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MINERAL COMPOSITION OF DURUM WHEAT GRAIN AND PASTA UNDER INCREASING ATMOSPHERIC CO_2 CONCENTRATIONS

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Running title: Effects of high CO_2 on durum wheat mineral content

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Abstract

The concentrations of 10 minerals were investigated in the grain of 12 durum wheat genotypes grown under free air CO_2 enrichment conditions, and in four of their derived pasta samples, using inductively coupled plasma mass spectrometry. Compared to ambient CO_2 (400 ppm; AMB), under elevated CO_2 (570 ppm; ELE), the micro-element and macro-element contents showed strong and significant decreases in the grain: Mn, -28.3%; Fe, -26.7%; Zn, -21.9%; Mg, -22.7%; Mo, -40.4%; K, -22.4%; and Ca, -19.5%. These variations defined the 12 genotypes as sensitive or non-sensitive to ELE. The pasta samples under AMB and ELE showed decreased mineral contents compared to the grain. Nevertheless, the contributions of the pasta to the recommended daily allowances remained relevant, also for the micro-elements under ELE conditions (range, from 18% of the recommended daily allowance for Zn, to 70% for Mn and Mo).

Key words: Free air carbon dioxide enrichment, mineral content, durum wheat, pasta

Abbreviations

AMB, ambient CO_2 (400 ppm); ELE, elevated CO_2 (570 ppm); FACE, free air CO_2 enrichment; PCA, principal component analysis

1. Introduction

The effects of increasing atmospheric CO_2 levels on the global climate are dramatically evident. As predicted by Easterling et al. (2007), the rise in atmospheric CO_2 is changing the global climate, with severe effects on mean temperatures and with altered patterns of global rainfall. According to the World Meteorological Organisation, the overall mean CO_2 concentration in the atmosphere in 2015 exceeded the symbolic limit of 400 parts per million (ppm) for the first time since measurements began (measured at Mauna Lowa Observatory, Hawaii). This was maintained through 2016, and was accompanied by a mean temperature increase of almost 1 °C (GHG-Bulletin 2016).

To be able to predict the effects of these increasing atmospheric CO_2 concentrations on crop yields and quality, several studies have been conducted under conditions of free air CO_2 enrichment (FACE). Many studies have shown that the grain yield of C3 crops, such as wheat, increases under FACE (DaMatta, Grandis, Arenque, & Buckeridge, 2010; Badeck, et al., 2013). Leakey, Ainsworth, Bernacchi, Rogers, Long, and Ort (2009) reviewed the earlier studies under FACE and calculated that with a CO_2 exposure of about 580 ppm, C3 plants (e.g., crops, trees) would increase their photosynthetic carbon uptake by 19% to 46%, even if their yield gain would be lower than expected. However, severe effects have been reported on the nitrogen and mineral concentrations in grain under these conditions: Fares et al. (2016) reported decreases of 7.0% and 13.3% for grain protein and gluten content, respectively; Myers et al. (2014) reported a similar decrease in grain protein content (6.3%), and also for Zn and Fe, with decreases of 9.3% and 5.1%, respectively. Fernando, Panozzo, Tausz, Norton, Fitzgerald, and Seneweera

(2012) instead reported greater decreases in both grain protein content, at around 12.7%, and grain Fe and Zn contents, at 10% and 22%, respectively. This might be of concern, as changes in grain composition are not only relevant for the nutritional value of cereal-based diets, but they might also affect the quality of the typical products that are obtained from the grain. This is the case in particular for durum wheat, where about 75% of the worldwide production (37 Mt; FAO, 2013) is used by the pasta industry. Similarly for the cous-cous and bread products that are intended for human consumption.

Recently, Fares et al. (2016) carried out FACE studies using CO_2 elevation to 570 ppm (ELE) for durum wheat, and they reported that the grain protein and gluten contents decreased (by 7.0%, 13.3%, respectively). Furthermore, the pasta quality worsened in terms of firmness under ELE (AMB, 406 g; ELE 373 g). These findings agree with other studies on wheat (*Triticum aestivum*). Fernando et al. (2015) established that the high-molecular-weight gluten protein sub-units in wheat grain decreased by up to 50%, and that this alteration in the grain protein quality was associated with lower bread quality. In an earlier study, the same authors reported an 11% decrease in the grain protein levels under ELE, which was associated with a bread volume decrease of 7% (Fernando et al., 2012). Similarly, studies conducted to date have shown that mineral concentrations can also be lower under ELE. Here, in C3 plants, Loladze (2014) showed that the overall mineral concentrations decreased by 8% (as the mean for 25 minerals), while Myers et al. (2014) reported mean decreases for Zn and Fe of 9.3% and 5.1%, respectively.

Accordingly, concerns have arisen for human nutrition, because dietary deficiencies of the basic micro-elements (e.g., Fe, Zn) represent a global public health problem (Myers et al., 2014). Above all, this indicates added risk for underdeveloped regions in countries such as India or

Africa, where wheat and pulses are the main dietary sources for a large part of the rural population. Under such conditions, the risk of not reaching the minimum daily requirements for protein, Fe and Zn intake might have severe effects on public health (Myers et al., 2014). Furthermore, owing to an increase in people who prefer a diet based only on vegetables in the developed countries, this concern might also become a serious problem for public health. Thus, global declarations about "hidden hunger" and obesity are under widespread discussion in the current literature (Loladze, 2014; Myers et al., 2014), and are destined to become more of a global challenge.

The present study completes the description of the potential effects on the grain and pasta quality of durum wheat varieties caused by rising concentrations of CO_2 in the atmosphere. The durum wheat was thus grown under ELE conditions according to our previously published details (Fares et al., 2016). To the best of our knowledge, there have not been any studies of the effects of FACE on micro-element and macro-element levels in grain and pasta across a large number of durum wheat varieties, as in our studies here. As pasta represents a cheap food that is consumed worldwide, any changes in its nutritional profile need to be carefully evaluated to estimate the consequent variations in dietary mineral intake. To do this, we evaluated the effects of pasta processing on durum wheat grown under ELE, and evaluated the contributions to the recommended daily allowance (RDA) of the pasta samples, for each mineral element.

