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Selection of Durum Wheat and SSR Markers for Organic Farming in Central Italy Using AMMI Analysis

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Abstract: Durum wheat is one of the main crops in the Mediterranean region, which is characterized as the hotspot of climate change, with large year-to-year weather fluctuations. Although chemical input reduction in agriculture is strongly demanded, as well as healthy food, there is still a lack of stable and high-yielding crop varieties specifically adapted for organic conditions. This study evaluates the performance of fifteen durum wheat varieties in terms of suitability for organic farming in central Italy and assesses the impact of the genotype–environment interaction (GEI) on productive and quality traits. Variety performance was evaluated in field experiments over four successive seasons. In addition, a genotypic diversity analysis of 38 microsatellites associated with traits important for organic farming was performed. The AMMI (additive main effects and multiplicative interaction) stability analysis revealed that the best and most stable genotype regarding quality traits, such as thousand-kernel weight, protein content, and test weight was the ancient variety, Senatore Cappelli. The most stable and high yield was determined for the Fuego, Iride, and Mv-Pelsodur genotypes. Moreover, SSR markers that could be used for plant breeding, targeting organic farming systems based on molecular markers and GEI results, were identified.

Keywords: durum wheat; organic farming; AMMI analysis; GEI analysis; SSR markers

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1. Introduction

Climate change, human population growth, and the negative impacts of intensive conventional agriculture have created interlinked problems that require solutions. The European Union (EU) has established many recommendations and directives aimed at reducing the excessive use of chemical inputs in agriculture [1,2]; however, it is well-known that a lower input of fertilizers, herbicides, and pesticides is the main factor limiting grain yield [3]. Although the EU Farm to Fork strategy seeks to reach at least 25% of the EU's agricultural land under organic farming by 2030 [2], there is still a lack of stable and high-yielding crop varieties specifically adapted to organic farming [4]. All the varieties used in organic agriculture are selected under conventional practices. Breeding high-yield crop varieties adapted to specific environments is a promising approach to address the problem of low yield under organic management [5,6]. However, significant year-to-year weather fluctuations during growing seasons require the selection of varieties with high and stable yields under changing conditions [7].

The Mediterranean basin is indeed one of the most vulnerable areas to climate change, with the highest increase in temperature and the lowest precipitation rates [8]. Durum wheat is a staple crop in the Mediterranean region, and its productivity is profoundly affected by rainfall and biotic (pests and diseases) and abiotic (drought, sunlight, cold, and salinity) stresses [8,9]. Italy is the largest producer of durum wheat in the European Union, with an average production of 4.26 million tons in the last decade [8]. The country is also the most important producer of organic durum wheat in the EU [10]. However, studies that compared conventional and organic durum wheat production in Italy reported that gross income for conventionally farmed durum wheat is still higher than organic production [3,11].

The selection of wheat varieties for organic farming is a complex process that involves several considerations, including high crop yield and quality, resistance to biotic and abiotic stresses, and weed competitiveness. Furthermore, a recent study has identified several important functional traits, including root nutrient absorption capacity, seedling vigor, and early growth rate, which can be used to select wheat varieties for organic farming [12].

Studying the genotype–environment interaction (GEI) is essential for understanding crop environmental adaptation and selecting varieties with high crop yield and quality suitable for a particular area [13]. Several statistical methods have been developed to analyze GEI data, including the additive main effects and multiplicative interaction (AMMI) model, the genotype main effect plus genotype-by-environment interaction (GGE) model, and the factor analytic model (FA) [14]. These models can help to identify the most stable and adaptable genotypes across different environments and provide insights into the underlying mechanisms of GEI [14]. The study by Mulugeta et al. [15] evaluated the performance and phenotypic stability of 385 Ethiopian durum wheat landraces and 35 cultivars. Their results showed that the AMMI and GGE models were able to identify stable and high-yielding landraces or cultivars that can be used for further breeding programs. Another five-year experiment performed in southern Italy evaluated the impact of N-deficient environments and organic farming on durum wheat yield, quality, and stability performances [16]. The results of this study revealed that the old cultivars may play a role in organic farming [16]. Iannucci et al. [17] recommended two varieties (Iride and Saragolla) for organic farming systems in Mediterranean areas, which showed high-stability responses and good seed yield under both conventional and organic farming.

Simple sequence repeat (SSR) molecular markers are highly effective molecular tools for genetic analysis and efficient selection in breeding programs [18]. They can help breeders to identify and select varieties with desirable traits, such as resistance to diseases, high yield and quality, which are essential for sustainable and profitable organic farming [19,20]. The combination of GEI analysis and SSR markers could improve durum wheat selection and breeding. Moreover, these markers could be used for marker-assisted selection (MAS) in plant breeding, targeting organic farming systems, which are not, to date, specially selected in combination with GEI and stability analysis.

Our study aims to evaluate the performance of 15 durum wheat varieties, in terms of suitability for organic farming in central Italy, using GEI analysis in four growing seasons (2018/2019, 2019/2020, 2020/2021, and 2021/2022). Variety performance was evaluated in the field experiment in terms of morphologic, productive, and quality traits. In addition, genotypic diversity analysis was carried out using 38 microsatellites associated with important traits for organic farming.

2. Materials and Methods

2.1. Experiment Location, Conditions, and Design

The four-year experiment was performed at the experimental farm at Tuscia University located in Viterbo, Italy (Figure 1) using a randomized block design with three replications. During the first year, 72 durum wheat accessions with different origins were evaluated. The plot size was 2.25 m^2, with a sowing density of 45 g per plot. The field, which had very low fertility, was fertilized with 7.7 t ha^{-1} of organic fertilizer (Fertile bio Ilsa), applied before sowing. From the first-year trial, 15 genotypes were selected according to two main traits, such as grain yield and protein content, and evaluated in the field trial for three more years. Plant material consisted of four durum wheat varieties from ICARDA (Azeghar2-1(56), Icajin 38(64), Sebatel2-45, HFN 94n), five varieties from Hungary (Mv-Makaroni, Mv-Pelsodur, Mv-Vekadur, MVTD15-19, MVTD20-19), three varieties from Italy (Cappelli called Senatore Cappelli, Iride, Saragolla), and three Fuego, Gibraltar, and Vulci varieties released by Syngenta Company and Sonno Agricoltura in the Mediterranean area (Table

S1). During the following three years, bigger plot sizes (7.8 m^2) with a sowing density of 234 g per plot were used.

Figure 1. Four-year durum wheat selection under organic conditions at the experimental farm field at Tuscia University, located in Viterbo, central Italy (42°25′12.0″ N, 12°04′48.0″ E, 326 m ASL).

2.2. Evaluation of Durum Wheat Characteristics during Vegetation Period

The durum wheat screening focused mainly on traits important to organic farming, including early vigor competitiveness with weeds, such as the ground cover (GC), growth habit (GH), and days to heading (HD) (Figure 2).

