

Article



Effect of Genotype, Environment, and Their Interaction on the Antioxidant Properties of Durum Wheat: Impact of Nitrogen Fertilization and Sowing Time

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Abstract: In this study, the influence of genotype (G), environment (E), and their interaction ($G \times E$) on the content of total free phenolic compounds (TPC) and the antioxidant capacity (AC) was investigated, using sixteen durum wheat genotypes cultivated under seven crop management systems in Mediterranean environments. Possible correlations between TPC and AC with protein content (PC) and vitreous kernel percentage (VKP) were examined. Gs that exhibited stability across diverse conditions were studied through a comprehensive exploration of $G \times E$ interaction using a GGE biplot, Pi, and KR. The results indicated significant impacts of E, G, and $G \times E$ on both TPC and AC. Across E, the mean values of G for TPC, ABTS (2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid), DPPH (2,2-diphenyl-1-picrylhydrazyl), and FRAP (ferric reducing antioxidant power) values were 48.8 mg Trolox equivalents (TE)/100 g, 121.3 mg TE/100 g, 23.0 mg TE/100 g, and 88.4 mg TE/100 g, respectively. E, subjected to splitting top-dressing N fertilization, consistently showed low values, while the late-sowing ones possessed high values. Organic crop management maintained a stable position in the middle across all measurements. The predominant influence was attributed to $G \times E$, as indicated by the order $G \times E > E > G$ for ABTS, DPPH, and FRAP, while for TPC, it was $E > G \times E > G$. For TPC, the superior Gs included G5, G7 and G10, for ABTS included G3, G5 and G7, and for protein included G1, G9, and G16. G7 and G5 had a high presence of frequency, with G7 being the closest genotype to the ideal for both TPC and ABTS. These results suggest that the sowing time, nitrogen fertilization, and application method significantly impact the various antioxidant properties of durum wheat. This study holds significant importance as it represents one of the few comprehensive explorations of the impact of various Es, Gs, and their interactions on the TPC and AC in durum wheat, with a special emphasis on crop management and superior Gs possessing stable and high TPC and AC among them, explored by GGE biplot, Pi and KR. Further experimentation, considering the effect of the cultivation year, is necessary, to establish more robust and stable conclusions.

Keywords: cultivars; antioxidant properties; phenolic compounds; protein; GGE biplot; stability indices; cultivation practices; adaptation to climate change



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1. Introduction

In 2020, durum wheat (*Triticum turgidum* L. var durum Desf.) accounted for 6.2% of the 219 million hectares of grain cultivated worldwide [1,2]. Among the Mediterranean basin countries, Greece, Italy, Algeria, and Tunisia have the highest proportions of durum wheat to bread wheat acreage, reaching up to 85%. This demonstrates the excellent adaptability of durum wheat to the climatic conditions of the Mediterranean area [2,3]. The durum wheat kernel, which consists of three parts (bran, endosperm, and embryo), is typically fractionated and, due to its vitreous nature, which differs from the bread wheat kernel, is referred to as semolina [4–6]. Bread, couscous, bulgur, frekeh, noodles, and, most importantly, pasta are the most renowned products made from durum wheat [5].

Consumer awareness of health-promoting foods has increased [7]. Many human degenerative diseases are linked to reactive oxygen species and oxidative stress, and antioxidant activity significantly impacts many diseases [8]. Polyphenols play a broader role in the human body by regulating antioxidant enzyme gene transcription and being involved in cell growth regulation, inflammation, etc. [9]. Furthermore, the activity of transcription factors or microRNA modulation could be influenced by polyphenols [10].

Many plants with significant antioxidant activity are cultivated in the Mediterranean basin, contributing to healthy living. These plants include *Sideritis scardica*, *Melissa officinalis* L., *Cannabis sativa* L., and the *Lamiaceae family*. Nevertheless, these plants are consumed in much smaller quantities than wheat [11–17]. Durum wheat contains significant amounts of antioxidants, with whole wheat flours exhibiting higher antioxidant activity than their corresponding white flours, primarily because the phenolic compounds are mainly found in the bran [18–21]. Some by-product fractions of durum wheat have shown antioxidant activity comparable to that of fruits and vegetables, likely due to fiber-bound phenolic compounds [22,23]. Thus, the consumption of whole wheat flour may be beneficial for health.

Significant differences have been observed among different wheat genotypes (Gs) in the amounts of phenolic acids, with trans-ferulic acid being the most abundant [7,18,24]. Beta et al. [25] noted an influence of the environment (E) on total phenolic compounds (TPC) and antioxidant capacity (AC). G, E, $G \times E$, and year all impact the TPC and the AC of durum wheat. Most notably, the year affects the free phenolic acids, the environment by year interactions affect the conjugated phenolic acids, and the G affects the bound phenolic acids [26,27].

The interaction between G and performance, which varies across different Es (G × E interaction-GEI), adds complexity to identifying superior genotypes. This complexity is referred to as the crossover concept [28]. The primary goal is to obtain a G that consistently has high and stable values for seed yield or other quality characteristics across a diverse range of tested Es. Only then can it be asserted that a genotype is a superior selection with high adaptability [26,29]. Various statistical tools have been developed to address G × E interaction and crop stability.

Commonly used parametric models helping in the identification of superior Gs [30] are the ASV_i stability measure [31] from Additive Main effect Multiplicative Interaction (AMMI) analysis [32], the G superiority P_i index [33], the stability variance (σ_i^2) [34], the variance of deviations from regression (s_{di}^2) [35] and the Kang's rank-sum method (*KR* index) [36]. The GGE biplot analysis visualizes the interaction outcomes between G and E. In its pursuit, the analysis has a dual objective: firstly, to pinpoint varieties that surpass the average performance and demonstrate stability across multiple Es, and secondly, to recommend the utilization of varieties exhibiting stability in specific Es [28].

Utilizing the GGE biplot model [28,37], a comprehensive visual representation of the entire $G \times E$ interaction is offered. This involves a biplot that encapsulates both the average yield performance and stability. Furthermore, the GGE plot distance between any given G and the ideal G can be utilized to measure its desirability [28,37].

Nevertheless, there is a gap in existing research, as no prior study fully explores the impact of these factors and their interactions on the antioxidant properties of durum wheat,

with a focus on different crop management systems and the identification of superior Gs possessing stable and high TPC and AC among them.

