects of late-season foliar nitrogen fertilisation in durum wheat

Abstract

BACKGROUND: With the increasing demand for high-quality foodstuffs and concern for environmental sustainability, late-season nitrogen foliar fertilization of common wheat is now an important and widespread practice.

RESULTS: We investigated the effects of late-season foliar vs. soil N fertilization on yield and protein content of four varieties of durum wheat, Aureo, Ariosto, Biensur and Liberdur, in a three-year field trial in northern Italy. Variations in LMW-GS, HMW-GS and gliadins were assessed by SDS-PAGE. We found that N applied to the canopy did not improve protein rate compared with N application to the soil (general mean: 138 mg g^{-1}), but moderately increased productivity in the high-yielding varieties Liberdur and Biensur (three-year means: 7.23 vs. 7.13 t ha^{-1} and 7.53 vs. 7.09 t ha^{-1}, in two varieties respectively). Technological quality was mainly related to variety choice, Aureo and Ariosto having higher protein rates and glutenin/gliadin ratios. We also found a strong 'variety × N application method' interaction in the proportions of protein sub-units within each class, particularly LMW-GS and gliadins. A promising result was the higher N uptake efficiency, although as apparent balance, combined with higher HMW/LMW-GS ratio in var. Biensur.

CONCLUSION: Late-season foliar N fertilization allows N fertilizer saving, potentially providing environmental benefits in the rainy climate of the northern Mediterranean area, and also leads to variety-dependent up-regulation of essential LMW-GS and gliadins. Variety choice is a key factor in obtaining high technological quality, although it is currently associated with modest grain yield. We provide evidence of high quality in the specific high-yielding variety Biensur, suggesting its potential as a mono-varietal semolina for pasta production.

Key words: durum wheat varieties, foliar nitrogen fertilization, gliadins, HMW-GS, LMW-GS, sustainable agriculture, technological quality in pasta production.

INTRODUCTION

Durum wheat (*Triticum turgidum* L., var. *durum*) plays a major role in human diets, and is used throughout the world, and particularly the Mediterranean region, to produce essential foods, like pasta, couscous, bread and bulgur. The technological value of durum wheat mainly depends on the quantity and quality of proteins and starch[1,2]. As protein content and composition are highly dependent on genotype, variety choice is essential for producing high-quality pasta.

The most important class of biochemical markers in determining wheat quality is the polymorphic group of seed storage proteins, represented by gliadins and glutenins. Gliadins are alcohol-soluble proteins, which can be classified into S-rich prolamins, i.e., α–β–γ–gliadins (molecular weight 36-44 kDa), and S-poor prolamins, i.e., ω-gliadins (molecular weight 44-78 kDa). Glutenins consist of different sub-units of high molecular weight (HMW-GS, 80-140 kDa) and low molecular weight (LMW-GS, 31-51 kDa), which form large aggregates joined by disulfide bonds. Gluten properties determine dough strength, which is greater when there are higher proportions of HMW-GS than LMW-GS polymers, although a high glutenin/gliadin ratio is also important in determining the functional proprieties of the gluten[3].

Environmental factors and agronomic management play an important role in determining wheat quality characteristics. For example, temperature and precipitation during grain filling, and N availability are key factors in seed protein accumulation[4]. Nitrogen fertilization can have a strong impact on yield and semolina quality, according to dosage, source, application timing and dose split[5].

The rate of N fertilization of durum wheat has a greater influence on quality than on yield[6,7], as the total available N required to obtain an appreciable protein content may be considerably higher than that needed simply to maximize yield[6,8]. Nitrogen is more effective in increasing grain proteins when applied late in the season[9,10], and foliar spraying can be more beneficial than soil application at appropriate stages[11-13]. Applied at flowering, N is allocated more efficiently to the grains, while a spray solution applied to the flag leaf and underlying leaf allows faster access of N to seeds[14]. Nitrogen uptake from foliar spraying has the advantage of being less dependent on soil moisture and may be effective when root uptake is impaired in dry soils or at late growth stages when there is less root activity[15]. Applied to the canopy, lower quantities of N are required, thus minimizing phytotoxicity, while the high efficiency of this method can reduce crop requirement[11,16,17], thereby lowering gaseous N emissions and nitrate leaching[16,18,19], making this a valuable method in a context of increasing demand for environmental sustainability.

Foliar N application is not yet a widespread practice and little information is available about its effects on durum wheat, especially in the unusual agricultural region at the northern limit of the Italian Mediterranean environment. There is a great need to improve the efficiency of N fertilization of this wheat species, which has a high grain protein rate and N requirement, in order to reach the required standards of quality while avoiding damage to the environment.

As nowadays late-season nitrogen supply has become a common practice in modern wheat cultivation, the aim of the present study was to analyze and compare the effects of two methods of N supply at the heading stage, soil vs. foliar spraying, on grain yield and gluten protein accumulation in four different commercial durum wheat varieties.

We characterized the gluten protein profiles of these varieties in order to identify suitable configurations for high technological quality for pasta production and to detect possible interactions between fertilization method and protein sub-unit composition.

MATERIALS AND METHODS

Field trial set-up

The trial was carried out in north-eastern Italy at the "L. Toniolo" Experimental Farm of the University of Padua at Legnaro (45°20’ N, 11°56’ E, 12 m asl) over 3 years, starting with the 2010-2011 growing season. The soil is silty-loam with pH 8.0, 1.7% organic matter, CEC of 11.4 cmol(+) kg^{-1}, and a total N content of 1.1 g kg^{-1} (arable layer, beginning of experiment). Temperatures and precipitation in the 3-year period 2010-2013 are reported in Fig. 1A. The first year was characterized by high temperatures and low rainfall in the last stage of the crop cycle; the second year (2011-12) had the driest growing season together with the highest maximum temperatures; the third year (2012-13) was characterized by high rainfall, especially in winter and spring, and low maximum temperatures.

The effects of the two methods of late-season N fertilization were investigated in 4 durum wheat varieties using a split-plot experimental design with three replicates. The fertilization factor was set in large plots, and varieties in randomized subplots (3 m wide × 8 m long). The previous crop had been sugar beet in all three years. Sowing generally took place in late October at a density of 350 seeds m^{-2} after careful soil tillage consisting of 30-cm deep ploughing and 15-cm deep disc harrowing.

Four different varieties were compared, two of them acknowledged as being of very high quality in terms of grain protein content, i.e., Aureo (Produttori Sementi Bologna, Italy, now Syngenta, Basel, Switzerland) and Ariosto (Apsovsementi, Voghera, Italy), and two of medium-high quality, i.e., Biensur (Apsovsementi) and Liberdur (S.I.S. Società Italiana Sementi, Bologna, Italy) (Table SI 1).