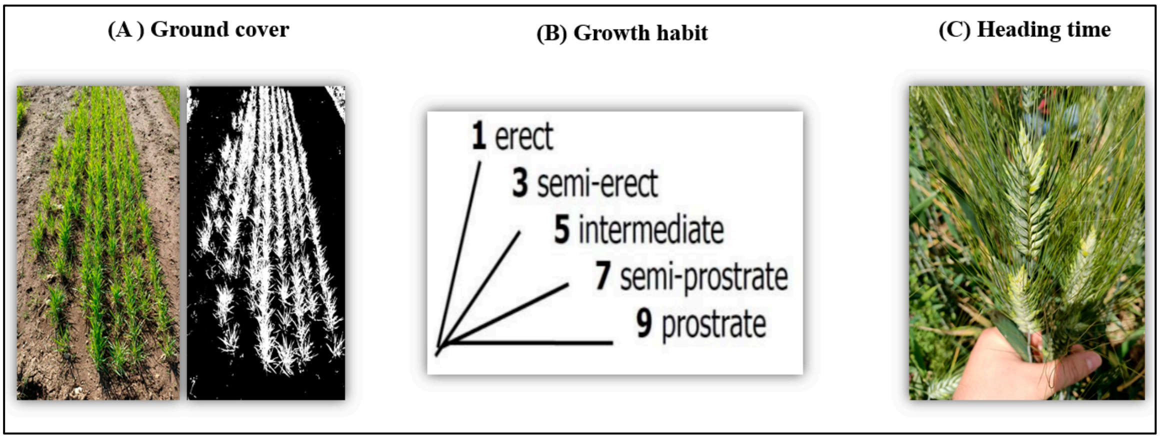

Figure 2. Evaluation of ground cover (**A**), growth habit (**B**), and days to heading (**C**).

Ground cover was evaluated using the Canopeo® mobile phone app and it is expressed in a percentage (Figure 2A). Canopeo® is an image analysis tool developed in the MATLAB programming language (MathWorks, Inc., Natick, MA, USA); it uses color values in the red–green–blue (RGB) system to analyze and classify all pixels in an image [21,22]. The result of the analysis is a binary image where white pixels correspond to the pixels that satisfy the selection criteria (green canopy) and black pixels correspond to the pixels that do not meet the selection criteria (not green canopy). The fractional green canopy cover ranges from 0 (no green canopy cover) to 1 (100% green canopy cover) [21]. The growth habit of durum wheat was determined at the beginning of stem elongation. A five-point (1–9) scale describes plant growth habits, where (1) means predominantly straight and (9) denotes curved leaves (Figure 2B). The evaluation of days to heading (HD), counted from the sowing date, can help identify varieties capable of finishing their life cycle in Mediterranean climate conditions before severe drought occurs (Figure 2C).

2.3. Postharvest Evaluation of Durum Wheat

Postharvest analysis for each plot consisted of the evaluation grain yield (GY, dt/ha), test or hectoliter weight (HLW, kg/hL), protein content (PROT, %), and thousand-kernel weight (TKG, g). The test weight (kg/hL) and protein (%) were analyzed using NIR spectroscopy analysis.

2.4. DNA Extraction, PCR Amplifications, and Gel Electrophoresis

To assess the genotypic diversity, 38 microsatellites associated with traits important for organic farming, i.e., 8 for biotic stress, 7 related to yield and quality traits, 10 root-related traits, and 13 for heading day and shoot length (Table S2), were chosen. The genomic DNA was extracted from fresh leaves per genotype using the extraction kit PureLink Plant Total DNA Purification kit (Invitrogen; Thermo Fisher Scientific, Waltham, MA, USA). The PCR amplifications were performed using 0.125 μL of GoTaqG2 DNA Polymerase (Promega, Madison, WI, USA), 5 μL of 5X Colorless GoTaq® Reaction Buffer, 0.5 μL of 10 μM dNTP mix, 2 μL of 10 μM/μL forward and reverse primer, 100 ng of gDNA, and sterile water for a total reaction amount of 25 μL. The PCR reaction was performed using SwiftMaxi Thermal Cyclers (Esco Technologies, St. Louis, MI, USA) in the following steps: an initial denaturation of 5 min at 94 °C followed by 35 cycles with 15 s at 94 °C, 20 s at different temperatures, according to the annealing temperature of each marker (Table S2), 20 s at 72 °C, and a final extension of 10 min at 72 °C. The electrophoresis of PCR products was carried out using a capillary electrophoresis device, QIAxcel Advanced Instrument (Qiagen, Hilden, Germania), using the QIAxcel DNA High-Resolution Kit. The allele analyses were performed using QIAxcel ScreenGel software 2.0.0.0 (Qiagen, Hilden, Germania).

2.5. Statistical Data Analysis

All statistical analyses were performed using R studio software [23,24]. All traits were evaluated for normality before undertaking further analyses. Data were analyzed as genotype means for all seasons. For each trait, a combined analysis of variance (ANOVA) using a mixed linear model for all the traits within and across the environments using the residual maximum likelihood algorithm (REML) through the lmer package [25] was performed. Then, the additive main effects and multiplicative interaction (AMMI) model [26], and the genotype main effect and genotype–environment interaction (GGE) model [27] were adopted and their biplots were constructed to analyze the genotype–environment interaction and stability. Both models were computed through multi-environment trial analysis using the metan package [28]. Stability analysis was performed using the metan package. The weighted average of absolute scores (WAASB) was computed using the best linear unbiased predictions for the GEI effects generated by a linear mixed-effect model [29]. This stability index can be used to jointly interpret stability and productivity.

It is estimated as follows:

$$\mathrm{WAASB_i} = \frac{\sum_{k=1}^{p} \left|\mathrm{IPCA}_{ik} \times \mathrm{EP}_k\right|}{\sum_{k=1}^{p} \mathrm{EP}_k}$$

where $\mathrm{WAASB_i}$ is the weighted average of absolute scores of the ith genotype; IPCA_{ik} is the score of the ith genotype in the kth interaction principal component axis (IPCA), and EP_k is the amount of the variance explained by the kth IPCA. The variety with the lowest WAASB value is considered the most stable. Simultaneous selection for the stability and performance is calculated as follows:

$$\mathrm{WAASBY_i} = \frac{(\mathrm{rG_i} \times \theta_\mathrm{Y}) + (\mathrm{rW_i} \times \theta_\mathrm{S})}{\theta_\mathrm{Y} + \theta_\mathrm{S}}$$

where $\mathrm{WAASBY_i}$ is the superiority index for the ith genotype with weights between performance and stability, θ_Y and θ_S are the weights assigned to the response variable and stability (WAASB). A biplot based on the WAASB and the response variable (WAASBY) is constructed to highly identify performance and stable varieties. The correlation matrix between all traits is conducted, and Pearson correlation coefficients are calculated using the corrplot function R package. Genetic analyses identifying the number of alleles, Shannon's information index, observed and expected heterozygosity, polymorphism information content, private alleles, and Nei's genetic distance are performed using GenAlEx [30] and GDA [31].

3. Results

3.1. Weather Conditions of Four Seasons in Central Italy

Weather conditions varied over the four seasons, particularly the distribution of precipitation from October to July, which covers the durum wheat growing cycle in central Italy (Table 1).