In our ongoing commitment to improving the health-related aspects of wheat, this study assesses the impact of G, E, and $G \times E$ on the TPC and AC (DPPH, ABTS, and FRAP) of durum wheat in Mediterranean environments. Specifically, the TPC and AC of sixteen durum wheat genotypes under seven high/low input crop management systems were investigated. Correlations between TPC and AC and their relationships with protein content (PC) and vitreous kernel percentages (VKP) were also studied. Moreover, the genotypes with the higher and more stable TPC and AC across the Es were identified through a comparative examination with GGE biplot analysis and five parametric stability models.

2. Materials and Methods

2.1. Plant Material and Experimental Design

Experiments were conducted in the 2020–2021 growing season, evaluating sixteen commercially available Gs (Table 1) across seven different Es (Table 2). These Gs were selected considering their popularity among Greek growers, their potential for high yields, and a comprehensive assessment of commercial factors to determine their adaptability.

Genotype Code	Name of Genotype	Country of Origin	Genealogy	Year Released
G1	Pigreco	Italy	Not available (NA)	NA
G2	Canavaro	Italy	Coloseo/Simeto	2008
G3	Maestrale	Italy	Iride/Svevo	2004
G4	M. Aurelio	Italy	D95241/Arcobaleno/Svevo	NA
G5	Meridiano	Italy	Simeto/WB881/Duilio/F21	1999
G6	Mexicali-81	Greece	Selection from Mexicali 75	1981
G7	Monastir	France	Not Available (NA)	NA
G8	Simeto	Italy	Capeiti 8/Valnova	1988
G9	Svevo	Italy	Linea Cimmyt/Zenith	1996
G10	Vendeta	Italy	Creso/Ofanto	2003
G11	Egeo	Italy	Claudio/v80	NA
G12	Elpida	Greece	Sifnos/Mexicali-81	2010
G13	Žoi	Greece	Simeto /Mexicali-81	2011
G14	Secolo	Italy	NA	NA
G15	Grecale	Italy	NA	NA
G16	Zeta E.	Greece	NA	NA

Table 1. Origin, genealogy, and release date of the 16 genotypes.

The seven Es (Table 2) that were evaluated include the following:

- 1. Thermi-typical fertilization/typical sowing time (mid-November). With typical fertilization (total N amount of 180 kg ha⁻¹), one-third of which was applied (ammonium phosphate 20-10-0) before sowing and two-thirds (ammonium nitrate 33.5-0-0) at full tillering (Zadok 29). (High-productivity environment);
- Thermi-organic field (no fertilization)/typical sowing time; low-productivity environment);
- Thermi-typical fertilization/late sowing time, i.e., end of January. All the other agronomic treatments were identically applied to all plots. (Low-productivity environment);
- 4. Thermi-splitting topdressing N fertilization/typical sowing time. Splitting topdressing N fertilization involved splitting one-third (ammonium phosphate 20-10-0) before sowing, one-third (ammonium nitrate 33.5-0-0) at full tillering (Zadok 29), and onethird during the first node (Zadok 31). (High-productivity environment);
- 5. Thermi-splitting topdressing N fertilization/late sowing time (as described above). (High-productivity environment);

Code	Location	Latitude/ Longitude	Climate Type	PrA ² (mm)	PrA-M ³ (mm)	Τ ⁴ (°C)	Prod. ⁵	Fertilization ⁶	Planting Date	Soil Texture ⁷	pH (1:1)	EC ⁸	SOM ⁹ %	P _{Olsen} mg kg ⁻¹
E1	Thermi/typical fertilization/typical sowing date	40°54′ N/ 23°00′ E	BSk ¹	343.6	45.4	12.8	HP	Typical	Typical	L	7.89	0.516	1.8	12.28
E2	Thermi- Organic/typical sowing date	40°54′ N/ 23°00′ E	BSk	343.6	45.4	12.8	LP	Organic	Typical	L	7.67	0.585	2.5	22.47
E3	Thermi-typical fertilization/late sowing	40°54′ N/ 23°00′ E	BSk	343.6	45.4	12.8	LP	Typical	Late sowing	L	7.84	0.681	1.8	18.29
E4	Thermi-splitting fertilization/typical sowing date	40°54′ N/ 23°00′ E	BSk	343.6	45.4	12.8	HP	Splitting topdressing application	Typical	L	8.14	0.564	1.7	20.63
E5	Thermi-late splitting fertilization/late sowing date	40°54′ N/ 23°00′ E	BSk	343.6	45.4	12.8	LP	Splitting topdressing application	Late sowing	L	7.99	0.497	1.8	23.21
E6	Nea Gonia/typical fertilization/typical sowing date	40°35′ N/ 23°08′ E	BSk	335.5	57.6	12.4	HP	Typical	Mid- November	CL	7.05	0.525	1.8	30.10
E7	Sindos/typical fertilization/late sowing date	40°68′ N/ 22°80′ E	BSk	376.2	41.6	12.7	LP	Typical	Late sowing	SL	7.85	0.485	1.7	28.50

Table 2. Soil and climatic	characteristics of seven	evaluation environments.
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¹ Köppen–Geiger climate types: BSk = arid, steppe, cold [38,39]; ² PrA = precipitation during all growing season (November to June); ³ PrA-M = precipitation of grain filling period i.e. April–May (this period mainly represents the beginning of flowering to grain filling); ⁴ T (°C) = the average temperature in the growing season (November to June); ⁵ All information has been described; ⁶ Fertilization (different ways of fertilization explained below); ⁷ Soil textures: L = loam, CL = clay loam, SL = sandy loam; ⁸ EC = electrical conductivity (Ms cm⁻¹); ⁹ SOM = soil organic matter.

- 6. Nea Gonia (typical sowing time) with typical fertilization (total N amount of 150 kg ha⁻¹), one-third of which was applied (ammonium phosphate 20-10-0) before sowing and two-thirds (ammonium nitrate 33.5-0-0) at full tillering (Zadok 29). (High-productivity environment);
- 7. Sindos-typical fertilization/late sowing time. (Low-productivity environment).

In each location, the Gs were arranged in plots across the trials using a randomized complete block design (RCBD) with three replicates. Each plot covered an area of 1 square meter and consisted of four rows, 1 m long and 0.25 m apart. Details on soil/climatic data and agronomic practices, such as the date of sowing and fertilization, are provided in Table 2. In each location, the trial daily mean air temperature and precipitation during the growing seasons were recorded by a wireless automatic weather station (Pessl iMetos OEM Model-1) installed and supported by the software DSS Legumini.net ver. 1.0 for better microclimate illustration (Table 2).

Each sample was milled in a laboratory mill (ZM-100; Retsch, Haan, Germany) to pass through a 0.5 mm sieve. All other chemicals and solvents used were of analytical grade.