Varieties were factorially combined with the two methods of late-season N fertilization: foliar vs. soil application at the heading stage. The overall N fertilization rate was 162 kg N ha^{-1} for foliar treatment and 182 kg N ha^{-1} for soil treatment. Prior to the late-season N supply, the same quantities of N were applied to the soil: 24 Kg at sowing (as ternary fertilizer), 52 kg during tilling (end February, as ammonium nitrate), and 71 kg at the beginning of stem elongation (mid-April, as urea). Foliar fertilization consisted of 15 Kg N ha^{-1} as UAN (urea ammonium nitrate, NSZ 26, Cifo) divided into two applications in order to avoid any possible phytotoxicity, half at heading and the other half fourteen days later at flowering. The soil treatment consisted in applying N as urea at the heading stage at a rate of 35 Kg ha^{-1}, roughly double that of the foliar treatment, in order to take into account a ~50% efficiency.

Harvesting was carried out with a plot combine harvester (Wintersteiger, Ried, Austria) at the beginning of summer: 21 June 2011, 29 June 2012, and 3 July 2013. Grain moisture was measured for each plot and yield adjusted to 13% moisture.

The thousand seed weight (TSW) was determined by weighting 1,000-kernel samples obtained from a counting-machine (Numigral Seed Counter, Chopin Technologies, Villeneuve-la-Garenne, France). The number of kernels per square meter was calculated by dividing the grain yield in a 0.5-m^2 sampling area by TSW in each plot.

The crop harvest index (HI) was calculated as grain-to-total aboveground biomass (dry matter) ratio in 0.5-m^2 sampling areas in each plot at harvest.

Analysis of grain quality

Grain protein content

The total grain protein content was determined by the Kjeldahl method (official analytical method 2001.11) [20] as total N rate multiplied by the coefficient 5.7.

Gluten protein extraction and quantification

A sample of 35 g of dried seeds from each cultivar-treatment combination was milled into fine flour with a KnifetecTM 1095 (Foss, Hillerød, Denmark). Gluten proteins, gliadins, high–molecular-weight glutenin subunits (HMW-GS) and low–molecular-weight glutenin subunits (LMW-GS) were determined using the modified sequential extraction procedure of Singh et al[21]. A 30-mg flour sample was subjected to extraction with 1.5 mL of 55% (v/v) propan-2-ol for 20 min with continuous mixing at 65 °C, followed by centrifugation for 5 min at 10,000 rpm. This step was repeated three times, and the supernatants were combined and dried in a vacuum centrifuge to obtain the protein gliadin fraction. The remaining pellet containing the GS fraction was suspended in a 400 µL solution of 55% (v/v) propan-2-ol, 0.08 M tris(hydroxymethyl)aminomethane hydrochloric acid (Tris HCl) pH 8.3, and 1% (w/v) 1,4-dithio-threitol (DTT) as reducing agent, and incubated for 30 min at 60 °C with continuous mixing. After centrifugation for 5 min at 14,000 rpm, the supernatant containing the HMW-GS and LMW-GS fractions was transferred to a new tube. To precipitate HMW-GS, acetone was added to obtain a final concentration of 40% (v/v), which was then centrifuged for 10 min at 14,000 rpm. The LMW-GS fraction was precipitated in the remaining supernatant by adding acetone to obtain a final concentration of 80% (v/v), and this was then centrifuged for 10 min at 10000 x*g*. The GS fractions and gliadins were dissolved in 50% (v/v) acetonitrile (ACN) with 0.1% (v/v) trifluoroacetic acid (TFA); relative quantification was determined by colorimetric Bradford assay (Biorad Hercules, CA). Three technical replicates were performed for each sample.

Protein separation by SDS-PAGE and densitometric analyses of gliadins and glutenins

SDS-PAGE was performed on a Mini-PROTEAN Tetra Cell (Bio-Rad) on 8%, 12% and 15% acrylamide gel for the HMW-GS, LMW-GS and gliadin fractions, respectively. 2.5 µg each of dried HMW-GS, LMW-GS and gliadins were suspended in 20 µL of loading buffer containing 2% (w/v) SDS, 0.02% (w/v) bromophenol blue, 0.1% β–mercaptoethanol, 0.05 M Tris-HCl pH 6.8, and 10% (v/v) glycerol, and boiled at 95 °C for 5 min before loading onto the gel. A ColorBurst™ Marker Electrophoresis High Range (Mw 30,000-220,000) was used to detect HMW-GS, and a Molecular-Weight Marker® (Mw 14,000-66,000; Sigma Aldrich, St. Louis, MO, USA) to detect LMW-GS and gliadins. After electrophoretic separation at 40 mA, the gels were stained with brilliant blue G-colloidal solution (Sigma Aldrich) fixed in 7% (v/v) acetic acid and 40% (v/v) methanol, and de-stained in 25% (v/v) methanol. Each protein sample (HMW-GS, LMW-GS, and gliadins) was analyzed in three technical replicates in all three experimental years. IMAGE lab 4.5.1 (Bio-Rad) software was used to identify protein molecular weights (MW) and for relative quantification of the gliadin, LWM-GS and HMW-GS single protein sub-units on each gel.

Statistical analyses

Analysis of variance (ANOVA) of the agronomic data was run with the Statgraphics Centurion XVII statistical software (Statpoint Technologies, Inc. Warrenton, Virginia, USA). Significant differences among means were verified with the Newman-Keuls test at $P \leq 0.05$.

To facilitate interpretation of the large dataset obtained from the three-year field trial, factorial discriminant analysis (MDA, Multigroup Discriminant Analysis with Wilks' lambda and Pillai's trace tests) and principal component analysis (PCA) were carried out to describe yield components and quality with respect to variety choice and method of late-season N supply. Multivariate data normality was first verified by Shapiro test. The data were standardized before analysis by subtracting the mean and dividing by the standard deviation within each variable. Analyses were performed with MS Excel XLSTAT (Addinsoft, Paris, France).

RESULTS AND DISCUSSION

Effects of N fertilization and variety choice on yield and protein content

Grain yield was significantly affected by cultivar choice. The two high-yielding varieties, Biensur and Liberdur, were found to have better potential (7.31 and 7.18 t ha^{-1} at the commercial humidity of 13%, respectively) than Ariosto (6.46 t ha^{-1}, with the highest kernel weight) and particularly Aureo (5.12 t ha^{-1}) (Fig. 1B). As the different varieties had very similar plant densities, grain yield was thought to be mainly related to spike fertility, as confirmed by the number of kernels per m^2 that was higher in Biensur than in Aureo (+36%) (Fig. 2A). The high-yielding varieties also had better harvest indexes vs. the high-quality ones (+7%, Fig. 2C), despite worse values of the thousand kernel weight (TKW, –14%) (Fig. 2B). There was no difference in yield between foliar and soil N application, although only slight, not significant, improvements were attributed to foliar fertilization, particularly in var. Liberdur (Fig. 1B). Considering that the amount of N applied in foliar spraying (two applications) was half that of the soil treatment, our data confirm previous work reporting the high level of efficiency and sustainability of foliar N application for crop requirements[11,16,17]. Recent studies referred to our agricultural region confirm that nitrogen use efficiency for granular soil-applied fertilizers may vary considerably, it depending on soil type and year, with an average value of 46% and maximum of 75%[22]. Again, grain protein content differed significantly across varieties: var. Aureo had the highest value (158 mg g^{-1}) followed by Ariosto (142 mg g^{-1}), Biensur (131 mg g^{-1}), and finally Liberdur with the lowest value (122 mg g^{-1}). While confirming the general rule that productivity is negatively correlated with grain protein content, we point out that the protein rates of three of the varieties tested were > 130 mg g^{-1}, a critical threshold for pasta processing. The method of late-season N application (foliar vs. soil) had no significant influence on protein content (Fig. 1B). There was a marked effect of cultivation year on yield and, to a lesser extent, on kernel protein content (Fig. 1C), suggesting that protein content has a strong genetic dependence[23]. The high rainfall in the third trial year (2012-13) reduced both grain yield and protein content.

