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Comparison of Agro-Phenological and Technological Traits in Advanced Durum Wheat Lines Differing in Reaction to Hessian Fly Infestation

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Abstract: The aim of this study was to compare agro-phenological and technological traits in advanced durum wheat lines differing in responses to Hessian fly larval attacks. A field experiment was carried out during the 2014/2015 cropping season at the main research stations of Marchouch and Tassaoute in a randomized completed block design. Twenty-two durum wheat genotypes comprising twenty advanced lines from the INRA breeding program and two commercial varieties were used in this study. Agro-phenological and grain quality determinations were concerned with plant height, grain yield, days to heading, protein content, wet gluten content, gluten strength, and yellow pigment content. The analysis of variance revealed significant environment, genotype, and genotype-by-environment (G $\times$ E) effects for the majority of the measured traits. A high level of heritability was also observed for grain yield (94.9%), followed by gluten strength (90.7%), yellow pigment content (79.3%), wet gluten content (77.79%), plant height (71.5), and protein content (70.02%); moderate levels of heritability were recorded for days to heading (65.8%). The data for Hessian fly resistance in both the field and infested greenhouse demonstrated three distinct group of genotypes: "resistant" with 4 genotypes, "susceptible without field escape to Hessian fly attack" with 16 genotypes, and "susceptible with field escape to Hessian fly attack" with 2 genotypes. The ANOVA analysis indicated the existence of significant differences between groups of genotypes for grain yield, days to heading, protein content, yellow pigment content, and gluten strength at the Tassaoute station, while at the Marchouch site, the differences were significant only for plant height and gluten strength. Protein content and wet gluten content revealed highly significant positive correlations, indicating the possibility of effective selection of the two traits simultaneously. Biplot analysis indicated that the first two Principal Components (PCs) accounted for 56.04% and 56.34% of the relationships between the genotypes and all of the attributes at the Marchouch and Tassaoute sites, respectively. The results of principal component analysis and Cluster based on agro-phenological and grain quality traits categorized the genotypes into three separate groups for the Marchouch site and four groups for the Tassaoute site. Individuals within each group are characterized by well-defined precocity, productivity, and technological criteria. The different behaviors of the lines studied in the present work are of great interest and can be exploited in breeding programs in order to improve agro-phenological and technological traits in durum wheat.

Keywords: breeding; Hessian fly; durum wheat; quality; yield; genetic resistance

1. Introduction

Durum wheat (*Triticum turgidum* subsp. *durum* (Desf.) is a tetraploid (AABB) cereal species that is an economically important crop worldwide. It is the preferred raw material for the production of pasta flour and semolina [1–3]. Durum wheats also represent the main sources of carbohydrates and proteins in the Mediterranean diet [4–6]. Considering that durum wheat is mainly grown under rainfed conditions in the Mediterranean basin, its productivity is profoundly affected by biotic and abiotic stress. Among these, the Hessian fly, *Mayetiola destructor* (Say), is one of the most destructive pests in wheat worldwide, particularly in North Africa, North Kazakhstan, South Europe, and North America [7,8]. In Morocco, this insect causes an average yield loss of 32% on durum wheat during dry years, and at worst, it may destroy entire crops, leading to a total loss of crop yield [9]. Antibiosis is the most desirable form of resistance in the case of host plant resistance to the Hessian fly, as it is controlled by a gene-for-gene relationship [10–12]. Damage to susceptible wheat caused by larval feeding manifests as a darker, almost blue-green foliage color, stunted growth, lodging, stem breakage, and either low seed production or a failure to produce seeds [10]. Several integrated pest management systems have been used to manage Hessian fly populations, combining biological, cultural, and chemical control methods [13]. However, growing resistant cultivars is the most effective approach to minimize Hessian fly damage, which is the most practical, environmentally sound, and economical option for Hessian fly control [8,12,14–16].