2.2. Vitreous Kernel Percentage

Visual estimation was applied to separate three sets of 100 kernels into vitreous and non-vitreous kernels. Those with a dark translucent appearance were considered vitreous kernels, while non-vitreous kernels appeared starchy and opaque. The results were expressed as percent (%) of vitreous kernels (VKP). VKP was only recorded in the central environment for a brief qualification of the genotypes.

2.3. Protein Content

The protein content of the grounded samples was determined using a Near-InfraRed (NIR) analyzer (PerCon Inframatic 8620, Perten Instruments, Hamburg, Germany) after a calibration curve was set using the Kjeldahl method [40].

2.4. Free Phenolic Extraction

We dissolved 0.25 g of durum wheat flour in 2 mL 70% aqueous methanol (MeOH/H₂O), vortexed this for 1 min, and then incubated it for 10 min in an ultrasound bath (frequency 37 kHz, model FB 15051, Thermo Fisher Scientific Inc., Loughborough, UK) at room temperature. Then, the extracts were centrifuged (Universal 320R, Hettich, Frankenberg, Germany) at 4000 rpm for 10 min, the supernatants were collected, and the residue was re-extracted one more time. Finally, the clear supernatants were mixed and stored at -20 °C until analysis. Three replications were conducted for each sample.

2.5. Total Phenolic Content (TPC)

The TCP determination was carried out based on the Folin–Ciocalteu method, according to Singleton et al. [41]. Briefly, 0.2 mL of the free extracts were mixed with 0.8 mL of diluted Folin–Ciocalteu reagent (diluted 10-fold in deionized water), vortexed, and allowed to rest for 2 min. Then, 2.0 mL of sodium carbonate (7.5% w/v) solution and distilled water up to 10 mL were added and incubated for one hour under dark conditions. The absorbance was recorded in a spectrophotometer (HITACHI U-1900, Tokyo, Japan) at 725 nm, and the results were expressed as mg of gallic acid equivalents (GAE) per 100 g of dry weight (dw).

2.6. Antioxidant Capacity

2.6.1. Radical Scavenging Activity (ABTS)

The activity of radical scavenging of the durum wheat extracts against ABTS (2,2azinobis-(3-ethylbenzthiazoline-6-sulphonic acid) radical cation was determined according to Re et al. [42]. For the ABTS^{•+} preparation, two mM ABTS were mixed with 0.73 mM potassium persulfate ($K_2S_2O_8$) and dissolved in distilled water. After the mixture was stored under dark and ambient temperature conditions, its absorbance was adjusted at 0.70 (±0.02) at 734 nm. A volume of 3.9 mL of the ABTS^{•+} solution was mixed with 0.1 mL of the durum wheat extract, and after 4 min, its absorbance was recorded at 734 nm. For the percent inhibition of the ABTS radical cation, the following equation was used:

Inhibition (%) =
$$\left[\frac{A_o - A_s}{A_0}\right] \times 100$$

where A_0 is the blank's absorbance and A_s is the sample's absorbance.

Trolox was used for the calibration curve, as a standard compound, and the results were expressed as mg of Trolox equivalents (TE) per 100 g dw.

2.6.2. Ferric Reducing/Antioxidant Power (FRAP)

The reducing power of the durum wheat extracts was determined according to Benzie and Strain's method [43]. The FRAP assay was prepared by mixing 20 mM ferric chloride solution (FeCl3.6H2O), 10 mM TPTZ (2-4-6-tripyridyl-s-triazine) in 40 mM HCl and 0.3 mM acetate buffer (pH 3.6), in a proportion of 1:1:10, respectively).

A volume of 3.0 mL of the FRAP solution was mixed with 0.1 mL of the durum wheat extract, and after 4 min of incubation at 37 °C under darkness, its absorbance was recorded at 593 nm against a blank. The results were expressed as mg of Trolox equivalents (TE) per 100 g dw.

2.6.3. Radical Scavenging Capacity Activity (DPPH)

Radical scavenging capacity activity was determined using the Yen and Chen method [44]. A 0.1 mM DPPH (2,2-diphenyl-1-picryhydrazyl) solution in methanol was prepared (DPPH[•]). A volume of 2.85 mL of DPPH[•] was mixed with 0.15 mL of the durum wheat extracts, and the absorbance was recorded at 516 nm after 5 min of incubation. The results were expressed as mg of Trolox equivalents (ET) per 100 g dw.

2.7. Statistical Analysis

Characteristics were subjected to an over environment two-way analysis of variance (ANOVA), using a mixed model considering environments as a random effect and genotypes as a fixed effect. The Shapiro–Wilk test was employed to assess the normal distribution of variables, whereas Levene's test checked the ANOVA's assumptions for the equality of the error variances and residual normality [45]. Differences between either genotypes or environments were identified with a post hoc Tukey HSD test. Pearson and Spearman's rank correlation coefficients were calculated and evaluated for their significance at three probability levels: 0.001 (indicating a strong correlation), 0.01 (indicating a moderate correlation), and 0.05 (indicating a weak correlation). All the statistical analyses were performed using IBM SPSS Statistics 28.0.0.0 (190) software.

2.8. Data Analysis

The AMMI models [32] were executed utilizing the GenStat (13th edition) statistical software. It relies on the calculation of the first and second principal component (PC) scores, denoted as PC1 (indicative of the first PCA's interaction) and PC2 (indicative of the second PCA's interaction), as outlined in Purchase's study [31,32]. The following equation details the computation process:

the
$$ASV_i = \sqrt{\frac{SSPC1}{SSPC2}(PC1)^2 + (PC2)^2}$$

where *SS* is the sum of squares. The genotype with the smallest ASV_i value was regarded the most stable.

Genotype superiority, P_i .

The computation of genotype superiority [46], labeled as P_i , entailed the assessment of the mean square distance between the genotype and the maximum response using the following equation:

$$twoP_i = \sum_i \left(\overline{y}_{ij} - max_j\right)^2 / 2e)$$

In this equation, max_j represents the maximum response observed among all genotypes in the given environment (j). The key takeaway is that the smallest P_i value indicates the better genotype.

Shukla's stability variance, σ^2_i .

In 1972, Shukla [34] proposed the stability variance of genotype *i*, defined as its variance across environments after accounting for the main effects of environmental means. According to this statistic, genotypes with the lowest values are more stable.

Deviation from regression, S^2_{di} .

The utilization of the variance of deviations from the regression (S^2_{di}) has been proposed as one of the prominent parameters in the selection of stable genotypes. Genotypes with an $S^2_{di} = 0$ are considered the most stable, whereas an $S^2_{di} > 0$ would indicate lower stability across all environments. Hence, genotypes with lower values are the most desirable.

Kang's Genotypes with a rank-sum, KR.