In terms of apparent N balance (i.e., the difference between N supply and grain N), foliar supply was more favorable (lower values) than soil application in all varieties, particularly Biensur, so we can expect nutrient foliar applications to have a generally lower environmental impact[24] (Fig. 3). Although incomplete, the apparent N balance is a good estimator of N uptake as the grains generally contain more than 75% of the total plant uptake, while straw has negligible N concentrations (~0.3%).

These data suggest that, besides Aureo and Ariosto, var. Biensur can also provide good quality with high both yield and nitrogen uptake, and may therefore be profitably considered for future use in pasta production. As regard the application method of N fertilizer, small agronomic variations were detected in these varieties, probably because similar plant N uptakes were achieved with the two methods and treatment differentiation started late in the season. However, improved grain N recovery is generally expected with foliar fertilization compared with soil application[25], with benefits in yield and protein contents[26-28] mainly due to retarded senescence of the flag leaf[7].

Characterization of gluten proteins

In accordance with other findings that N plays a key role in wheat plant nutrition and grain quality[29], we found a significant correlation between N accumulation in the kernels and the final total gluten protein content measured by quantifying the various protein fractions (HMW-GS, LMW-GS and gliadins) across the three experimental years (R^2 = 0.75). Nitrogen fertilization has been reported to enhance the synthesis of GS, resulting in a higher GS/gliadin ratio, due to different expressions of the gluten gene families[30-34-28]. However, modulation of the gluten content by N fertilizers is also expected to be cultivar dependent[31]. In this regard, var. Aureo and Ariosto had higher total protein contents, while Biensur exhibited the highest HMW/LMW-GS ratio under both N treatments, and a higher GS/gliadin ratio than Liberdur (Fig. 4). Foliar fertilization slightly increased, although not significantly, the GS/gliadins ratio in all varieties, a part from Biensur, and the HMW/LMW-GS ratio in Ariosto only.

Previous studies have found a positive correlation between the GS/gliadin ratio and dough strength[34,35]. Edwards and collaborators[36] showed that addition of a glutenin-rich fraction consisting of HMW-GS and LMW-GS to the base semolina increases the mixograph dough strength. In common wheat, improvements of bread-making quality of flour by late foliar N supply have already been demonstrated[37]. Studies have also suggested that foliar N is mainly incorporated in storage proteins[38].

Principal component analysis

PCA was conducted on the whole dataset of the three-year trial in order to establish the relevance of each variable and treatment. PCA identified two synthetic variables, which explained an overall variability of 91.4%, mainly represented by F1 (78.87%) (Fig. 5). Most of the relevant variables (loadings > |0.5|) were assigned to the F1 variable, such as total grain protein rate, HMW-GS and LMW-GS content, and the GS/gliadin ratio, while the F2 variable included the HMW/LMW-GS ratio as a significant variable.

The vector direction of the variables enabled us to establish a general strong negative correlation between yield and quality parameters (i.e., total gluten proteins, and both HMW-GS and LMW-GS), which highlights the difficulty in obtaining high yield and high quality together. A positive correlation was found between yield and the polymeric-to-monomeric protein ratio (i.e., HMW/LMW-GS), a selection criterion for pasta production[2].

Marked differences between the varieties were found, as clearly evidenced by the separate plotting of groups in the MDA chart (circles and centroids; Fig. 5). Results confirmed var. Ariosto and particularly Aureo as superior-quality varieties with high levels of total proteins and HMW-GS and LMW-GS, while Biensur exhibited a high HMW/LMW-GS ratio, and, along with Liberdur, high yield. Regarding fertilization method, foliar application constantly diverged from soil application, improving yield in Liberdur, Biensur, and, unexpectedly, Ariosto, and further improved the already good technological value of Aureo.

Protein indicators for wheat quality

Varieties Biensur and Ariosto had HMW-GS compositions typical of the allelic configuration Bx7 and By8 at locus *Glu-B1* (Fig. 6), in accordance with published data[39]. The HMW-GS of var. Aureo had the typical configuration Bx6 and By8 at the same locus. It has been suggested that both HMW-GS configurations make a favorable contribution to the technological quality of gluten in durum wheat[40]. Liberdur exhibited only the Bx20 allele expression while By was null (Fig. 6). There is scientific evidence that durum wheat with this protein profile is weak[41,42], while Shewry and collaborators[43] used wheat transformation to confirm that HMW-GS Bx20 is associated with lower dough strength. Lower overall strength of this protein subunit is thought to depend on lower density of intermolecular disulfide bonds (fewer cysteine residues at the N-terminal), resulting in lower polymeric protein content.

In all the varieties tested, LMW-GS protein patterns showed the typical configuration of the LMW-2 protein groups, which are coded at the locus *Glu-B3* (Fig. 6). This configuration is characterized by a 42 kDa LMW-GS, the most abundant GS of this class[44]. An additional band was present in var. Biensur, suggesting a putative LMW-2 allelic variant[42]. Lastly, Figure 6 also shows the gliadin configurations of all the varieties; the γ-45 gliadin group is associated with the LMW-2 GS pattern due to genetic linkage[45].

Var. Biensur had the best HMW-GS configuration for high gluten strength and an optimal response in term of the GS/gliadin ratio, both of which are associated with significant differences in the dough strength of durum wheat[2,35,44,46].

Members of the gliadin, LMW-GS and HMW-GS families were quantified by densitometric analysis to examine varietal differences and possible up-regulation by the late nitrogen supply method. Statistical analysis of the data from the three experimental years revealed significant differences among varieties with respect to the relative abundances of all the members of each gluten protein class (Table 1). Significant differences between the N fertilization methods (foliar vs. soil treatment) were also observed in all the LMW-GS and gliadin classes, with the exception of gliadin 35 kDa, while HMW-GS were not affected (Table 1). The most important result was the significant ‘cultivar x N supply method’ interaction found in all gluten protein classes (Table 1). Variations in HMW-GS composition can be mainly ascribed to variety choice, while the effect of fertilization method was very low. On average, var. Aureo contained ~50% subunit Bx6 and ~50% sub-unit By8, while varieties Ariosto and Biensur contained ~65-70% Bx7 and ~30-35% By8 (Fig. 7). Liberdur was not included in the densitometric analysis for HMW-GS as it exhibited only the Bx20 sub-unit, while By was a null allele.