Durum wheat breeders all over the world are trying to develop high-yield and adapted genotypes to their specific environments [17]. This has resulted in the release of various commercial varieties. Obtaining high-yield genotypes of durum wheat for different environments, especially for Mediterranean rainfed areas, is considered difficult due to high genotype by environment interaction [13,18–20]. Consequently, improving resistance to abiotic and biotic stresses and increasing grain yield and adaptation are always targets for the genetic improvement of this crop. In Morocco, durum wheat germplasm has constituted and greatly contributed to the improvement of modern wheat varieties [13]. Characterization of these cultivars is essential for their evaluation, conservation, and end-use in breeding programs.

In the recent past, due to demands of high quality wheat in domestic and international markets, end-use quality has also become one of the major targets of various wheat breeding programs around the world [21,22]. Thus, farmers have become extremely interested in these characteristics that ensure premium prices at the time of sale, obliging breeders to often prefer them over other mere agronomic traits [23,24]. In Morocco, durum wheat gain quality is one of the key priorities of the INRA breeding programs [13,25]. There is growing interest in the use of available genetic resources in the development of new durum wheat cultivars with high grain yield and end-use quality as well as resistance or tolerance to diseases and Hessian fly larval attacks [25]. This development will require a deeper understanding of the available tools for measuring genetic variation and the strong influence of environmental conditions [26,27]. Hence, the determination of grain quality and agro-phenological traits in genotypes that present resistance or susceptible response to Hessian fly larval attack needs several measurements to yield results, since they are greatly affected by environmental conditions. From this perspective, the objectives of this research were (i) to study the effect of genotype (G), environment (E) and interaction genotype by environment (GEI) toward the variation in agro-phenological and technological traits across two locations amongst 22 durum wheat genotypes, and (ii) to understand the association between these traits with response to Hessian fly larvae attacks.

2. Materials and Methods

2.1. Plant Material, Locations, and Experimental Design

Twenty-two durum wheat genotypes were included in this study. Twenty advanced durum wheat lines were derived from the INRA breeding programs, which were selected for their responses to Hessian fly larval attack and their good performances in Moroccan conditions. Additionally, two Moroccan durum varieties 'Tomouh' and 'Cariouca' were used for their susceptibility to Hessian fly infestation. The full list of pedigrees is reported in Table 1. The experiments were conducted during the 2014/2015 cropping season in two experimental sites of the National Institute of Agricultural Research: Tassaoute (32°03′ N/7°24′ W) and Marchouch (33°60′ N/6°71′ W) locations, representing two contrasting regions of Morocco in term of climate, soil type, and agronomic practices (Table 2). The experimental design was a randomized completed block with four replicates. Each entry was planted in plots of six rows of five meters in length with an inter-row spacing of 0.3 m. Sowing was carried out by hand at a depth of approximately three to five cm.

Table 1. Pedigree and list of 22 durum wheat genotypes used in this study and their simplified code.

Entry	Pedigrees	Type
G1	Marzaq/DON MTD 14	Advanced line
G2	Ourgh/DON MTD 14	Advanced line
G3	ICAMOR-TA04-40/Ourgh (Tarik)	Advanced line
G4	Aus 27 689/ICAMOR-TA04-9	Advanced line
G5	LD357E/2*TC60//JO69/3/FGO/4/GTA/5/SRN-1/6/	Advanced line
G6	SOOTY-9/RASCON-37//SOMAT-3,1	Advanced line
G7	SOOTY-9/RASCON-37//GUAYACAN INIA	Advanced line
G8	SILK-3/DIPPER-6/3/AC089/DUKEM-4//5*AC089/4/	Advanced line
G9	PLATA-6/GREEN-17/3/CHEN/AUK//BISU*2/5/	Advanced line
G10	CNDO/PRIMADUR//HAI-OU-17/3/SNITAN/4/SOMAT	Advanced line
G11	LD357E/2*TC60//JO69/3/FGO/4/GTA/5/SRN-1/6/	Advanced line
G12	CNDO/VEE//7*PLATA-8/3/GUANAY/10/PLATA-10/6/	Advanced line
G13	RANCO//CIT71/CII/3/COMDK/4/TCHO//SHWA/MALD	Advanced line
G14	AINZEN-1/6/2*CMH82A,1062/3/GGOVZ394//SBA81	Advanced line
G15	STORLON/3/RASCON-37/TARRO-2//RASCON-37/4	Advanced line
G16	PLATA-7/ILBOR-1//SOMAT-1/3/CABECA-2/PATKA	Advanced line
G17	AJAIA/LOTUS-4/3/SOMAT-3/PHAX-1//TILO-1	Advanced line
G18	RANCO//CIT71/CII/3/COMDK/4/TCHO//SHWA/MALD	Advanced line
G19	ARMENT//SRN-3/NIGRIS-4/3/CANELO-9,1/4	Advanced line
G20	GODRIN/GUTROS//DUKEN/3/THKNEE-11/4/DUKEM-	Advanced line
G21	CARIOUCA: Oum Rabia 6 ICARDA	Cultivar
G22	TOMOUH	Cultivar