Table 1. The rainfall distribution according to the growth stage of durum wheat in central Italy for four seasons.

Season	Growth Stage	Rainfall, mm	Total, mm
2018/2019	Vegetation stage	476	817
	Stem elongation	129	
	Heading/Maturity	211	
2019/2020	Vegetation stage	372	642
	Stem elongation	114	
	Heading/Maturity	156	
2020/2021	Vegetation stage	687	909
	Stem elongation	123	
	Heading/Maturity	99	
2021/2022	Vegetation stage	159	258
	Stem elongation	65	
	Heading/Maturity	35	

Vegetation stage (from October to February), stem elongation (March and April), and heading/maturity (from May to July).

The 2021/2022 season was the driest season, with a total rainfall content of 258 mm, and drought appeared in the most important durum wheat development periods, such as vegetative and stem elongation stages. The seasons 2018/2019 and 2020/2021 were the wettest, with a total of 817 mm and 909 mm of precipitation, respectively. Moreover, in the 2020/2021 season, 75% of total rainfall fell in the vegetative growth stage. During the vegetative stage, the amount of precipitation was similar in the three seasons (2018/2019, 2019/2020, and 2021/2022) and reached about 60% of the total rainfall amount. In addition, in January of 2018/2019 and 2020/2021, crops were affected by frost temperatures (Figure S1).

The environmental index (EI) reflects the environmental effects on durum wheat performance in different years using negative and positive values (Table 2). The 2020/2021 season—with the highest amount of precipitation—had a negative impact on yield and all quality traits. Drier years (2019/2020 and 2021/2022) were significantly better for durum wheat grain yield (GY) than years with a lot of rainfall during the growing season. In addition, grain yield incurred losses due to the frost in 2018/2019 and 2020/2021. Thousand-kernel weight (TKG) and test weight (HLW) had the same trend, and in the first two seasons, they had a positive EI. The climate data analysis revealed that those seasons had more precipitation at the heading and maturity stages. Protein content (PROT) showed a positive value of EI only in the last season (2021/2022) when durum wheat suffered drought.

Table 2. The environmental index (EI) obtained as the mean of all varieties under all environments minus the grand mean.

Traits	Seasons			
	2018/2019	2019/2020	2020/2021	2021/2022
Grain yield (GY)	−2.67	5.51	−6.76	3.91
Test weight (HLW)	0.70	0.35	−1.01	−0.05
Thousand-kernel weight (TKG)	3.45	5.47	−4.84	−4.08
Protein content (PROT)	−2.21	−0.88	−0.77	3.85

3.2. The Analysis of Variance (ANOVA)

The analysis of variance (ANOVA) revealed a significant genotype (G) effect for all traits. In addition, a significant GEI for traits such as grain yield (GY), test weight (HLW), protein content (PROT), and thousand-kernel weight (TKG) were obtained (Table 3). The heading day (HD), ground cover (GC), and growth habit (GH) did not show significant GEI, accordingly.

Table 3. ANOVA significance of agronomical, yield, and quality parameters of 15 durum wheat genotypes over four growing seasons.

Source of Variance	df	HD	GY	HLW	TKG	PROT	GC	GH
(E)	3	$p < 0.001$	0.321	$p < 0.001$	$p < 0.001$	$p < 0.001$	0.013	$p < 0.001$
(G)	14	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	0.01	$p < 0.001$
GEI	42	0.137	$p < 0.01$	$p < 0.001$	$p < 0.001$	$p < 0.001$	0.186	0.176

HD—heading day; GY—grain yield; HLW—test weight; TKG—thousand-kernel weight; PROT—protein content; GC—ground cover (%); GH—growth habit.

The days to heading (HD) significantly differ across varieties; they are divided into three groups. The first group, which reached HD earliest, consisted of three varieties from ICARDA, such as Sebatel2-45, Icajin 38(64), Azeghar2-1(56), and two from Italy, Senatore Cappelli, and Iride (Figure 3).

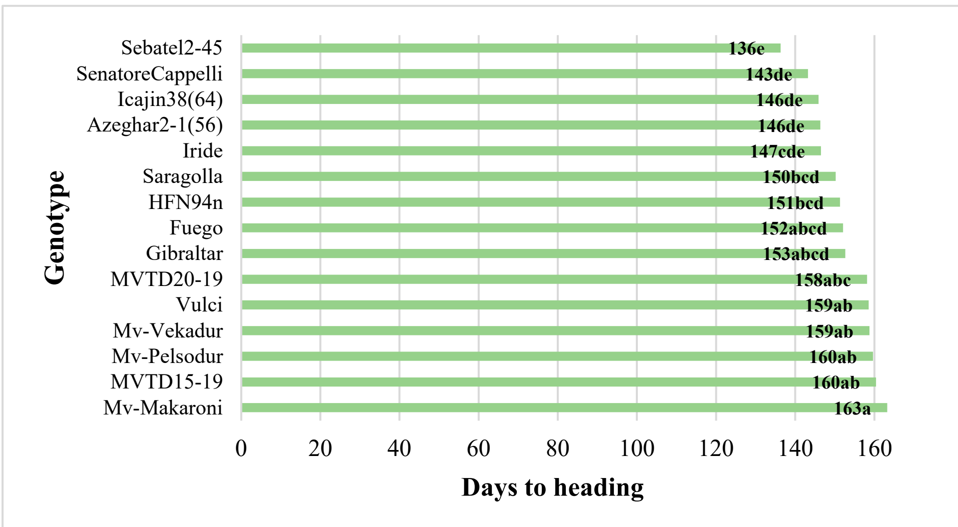

Figure 3. The days to heading for durum wheat. Values are a means of four growing seasons.

The second group, which showed the latest HD, consisted mostly of winter wheat varieties from Hungary, such as Mv-Makaroni, MVTD15-19, Mv-Pelsodur, and Mv-Vekadur, as well as Vulci from Italy. The difference between varieties that reached the heading days the earliest (Sebatel2-45) and the latest (Mv-Makaroni) was 27 days.

The ground cover that was evaluated using the Canopeo® mobile phone app and expressed in percentage ranged from 59.3% to 72.5% (Figure 4). The ground cover of the landrace Senatore Cappelli was significantly higher than four winter durum wheat varieties, e.g., MVTD15-19, Mv-Makaroni, Mv-Pelsodur, and Mv-Vekadur, and two Italian varieties, e.g., Vulci and Saragolla.