Regarding the LMW-GS, there are evident differences among cultivars, particularly in the 42 kDa and 37 kDa sub-units, which are the most abundant ones (Fig. 8) and were generally up-regulated by N foliar treatment (main effect +0.8% and +0.3%, respectively, compared with N supply to the soil). The less abundant sub-units 32 and 31 kDa were instead generally down-regulated by N foliar treatment (Fig. 8). Aureo, in particular, responded better than the other varieties to foliar N treatment, up-regulating both LMW-GS 42 kDa and LMW-GS 37 kDa (up to 2%). LMW-GS 37 kDa also improved in Ariosto and Biensur, although to a lesser extent (Fig. 8). Var. Liberdur had the highest relative abundance of LMW-GS 42 kDa (> 50% of the total LMW-GS) and exhibited a significant increase in this sub-unit under foliar N treatment (up to 1%). Biensur had the highest relative abundance of LMW-GS 37 kDa (> 35% of the total LMW-GS) and the greatest increase due to N foliar treatment (up to 2%).

Regarding the gliadins, we carried out relative quantification of the fractions in the MW range 28-35 kDa, which corresponded to the overlapping α/β– and γ-gliadin MWs. These gliadins account for up to 64% of total gliadin content and are those mainly involved in gluten technological proprieties due to the presence of six (α/β–) and eight (γ–) cys residues in the C-term domain[47]. In particular, γ-gliadins can form intra- and inter-chain disulfide bonds interacting with the HMW and LMW polymers[47]. In the range of 39-35 KDa, where most of the γ-gliadins usually occur, var. Biensur had the highest content of 39 kDa gliadin under both N fertilization methods, but it was slightly lower with foliar N; although this sub-unit is less abundant in Aureo and Liberdur, it was significantly up-regulated by foliar N (+1 and +2%, respectively) (Fig. 9). The 35 kDa gliadin was also positively regulated by foliar N supply in varieties Aureo, Biensur and Liberdur (average increase of 1,5-2%), but the opposite pattern was found with var. Ariosto. In the range of 31-28 kDa, in which most of the α/β gliadins fall, the 30 kDa gliadin was up-regulated by foliar N treatment in Biensur (up to 2%), and in Ariosto (up to 3%); in the latter variety this protein band accounted for more than 40% of gliadins (Fig. 9). Gliadins 31 kDa and 28 kDa were both less modulated by the N supply method. These data indicate that the proportions of the different members of each GS-protein class is a varietal trait, although it is possible to obtain a significant positive effect of N foliar treatment on the most abundant LMW-GS sub-units and the gliadin fractions in the range 39 to 30 kDa. Nitrogen uptake from foliar spraying can be more efficient than from soil supply when root activity is reduced, as in the case of late growth stages (post-anthesis), and increases protein accumulation in developing grains. Previous studies demonstrated that late-season urea spraying of wheat consistently resulted in higher yield and a cultivar-dependent increase in protein content[48]. In this study, technological quality was mainly related to variety choice, but our data support the important influence of the method of N fertilizer supply on durum wheat gluten protein sub-unit composition, and consequently on grain quality. The amount of N and the method of late-season application, even when the amount of N taken up by the plant is similar, are key factors in determining wheat productivity and quality. PCA and MDA of our 3-year dataset did not support the significance of rainfall and temperature recorded during grain filling, although agronomically important, probably because our experimental site is located at the northern border for durum wheat in the Mediterranean area, where water deficits and heat stress are unusual events. In this regard, both air temperature and humidity affect the effectiveness of foliar N supply and phytotoxicity, but not in this trial probably because of the small amount of fertilizer supplied and the favorable (i.e., moderate temperature, high air humidity) climatic conditions at heading. Nonetheless, modulation of HMW-GS, LMW-GS, and α-gliadins, γ-gliadins, and ω-gliadins in response to high temperatures, drought and also nitrogen fertilization are documented in the literature[31,46,49-51-44]. In particular, Hurkman and collaborators[30] demonstrated that the proportions of ω-gliadins and HMW-GS, and a few α-gliadins and LMW-GS were modified in developing endosperm in response to fertilizer and high temperature during anthesis, while most of the γ-gliadins and LMW-GS were unchanged. Gluten protein accumulation is a complex process subject to spatial and temporal regulation as well as environmental signaling. Individual proteins within each gluten protein class accumulate to different levels and can be influenced by environmental changes to different extents, suggesting that the corresponding genes have different basal levels of expression and possibly different N response regulatory elements in the promoter sequences.

CONCLUSIONS

This study demonstrates that N fertilizer applied to the canopy of durum wheat at heading-flowering guarantees faster N access to seeds, has appreciable effects on grain quality and gives moderate improvement in yield. Foliar treatment is of environmental importance as lower amounts of N need to be applied to the crop and greater N uptake is expected; this has potential benefits in terms of reducing nitrate leaching in the rainy climate of NE Italy, which lies at the northern Mediterranean border for durum wheat cultivation. This study demonstrates that an amount of at least 20 kg N ha^{-1} can be saved in the fertilization plan with foliar supply compared with soil fertilization, and that the greater amount of N taken up by the crop (varietal mean: +19 kg ha^{-1}) may reduce further the leaching risk.

Among the genotypes, the high-yielding var. Biensur was, unexpectedly, found to have high protein quality, acceptable total gluten protein content, high GS/gliadin and HMW/LMW-GS ratios, and an optimal allelic GS configuration (Bx7 and By8), suggesting that high productivity can be combined with good quality through suitable breeding programs. Aureo, followed by Ariosto, were confirmed as being high-quality varieties with high contents of total proteins, HMW-GS, and LMW-GS, supporting their current use as mono-varietal semolina for pasta production. Although each variety has a specific protein fingerprint, late-season foliar N supply can positively up-regulate important LMW-GS and gliadins, although the effect is variety-dependent. This suggests that fine-tuning of grain quality, especially in weak genotypes, would require assessment of their individual responses to late foliar N supply. Future work will require to evaluate dough rheological and pasting properties of semolina obtained from cv. Biensur cultivated in Northern Italy for its possible use as monovarietal source in pasta production.

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Table 1: Significance of main effects of 'variety' and 'nitrogen fertilization method' (late-season foliar vs. soil supply) and their interaction (Newman-Keuls Test) on proportions of HMW-GS, LMW-GS, and gliadins in each protein class.

Protein class	KDa	Variety (*P* level)	N supply method (*P* level)	Interaction (*P* level)
HMW-GS	X-type	0.0000 ***	0.1350 n.s.	0.0120 *
	Y-type	0.0000 ***	0.1350 n.s.	0.0120 *
LMW-GS	42	0.0000 ***	0.0126 **	0.0000 ***
	37	0.0000 ***	0.0000 ***	0.0000 ***
	32	0.0000 ***	0.0000 ***	0.0000 ***
	31	0.0000 ***	0.0007***	0.0000 ***
Gliadins	39	0.0000 ***	0.0027 ***	0.0000 ***
	35	0.0000 ***	0.2267 ns	0.0000 ***
	31	0.0000 ***	0.0010 **	0.0003 ***
	30	0.0000 ***	0.0001***	0.0000 ***
	28	0.0000 ***	0.0000 ***	0.0000 ***