2. Materials and Methods

2.1. Field materials

Ten durum wheat *(Triticum durum)* varieties with contrasting quality characteristics were selected for this study: Simeto, Ciccio, Claudio, Anco Marzio and Saragolla, as modern high-yielding varieties; Svevo and Aureo, as high-protein content varieties; and Cappelli, Creso and Ofanto, as varieties with a relevant role in the history of Italian durum wheat breeding. In addition, two lines selected from an Ofanto × Cappelli recombinant inbred line population were included (RIL11, RIL28). The ELE conditions were installed on the experimental farm of the Genomics Research Centre of the *Consiglio per la Ricerca in Agricoltura e l'Analisi dell'Economia Agraria* (CREA) in Fiorenzuola d'Arda (44.927°N, 9.893°E). The soil was classified as silt-clay-loam (15% sand, 51% silt, 34% clay). Total nitrogen content was about 0.9%, organic matter was 1.8%, and K_2O and P_2O_5 were 350 ppm and 12 ppm, respectively. The experimental units were plots of 2.2 m × 1.36 m. The ELE treatment (of 570 ppm CO_2) was applied to four octagons inscribed in circles of 14-m diameter. Each of these four ELE systems, and the four controls at ambient CO_2 (AMB 400 ppm), contained two replicates of the 12 genotypes. The ELE treatment was started on 16 November, 2011, and stopped when the leaves were senescent, on 14 June, 2012. The plots were fertilised with application of a N:P:K fertiliser at pre-seeding (45 kg for each N:P:K) and two top dressings with ammonium nitrate, of 52 kg N each. Treatments with herbicides and fungicides were applied according to local standard practices. The final harvest was on 2 July, 2012 (Fares et al., 2016).

2.2. Grain milling and pasta processing

The full description of the grain milling and pasta processing were published by Fares et al. (2016). In the present study, the grain of all of the genotypes previously described were analysed, while for the pasta samples, four were analysed accordingly: Saragolla and Simeto, as two varieties that were chosen from among those that did not shown significant differences in mineral compositions across these two environments; and Cappelli and Ciccio, which instead significantly differed for the same minerals. Moreover, we evaluated the mineral content of 100 g of pasta serving derived from the grain produced under AMB and ELE, while noting the reference values for each mineral (i.e., RDA) (EEC, 2008).

2.3. Determination of macro-elements and micro-elements

For the determination of the contents of the mineral macro-elements Na, K, P, Ca and Mg and micro-elements Mn, Fe, Cu, Zn and Mo of both the grain and the pasta, the dried samples were milled (Pulverisette 7; Planetary Micro Mill, Classic Line, Fritsch) using an agate jar and balls. Then 20 mg of each sample was used for the analysis (as four replicates for the grain samples, and three replicates for the pasta samples).

The macro-elements and micro-elements were determined on samples digested and diluted to 50 mL with high purity deionised water, in polypropylene disposable tubes, as reported by Ficco et al. (2009). Then, their content was analysed using inductively coupled plasma mass spectrometry (Agilent 7700x; Agilent Technologies, Italy), equipped with an auto-sampler (ASX-500), as described by Hansen, Laursen, Persson, Pedas, Husted, and Schjoerring, (2009), with minor modifications. The inductively coupled plasma mass spectrometry was tuned to standard mode and with collision gas (He) to remove many of the simple solvents and the argon-based polyatomic spectral interference. The plasma power was operated at 1550 ±50 W, and the carrier and make-up gases were typically set at 0.83 L min^{-1} and 0.17 L min^{-1}, respectively. Sample uptake was maintained at approximately 0.1 mL min^{-1} using a self-aspirating nebuliser. A reference material was included randomly in the analytical batches, from digestion onwards (RM 1567b; from the National Institute of Standards and Technology). The data were processed using the MassHunter WorkStation software (Agilent Technologies, Italy).

2.4. Statistical analysis

Analysis of variance (ANOVA) was performed on the macro-element and micro-element contents to separate the effects due to genotype, environment, and their interaction. The significant differences among the means were defined according to Tukey's multiple range tests ($P \leq 0.05$). To obtain general and comprehensive characterisation of the samples, the data for the elements detected were subjected to principal component analysis (PCA), based on their correlations. All of the statistical analysis was performed using JMP version 8.0 (SAS Institute Inc., Cary, NC, USA).

3. Results and discussion

3.1. Grain mineral content

In our previous study (Fares et al., 2016), we highlighted that the positive influence of ELE on grain yield was counterbalanced by lower grain quality, which when processed as pasta, was of inferior cooking quality. Here, we investigated the effects of ELE on the mineral contents of durum wheat grain, in comparison to AMB, and then investigated the variations in the pasta derived from this ELE environment.

ELE significantly impacted on almost all of the macro-elements and micro-minerals recorded for the grain (Table 1). Conversely, significant effects were only seen for the genotypes for Fe and Mn, with higher and lower levels for Svevo and Anco Marzio, respectively (Supplementary Figure 1). Selective changes in the grain mineral concentrations in response to ELE were recorded (Table 2). Cu showed a positive but non-significant increase (+8.1%), while almost all of the micro-elements showed strong and significant decreases, as: Mn, -28.3% ($p < 0.0001$); Fe, -26.7% ($p < 0.0001$); Zn, -21.9% ($p = 0.0004$); and Mo, -40.4% ($p = 0.0469$). For the macro-elements, only Na increased significantly ($p < 0.0001$) under ELE (as a doubling), while significant decreases were seen for Mg (-22.7%; $p < 0.0001$), K (-22.4%; $p < 0.0001$) and Ca (-19.5%; $p = 0.0183$). For P, the decrease was not significant (-4.7%). Högy et al. (2013) reported significant decreases only for Ca, Mg, Fe, Co and B in wheat grown under FACE conditions. In a previous study, Högy et al. (2009) reported no significant effects of CO_2 on grain mineral composition of a single wheat variety (Triso), while Erbs, Manderscheid, Jansen, Seddig, Pacholski, and Weigel (2010) reported significant decrease of only the S levels (-5%).