Kang's rank-sum method [26,47] utilizes yield and σ_i^2 (variance) as selection criteria. This approach assigns equal weight to yield and stability statistics in identifying high-yielding and stable genotypes. The genotype achieving the highest yield and the lowest σ_i^2 is given a rank of one. Subsequently, the ranks for yield and stability variance are combined for each genotype. Genotypes with the lowest rank-sum are regarded as the most desirable.

The GGE biplot model is grounded in the singular value decomposition of the first two principal components [28], as follows:

$$y_{ij} - \mu - \beta_j = \lambda_1 \xi_{i1} \eta_{j1} + \lambda_2 \xi_{i2} \eta_{j2} + \varepsilon_{ij}$$

where y_{ij} is the measured mean of genotype *i* in environment *j*, μ is the grand mean, β_j is the main effect of environment *j*, λ_1 and λ_2 are the singular values for the first and second principal component (*PC*1 and *PC*2, respectively), ξ_1 and ξ_2 are eigenvectors of genotype *i* for *PC*1 and *PC*2, η_1 and η_2 are eigenvectors of environment *j* for *PC*1 and *PC*2, and ε_{ij} is the residual associated with genotype *i* in environment *j*.

3. Results and Discussion

The study investigated the effect of G, E, and $G \times E$ on durum wheat's TPC and the AC. It also examined the correlations among TPC, AC, PC, and VKP percentages.

3.1. Effect of Genotype, Environment, and Genotype by Environment

The analysis of variance showed that all sources of variation were highly significant in all antioxidant-related traits (Table 3). $G \times E$ had the most significant effect on all the analyses referred to AC. For ABTS, DPPH, and FRAP, the contributions of $G \times E$ to the variation were 45.3, 49.9, and 64.9%, respectively (Table 3). Regarding TPC, E contributed a large portion (42.4%), followed by $G \times E$ (42.2%). G showed a low contribution (from 6.7 to 12.7) to the variation for all the traits, while E, for the AC, had a moderate contribution from 20.7 to 33.1%.

In accordance with these results, other studies reported a high contribution by the E for TPC [48,49]. A high contribution by $G \times E$ for TPC is also reported by Martini et al. [50]. Regarding the AC by DPPH test, other studies reported a higher contribution to the variation by either E or G [48,49]. However, Irakli et al. reported a higher contribution by $G \times E$ to the total variation in ABTS values for *lens culinaris* L. [51]. In hemp seeds, the cultivation year had a higher effect on TPC, ABTS, and FRAP [12]. Other studies exploring the impact of genotype and environment on soft winter wheat reported a significantly higher effect of E on ABTS, followed by $G \times E$ [50,52].

			TPC			ABTS			DPPH		FRAP		
	df	MS	SS	SS%	MS	SS	SS%	MS	SS	SS%	MS	SS	SS%
Environment	6	1787.6 ***	10,725.5	42.4	7348.9 ***	44,093.6	33.1	2664.9 ***	3992.1	38.3	25,886.9 ***	155,321.4	20.7
Genotype	15	154.7 ***	2320.2	9.2	594.0 ***	8910.0	6.7	266.1 ***	3992.1	9.6	6325.1 ***	94,876.7	12.7
G×Ê	90	118.6 ***	10,670.6	42.2	671.0 ***	60,389.3	45.3	231.6 ***	20,844.8	49.9	5403.5 ***	486,314.2	64.9
Error	224	6.9	1551.4	6.2	89.4	20,016.5	15.0	4.1	926.3	2.2	56.9	12,746.9	1.7

Table 3. Two-way ANOVA for total phenolic compound (TPC), ABTS scavenging activity, DPPH scavenging activity, and ferric reducing/antioxidant power (FRAP) was determined for sixteen durum wheat genotypes cultivated under seven environments.

df: degrees of freedom, MS: Mean Square, SS: Sum of Squares. ***: *p* < 0.001.

The effect of E on the antioxidants in cereals is significant [53]. Growing conditions, particularly solar radiation during the grain-filling period, contribute to free radical formation that increases oxidative stress. This triggers the biosynthesis of antioxidants for self-defense against environmental stress [54]. It has been reported that droughts reduce grain size by shortening the filling phase, and the high temperature and drought jointly affect the duration of grain filling, rather than individually [55]. The soluble forms of polyphenols are affected mainly by the climatic conditions occurring during the different years of experimentation. The interaction between weather conditions and location can induce a diverse response in accumulating compounds in the kernel [56]. Except for the crop management and the cultivation location, a year-to-year variation in the content of phenolic compounds in durum wheat has also been reported [57]. In general, there is a small impact of G and large effects of both year and $G \times E$ interaction on the metabolite composition (amino acids, sugars, organic acids, fatty acids, and sterols) and the quality of durum wheat grains [58]. Given these complexities, further investigations that consider the year-to-year variations in the antioxidant properties of durum wheat are necessary. Such studies would significantly contribute to the ongoing discourse on understanding and enhancing the quality of durum wheat in varying environmental contexts.

3.2. Total Phenolic Compounds

Phenolic compounds are among the most extensively researched phytochemical classes derived from plants, due to their capability to function as radical scavengers. Thus, they have garnered significant attention for their potential in preventing cancer and various chronic diseases [59,60]. In Table 4, the mean values \pm SE of TPC, ABTS, DPPH, and FRAP for different Es and Gs are displayed and grouped using the Tukey HSD a, b test ($\alpha = 0.05$). The mean TPC value was 48.8 ± 0.5 mg GAE/100g dw. Generally, E4 and E5 had significantly lower TPC values than the rest of the Es. In contrast, the significantly highest values were observed in E3, followed by E7, where the same cultivation practices (late sowing) were applied in a different region. E1, E2, and E6 had medium TPC values. This suggests that using a splitting fertilization approach over conventional practices negatively affected the TPC content of durum wheat. There was also a significant difference between E1 and E7, which were both environments with typical fertilization and typical sowing dates that were cultivated in different regions. This variation in phenolic compounds across locations might be due to pedoclimatic differences or how different genetic backgrounds of the plant material are adjusted in different environments [51].

The G with the highest mean TPC content was G9 (Svevo), but no significant differences were observed with other varieties (G5, G6, G7, and G15), while the lowest was observed in G2. A similar range of TPC for cv. Simeto (G8 in this study) was reported by Laus et al. [61], ranging from 14.6 ± 0.60 to 74.5 ± 2.40 mg GAE/100g dw. The lowest value was observed in an irrigated field fertilized with 33 kg. ha⁻¹ of sulfur, while the highest value was found in a non-irrigated, no-sulfur field, the only one exceeding the values presented in this report. The TPC for whole meals of durum wheat for the extractable phenolic compounds, Duilio, Sant'Agata, and Simeto, were 192.3 ± 0.5 , 144.5 ± 1.8 , and 181.9 ± 3.2 mg GAE/100g dw, more than double the values reported here. Similar values have been reported for old and modern varieties [19,62–64]. These differences are likely

attributable to the extraction method and the fact that bound phenolic compounds were not extracted [21].