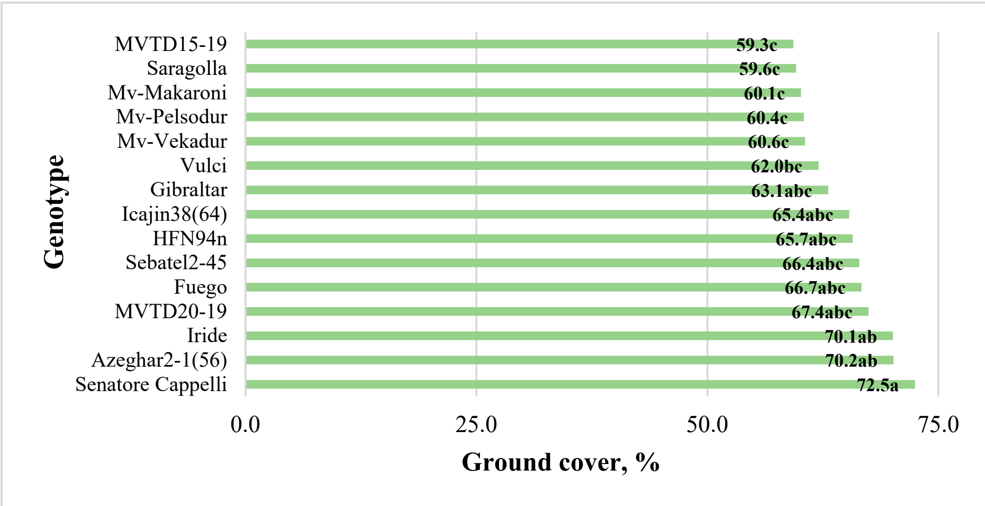

Figure 4. Ground cover (%), values are means of four growing seasons.

The growth habit (GH) significantly differed between varieties, GH varied from intermediate to erect, while semi-prostrate or prostrate GH has not been determined (Figure 4). Most varieties from ICARDA and Italy, such as Senatore Cappelli, Gibraltar, Saragolla, Iride, Sebatel2-45, Icajin 38(64), Azeghar2-1(56), and Fuego, had erected or semi-erected GH. The GH of varieties from Hungary, such as Mv-Makaroni, MVTD15-19, MVTD20-19, Mv-Pelsodur, Mv-Vekadur, Vulci from Italy, and HFN 94n from ICARDA, was intermediate between semi-erect and semi-prostrate (Figure 5).

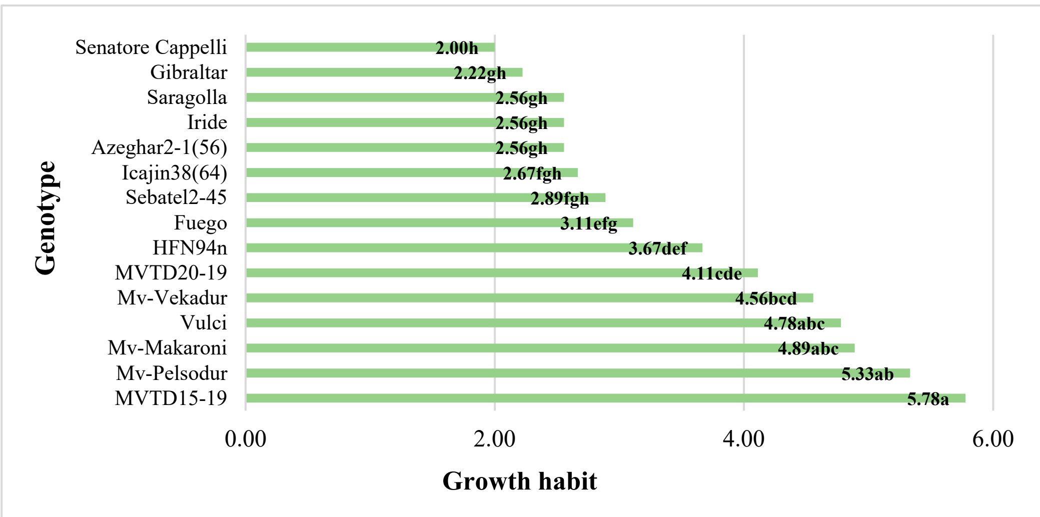

Figure 5. Growth habit (GH), (1–9) scale for plant growth habits, where (1) means predominantly straight and (9) curved leaves. Values are the means of four growing seasons.

3.3. Additive Main Effects and Multiplicative Interaction (AMMI)

The AMMI model, i.e., the additive main effects and multiplicative interaction (AMMI 1) biplot, was designed to present the first principal component (PCA1) and the main effects of TKG (Figure 6A).

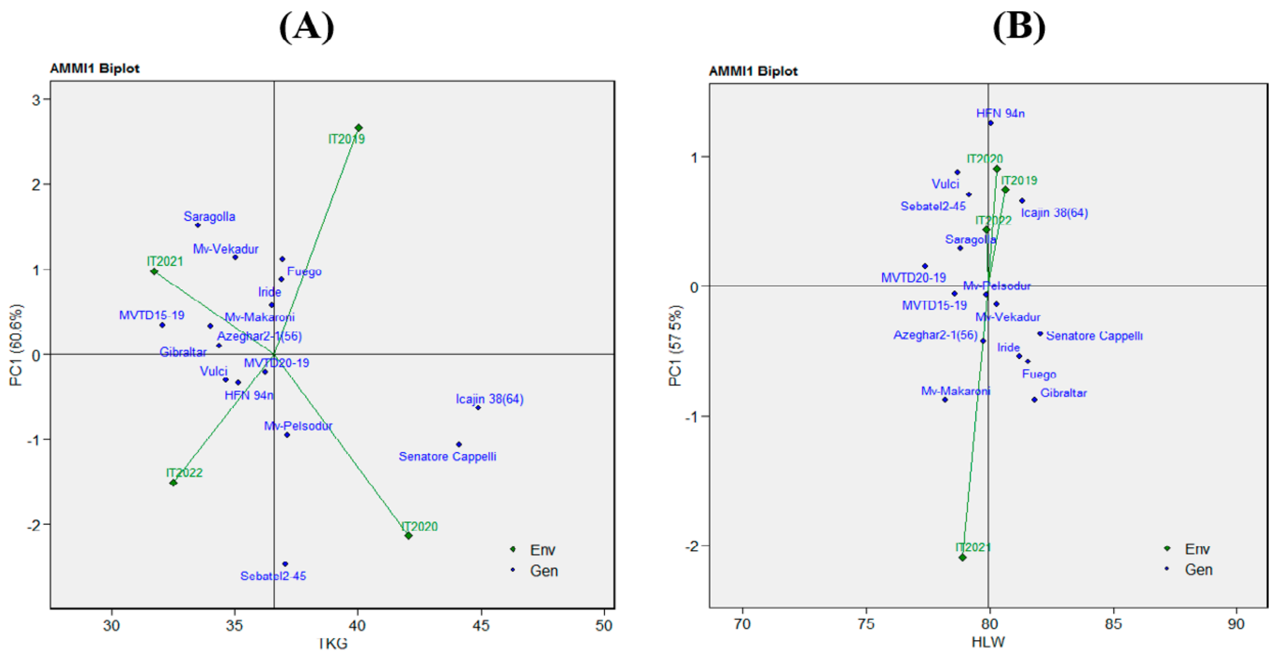

Figure 6. Additive main effects and multiplicative interaction (AMMI 1) biplots show the GEI of the 15 durum wheat varieties under 4 environments for (**A**) thousand-kernel weight (TKW) and (**B**) test weight (HLW).