* $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$

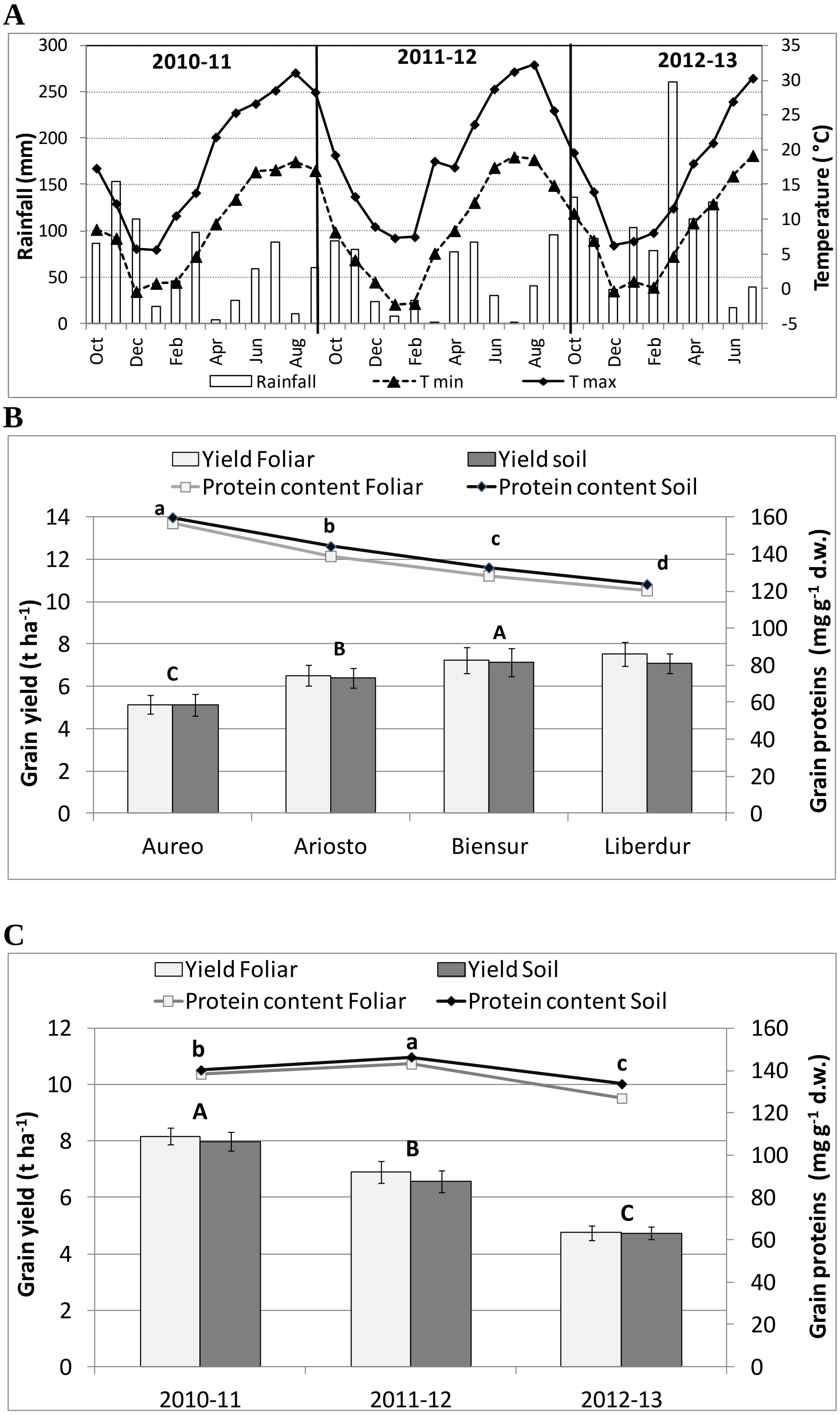
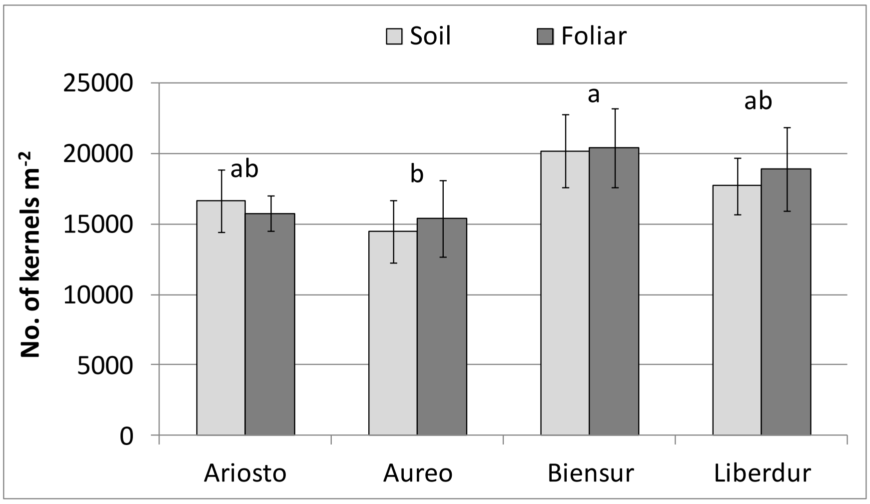


Figure 1. (A) Monthly rainfall and mean temperatures; (B) grain yield (at 13% moisture) and protein content (mg g^{-1} DW) (3-year means ± S.E.; n=9) in four varieties of durum wheat and (C) across three-year trial under late-season foliar vs. soil N fertilization. Lower case letters: differences in grain protein contents among varieties/years. Capital letters: differences in yield among varieties/years (Newman-Keuls test, *P* <0.05).

A



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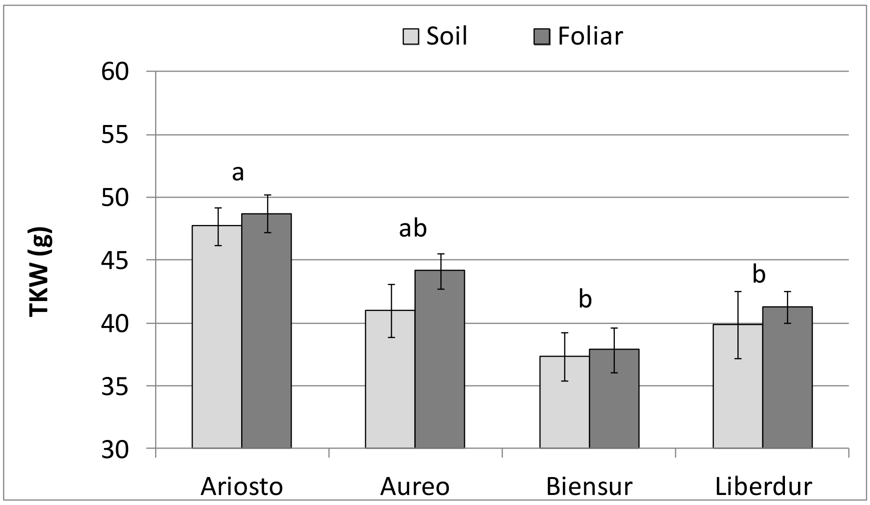