Table 4. Mean values \pm SE, for 16 durum wheat genotypes and seven environments of total phenolic compounds (TPC), ABTS and DPPH radical scavenging activity, and ferric reducing/antioxidant power (FRAP).

	TPC *	ABTS	DPPH	FRAP
		Environment		
E1	48.8 ± 1.1 ^c **	$118.3 \pm 2.2 \ ^{\rm c}$	14.4 ± 1.7 ^g	$75.7\pm4.6^{\rm ~d}$
E2	$49.5\pm1.1~^{ m c,d}$	$122.2\pm2.2~^{\rm c}$	$18.7\pm1.8~^{\rm e}$	$91.2\pm9.6~^{ m c}$
E3	57.3 ± 1.2 ^a	142.6 ± 2.8 $^{\rm a}$	21.5 ± 2.3 ^d	106.7 \pm 10.0 ^b
E4	42.0 ± 1.0 ^d	110.8 ± 2.6 ^d	$16.6\pm0.9~^{\rm f}$	$88.7\pm2.5~^{\rm c}$
E5	$39.8\pm0.7~^{\rm e}$	$104.8\pm2.3~^{\rm e}$	34.4 ± 0.6 ^a	$52.4\pm3.0~^{\mathrm{e}}$
E6	51.7 ± 0.7 ^b	$128.4\pm2.4~^{\rm b}$	$24.5\pm0.5~^{\rm c}$	79.2 ± 1.6 ^d
E7	52.8 ± 0.8 ^b	$122.4\pm2.1~^{ m c}$	31.1 ± 0.8 ^b	125.1 ± 5.7 $^{\rm a}$
Average	48.8 ± 0.5	121.3 ± 1.1	23.0 ± 0.6	88.4 ± 2.6
		Genotype		
G1	49.9 ± 2.4 ^{b,c} **	$122.8 \pm 5.1 \ ^{\rm a,b}$	$26.1\pm2.1~^{\rm b}$	$106.6\pm9.7~^{\rm b}$
G2	$43.8\pm1.9~^{ m f}$	$112.8\pm3.4~^{ m c,d}$	20.0 ± 2.3 $^{ m e}$	$77.1\pm8.3~^{ m e,f}$
G3	46.5 ± 2.1 d,e	124.7 ± 5.0 ^{a,b}	$22.7\pm1.6~^{ m c,d}$	$80.0\pm5.6~^{ m d-f}$
G4	49.4 ± 1.7 ^c	124.0 ± 4.0 ^{a,b}	23.6 ± 1.8 ^c	$109.2\pm12.3~\mathrm{b}$
G5	52.4 ± 1.0 ^{a,b}	$129.0\pm5.1~^{\mathrm{a}}$	$22.7\pm1.5~^{ m c,d}$	72.9 ± 4.9 ^{f,g}
G6	49.0 ± 2.2 ^{c,d}	$121.1 \pm 5.3 \ ^{\mathrm{a-c}}$	27.0 ± 4.2 ^b	$112.0\pm19.7~^{\mathrm{b}}$
G7	52.4 ± 1.7 ^{a,b}	125.5 ± 3.2 ^{a,b}	$23.9\pm2.0~^{\rm c}$	$108.0\pm8.6~^{\rm b}$
G8	46.9 ± 1.2 d,e	107.0 ± 3.4 ^d	$20.3\pm2.1~^{\mathrm{e}}$	$69.9 \pm 5.5 {}^{ m g}$
G9	53.1 ± 2.6 ^a	122.4 ± 5.0 ^{a,b}	31.7 ± 4.1 ^a	$90.4\pm9.8~^{ m c}$
G10	50.4 ± 1.7 ^{b,c}	$120.2 \pm 3.4 \ ^{\rm a-c}$	20.9 ± 2.0 d,e	86.3 ± 6.0 ^{c,d}
G11	47.0 ± 2.0 d,e	$121.3 \pm 3.5 \ ^{\mathrm{a-c}}$	$18.9\pm2.0~^{ m e,f}$	81.0 ± 5.3 ^{d,e}
G12	46.9 ± 1.2 d,e	116.7 ± 3.1 ^{b,c}	$17.8\pm2.0~^{\rm f}$	72.9 ± 4.3 $^{\mathrm{f,g}}$
G13	49.6 ± 1.4 ^c	125.4 ± 2.9 ^{a,b}	$22.9\pm1.5~^{\rm c}$	65.8 ± 2.6 $^{ m g}$
G14	45.7 ± 1.7 e,f	$120.1 \pm 3.7~^{\rm a-c}$	$20.1\pm1.3~^{ m e}$	$80.0\pm4.5~^{ m d-f}$
G15	$52.0\pm2.3~\mathrm{a-c}$	124.2 ± 6.1 ^{a-c}	27.2 ± 2.9 ^b	122.1 ± 21.1 a
G16	46.5 ± 1.7 d,e	124.2 ± 5.7 ^{a,b}	22.4 ± 1.7 ^{c,d}	80.5 ± 7.1 d,e
Average	48.8 ± 0.5	121.2 ± 1.1	23.0 ± 0.6	88.4 ± 2.6

* TPC: total phenolic compound (expressed as mg GAE/100 g dw), ABTS: ABTS + scavenging activity (expressed as TE/100 g dw), DPPH: DPPH scavenging activity (expressed as TE/100 g dw) and FRAP: ferric reducing/antioxidant power (expressed as TE/100 g dw). ** Different letters within a column indicate significant differences (among environments or genotypes) according to the Tukey HSD test (p < 0.05).

In the present study, organic crop management did not show significant differences in TPC compared to the two conventional methods (E1 and E6). However, in one of the two years studied by Nocente et al. [65], there was a significant difference between conventional and organic crop management, with organic practices resulting in higher TPC values. Similar findings with significantly higher TPC amounts have also been reported in other studies [49,66]. On the other hand, nitrogen fertilization had a positive and analogous effect on the TPC of winter wheat grains in Ma et al. [67,68]. The E has a high impact on the antioxidants in cereals [53]. For example, sunny days, soil type, and precipitation can affect the TPC of plants [69,70]. More factors have been reported to impact the TPC of plant material, such as prolonged exposure to Ultraviolet (UV) radiation, high altitudes, and water-deficit conditions that positively influence its synthesis [71,72]. Other factors that influence TPC come after the durum wheat kernel processing. Abdel-Aal and Rabalski [73] reported that the TPC in einkorn bread, cookies, and muffins increased after baking due to the degradation of conjugated and bound phenolic acids.