The PCA1 scores for both environments and genotypes are plotted, where genotypes are shown in blue and seasons in green colors, with vectors connecting the environments with the origin. The PCA1 accounts for about 60.6% of the TKG variation across four seasons and 15 genotypes (Figure 6A). Regarding TKG, the seasons are grouped into two groups. On the negative PCA1 side, the wettest years (2018/2019 and 2020/2021) are plotted, and on the positive PCA1 side, the driest years are plotted (2019/2020 and 2021/2022). However, according to TKG values, the seasons are divided into two groups. The highest values (40 g and 42 g) of TKG were obtained in seasons 2018/2019 and 2019/2020, which had more precipitation at the heading and maturity stages (Figure S2). The highest TKG overall was obtained for Icajin 38(64) at 44.9 g under favorable, and Senatore Cappelli at 44.12 g under unfavorable environments. The PCA1 accounts for about 57.5% of the grain yield (HLW) variation for four seasons and 15 genotypes (Figure 6B). On the negative PCA1 side, the wettest years (2020/2021) are plotted, and on the positive PCA1 side, the other seasons (2018/2019, 2019/2020, and 2021/2022) are plotted. However, for TKG, the highest values (80.6 kg/hL and 80.3 kg/hL) of HLW were obtained in seasons 2018/2019 and 2019/2020 (Figure S2). The highest HLW overall was obtained in Senatore Cappelli (82 kg/hL), Gibraltar (81.8 kg/hL), and Fuego (81.6 kg/hL).

The PCA1 accounts for about 60.2% of the grain yield (GY) variation across four seasons and 15 genotypes (Figure 7A). The PCA1 accounts for about 60.2% of the grain yield (GY) variation across four seasons and 15 genotypes (Figure 7A). The highest GYs were obtained in the driest seasons, 2019/2020 and 2021/2022, with 46.58 dt/ha and 44.98 dt/ha, respectively (Figure S3). The highest GYs overall were obtained for Fuego 48.2 dt/ha, Iride 45.5 dt/ha, and Mv-Pelsodur 44.5 dt/ha. The results show that Iride produced the highest grain yield under the 2021/2022 season when durum wheat suffered from drought.

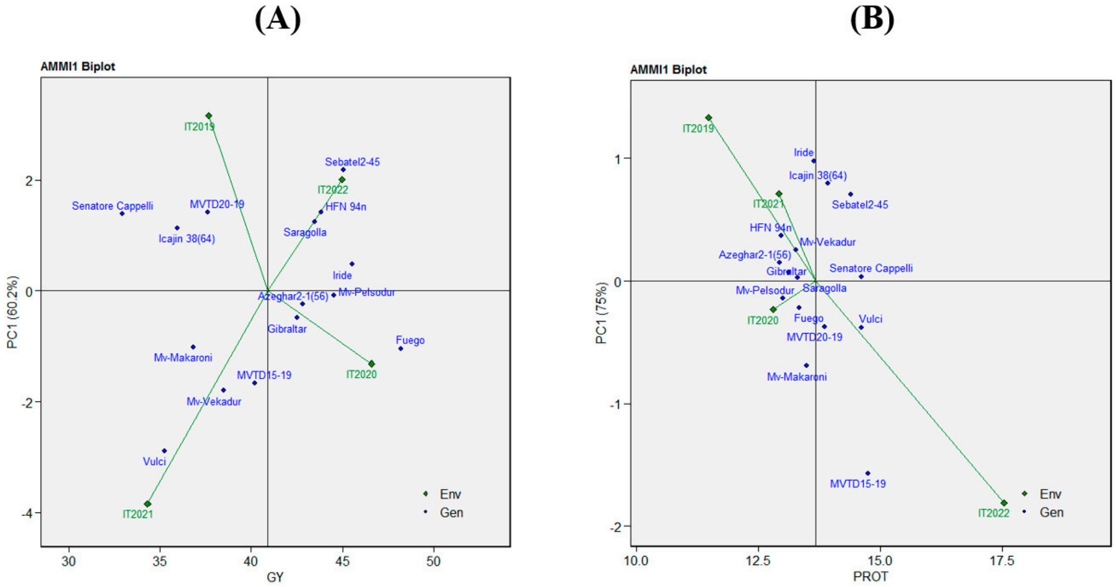

Figure 7. Additive main effects and multiplicative interaction (AMMI 1) biplots showing GEI of the 15 durum wheat varieties under 4 environments for (**A**) grain yield (GY) and (**B**) protein content (PROT).

In addition, drought in the 2021/2022 season had a positive effect on protein content (Figure 7B). The PCA1 accounts for about 75% of the protein content (PROT) variation across the four seasons and 15 genotypes. The highest PROT (17.53%) overall was obtained in the driest 2021/2022 season for Senatore Cappelli, following Vulci, and Sebatel2-45.

3.4. Assessment of Durum Wheat Performance Stability

A best linear unbiased prediction (BLUP)-based mixed model, WAASBY, was employed to identify stable varieties under organic farming conditions. Senatore Cappelli, Azeghar2-1(56), HFN 94n, MVTD19-20, Icajin 38(64), Saragolla, and Iride, had the highest ground cover stability among durum wheat varieties (Figure 8). Regarding growth habits, most of the stable varieties were from Hungary, such as MVTD 15-19, Mv-Pelsodur, Mv-Vekadur, Mv-Makaroni, HFN 94n, MVTD19-20, and Icajin 38(64).

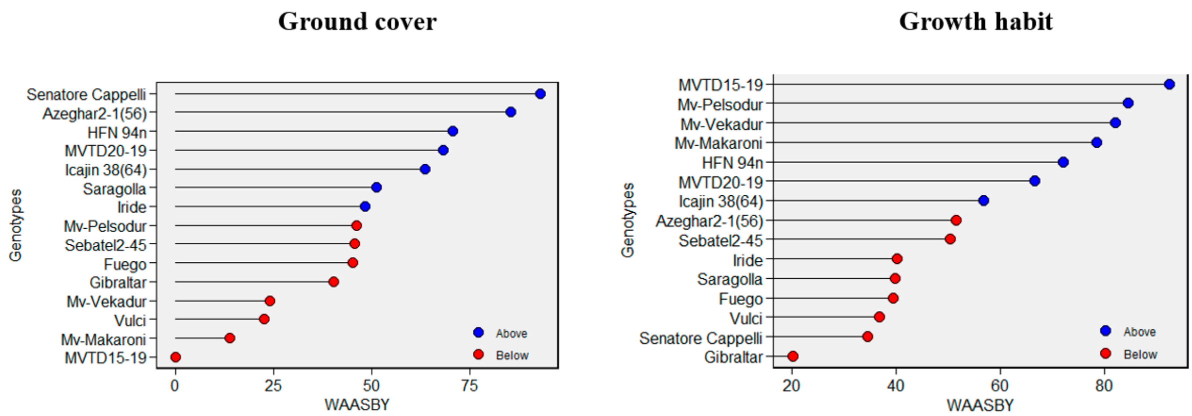

Figure 8. Estimated values of the weighted average of stability (WAASB) and the mean performance (Y) (WAASBY) for the ground cover and growth habit of 15 durum wheat varieties across the 4 environments. Circle points with different colors represent the values of the index above and below the grand mean.