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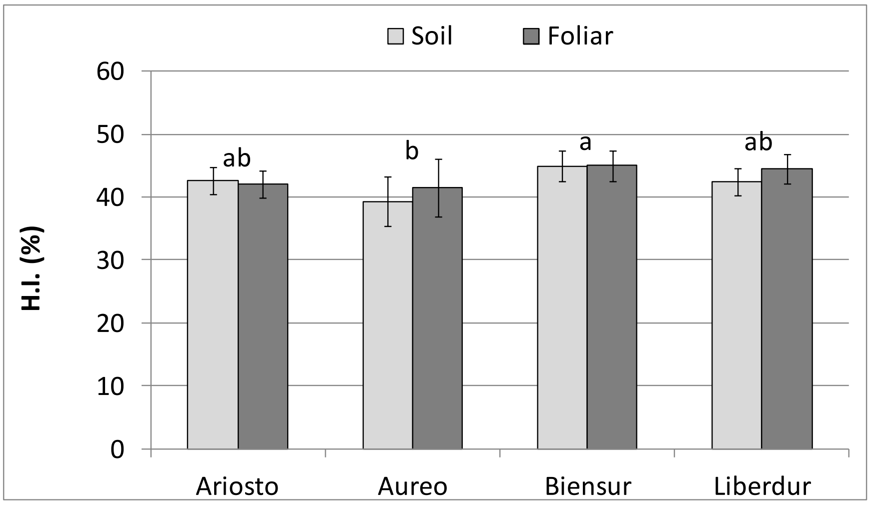


Figure 2. Three-year means (± S.E.; n=9) of yield components, i.e., number of kernels per unit surface area (A) and thousand kernel weight TKW (dry weight) (A) and harvest index H.I (C) in four varieties of durum wheat. Letters: differences among varieties (Newman-Keuls test, $P < 0.05$).

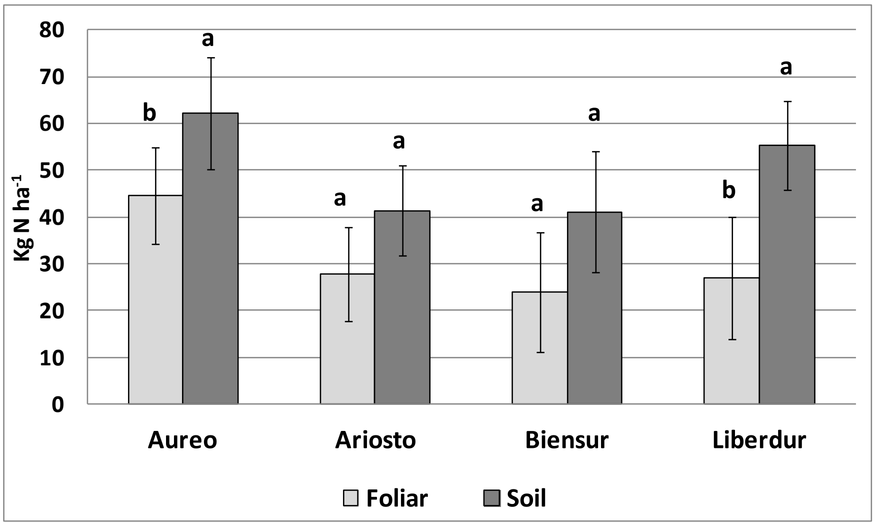


Figure 3. Apparent N balance (3-year average; ± s.e.; n=9) calculated as the difference between total N fertilization (foliar or soil application) and grain N content in four durum wheat varieties. Letters: differences between late-season foliar vs. soil nitrogen fertilization within same variety (Newman-Keuls test, *P* < 0.05). Note that smaller values indicate greater N uptake efficiency.

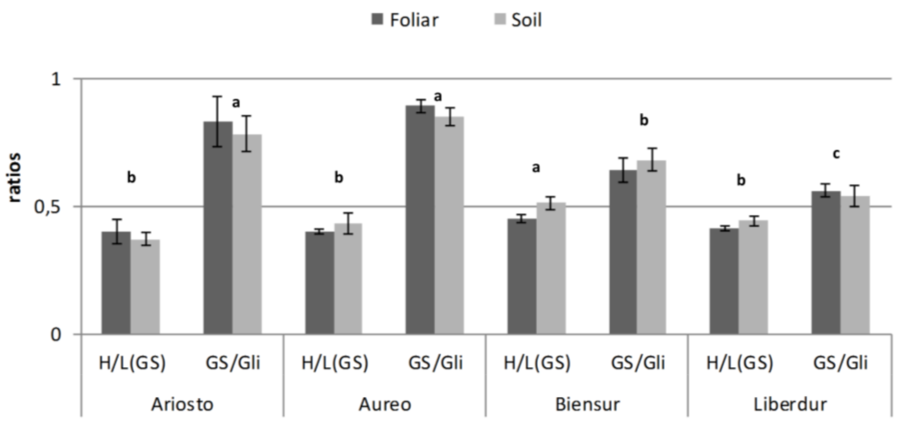
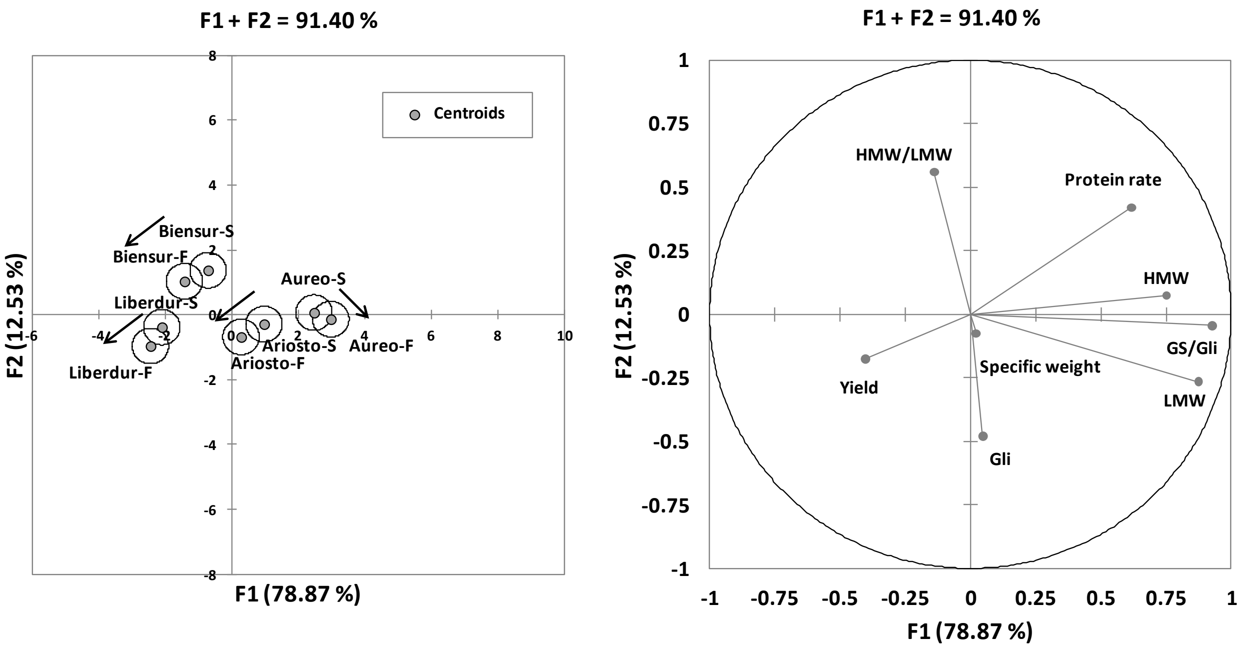


Figure 4. HMW-to-LMW-GS (H/L) and glutenin-to-gliadin (GS/Gli) ratios (3-year average; ± S.E.; n = 9) in four durum wheat varieties under late-season foliar vs. soil N fertilization. Letters: differences among varieties within each parameter (Newman-Keuls test, $P < 0.05$).