Table 2. Characteristics of the experiment sites used for field testing during 2014/2015.

Site Name	Climate	Annual Rainfall	Agro-Ecological Zones	Site Characteristics
Marchouch	Mediterranean temperature	449 mm	Mid favorable rainfed mid-season length	Central region, heat, drought, leaf rust, root rot, tan spot, medium infestation by Hessian fly.
Tassaoute	Hot steppe	250 mm	Fully irrigated south. Short season	South central, dry land, heat, leaf stem and stripe rusts, tan spot, lodging, medium infestation by Hessian fly.

2.2. Resistance/Susceptibility Screening

Testing for Hessian fly resistance was conducted in a greenhouse with reared Hessian fly populations. The flies were taken from a culture maintained at the Entomology's laboratory of INRA-CRRA-Settat, Morocco. Advanced wheat lines were grown in standard wooden fat (0.54 m × 0.36 m × 0.08 m) containing a mixture of 1/3 peat and 2/3 soil, at a rate of 10 rows per flat and 20 seeds per row. Each wheat line had 15 plants, which were grown in a greenhouse at 20 °C (daytime) and 18 °C (nighttime) with a photoperiod of 16:8 (L:D) h. At the one- or two-leaf stage, they were infested by about 200 newly emerged and mated females of Hessian fly under cheesecloth tent. The flat was caged with a cheesecloth tent and releases were made for two days and then removed. Three weeks after infestation (equivalent to 16 d after the initial Hessian fly larval attack), resistant and susceptible plants were categorized and recorded.

The combination of data from both greenhouse and field experiments allowed us to classify the entries as "resistant" or "susceptible without field escape to Hessian fly attack" and "susceptible with field escape to Hessian fly attack".

2.3. Productivity and Grain Quality Traits Assessment

2.3.1. Agro-Phenological Traits

The number of days to heading was recorded when about half of the culms showed emerging spikes [28]. Plant height was measured during the maturation stage, when the maximum height level was achieved. The lines of the plot were harvested mechanically and evaluated for grain yield and grain quality traits.

2.3.2. Technological Parameters

Harvested grains from two experiment stations were used for laboratory analysis of technological traits. Whole grain flour samples were obtained with a whole mill grinder (Udy-Cyclone 0.5 mm sieve). Six quality parameters were considered in this study.

Gluten Strength by SDS-Sedimentation Test

Gluten strength was determined via the SDS-sedimentation test as described in Moroccan standard method [29]. Samples of milling fractions of 6.3 g were suspended in Bromophenol blue solution (1%); the protein hydration was facilitated by addition of 50 mL of a mixture of SDS and lactic acid solution. After a series of agitations, sedimentation volume was read. Results are expressed in millimeters in the interface line between solid and liquid into a measuring cylinder.