3.3. Antioxidant Capacity

The mean value of the ABTS scavenging activity among the different Es was $121.3 \pm 1.1 \text{ mg TE}/100 \text{ g}$ dw. Like TPC, the significantly lowest mean values were observed for E4 and E5, while the highest values were found in E3, with a significant difference from the other Es (Table 4) [74]. For the DPPH values, different observations were made. The E with the highest mean value was E5, followed by E7 (both late sowing), while E1 and E4 had the lowest values (Table 4). Regarding the genotype, there were significant variations among them, with G9 giving the highest value and showing a significant difference from the rest. Different levels of phenolic compound biosynthesis in two sowing times can be attributed to the induction caused by diverse biotic stresses. Moreover, the variability between the two locations could be linked to the varying levels of severity of plant pathologies present across these locations [75]. Variations in temperature conditions before the harvesting of wheat seeds have also been reported as a major factor influencing the profile of AC [74].

Di Loreto et al. [7], in their analysis of 22 old and modern durum wheat varieties, reported DPPH values of 186.2 mg TE/100g dw ($7.4 \pm 0.3 \mu mol/g$) for the old durum wheat cv. Inglesa and 101.6 mg TE/100g dw ($4.1 \pm 0.2 \mu mol/g$) for the modern durum wheat cv. Claudio [7]. Similar results have been reported by Truzzi et al. [64].

In accordance with the results presented in this study, Fares et al. (2019) [66] reported no significant differences between ABTS values for conventional and organic crop management. However, in one of the two years studied by Nocente et al. [65], there was a significant difference between conventional and organic crop management, with conventional practices resulting in higher total antioxidant capacity, as measured by the ABTS radical solution.

Consistent with our findings, other studies mentioned that organic cultivation possessed higher DPPH values than conventional one [49]. For broccoli, cauliflower, and red cabbage, DPPH values were significantly higher in organically cultivated vegetables, and the same trend was observed for ABTS values in kohlrabi. In contrast, broccoli showed higher ABTS values under conventional cultivation [76]. For other crops, there were no significant differences in AC between organic and conventional cultivation methods [77]. On the other hand, nitrogen fertilization had a positive and analogous effect on the AC of winter wheat grains, as reported by Ma et al. [67]. More post-processing factors can influence the AC of durum wheat. For example, for raw and cooked macaroni, it was reported that the antioxidant capacity increased after cooking because of Maillard reaction products, like Amadori compounds [78]. Similar statements were made by some researchers about boiled, microwaves, or steam-cooked vegetables [79].

For FRAP, E5 had a significantly lower mean value than the rest of the Es, while E7 and E3 had the highest mean values, with a significant difference from the rest (Table 3). G15 had the significant highest mean values among the Gs. Di Loreto et al. [7] reported a mean value of 357.9 mg TE/100g dw for 22 durum wheat varieties $(1.4 \pm 0.05 \text{ mmol}/100g)$. Truzzi et al. [64] reported almost one-third of those values, both for old and modern durum wheat varieties, even though the opposite was observed for TPC and DPPH values. Significantly higher FRAP values were reported for broccoli and kohlrabi cultivated under organic crop management than in conventional conditions [76]. Differences in climatic conditions, such as temperature and amount of rainfall before harvest, can impact both the TPC and AC of plants [74].

3.4. Organic and Late Sowing Environments

An interesting observation is that in almost all the Es but E2 (organic), even when high values were observed in one analysis, low values were observed in another when compared with the rest. In all the analyses (TPC, ABTS, DPPH, FRAP), E2 consistently yielded values falling between those of the other Es (Table 3). This provides evidence that nitrogen fertilization and its application method significantly impact the various antioxidant properties of durum wheat. Phenolic compounds are the ecological response of the plant to external factors. The influence of these parameters may also be higher when considering an agronomic system without inputs, such as the organic crop management presented here, to be introduced to improve the crop's nutritional status and protect the plant against diseases [57]. A detailed examination of the specific compounds, their quantities, and their activity in different environments would shed more light on this observation.

In the case of late sowing Es, mainly E7, high values were observed significantly in all the antioxidant analyses. This could be attributed to the harsh or non-optimal conditions the plants faced, leading them to produce higher levels of antioxidants for protection [1]. Further research is needed to gather clear evidence. However, this could also be related to the dilution effect, as late sowing environments had lower productivity.

3.5. Vitreous Kernel and Protein

The samples' PC and VKP (only for E1) were determined to extract possible correlations with their antioxidant properties. NIR spectroscopy was used for the former, while visual observation was employed for the latter.

Regarding the VKP results, only one repetition was conducted in E1, and the results are presented in Table 5. G2, G4, G9, and G10 possessed high VKP percentages; however, no significant differences could be calculated.

Table 5. Mean protein \pm SE percentages of sixteen durum wheat genotypes cultivated under seven environments, and percentages of vitreous kernels of sixteen durum wheat genotypes only under the central environment.

	Mean Protein %	Vitreous %
Environment		
E1	13.3 ± 0.1 ^c *	Not Availiable (NA)
E2	10.7 ± 0.3 ^{d,e}	NA
E3	11.6 ± 0.2 ^d	NA
E4	15.1 ± 0.2 a	NA
E5	13.1 ± 0.2 ^c	NA
E6	$10.3\pm0.1~^{ m e}$	NA
E7	$14.1\pm0.1~^{\mathrm{a,b}}$	NA
Genotype		
G1	13.9 ± 0.5 a	62.25
G2	12.9 ± 0.4 a	87.5
G3	12.2 ± 0.5 a	74.8
G4	13.1 ± 0.4 a	86.7
G5	12.2 ± 0.4 a	69.3
G6	11.8 ± 0.3 a	66.6
G7	12.7 ± 0.4 a	55.1
G8	12.1 ± 0.5 a	73.1
G9	13.0 ± 0.5 a	83.5
G10	12.6 ± 0.5 a	92.2
G11	12.7 ± 0.5 a	63.6
G12	11,9 \pm 0.4 $^{ m a}$	56.6
G13	12.5 ± 0.3 a	77.4
G14	12.5 ± 0.4 a	45.6
G15	12.1 ± 0.4 a	72.7
G16	13.3 ± 0.4 ^a	76.9
Average	12.6 ± 0.1	71.5

* Different letters within a column indicate significant differences according to the Tukey HSD test (p < 0.05).