Variables	F1	F2
Yield	-0.401	-0.175
Specific weight	0.022	-0.075
Protein rate	0.618	0.420
HMW	0.752	0.075
LMW	0.875	-0.265
HMW/LMW	-0.139	0.561
Gli	0.047	-0.481
GS/Gli	0.929	-0.045

Figure 5: PCA with F1 and F2 loadings (highlighted values > |0.5|) and DA for variety classification considering yield, specific weight of kernels, protein content, gluten proteins [HMW-GS, LMW-GS and gliadins (Gli)], and HMW/LMW-GS and GS/Gli ratios. In DA arrows indicate variations from late foliar (F) to soil (S) nitrogen fertilization within same variety.

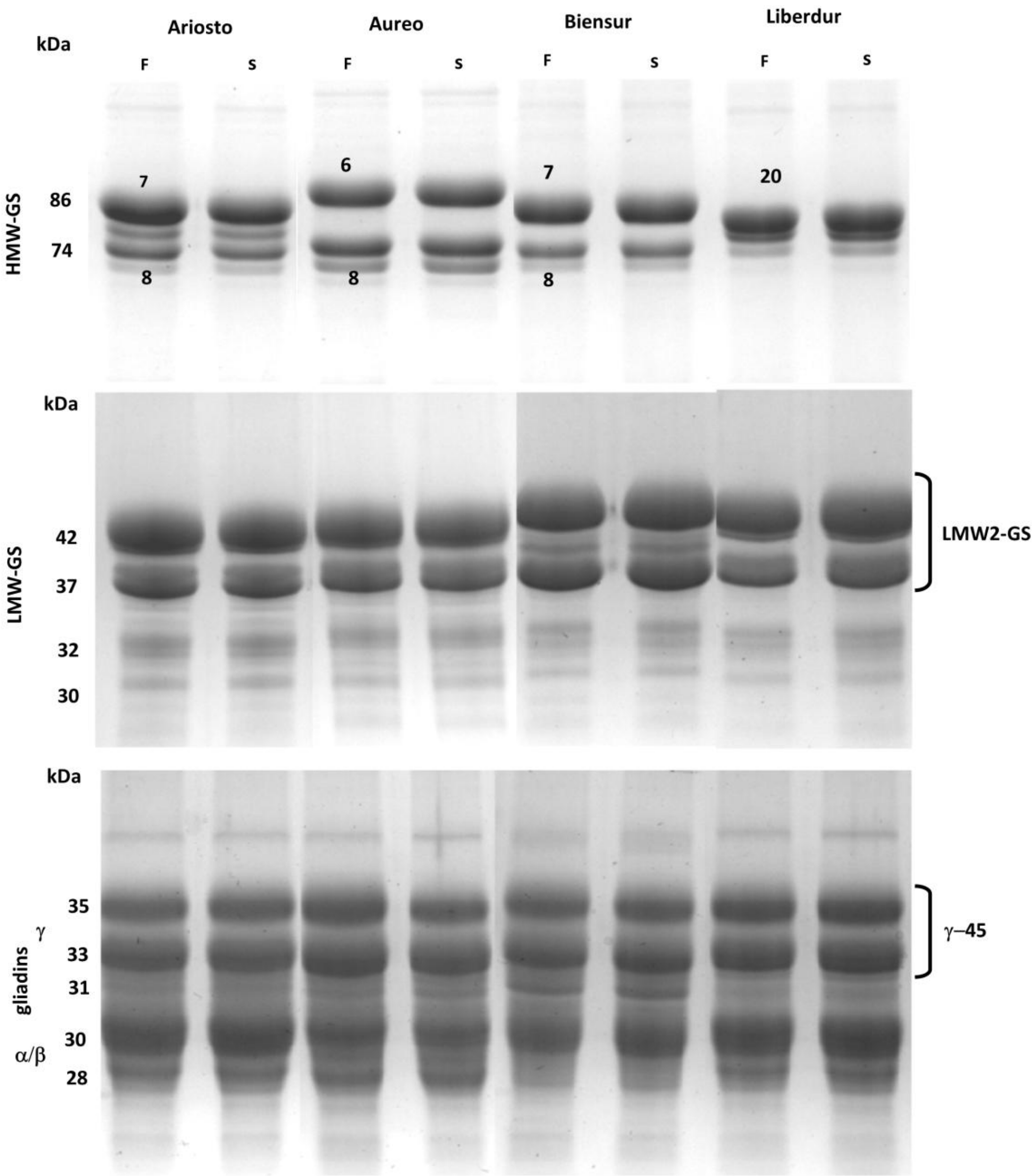


Figure 6. SDS-PAGE of HMW-GS (top), LMW-GS (middle) and gliadins (bottom) with their molecular weights of four durum wheat varieties under late-season foliar (F) vs. soil (S) nitrogen fertilization.