The means and the variations in the mineral contents of the 12 genotypes tested (Table 2) were then determined without considering Na (which showed the opposite behaviour with respect to the other minerals). This defined a group of seven genotypes (six varieties, one line), as

Claudio, Creso, Ofanto, Saragolla, Simeto, Svevo and RIL 28, that did not show any significant variations for any of the minerals examined (i.e., for both micro-elements and macro-elements). The remaining five genotypes, showed significant variations for some elements, which identified this group as potentially sensitive to the effects of the ELE environment. In particular, for Aureo, significant decreases were seen for Mg ($p = 0.0140$) and Zn ($p = 0.0019$), and for Anco Marzio, for Fe ($p = 0.008$) and Mo ($p = 0.0386$). For Cappelli and Ciccio, there were significant decreases for two minerals, Mg and Mn (slightly higher for Cappelli; Table 2), and for RIL 11, significant decreases were seen for K ($p = 0.0485$), Ca ($p = 0.0375$) and Mn ($p = 0.0240$). According to Broberg, Högy, and Pleijel, (2017), the present data show that the dilution hypothesis cannot fully explain these decreases in minerals, particularly with a significant mean increase in yield of 16.7% under ELE (Badeck et al. 2013). Thus, the significant genotypic variability observed in the yield, with increases that ranged from 4.4% (Ciccio) to 20.4% (RIL28), together with the decreases in the mineral contents, demonstrated that other mechanisms are involved. If the dilution hypothesis was the main process that acted to reduce these minerals, the highest yield that was seen for RIL28 should also have been accompanied by the greatest mineral loss under ELE; however, RIL28 was grouped among the non-sensitive genotypes. Similarly, as Ciccio showed the lowest yield, it should not have shown any decreases in the minerals, while it was instead grouped among the sensitive genotypes. Therefore, the ELE condition might indirectly affect the mechanisms of translocation of these minerals as a consequence of different genetic patterns.

As there is no information available relating directly to durum wheat varieties grown under ELE, here we focus the discussion on data reported for common wheat. Fernando et al. (2012) studied the effects of ELE conditions on normal and late seeding dates in the Australian common wheat cultivar Yitpi. They reported that the minerals Zn, Ca, Fe and S significantly decreased under ELE (i.e., about -22%, -15%, -10% and -5%, respectively). In the present study, Zn (-21.9%), Ca (-19.5%) and Fe (-26.7%) showed more severe decreases under ELE. Högy and Fangmaier (2008) reviewed a large dataset on wheat for a range of CO_2 exposures and reported that the decreases in macro-elements due to their FACE conditions, which were seen for Na, Ca, Mg and S, were consistent across the different cultivars examined. For the micro-elements Fe, Zn and Mn, they reported again that the predominant trend was towards decreases.

To define further these low effects of the Genotype × Environment interactions, the dataset for the grain in the present study was subjected to PCA. Figure 1 shows the comparison of the diversity of the samples (Fig. 1A, PCA scores plot), and also the identification of the minerals responsible for the separation (Fig. 1B, PCA loadings plot), with PC1 and PC2 accounting for 50.9% and 19.1% of the variation, respectively. The genotypes positioned on the right-hand side of the PCA scores plot were those grown under normal conditions (AMB), and these showed higher levels of the minerals, with the exception of Na, which was negatively correlated to PC1. On the contrary, the genotypes positioned on the left-hand side were grown under the elevated CO_2 conditions (ELE), and these showed decreased levels of Mg, Ca, K, Mn, Fe and Zn, with a higher level of Na. PC2 showed the separation of the samples in the vertical direction, and the genotypes positioned in the lower half of the PCA scores plot (Fig. 1A), such as Claudio and Creso, contained higher and lower Mo and Cu, respectively. Considering that the mineral content of grain reflects the soil composition and the weather conditions (Maathuis & Diatloff, 2013), these findings provide an example of genetic diversity for the mineral contents in response to ELE. Nevertheless, because the Genotype × Environment (i.e. AMB and ELE) interactions observed here were mainly due to the environment systems, it would be of interest to use a wider panel of different durum wheat genotypes, or to explore wild and exotic germplasm. This biodiversity within a given species, as is initially defined here for durum wheat, can be used to investigate how the regulatory mechanisms of individual elements might interact, and to identify the genes that are important for these processes (Baxter et al., 2012). Watanabe et al. (2007) reported that over 25% of variance can be assigned as higher-level phylogenetic effects (i.e., at the family level) for 21 elements during metabolism, which included those with lesser quantitative roles than for N, and also for elements that might not be essential for plant growth.

3.2. Pasta mineral content

Four pasta samples that were prepared from single cultivars from the 12 durum wheat varieties cultivated under ELE were also analysed (Table 1). The interaction was significant ($p < 0.0001$) for all of the macro-elements except Ca, while it was significant only for Mn and Fe among the micro-elements. Significant effects were ascribed to genotype for almost all of the minerals, except for Ca, Cu and Mo (Table 1). Overall, ELE had a significant impact on two macro-elements (P decrease, $p < 0.0001$; K increase, $p = 0.0094$) and four micro-elements (Mn increase, $p = 0.0004$; Cu, Zn, Mo decreases, $p = 0.0061$, $p = 0.0369$, $p = 0.0469$, respectively); Fe also showed an increase, but this did not reach significance (Table 3).

This behaviour can most likely be ascribed to different and selective patterns of mineral distributions across the grain of the four varieties (Fares,Troccoli, & Di Fonzo, 1996; Singh, Vogel-Mikuš, Vavpetič, Jeromel, Kumar, & Tuli, 2014), which might be altered under ELE. Indeed, Mn was significantly increased under ELE only in the Cappelli and Simeto pasta, while Fe was also increased in Saragolla, although this did not reach statistical significance. It is therefore conceivable that in these varieties the pattern for the mineral distributions for Mn, and Fe (which can only be evaluated after milling) under ELE is similar and selectively concentrated towards the inner layers of the endosperm. On the contrary, Ciccio showed a reduction in all of the minerals; for the other varieties, the behaviours were less homogeneous. With Cappelli, ELE resulted in significant increases for Mg, K and Mn ($p = 0.0121$, $p = 0.0009$, $p = 0.0012$, respectively), while P and Cu were significantly decreased ($p = 0.0009$, $p = 0.0485$, respectively). In Saragolla, ELE decreased Mg, P, Cu and Zn ($p = 0.0056$, $p = 0.0002$, $p = 0.0078$, p = ns, respectively), while for Simeto there were significant decreases for Mg, P and Zn ($p = 0.0026$, $p = 0.0059$, $p = 0.0086$, respectively).