The mean protein content of the samples was $12.6 \pm 0.1\%$, which is slightly lower than the value reported by Žilić et al. (2010) for durum wheat (13.89%) and somewhat higher than that reported for bread wheat (11.7%) (Table 5) [80]. The highest protein percentages, significantly different from the rest, were found in E4, but were not significantly higher than in E7. E6 had the lowest content, which was not significantly different from E2. Both environments with splitting fertilization (i.e., E4 and E5) had higher protein content than their conventionally fertilized counterparts. Among the Gs, no significant differences were observed. Products made from durum wheat are considered staple foods due to their significant contribution to energy and nutrition, coming primarily from their carbohydrate and protein content. Moreover, wheat contains essential nutrients and phytochemical compounds notable for their significant biological impact [61,81,82]. The antioxidant properties of proteins are also noteworthy; the AC of wheat gluten protein hydrolysates has been previously reported [20,23].

3.6. Correlation among Traits

Due to the large quantity of samples, making it easier for correlations to emerge among the data, a correlation was considered significant only if it exceeded 0.4 (Table 6). A highly significant positive correlation was observed between FRAP and TPC, while a moderate correlation was observed between FRAP and each ABTS and DPPH value. TPC and ABTS also demonstrated a strong significant correlation (Table 6). No significant correlations were found among vitreous kernel percentages with TPC, DPPH, and ABTS values. However, FRAP had a weak negative correlation with VKP (-0.351). VKP showed a high correlation with protein content. A significant correlation between TPC and DPPH was reported by Pandino et al. [49]. On the other hand, when AC was measured with DPPH assay, it was not correlated with the phenolic content [74]. In other studies, TPC and the AC were also strongly correlated by the strong correlation between TPC and each ABTS and FRAP found here [61,83,84]. The only weak negative correlation, between TPC and DPPH, could be attributed to the extraction and measurement of only the free phenolic compound of the samples. Phenolic compounds in insoluble-bound form are the major contributors to the AC of wheat grains [20–22]. In durum wheat-based food products, this can be observed in white wheat flour and white flour-based products with low levels of phenolic acids. This is due to removing components during the milling process, such as bran, aleurone, and hyaline layers, which typically contain the highest concentration of phenolic acids [85].

Table 6. Pearson correlations for TPC (total phenolic compound), ABTS scavenging activity, DPPH scavenging activity, and ferric reducing/antioxidant power (FRAP), protein content (PC), and vitreous kernel percentage (VKP) of durum wheat for sixteen DW genotypes cultivated under seven environments.

	TPC	ABTS	DPPH	FRAP	Protein	Vitreous %
TPC	1	0.676 **	0.273 **	0.525 **	-0.333 **	-0.052
ABTS		1	0.110	0.452 **	-0.313 **	0.097
DPPH			1	0.443 **	0.077	-0.112
FRAP				1	0.113	-0.351 *
Protein					1	0.706 **
Vitreous						1

** = significance at 0.01 and * = significance at 0.05.

3.7. $G \times E$ Interaction Analysis

As $G \times E$ possessed a high contribution for all the analyses, the results were further analyzed by a GGE biplot analysis to visualize the interaction outcomes between genotypes and environments. Thus, varieties that surpassed the average performance and demonstrated stability across multiple environments were pinpointed. The GGE biplot analysis was explicitly applied to TPC, indicating the antioxidant profile of the durum wheat samples. TPCs are considered important bioactive compounds due to their potential biological activities. They are found in plants with antioxidant, anticancer, and anti-inflammatory properties [86]. Notably, in Section 3.5, ABTS exhibited the highest correlation with TPC, leading to its selection as an indicator of antioxidant activity. The objective was to pinpoint genotypes with higher and more stable phenolic profiles and antioxidant activity across Mediterranean farming systems and with high protein content. Both parametric and nonparametric indices were computed to assess genotypes for their suitability across diverse environments. Table 7 illustrates the genotypes occupying the first and last five positions based on rankings derived from each statistical measure. In the overall evaluation across all environments, G5, G7, and G10 consistently secured the top five rankings with a presence frequency of 4/6, 3/6, and 5/6, respectively, establishing them as the most stable genotypes for TPC. Conversely, G2 and G8 occupied the least favorable positions in the bottom five rankings, with frequencies of 3/6 and 5/6, respectively.

Regarding ABTS, the most stable genotypes appeared to be G3, G5, G7, and G13, which consistently secured top five rankings with a presence frequency of 4/6, 3/6, 4/6, and 4/6, respectively. On the other hand, G8 and G12 occupied the least favorable positions in the bottom five rankings, with frequencies of 3/7 and 3/7, respectively. G1, G9, and G16, possessing 3/7, 4/7, and 5/7 presence frequencies, were the most stable genotypes for protein content.

According to the GGE biplot (Figure 1), for TPC in the total comparison of all Es, genotype G7 was the only one relatively close to the ideal genotype, followed by G10, G9, and G5. Similarly, the GGE biplot analysis revealed that for the total evaluation of ABTS in all environments, G7 was the only one relatively close to the ideal genotype, followed by G4, G13, and G5. For protein, G1 was the only one relatively close to the ideal genotype, followed by G9 and G16. The GGE biplot analysis explained 61.02%, 46.58%, and 67.14% of the total variability for TPC, ABTS, and protein.



Figure 1. Genotype and genotype by environment (GGE) comparison biplot of sixteen genotypes evaluated in seven environments for TPC (**left**), ABTS (**center**), and protein (**right**).

G7 and G5 had high frequency presences, with G7 being the closest genotype to the ideal for both TPC and ABTS. However, this trend was not observed for protein. This aligns with the strong positive correlation between TPC and ABTS and their weak negative correlation with protein. These data can contribute to the decision-making regarding the selection of genotypes and crop management practices, potentially offering increased health benefits through antioxidant and antiplatelet properties. These properties play a role in mitigating the development of various chronic diseases [87,88].

Table 8 displays the rank correlations among the statistical measures assessed across six environments. For TPC, the mean demonstrates a weak positive correlation with the measures of GGE. Among the statistical measures that had been estimated, strong positive correlations were recorded for σ_i^2 with s^2d_i and a weak positive correlation existed between *KR* and both σ_i^2 and s^2d_i . More correlations have been found among the metrics for ABTS. The mean had a moderate correlation with the measures of GGE, a strong correlation with Pi, and a weak one with σ_i^2 . GGE was positively correlated weakly with Pi and moderately with *KR*, which was moderately positively correlated with Pi. A moderate correlation was also observed between σ_i^2 and s^2d_i .