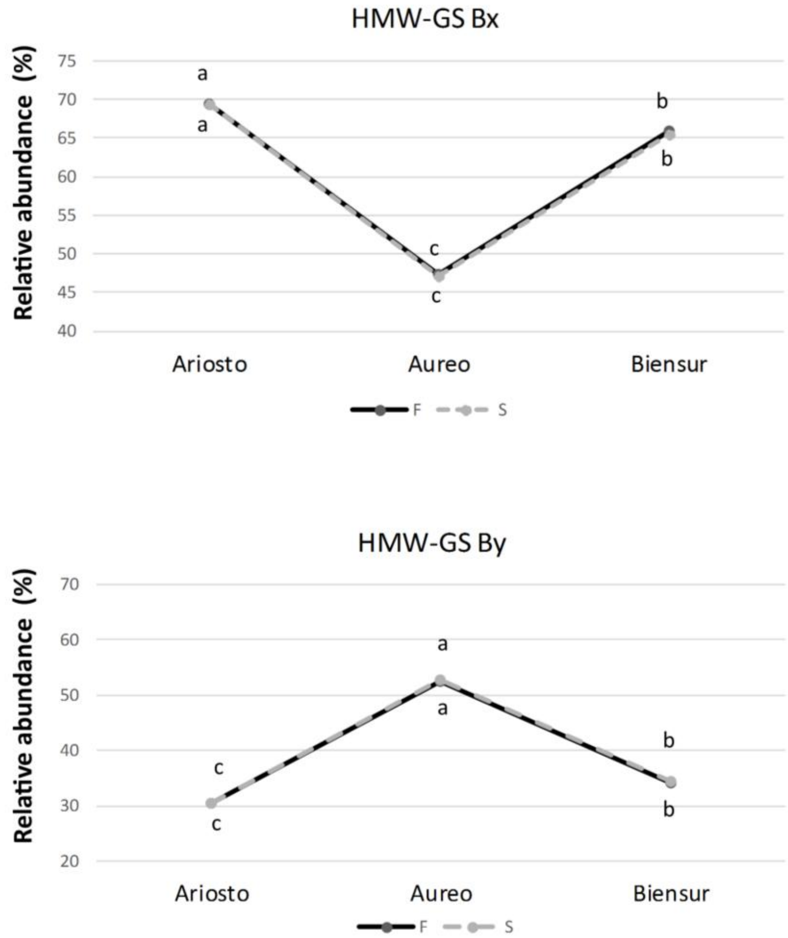


Figure 7. Relative abundances (%) of HMW-GS sub-units in three durum wheat varieties obtained by densitometric analysis. Data are means of three replicates and three years (n = 9). Letters: differences between late-season foliar (F) and soil (S) nitrogen fertilization within same variety (Newman-Keul test, $P < 0.05$). Variety Liberdur is not included as By expression was null.

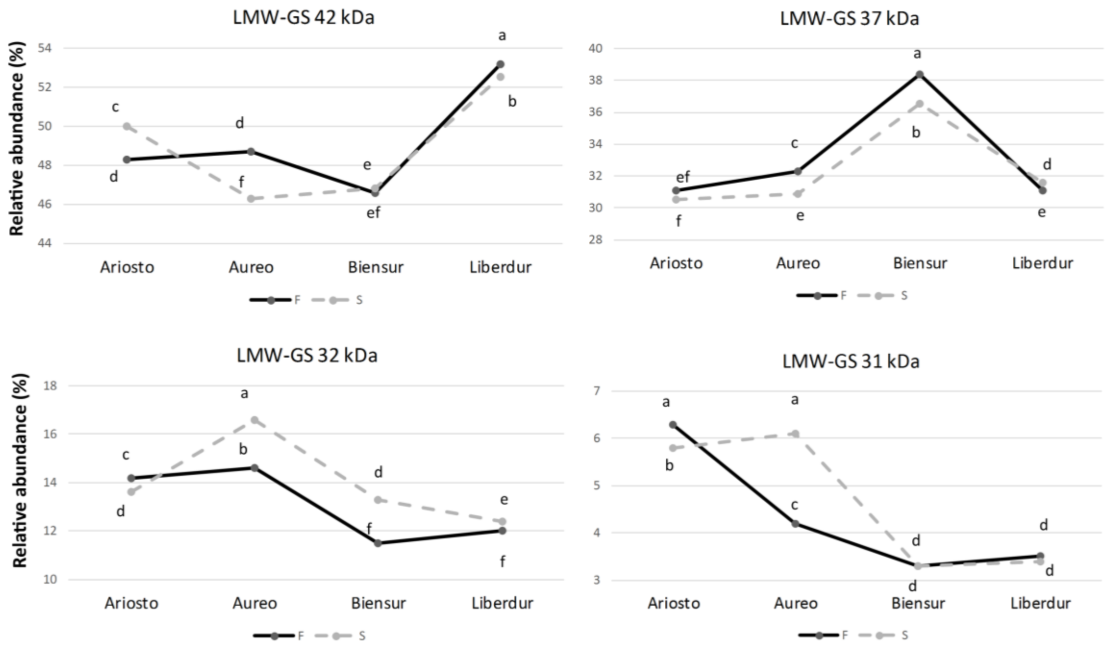


Figure 8. Relative abundances (%) of LMW-GS in four durum wheat varieties obtained by densitometric analysis. Data are means of three replicates and three years (n = 9). Letters: differences between late-season foliar (F) and soil (S) nitrogen fertilization within same variety (Newman-Keul test, $P < 0.05$).

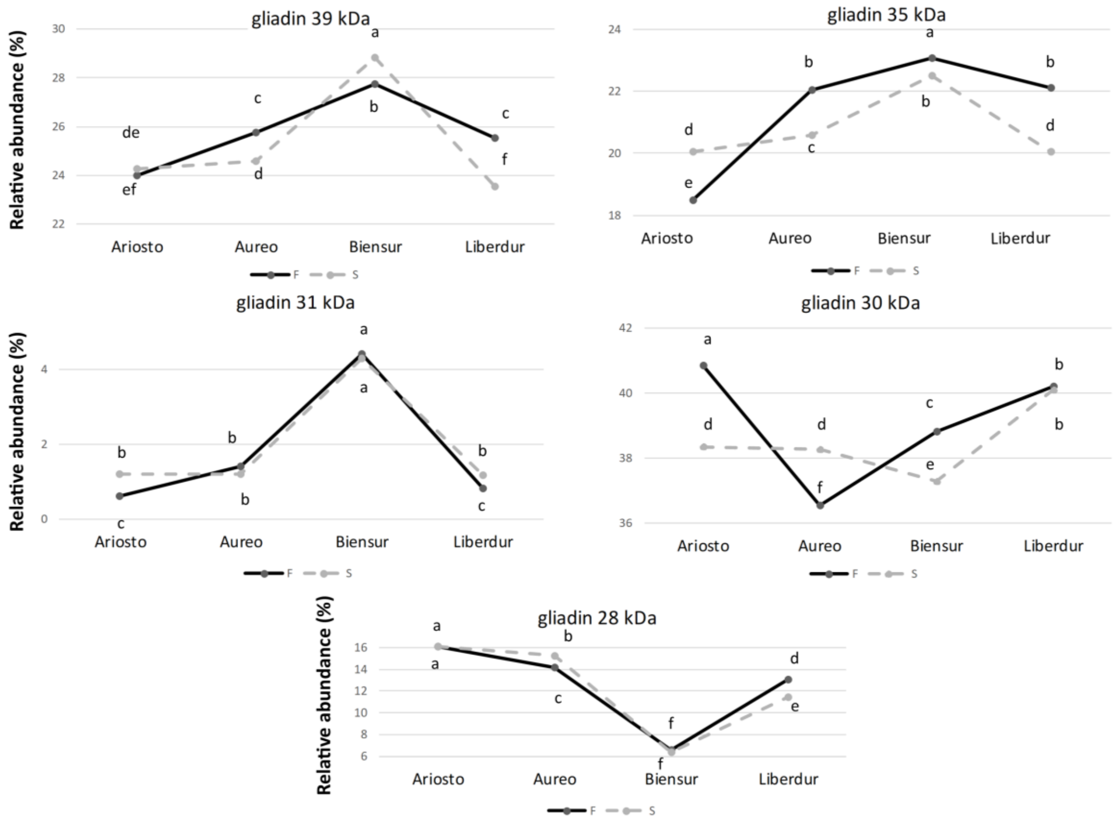


Figure 9. Relative abundances (%) of γ and α–β gliadins in four durum wheat varieties obtained by densitometric analysis. Data are means of three replicates and three years (n = 9). Letters: differences between late-season foliar (F) and soil (S) nitrogen fertilization within same variety (Newman-Keuls test, $P < 0.05$).