Icajin 38(64), Senatore Cappelli, MVTD19-20, Vulci, HFN 94n, Mv-Makaroni, and Gibraltar were the most stable regarding thousand-kernel weight (Figure 9). However, regarding test weight, Mv-Pelsodur, Senatore Cappelli, Iride, Mv-Vekadur, Fuego, Gibraltar, Azeghar2-1(56), and Icajin 38(64) were the most stable varieties (Figure 9). The most stable grain yield was determined for Mv-Pelsodur, followed by Iride, Fuego, Azeghar2-1(56), Gibraltar, Saragolla, and HFN 94n (Figure 10).

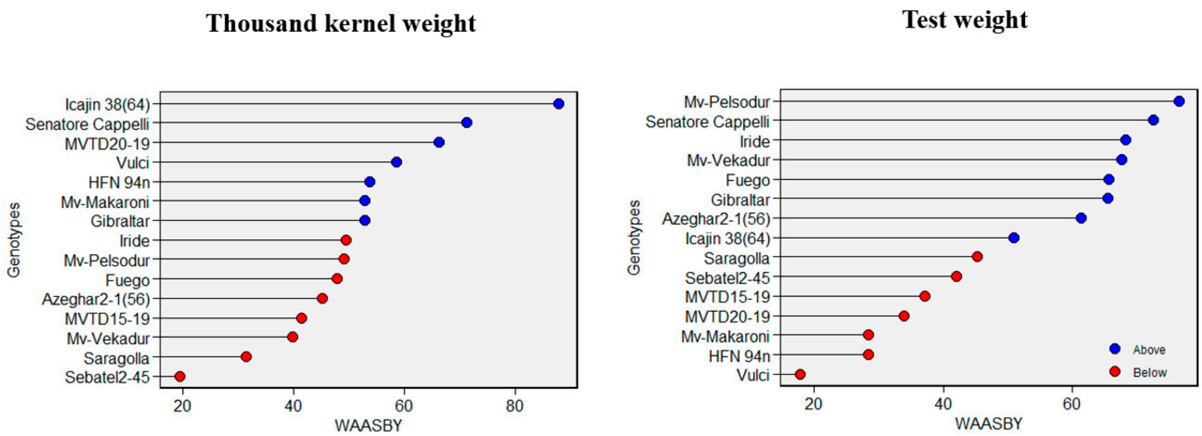

Figure 9. Estimated values of the weighted average of stability (WAASB) and the mean performance (Y) (WAASBY) for the thousand-kernel weight and test weight of 15 durum wheat varieties across the 4 environments. Circle points with different colors represent the values of the index above and below the grand mean.

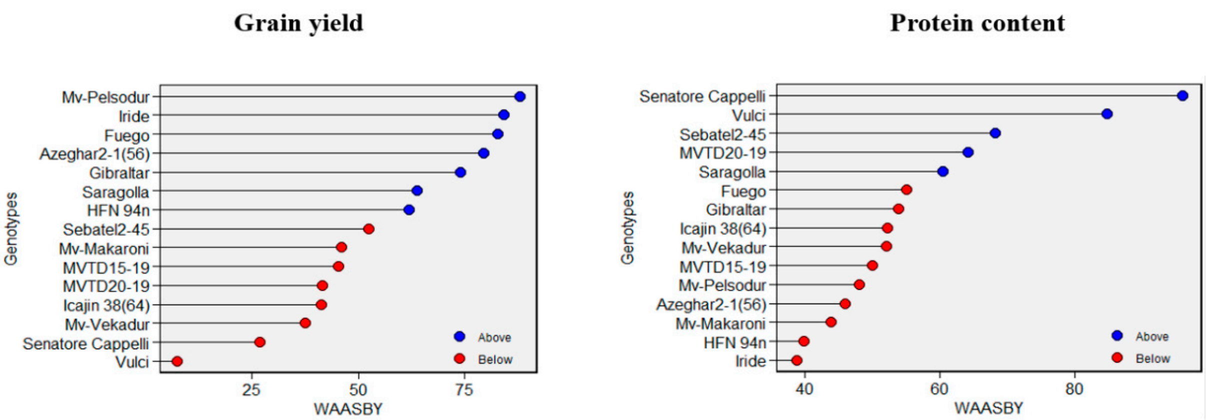

Figure 10. Estimated values of the weighted average of stability (WAASB) and the mean performance (Y) (WAASBY) for grain yield and protein content of 15 durum wheat varieties across the 4 environments. Circle points with different colors represent the values of the index above and below the grand mean.

Interestingly, the varieties that were not stable in terms of grain yield were the most stable for protein content, such as Senatore Cappelli, Vulci, Sebatel2-45, and MVTD19-20, except Saragolla, which was stable for both grain yield and protein content (Figure 10).

3.5. Genetic Diversity Based on SSR Markers

The 38 co-dominant SSR markers associated with traits important for organic farming were grouped into main groups, e.g., 8 markers associated with biotic stress [32–37], 7 related to yield and quality traits [38–40], 10 root-related traits [41,42], and 13 for heading day and shoot length [41,43] (Table S2). The number of alleles was very variable, ranging from 2 to 9 for wms493 and gwm637, with an average of about 4.5 alleles per marker (Table 4).

Table 4. Genetic diversity in durum wheat based on 38 co-dominant SSR markers.

Locus	Na	I	Ho	He	PIC
Grain yield and quality traits					
barc170	7	1.45	1.00	0.70	0.72
xgwm413	2	0.39	0.00	0.23	0.24
barc117	2	0.24	0.00	0.12	0.13
barc147	5	1.44	0.00	0.73	0.75
wms493	9	1.94	0.53	0.83	0.86
cfa2129	3	1.09	1.00	0.66	0.68
Biotic stress					
xwmc75	6	1.42	0.73	0.73	0.75
xgwm18	2	0.64	0.00	0.44	0.72
xbarc187	5	1.52	1.00	0.77	0.80
xgwm282	7	1.34	0.07	0.61	0.63
gpw7425	3	0.95	0.00	0.56	0.58
barc340.2	6	1.49	0.00	0.71	0.74
xbarc133	5	1.44	0.00	0.73	0.75
xgwm285	6	1.49	0.00	0.71	0.74
Root traits					
wms5	4	1.02	0.00	0.56	0.58
xgwm234a	2	0.24	0.00	0.12	0.13
xgwm234b	3	0.85	0.00	0.52	0.54
xgwm636	4	1.02	0.00	0.56	0.58
wms205	2	0.58	0.00	0.39	0.40
xcfa2257	6	1.50	0.20	0.74	0.77
xwmc727	4	0.94	1.00	0.56	0.58
xgwm637	9	1.95	0.00	0.82	0.85
gwm459	2	0.64	0.00	0.44	0.46
gwm499	4	1.22	1.00	0.66	0.68
Shoot length—heading day					
xgwm408	6	1.53	0.00	0.74	0.76
xgwm389	3	0.99	0.00	0.60	0.63
xcfa2086	5	1.20	0.00	0.60	0.62
xgwm566	3	0.86	0.00	0.50	0.51
xgwm99	3	1.09	0.07	0.66	0.68
xgwm573.2	5	1.55	0.93	0.77	0.80
xwmc505	4	1.17	0.00	0.65	0.67
xbarc134	3	0.93	0.00	0.55	0.57
xgwm155	5	1.30	0.80	0.68	0.71
xgwm46	5	1.36	0.00	0.70	0.73
xcfd50	5	1.53	0.00	0.77	0.80
xgwm332	8	1.58	0.93	0.72	0.74
gwm213	3	0.95	0.00	0.56	0.58
Mean	4.5	1.20	0.25	0.61	