β-Carotene Pigment Content (ppm)

The micro-procedure for measuring total yellow pigment was determined according to the AACC 14–50 modified method [30] using water saturated butanol (WSB) in (8:2) ratio as a solvent [31]. Samples of milling fractions of 0.25 g were extracted with 1.25 mL water saturated butanol (WSB) in 1.5 mL capacity micro-centrifuge tubes. The mixture was vortexed for 5 s and then rested for 16–18 h in the dark at room temperature, and vortexed for 15 s before centrifugation for 10 min at 10,000 rpm. Absorbance of supernatants was measured at 440 nm using a spectrophotometer and converted into yellow pigment concentration using the following formula:

$$C = 30.1 \times ABS \times \frac{100}{(100 - MC)}$$

where, C: Yellow pigment concentration; ABS: absorbance; MC: moisture content.

Moisture Content, Protein Content and Wet Gluten Content

Moisture content, protein content, and wet gluten content were measured using a Chopin Technologies Infraneo near-infrared spectroscope (NIRs) previously calibrated for quality traits.

2.4. Statistical Analysis

The genotypes were assessed in two different agro-climatic conditions as described in Table 2. All analyzed data were reported as means ± standard deviation and coefficients of variation (%). Significant difference among groups of genotypes was evaluated through an analysis of variance (ANOVA) using statistical software SPSS version 20.0 (2007). With the same software, assessment of the effect of genotype, environment and genotype by environment interaction (G × E) were carried for all traits. However, correlations, principal component analysis, and cluster analysis (Ward's method and Euclidean distance method) were applied through statistical software XLSTAT version 2016. Broad-sense heritability was calculated as follows:

$$H^2 = \frac{\delta G}{\delta G + \frac{\delta G \times E}{nE} + \frac{\delta e}{nE \times nr}}$$

where, δG: sum square (SS) value for genotype; δG × E: sum square of the G × E interaction; δe: sum square of the error; nE: number of environments; nr: number of replicates.

3. Results

3.1. Response to Hessian Fly Infestation

The data for Hessian fly resistance in both field and infested greenhouse revealed that only four of the twenty advanced durum lines had field resistance and had dead first instar larvae in the greenhouse. These lines confirmed their resistance to Hessian fly infestation and can be described as "resistant lines". The remaining 16 durum lines were phenotypically resistant in the field and susceptible in the greenhouse. These lines can be described as "susceptible with escape to Hessian fly larvae attack", whereas two Moroccan commercial varieties, Cariouca and Tomouh, were susceptible in both field and greenhouse experiments. Further analysis was carried out considering these three groups as "resistant", "susceptible with escape to Hessian fly larval attack", and "susceptible with no escape to Hessian fly larval attack" (Table 3, Figure 1).

Table 3. Status of Hessian fly resistance in field and infested greenhouse conditions.

Entry	Reaction to Hessian Fly Attack	Entry	Reaction to Hessian Fly Attack
G1	Resistant	G12	Susceptible with escape
G2	Resistant	G13	Susceptible with escape
G3	Resistant	G14	Susceptible with escape
G4	Resistant	G15	Susceptible with escape
G5	Susceptible with escape	G16	Susceptible with escape
G6	Susceptible with escape	G17	Susceptible with escape
G7	Susceptible with escape	G18	Susceptible with escape
G8	Susceptible with escape	G19	Susceptible with escape
G9	Susceptible with escape	G20	Susceptible with escape
G10	Susceptible with escape	G21	Susceptible with no escape
G11	Susceptible with escape	G22	Susceptible with no escape