Figure 2 summarises the variations in the mineral contents under AMB and ELE in going from the grain to the pasta. As is known, during durum grain milling, the bran and the germ from the endosperm are separated, and the semolina is obtained from the successive step of grinding, sieving and re-milling. This finally provides the product that is used for the pasta processing. During milling, the mineral contents of the grain decrease, as they are concentrated in the outer layers of the kernels that are removed during the milling (Hemery, Rouau, Lullien-Pellerin, Barron, & Abecassis, 2007; Fares et al., 1996). Therefore, a decrease in the mineral contents is expected for the pasta samples under both of these environments (i.e., AMB, ELE) as a consequence of the milling process.

Comparing the two environments, a general reduction in the significant variations was observed under ELE, compared to AMB (Figure 2). For ELE, the pasta of Cappelli showed significantly decreased Na, P, Mn and Cu. For Ciccio, significant decreases were seen only for Na, P and Cu. Saragolla showed significant decreases in Na, P and Mn, and Simeto in Na and Cu. For all four of these varieties studied, the Fe and Zn contents of the pasta from the grain grown under ELE were not affected, even if the grain showed significant decreases from AMB to ELE (Table 2). As under ELE there were lower levels of all of the minerals compared to AMB, it is likely that the depletion of the levels of these minerals due to the milling loss became less evident and dramatic in the ELE pasta samples. On the contrary, for the grain grown under AMB, there were significant decreases in the levels of almost all of the minerals in the pasta samples, except for Ca. For the micro-elements (Fig. 2), Fe decreased significantly in Cappelli ($p < 0.001$) and Saragolla ($p < 0.001$), and for Zn, a significant decrease was seen in Ciccio ($p < 0.05$). Significant and severe decreases in Mn ($p < 0.001$) were seen for all of the varieties (i.e., >50%), while Cu decreased significantly in Cappelli ($p < 0.001$), Saragolla ($p < 0.001$) and Simeto ($p < 0.05$), and Mo only showed a significant decrease in Simeto ($p < 0.05$).

The present study thus demonstrates that also in durum wheat the mineral levels (as both macro-elements and micro-elements) are decreased under ELE conditions in a similar way to that seen for common wheat (Loladze, 2014; Myers et al., 2014). As a consequence, some concerns can be raised for human nutrition, because dietary deficiencies of the basic micro-elements (e.g., Fe, Zn) represent a global public health problem (Myers et al., 2014). For the AMB grain, the levels of each mineral in one pasta serving of 100 g were seen to generally be very close to or greater than the 15% recommended, as the threshold that represents significant daily intake as recommended in the Annex of EEC

(2008) (Table 4). Surprisingly, for the pasta from the ELE grain, some of the minerals still abundantly exceeded these recommendations. In particular, for Fe, the levels in the ELE pasta represented 44.5% of the RDA, and for Zn, this was 18.7%. For Mn and Mo, the levels in the ELE pasta reached 73% and 60% of the RDA, respectively, while for Cu and P, these were 23% and 17.4%, respectively. Mg and K each accounted for about 13.5%, while the lowest levels were seen for Ca, at 3.4%, which confirms that pasta does not represent an adequate source of this macro-element.

4. Conclusions

This study has highlighted the overall depletion of micro-elements and macro-elements in durum wheat grown under ELE, compared to AMB, and that the variations in grain mineral concentrations of the 12 genotypes do not follow any unique behaviour. Significant differences among the genotypes tested allowed the identification of sensitive and non-sensitive cultivars in response to ELE.

After pasta processing, lower mineral levels were expected as a consequence of the milling process, so for the grain under both AMB and ELE. However ELE pasta showed increases for Fe and Mn, which were probably due to altered distribution patterns for minerals in seeds under ELE conditions. In addition, for the two micro-elements for which there is a risk of dietary deficiency (i.e., Fe, Zn), their levels in the pasta from ELE more than covered the basic levels of RDA.

Further efforts to study and optimise the remobilisation of the micro-elements and macro-elements from vegetative structures might provide a target for durum wheat breeding, so as to retain the current grain quality standard also under future ELE scenarios.

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The authors declare that they have no conflicts of interest.

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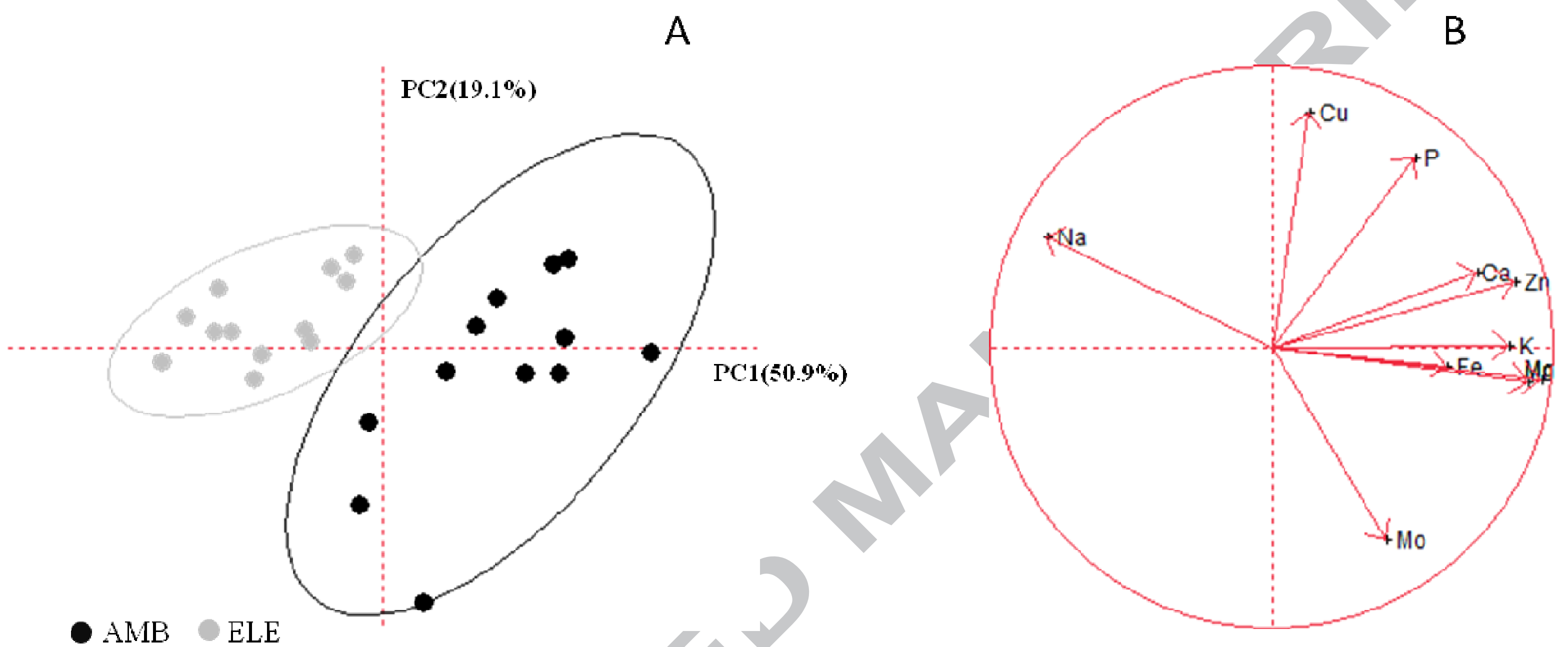
Figure 1. PCA analysis score plot (A) and loadings plot (B).