ТРС								ABTS							Protein						
All	Mean	GGE	ASVi	Pi	σ_{i}^{2}	$s^2 d_i$	K R	Mean	GGE	ASVi	Pi	σ_{i}^{2}	$s^2 d_i$	K R	Mean	GGE	ASVi	Pi	σ_{i}^{2}	$s^2 d_i$	K R
	G9	G7	G14	G5	G16	G3	G10	G5 ³	G7	G2 ³	G5	G2	G3	G3	G1 ³	G1	G13 ⁴	G1	G13	G13	G16
T	G5 ⁴ *	G10	G4	G3 ³	G10	G16	G5	$G7^4$	$G4^3$	G7	G3	G11	G2	G4	G16 ⁵	G9 ⁴	$G3^4$	G16	G16	G3	G9
lop	G7 ³	G9	G10	G13	G4	G10	G4	G13 ⁴	G13	G11 ³	G13	G12	G12	G11	G4	G16	G7	G4	G3	G16	G2
five	G15	G5	G16 ³	G4	G3	G5	G15	G3 ⁴	G5	G14	G4	G10	G11	G13	G9 ⁴	G7 ²	G12 ³	G9	G12	G12	G13
	G10 ⁵	G4 ⁶	G2	G7	G14	G4	G9	G16	G16	G10	G7	G4	G13	G7	G2	G14	G14	G10 ¹	G2	G9	G3
	G8 ⁵	G15 ²	G1	G6	G9	G15	G11	G10	G12	G6	G6	G15	G16	G14	G5 ⁵	G6	G6	G14	G6	G10	G10
Dattana	G3	G12	G7 ³	G16	G13	G13	G2	G14	G14	G16	G9	G16	G1	G15	G8 ⁴	G8	G1 ³	G3	G5	G5	G8
fine	G16	G3	G13 ⁴	G13	G7	G11	G12	G12 ³	G15 ³	G1	G12	G9	G5	G9	G15 ⁵	G12	G5	G8	G1	G1	G5
nve	G14	G2	G8	G8	G8	G7	G6	G2	G6 ⁶	G6	G2	G5	G9	G8	G12 ³	G5	G15	G12	G4	G4	G6
	$G2^3$	G6 ⁶	G6	G2	G6	G6	G8	G8 ³	G8	G9 ⁵	G8	G6	G6	G6	G6 ⁶	G15	$G4^3$	G6	G15	G15	G15

Table 7. Genotypes were categorized into top five and bottom five groups based on mean value, stability, and parametric measures. Top five and bottom five genotypes that occurred \geq 3 times within a group are presented in **bold**.

* Refers to the frequency of a genotype that occurs \geq 3 times within a group. Number equals the times of appearance.

Table 8. Spearman's rank correlation coefficients were computed between the statistics of mean TPC, ABTS, and protein with cultivar superiority, the GGE biplot rank, and parametric measures (*ASVi*, *Pi*, σ^2_{ij} , s^2d_{ij} , and *KR*).

		TPC	ABTS									Protein						
	GGE	ASVi	Pi	$\sigma^2{}_i$	$s^2 d_i$	K R	GGE	ASVi	Pi	$\sigma^2{}_i$	$s^2 d_i$	KR	GGE	ASVi	Pi	σ_{i}^{2}	$s^2 d_i$	KR
Mean	0.609 *	ns+	ns	ns	ns	ns	0.712 **	ns	0.785 ***	-0.506 *	ns	ns	0.865 ***	ns	0.915 ***	ns	ns	0.703 **
GGE	1	ns	ns	ns	ns	ns	1	ns	0.559 *	ns	ns	0.714 **	1	ns	0.659 **	ns	ns	0.773 **
ASVi		1	ns	ns	ns	ns		1	ns	ns	ns	ns		1	ns	0.903 ***	0.888 ***	0.582 *
Pi			1	ns	ns	ns			1	ns	ns	0.677 **			1	ns	ns	ns
σ_{i}^{2}				1	0.815 **	0.599 *				1	0.865 **	ns				1	0.988 **	0.718 **
$s^2 d_i$					1	0.587 *					1	0.685 **					1	0.696 **

*, **, *** significant at the p < 0.05, p < 0.01 and p < 0.001 levels of probability, respectively, + non-significant (ns)

On the other hand, GGE was strongly positively correlated with the mean and Pi for protein and moderately correlated with *KR*. Pi possessed a positive correlation with mean and ASVi, strong positive correlations with σ^2_i and s^2d_i and a weak positive correlation with *KR*. Moderate positive correlations were also observed between σ^2_i and both s^2d_i and *KR*, which were also correlated between them.

From statistic tools that consider both G and $G \times E$, the means (of TPC, ABTS, and protein) were positively correlated with GGE biplot analysis. Pi was positively correlated with the mean of ABTS and protein, while *KR* only with the mean of protein. Similar results about the effectiveness of the GGE biplot, Pi, and *KR* in handling $G \times E$ interaction, were found by other researchers for wheat yield [30,89,90].

4. Conclusions

This research evaluated the impact of G, E, and their interaction (G × E) on durum wheat's TPC and AC (DPPH, ABTS, and FRAP) in Mediterranean environments. High productivity Es exhibited reduced antioxidant quantities, whereas low productivity ones, growing under harsh conditions, had better profiles. Organic cultivation consistently yielded values falling between those of the other Es. In contrast, high values were observed in both TPC and AC for the late-sowing Es. G × E interaction was the most influential factor, significantly impacting TPC and AC. Notably, G7 emerged as a potential superior G, displaying high and stable TPC and AC across various crop management systems. These findings are significant as they represent one of the few comprehensive explorations focusing on the effects of different crop management systems on TPC and AC in durum wheat and identifying superior Gs possessing stable and high values among them. However, it is essential to note that the available results are solely derived from the specific cultivation year under investigation. Therefore, additional experimentation is necessary to establish more robust and stable conclusions.

Therefore, the results could advocate for policies supporting sustainable cultivation practices and incentivizing superior cultivars to enhance crop quality. Gs demonstrating high values and stability across conditions could potentially be adopted to improve durum wheat quality. Stakeholders should encourage farmers to adapt their farming strategies by considering the impact of $G \times E$ interactions on their crops' nutritional quality. Furthermore, promoting collaborations with industry stakeholders, such as food processors or manufacturers, to develop durum wheat-based products capitalizing on enhanced nutritional and antioxidant qualities is crucial. In conclusion, understanding these influences provides valuable insights into factors impacting durum wheat's nutritional and antioxidant quality, with potential implications for the agricultural industry and the production of healthier durum wheat-based products.

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