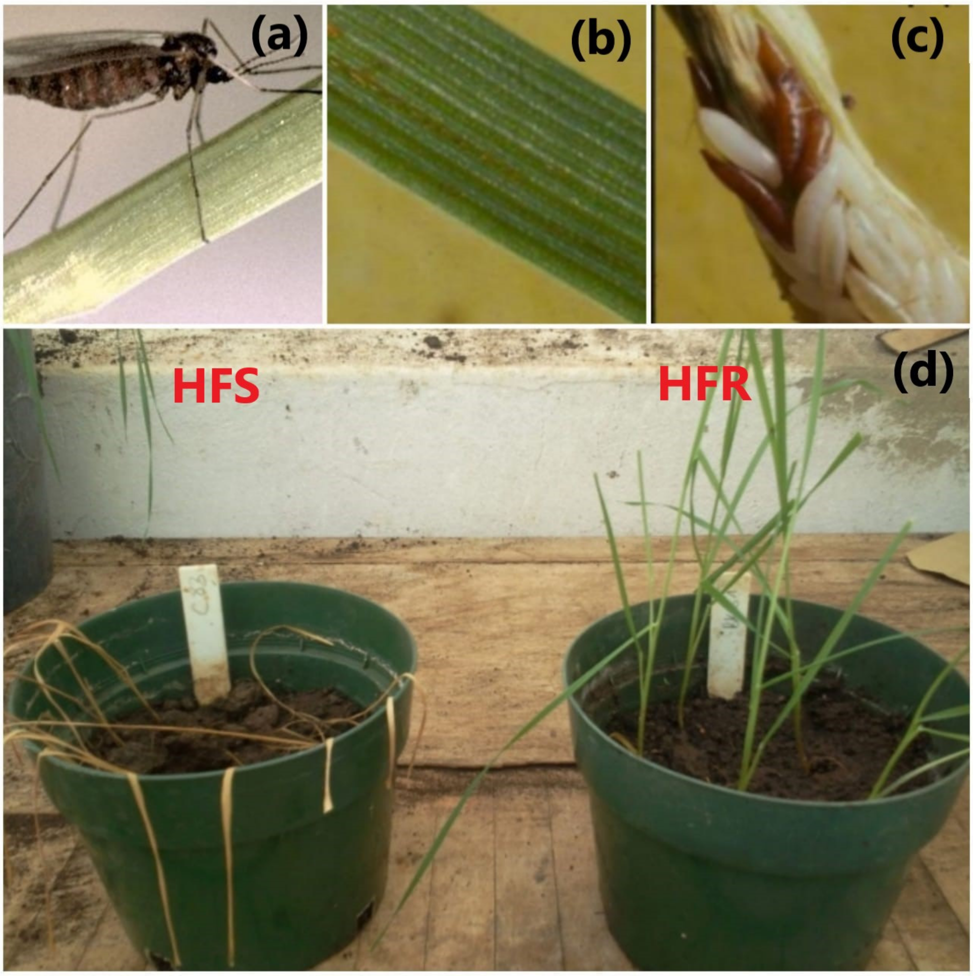

Figure 1. Effect of the Hessian fly (*Maytiola destructor* (Say)) on durum wheat. (**a**) Hessian fly adult. (**b**) Eggs on upper leaf. (**c**) Larvae (white maggots) and puparia on a stem. (**d**) The clear distinction between susceptible and resistant durum genotypes in seedling screening under greenhouse conditions. HFR: Hessian-fly-resistant genotype; HFS: Hessian-fly-susceptible genotype.

3.2. Genotype and Environment Effects and Their Interaction (GE), and Heritability (H)

An analysis of variance was run for grain yield (GY), plant height (PH), days to heading (DtH), wet gluten content (WGC), yellow pigment content (YPC), protein content (PC), and gluten strength (SDS-test) over two studied environments to confirm the significant effect of genotype, environment, and their interaction for all sources of variation ($p < 0.001$). However, there was not a significant contribution of environmental factor term to phenotypic variation observed among genotypes for protein content trait ($p = 0.676$). Our results indicate that the most heritable trait was grain yield (GY) with a value of 0.949, and the least heritable was days to heading (DtH) with 0.658. High level of heritability was also determined for grain yield (94.9%), followed by gluten strength (90.7%), yellow pigment content (79.3%), wet gluten content (77.79%), plant height (71.5), and protein content (70.02%); and moderately level for days to heading (65.8%) (Table 4). Based on data from two experiment sites, the three groups of genotypes showed significant differences ($p < 0.05$) for all quantitative traits, as was proved using the ANOVA (Table 5). For the Tassaoute site, significant differences between groups were observed for grain yield ($p = 0.033$), days to heading ($p = 0.010$), protein content ($p = 0.048$), yellow pigment content ($p = 0.021$), and gluten strength ($p = 0.001$). However, for the Marchouch experiment site, the differences between groups were statistically significant only for plant height ($p = 0.033$) and gluten strength ($p = 0.000$) (Table 4).