Figure 2. Variations in the mineral contents of the grain and pasta samples under AMB (left; black bars) and ELE (right; grey bars).

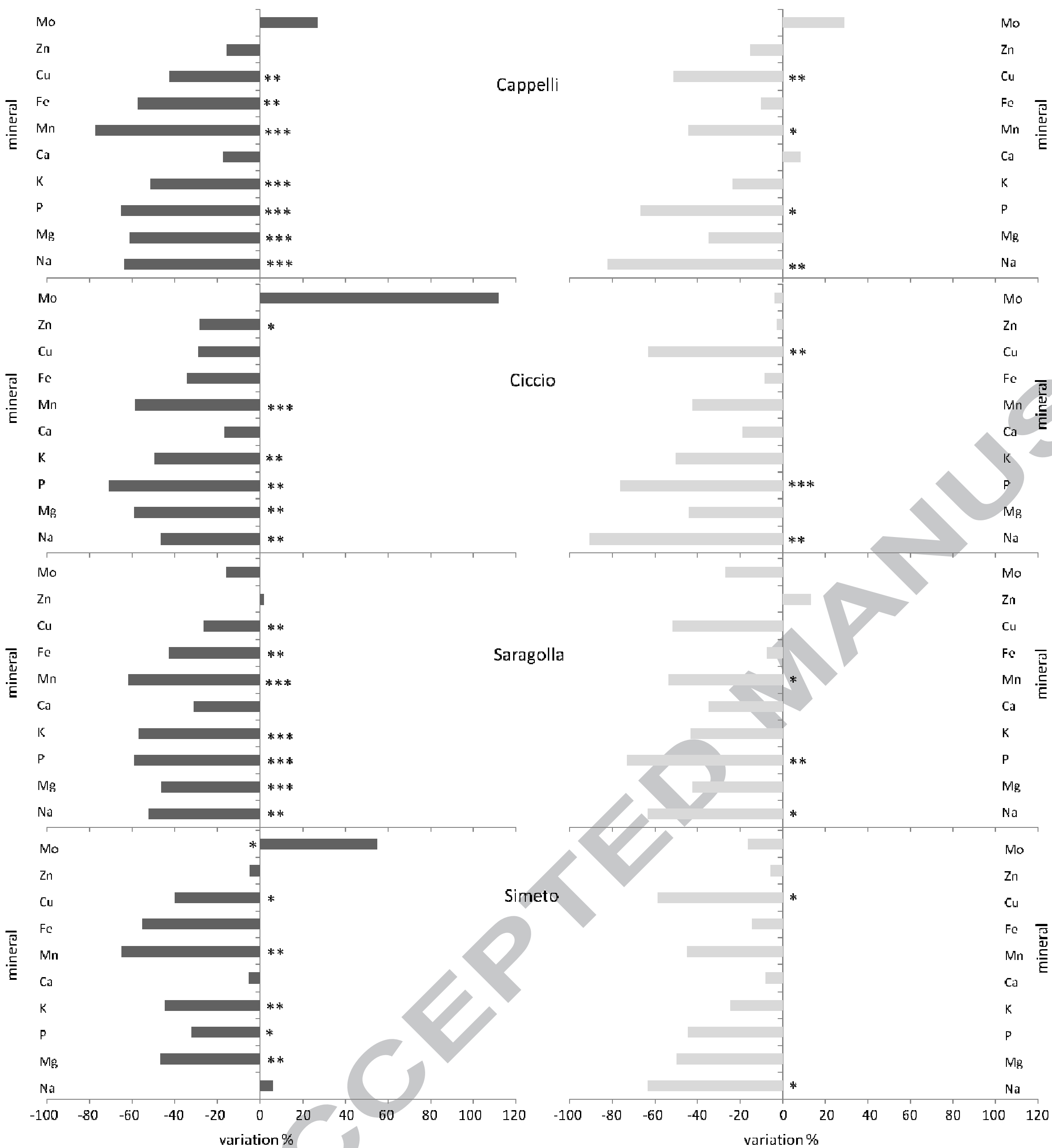
Supplementary Figure 1. Mean grain contents under ELE for Mn (A) and Fe (B) for each genotype. Different letters indicate statistical differences ($p < 0.05$; LSD tests).

Figure 1.



314 **Figure 2**

315



Cappelli
Ciccio
Saragolla
Simeto
mineral
Mo
Zn
Cu
Fe
Mn
Ca
K
P
Mg
Na
variation %
-100 -80 -60 -40 -20 0 20 40 60 80 100 120

316

Table 1. Analysis of variance of the mineral content in the matching grain and pasta samples.

Preparation	**Interaction**	**Significance**									
		Na	**Mg**	**P**	**K**	**Ca**	**Mn**	**Fe**	**Cu**	**Zn**	**Mo**
Grain	E	<0.0001	<0.0001	n.s	<0.0001	0.0183	<0.0001	<0.0001	n.s	0.0004	0.0339
	G	n.s.	n.s.	n.s.	n.s.	n.s.	0.0055	0.0002	n.s.	n.s.	n.s.
	G × E	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.02	n.s.	n.s.	n.s.
Pasta	E	n.s.	n.s.	<0.0001	0.0094	n.s.	0.0004	n.s.	0.0061	0.0369	0.0469
	G	0.0007	0.0162	<0.0001	<0.0001	n.s.	0.0008	<0.0001	n.s.	0.0049	n.s.
	G × E	0.046	<0.0001	<0.0001	0.0041	n.s.	0.0007	0.0046	n.s.	n.s.	n.s.