Locus includes marker and in boald the traits coded by the markers, Na—no. of alleles, I—Shannon's Information Index = $-1 \times$ Sum (pi $\times$ Ln (pi)), Ho—observed heterozygosity, he—expected heterozygosity, PIC—polymorphism information content. Different locus of the same marker is highlighted as "a" and "b".

The observed heterozygosity (Ho) was 0 for most of the markers, with 24 in total, while 10 markers had high Ho and ranged from 0.53 to 1. Gene diversity, computed as polymorphism information content (PIC) values, ranged from 0.13 for gwm234a and barc117 to 0.86 for wms493.

In total, 63 private alleles were obtained, with 25 private alleles for six selected genotypes demonstrating high and stable performance under organic farming (Tables S3 and S4). Private alleles per genotype ranged from 2 (Fuego, and Iride) to 8 (Icajin 38(64)). Fuego, Iride, and Mv-Pelsodur had the highest and most stable grain yield (GY) under organic farming conditions. Together, they have 8 private alleles from six SSR markers, namely, barc117, xgwm636, wmc75, gwm46, wms493, and wmc727. Two markers (xgwm636 and wmc727) are associated with primary root length, two (barc117 and wms493) with yield and quality traits, gwm46 with shoot length and heading day, and wmc75 with resistance to powdery mildew. Regarding quality traits, S. Cappelli had the highest and most stable quality characteristics, including TKW, HLW, and PROT, and four private alleles. Wms5 and gwm637 are associated with root angle, cfa2086 is associated with heading date, and gwm285 is associated with resistance to *Pyrenophora tritici-repentis* (Died.). Icajin 38(64) had the highest and most stable TKW and eight private alleles. Three markers (wms5, cfa2257, and gwm637) are associated with root-related traits, three (cfd50, gwm332, and wmc505) with shoot length, gwm408 with flowering time, and wms493 with yield and quality traits. Vulci had a high and stable protein content and five private alleles; three of them (two for gwm282 and Barc3402) are associated with resistance to biotic stress, gwm332 with shoot length, and gwm637 with root angle.

The UPGMA phenogram of the Nei (1972) genetic distance cluster for 38 SSR markers discriminated the tested genotypes into three main clusters and four subclusters (Figure 11).

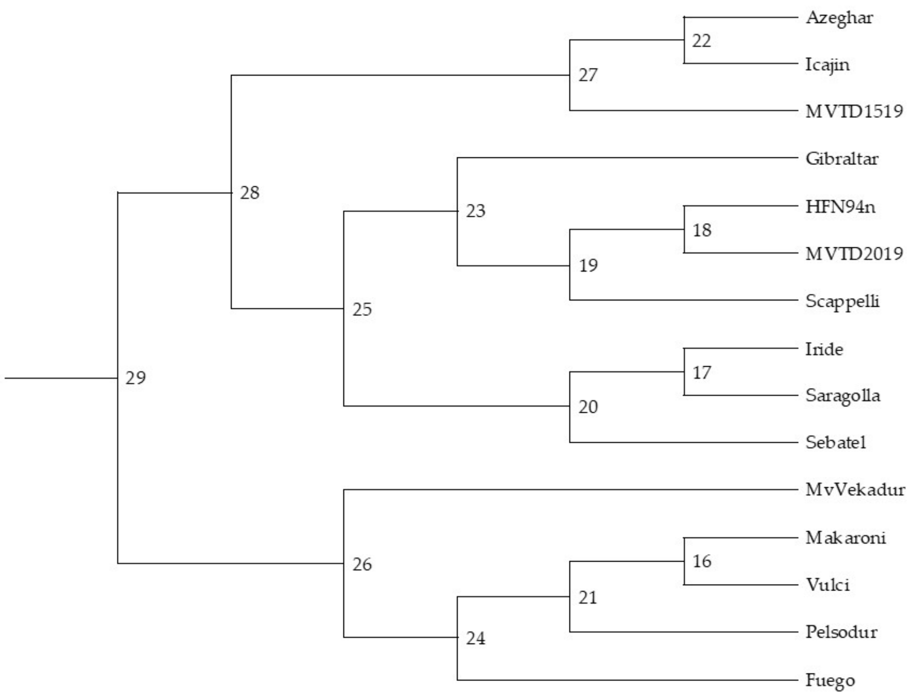

Figure 11. UPGMA phenogram of Nei's genetic distance among 15 durum wheat genotypes based on 38 SSR markers.

The reliability of the selected SSR markers was proven by the fact that Iride and its progeny Saragolla were grouped in one subcluster. One of the main clusters consisted of three varieties selected from Hungary, e.g., Mv-Vekadur, Mv-Makaroni, Mv-Pelsodur, and two varieties released in the Mediterranean area, Fuego and Vulci. The first subcluster consisted of two varieties from ICARDA (Azeghar and Icajin 38(64)), and MVTD15-19 from Hungary. The second subcluster consisted of four varieties with different origins, including the Italian Senatore Cappelli, Gibraltar released in the Mediterranean area, MVTD20-19 from Hungary, and HFN 94n from ICARDA. The third subcluster consisted of two related Italian varieties (Iride and Saragolla), and Sebatel2-45 from ICARDA.

4. Discussion

Most plant breeders focus on developing varieties with both a stable and high yield, as well as a wide adaptation, namely stability among geographically different regions with diverse climate conditions. However, due to weather fluctuations year to year, for farmers, it is more important to have a variety with high and stable yields in the given location. Our results indicate that climate conditions did not have a significant impact on grain yield (GY); however, in drier years, GY was higher. Albers et al. [44] reported that climatic impacts explain only a limited proportion of the modern German varieties' year-to-year grain yield variability. Moreover, it was shown that years and decades with cold springs and excessively wet summers were associated with unfavorable conditions for crop growth north of the Mediterranean region [45]. Protein content (PROT) had a positive environment index (EI) value only in the driest season when durum wheat suffered from drought. Several studies also revealed that moderately high temperatures and heat shocks during the grain-filling phase are associated with an increase in grain protein concentration in wheat [46–48]. Thousand-kernel weight (TKG) and test weight (HLW) had a positive environment index (EI) in seasons that had more precipitation at the heading and maturity stages. It was reported that drought and heat stresses have a very significant impact on durum quality, as indicated by a lower test weight, smaller kernels, lower milling yield, and higher semolina ash [49]. In our study, the most stable and highest TKG under organic farming was obtained for Icajin38 (64), and Senatore Cappelli, genotypes that had the heading date the earliest. A significant negative correlation was obtained between HD and TKG (−0.62), showing that early flowering genotypes were able to produce higher TKG under study conditions (Figure S4). Moreover, it was demonstrated that allele GS100 at Ppd-A1, which causes photoperiod insensitivity and results in early flowering genotypes, tended to increase TKW and yield [50].