E,environment (ambient [AMB] or elevated CO_2 [ELE]);G, durum wheat genotype; n.s., not significant

Table 2. Mean mineral contents and variations of the grain samples of each genotype grown under AMB and ELE conditions.

Genotype	Condition/ factor	Grain mean mineral contents (mg/kg)									
		Na	**Mg**	**P**	**K**	**Ca**	**Mn**	**Fe**	**Cu**	**Zn**	**Mo**
Total Samples	AMB	45.0 ± 0.8b	1077.5 ± 17.0a	3414.1 ± 77.0	5280.5 ± 91.6a	410.8 ± 30.1a	38.8 ± 1.1a	88.2 ± 5.9a	4.9 ± 0.1	23.0 ± 0.6a	0.5 ± 0.1a
	ELE	94.6 ± 4.4a	832.9 ± 37.4b	3253.4 ± 114.8	4096.7 ± 185.3b	330.8 ± 16.3b	27.9 ± 1.5b	64.7 ± 3.1b	5.3 ± 0.2	18.0 ± 1.2b	0.3 ± 0.04b
	variation %	**110.2**	**-22.7**	**-4.7**	**-22.4**	**-19.5**	**-28.3**	**-26.7**	**8.1**	**-21.9**	**-40.4**
	p	<0.0001	<0.0001	n.s.	<0.0001	0.0183	<0.0001	<0.0001	n.s.	0.0004	0.0469
A. Marzio	AMB	36.8 ± 2.0	1001.6 ± 17.3	3355.1 ± 129.2	4893.8 ± 136.3	287.5 ± 35.0	27.2 ± 1.1	70.5 ± 4.4a	4.6 ± 0.2	16.9 ± 0.1	0.6 ± 0.1a
	ELE	79.7 ± 22.6	742.9 ± 138.5	3258.7 ± 365.4	3582.4 ± 599.8	251.5 ± 32.5	20.0 ± 4.7	44.2 ± 5.1b	4.6 ± 0.4	14.1 ± 3.1	0.2 ± 0.1b
	variation %	**116.6**	**-25.8**	**-2.9**	**-26.8**	**-12.5**	**-26.5**	**-37.3**	**0.8**	**-16.4**	**-56.4**
	p	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.0080	n.s.	n.s.	0.0386
Aureo	AMB	47.4 ± 3.2b	1161.6 ± 47.7a	3487.1 ± 275.9	5050.8 ± 260.0	545.3 ± 124.3	41.8 ± 3.3	70.8 ± 1.2	4.8 ± 0.2	25.7 ± 10.9a	0.7 ± 0.3
	ELE	103.4 ± 15.9a	793.7 ± 96.1b	3273.1 ± 435.2	3731.4 ± 531.0	388.0 ± 55.4	31.3 ± 6.4	63.3 ± 4.4	4.7 ± 0.4	14.2 ± 2.0b	0.2 ± 0.1
	variation %	**118.0**	**-31.7**	**-6.1**	**-26.1**	**-28.8**	**-25.1**	**-10.7**	**-1.7**	**-44.7**	**-70.2**
	p	0.0137	0.0140	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.0019	n.s.
Cappelli	AMB	43.3 ± 1.9b	1253.0 ± 48.1a	3822.2 ± 201.0	4853.7 ± 152.6	324.4 ± 59.8	48.0 ± 1.5a	88.3 ± 6.6	4.8 ± 0.2	25.7 ± 1.6	0.4 ± 0.1
	ELE	100.1 ± 16.4a	778.6 ± 102.3b	3199.8 ± 501.9	3350.1 ± 614.7	245.6 ± 13.6	25.0 ± 2.6b	67.2 ± 8.3	4.8 ± 0.3	21.6 ± 5.3	0.2 ± 0.1
	variation %	**131.0**	**-37.9**	**-16.3**	**-31.0**	**-24.3**	**-47.8**	**-23.9**	**-0.3**	**-15.8**	**-46.3**
	p	0.0137	0.0057	n.s.	n.s.	n.s.	0.0003	n.s.	n.s.	n.s.	n.s.
Ciccio	AMB	45.5 ± 2.1b	1122.1 ± 55.2a	3178.3 ± 255.2	5329.9 ± 219.7	453.4 ± 72.0	35.5 ±1.3a	156.0 ± 42.5	4.8 ± 0.5	22.7 ± 1.6	0.3 ± 0.02
	ELE	107.0 ± 18.8a	771.5 ± 127.8b	3450.1 ± 46.8	3831.5 ± 656.9	287.7 ± 38.8	20.2 ± 3.6b	60.9 ± 9.6	4.8 ± 0.5	14.3 ± 3.2	0.3 ± 0.1
	variation %	**134.9**	**-31.2**	**8.6**	**-28.1**	**-36.6**	**-43.2**	**-61.0**	**0.4**	**-37.1**	**8.7**

	p	0.0174	0.0454	n.s.	n.s.	n.s.	0.0068	n.s.	n.s.	n.s.	n.s.
Claudio	AMB	44.0 ± 3.2	1005.7 ± 46.4	2726.1 ± 276.1	4673.2 ± 169.3	263.2 ± 16.7	41.2 ± 1.6	84.1 ± 12.5	4.0 ± 0.4	20.1 ± 0.4	1.3 ± 1.0
	ELE	76.9 ± 15.5	890.6 ± 144.9	3147.8 ± 434.4	4238.2 ± 735.8	330.8 ± 60.7	35.4 ± 6.9	47.5 ± 16.4	5.4 ± 1.1	18.9 ± 4.7	0.4 ± 0.1
	variation %	**74.9**	**-11.5**	**15.5**	**-9.3**	**25.7**	**-14.1**	**-43.6**	**34.8**	**-5.9**	**-72.8**
	p	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Creso	AMB	46 ± 2.7b	983.5 ± 14.4	2878.0 ± 213.7	4833.3 ± 150.4	322.2 ± 23.1	33.6 ± 1.0	72.5 ± 8.2	3.5 ± 0.03	18.6 ± 0.8	0.5 ± 0.2
	ELE	90.2 ± 13.7a	904.15 ± 159.6	3233.4 ± 472.7	4423.2 ± 723.6	322.5 ± 67.5	28.7 ± 4.6	63.3 ± 6.3	5.1 ± 0.9	20.4 ± 4.1	0.3 ± 0.1
	variation %	**96.1**	**-8.1**	**12.3**	**-8.5**	**0.1**	**-14.7**	**-12.7**	**48.3**	**9.8**	**-31.6**
	p	0.0193	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Ofanto	AMB	43.3 ± 1.6b	1038.2 ± 24.3	3810.6 ± 229.9	6325.7 ± 385.2	403.5 ± 87.2	39.6 ± 1.7	69.9 ± 1.9	5.8 ± 0.3	26.3 ± 1.6	0.4 ± 0.1
	ELE	105.7 ± 15.9a	825.9 ± 143.4	3274.9 ± 432.4	4796.3 ± 793.5	406.4 ± 66.9	30.3 ± 5.2	72.1 ± 10.7	5.8 ± 0.9	21.8 ± 5.0	0.3 ± 0.1
	variation %	**143.9**	**-20.4**	**-14.1**	**-24.2**	**0.7**	**-23.4**	**3.2**	**1.2**	**-17.3**	**-17.4**
	p	0.0080	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
RIL 11	AMB	46.0 ± 2.8b	1104.2 ± 63.8	3754.6 ± 205.6	5455.1 ± 268.5a	452.6± 42.6a	43.7 ± 2.5a	73.7 ± 4.6	5.9 ± 0.2	27.7 ± 2.6	0.4 ± 0.06
	ELE	100.1 ± 16.7a	766.6 ± 149.6	3367.2 ± 588.3	3665.3 ± 673.3b	316.6 ± 28.2b	26.0 ± 5.3b	75.4 ± 16.0	5.4 ± 1.1	15.5 ± 4.6	0.2 ± 0.1
	variation %	**117.9**	**-30.6**	**-10.3**	**-32.8**	**-30.0**	**-40.6**	**2.3**	**-8.5**	**-44.1**	**-54.1**
	p	0.0187	n.s.	n.s.	0.0485	0.0375	0.0240	n.s.	n.s.	n.s.	n.s.
RIL 28	AMB	47.4 ± 2.7	993.4 ± 41.7	3521.2 ± 271.4	5708.9 ± 292.9	460.0 ± 38.3	35.4 ± 1.2	69.4 ± 2.6	5.5 ± 0.7	25.2 ± 1.9	0.4 ± 0.1
	ELE	85.9 ± 18.8	807.7 ± 142.6	3148.9 ± 467.4	4609.6 ± 800.9	313.3 ± 51.9	26.5 ± 4.2	61.7 ± 10.1	4.8 ± 0.5	17.6 ± 4.4	0.3 ± 0.1
	variation %	**81.3**	**-18.7**	**-10.6**	**-19.3**	**-31.9**	**-25.0**	**-11.0**	**-12.5**	**-30.2**	**-29.1**
	p	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Saragolla	AMB	46.0 ± 2.4b	995.4 ± 30.8	3457.6 ± 87.1	5666.7 ± 71.4	375.5 ± 55.4	38.3 ± 1.5	70.2 ± 2.7	4.4 ± 0.2	19.9 ± 0.9	0.3 ± 0.04
	ELE	94.7 ± 16.5a	810.5 ± 128.0	3048.9 ± 421.7	4527.8 ± 684.7	338.2 ± 78.3	29.6 ± 5.1	55.1 ± 8.3	4.7 ± 0.8	16.4 ± 4.0	0.4 ± 0.1

	variation %	**105.7**	**-18.6**	**-11.8**	**-20.1**	**-9.9**	**-22.7**	**-21.5**	**5.7**	**-17.5**	**22.6**
	p	0.0267	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Simeto	AMB	45.5 ± 2.4b	1087.2 ± 57.8	3480.1 ± 350.01	5323.6 ± 417.1	361.1 ± 40.3	33.6 ± 2.8	84.3 ± 15.9	5.2 ± 0.4	24.3 ± 2.5	0.3 ± 0.04
	ELE	90.1 ± 14.9a	959.1 ± 190.7	3386.4 ± 550.7	4196.1 ± 702.0	349.8 ± 75.9	27.7 ± 6.4	75.1 ± 8.7	6.3 ± 1.2	21.0 ± 5.8	0.6 ± 0.4
	variation %	**97.9**	**-11.8**	**-2.7**	**-21.2**	**-3.1**	**-17.5**	**-10.9**	**20.8**	**-13.4**	**57.9**
	p	0.0256	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Svevo	AMB	48.7 ± 2.5b	1183.6 ± 65.5	3498.9 ± 260.8	5251.2 ± 282.5	680.5 ± 277.7	47.9 ± 6.3	148.7 ± 26.1	5.1 ± 0.4	23.2 ± 2.3	0.7 ± 0.2
	ELE	101.1 ± 11.6a	943.2 ± 159.4	3251.0 ± 460.3	4207.9 ± 696.9	418.8 ± 71.5	33.5 ± 5.2	90.4 ± 7.4	6.5 ± 1.5	19.9 ± 5.2	0.3 ± 0.1
	variation %	**107.5**	**-20.3**	**-7.1**	**-19.9**	**-38.5**	**-30.2**	**-39.2**	**28.4**	**-14.4**	**-46.9**
	p	0.0045	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Data are means ± SE (n = 4; expressed as dry matter) and variations from AMB for ELE (%; in bold); p, probability level (<0.05); n.s., not significant

Table 3. Mean mineral contents and variations of the pasta samples of each genotype grown under AMB and ELE conditions.