Very important traits are genotype seedling vigor and early growth rate, which are related to being competitive against weeds and can help reduce the need for herbicides in organic farming systems [51]. Our results showed that genotypes with high and stable yields and quality parameters, such as Senatore Cappelli, Icajin, Iride, and Fuego, had significantly high ground cover (GC). Also, some positive correlations were obtained between GC and TKG (0.64), as well as GY (0.49) (Figure S4). A significant negative correlation was obtained between GC and HD (−0.62), showing that early flowering genotypes had higher green canopy cover at early stages.

Moreover, the same genotypes with high ground cover had erected or semi-erected growth habits (GHs), which had a significant positive correlation with protein content (PROT). The angle of the tiller during the juvenile growth stage is a crucial agronomic trait. The transition from a prostrate to semi-prostrate and erect growth habit is especially important as dense ground cover, as it can affect the crop's competitiveness against weeds, light availability for photosynthesis, and water evaporation reduction. Additionally, Marone et al. [52] showed that the erect canopy architecture of spring wheat can increase yield by 13%, resulting in 24% more grains per unit area. The study on the long-term effects of conventional and organic cropping systems, tillage management, and weather conditions on the yield and grain quality of durum wheat in the Mediterranean environment of central Italy revealed that weed control and nitrogen supply appear to be the main factors limiting yield production and grain quality [53].

From our investigated genotypes, we can provide information about stable and high-yielding varieties, or varieties with high and stable quality parameters, but not both in one genotype. A multi-location study found that grain yield and protein content are of major importance in durum wheat breeding, but their negative correlation has hampered their simultaneous improvement [54]. In our study, Fuego, Mv-Pelsodur, and Iride had high and stable GY and HLW, but low and unstable PROT and TKG. Iride was already recommended for organic farming systems in Mediterranean areas and showed high-stability responses and good seed yield under both conventional and organic farming [17]. Senatore Cappelli had high and stable quality characteristics, including PROT, TKG, and HLW, but low and unstable GY. Senatore Cappelli is an old variety, released in 1915, and is characterized by high and stable protein content, dietary fiber, and antioxidants, but with low yield under organic or low input agricultural systems [6,55]. Icajin 38(64) had high and stable TKG and HLW, but average and unstable GY and PROT. Vulci could be used as the candidate parental line for breeding for high and stable protein content.

SSR markers were used to reveal genetic diversity in durum wheat and identify markers associated with seed quality traits [20]. SSR markers were used to investigate the genetic diversity of Tunisian durum wheat varieties and identify the most informative markers for quality selection [20]. The results showed that SSR markers were highly informative and could be used to distinguish between the different varieties [56].

Based on the private allele data of genotypes with high and stable yields (Fuego, Iride, and Mv-Pelsodur), xgwm636, xwmc75, wms493, xgwm46, and xwmc727 markers can be used for selecting durum wheat for grain yield, except barc117, which has a very low PIC value (0.13). A marker with a high PIC value is more informative than a marker with a low PIC value, as it can distinguish between more alleles [57]. These markers can be used to select durum wheat varieties for high and stable yields under organic farming. Two of these markers are associated with root-related traits (xgwm636 and xwmc727), one for resistance to biotic stress (xwmc75), one for yield and protein content (wms493), and xgwm46 for shoot length and heading day.

For the selection of high and stable quality parameters, a total of 17 private alleles with 12 SSR markers were obtained. Markers can be divided into two groups; in the first group, markers with lower than 0.7 PIC values were assigned, including wms5 (0.58), xcfa2086 (0.62), xgwm282 (0.63), and xwmc505 (0.67). PIC values of 0.7 or larger are required to show good linkage. In our study, 8 SSR markers with PIC values higher than 0.7 were identified, e.g., barc3402 (0.74), wms493 (0.86), xcfa2257 (0.77), xcfd50 (0.8), xgwm285 (0.74), xgwm332 (0.74), xgwm408 (0.76), and xgwm637 (0.85).

From the evaluated SSR markers suitable for selecting organic farming, four were associated with resistance to biotic stress, e.g., leaf rust (barc3402), fusarium (xgwm282), powdery mildew (xwmc75), and *Pyrenophora tritici-repentis* (died.) (xgwm285). Five SSR markers that were associated with root-related traits (wms5, xcfa2257, xgwm637, xgwm636, and xwmc727) were identified. Wheat root traits can play a crucial role in determining plant performance under organic farming conditions in terms of abiotic stress tolerance [58,59] and nitrogen uptake efficiency [60]. The published study revealed that genotypes with higher root/shoot ratios responded more positively to compost additions compared to those with lower ratios, particularly in terms of plant aboveground biomass, nitrogen uptake, and soil nitrogen cycling, and exhibited greater plasticity in root morphology [60]. Another study suggested that vigorous root growth is a better indicator of early crop performance than shoot growth and that the development of genotypes with appropriate root traits might increase crop yields in infertile soils [61]. Moreover, from 48 QTLs detected for root system architecture, 15 overlapped with QTLs for agronomic traits and/or grain yield in two or more environments [42].

5. Conclusions

The combination of GEI analysis and SSR markers can improve durum wheat selection and breeding. Our investigated genotype analysis indicated that we could provide information about either stable and high-yielding varieties, such as Fuego, Iride, and Mv-Pelsodur, or Senatore Cappelli with high and stable quality parameters, but not both in a single genotype. However, 12 SSR markers were identified as suitable for durum wheat selection for stable and high yields, as well as meeting quality parameters under organic farming conditions. In addition, the determined genotypes can be useful candidate parental lines for breeding new varieties suitable for organic agriculture, characterized by stable, high yield, and quality parameters.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/agronomy14030458/s1, Table S1: Origin and Pedigree of durum wheat varieties; Table S2: The 38 co-dominant SSR markers associated with traits important for organic farming; Figure S1: Climate data for four seasons; Figure S2: Additive main effects and multiplicative interaction (AMMI 1) biplots show GEI of the 15 durum wheat varieties for the thousand-kernel weight (TKG), and the test weight (HLW) under 4 environments; Figure S3: Additive main effects and multiplicative interaction (AMMI 1) biplots show GEI of the 15 durum wheat varieties under 4 environments for grain yield (GY), and protein content (PROT); Table S3: The total Private alleles; Table S4: The Private alleles for six selected genotypes; Figure S4: Correlation matrix.

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