Genotype	Environment	Pasta mean mineral contents (mg/kg)									
		Na	**Mg**	**P**	**K**	**Ca**	**Mn**	**Fe**	**Cu**	**Zn**	**Mo**
Total	AMB	27.6 ± 4.1	515.0 ± 14.4	1506.2 ± 161.0a	2607.7 ± 73.7b	311.8 ± 27.2a	13.0 ± 0.6a	54.5 ± 8.7	3.1 ± 0.2a	20.3 ± 0.9a	0.5 ± 0.1a
Samples	ELE	24.4 ± 3.8	506.6 ± 12.7	1214.9 ± 121.4b	2707.2 ± 81.0a	273.4 ±17.1b	14.6 ± 0.3b	62.3 ± 4.3	2.3 ± 0.1b	18.7 ± 0.4b	0.3 ± 0.03b
	variation %	**-11.7**	**-1.6**	**-19.3**	**3.8**	**-12.3**	**11.7**	**14.3**	**-26.2**	**-8.0**	**-29.7**
	p	n.s.	n.s.	<0.0001	0.0094	n.s.	0.0004	n.s.	0.0061	0.0369	0.0469
Cappelli	AMB	15.7 ± 1.6	486.7 ± 3.7b	1324.8 ± 25.6a	2357.7 ± 20.6b	268.9 ± 15.8	11.0 ± 10.1b	37.6 ± 9.9	2.8 ± 0.1a	21.6 ± 1.8	0.6 ± 0.2
	ELE	17.8 ± 3.8	506.4 ± 2.6a	1067.6 ± 13.7b	2568.7 ± 12.3a	266.3 ± 16.4	13.9 ± 0.3a	60.3 ± 3.2	2.3 ± 0.1b	18.3 ± 0.8	0.3 ± 0.1
	variation %	**13.5**	**4.1**	**-19.4**	**9.0**	**-1.0**	**26.7**	**60.3**	**-15.6**	**-15.2**	**-45.5**
	p	n.s.	0.0121	0.0009	0.0009	n.s.	0.0012	n.s.	0.0485	n.s.	n.s.
Ciccio	AMB	24.3 ± 3.6a	460.6 ± 17.5b	920.8 ± 33.6	2678.6 ± 90.8	377.3 ± 78.9	14.7 ± 1.1	102.5 ± 2.0a	3.4 ± 1.0	16.2 ±1.4	0.6 ± 0.2
	ELE	11.8 ± 2.2b	433.4 ± 8.4a	817.9 ± 46.5	1915.8 ± 35.1	233.4 ± 4.0	11.7 ± 0.2	55.8 ± 1.5b	1.8 ± 0.1	13.9 ± 0.6	0.3 ± 0.1
	variation %	**-59.8**	**-5.9**	**-11.2**	**-28.5**	**-38.1**	**-20.5**	**-45.6**	**-47.6**	**-14.3**	**-50.7**
	p	0.0420	0.0045	n.s.	n.s.	n.s.	n.s.	0.0003	n.s.	n.s.	n.s.
Saragolla	AMB	22.1 ± 2.7	535.3 ± 7.6a	1413.4 ± 22.5a	2443.1 ± 46.9	258.7 ± 24.8	14.7 ± 0.3	40.2 ± 1.3	3.2 ± 0.1a	20.3 ± 1.02	0.3 ± 0.01
	ELE	34.7 ± 10.6	467.0 ± 10.0b	827.8 ± 37.3b	2573.5 ± 36.1	220.2 ± 18.2	13.7 ± 0.4	51.0 ± 5.7	2.2 ± 0.1b	18.6 ± 0.6	0.3 ± 0.02
	variation %	**57.1**	**-12.8**	**-41.4**	**5.3**	**-14.9**	**-6.2**	**26.7**	**-30.5**	**-8.2**	**6.6**
	p	n.s.	0.0056	0.0002	n.s.	n.s.	n.s.	n.s.	0.0078	n.s.	n.s.
Simeto	AMB	48.3 ± 6.9	577.6 ± 11.2a	2365.6 ± 83.3a	2951.6 ± 45.2b	342.2 ± 64.9	11.8 ± 0.2b	37.8 ± 3.8	3.1 ± 0.02	23.2 ± 0.5a	0.5 ± 0.03

ELE	33.1 ± 0.9	480.8 ± 9.2b	1884.7 ± 34.3b	3162.5 ± 50.9a	322.1 ± 56.2	15.3 ± 0.3a	64.3 ± 15.3	2.6 ± 0.2	19.8 ± 0.5b	0.5 ± 0.1
variation %	**-31.4**	**-16.7**	**-20.3**	**7.1**	**-5.9**	**30.3**	**70.2**	**-16.5**	**-14.5**	**-14.7**
p	n.s.	0.0026	0.0059	0.0363	n.s.	0.0009	n.s.	n.s.	0.0086	n.s.

Data are means ± SE (n = 4; expressed as dry matter) and variations from AMB for ELE (%; in bold); p, probability level (<0.05); n.s., not significant

331 **Table 4.**Mineral content and estimated dietary intake according to reference values for recommended daily allowance (RDA; EEC, 2008) in one

332 pasta serving for each mineral.

Mineral	RDA (mg/day)	Content and estimated dietary intake for one pasta serving (100 g)					
		AMB			ELE		
		Mean (mg)	Range (mg)	%RDA	Mean (mg)	Range (mg)	%RDA
K	2000	260.77	235.8-295.2	13.0	270.72	191.6-316.2	13.5
Ca	800	31.18	25.8-37.7	3.9	27.34	22-32.2	3.4
P	700	150.62	92.1-236.6	21.5*	121.49	81.8-188.4	17.4*
Mg	375	51.5	46.1-57.8	13.7	50.66	43.3-50.6	13.5
Fe	14	5.45	3.8-10.2	38.9*	6.23	5.1-6.4	44.5*
Zn	10	2.03	1.6-2.3	20.3*	1.87	1.39-1.98	18.7*
Cu	1	0.31	0.28-0.34	31.0*	0.23	0.18-0.26	23.0*
Mn	2	1.3	1.1-1.5	65.0*	1.46	1.17-1.54	73.0*
Mo	0.05	0.042	0.03-0.06	84.0*	0.03	0.03-0.05	60.0*

333 *, significant daily intake (>15%)

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Highlights

Durum wheat genotypes were evaluated for free air CO_2enrichmentat 400 and 570 ppm;

Grain from ELE (570 ppm) showed significant decreases in micro- and macro-elements;

In pasta from ELE, Ca, Cu, Zn and Mo decreased, Fe and Mn increased;

The increases in Fe and Mn reflect different genotypic mineral distributions in grain;

For mineral recommended daily allowances, pasta from ELE contributed less than AMB.