Table 4. Analysis of variance and heritability for seven traits in twenty-two durum wheat genotypes.

Fixed Term	df.	D Statistic	*p* Value	H2
PH				
Genotype (G)	21	12.206	0.000	0.7154
Environment (E)	1	49.088	0.006	
G × E	21	14.452	0.000	
GY				
Genotype (G)	21	2.844	0.001	0.949
Environment (E)	1	162.681	0.001	
G × E	21	3.012	0.000	
DtH				
Genotype (G)	21	148.026	0.000	0.658
Environment (E)	1	49,823.473	0.000	
G × E	21	160.956	0.000	
PC				
Genotype (G)	21	2.686	0.001	0.7002
Environment (E)	1	0.213	0.676	
G × E	21	2.485	0.003	
WGC				
Genotype (G)	21	11.578	0.000	0.7779
Environment (E)	1	182.952	0.001	
G × E	21	7.166	0.000	
YPC				
Genotype (G)	21	18.458	0.000	0.7933
Environment (E)	1	20.402	0.020	
G × E	21	19.529	0.000	
GS				
Genotype (G)	21	56.546	0.000	0.907
Environment (E)	1	0.377	0.583	
G × E	21	36.255	0.000	

Significance level $p \leq 0.05$. df.: degree of freedom; GY: grain yield; PH: plant height; DtH: days to heading; PC: protein content; WGC: wet gluten content; YPC: yellow pigment content; GS: gluten strength (SDS-test); H2: broad-sense heritability.

Table 5. ANOVA data for seven characters in twenty-two durum wheat genotypes across the two study sites.

Sites	Trait	df.	F	*p*-Value
Marchouch	GY	2	1.498	0.249
	PH	2	4.090	0.033
	DtH	2	0.061	0.941
	PC	2	0.393	0.681
	WGC	2	0.260	0.774
	YPC	2	3.182	0.064
	GS	2	13.131	0.000
Tassaoute	GY	2	4.093	0.033
	PH	2	0.036	0.965
	DtH	2	5.999	0.010
	PC	2	3.573	0.048
	WGC	2	0.609	0.554
	YPC	2	4.743	0.021
	GS	2	11.403	0.001

Significance level $p \leq 0.05$. df.: degree of freedom; GY: grain yield; PH: plant height; DtH: days to heading; PC: protein content; WGC: wet gluten content; YPC: yellow pigment content; GS: gluten strength (SDS-test).

3.3. Comparison of Groups of Genotypes According to the Measured Traits

The comparisons of mean values and coefficients of variation (%) within each group in both environments are summarized in Table 6. Comparing mean values for all traits revealed a considerable variation among groups in two experiment sites. Our results indicated that some traits were more variable than others. The highest variation (%) was obtained for gluten strength (SDS-test), yellow pigment content, and grain yield, whereas the lowest variations were recorded for days to heading and plant height.

Table 6. Arithmetic mean, minimum and maximum values, and coefficients of variation (%) for the seven traits of the three group of genotypes across two experiment sites.

Sites	Traits	Group of Durum Wheat Genotypes									Variation %
		Susceptible with Escape to Hessian fly Larval Attack			Susceptible with No Escape to Hessian Fly Larval Attack			Resistant Genotypes to Hessian Fly Larval Attack			
		Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	
TST	GY	2958.75	4144.75	3552.09 ± 77.44	3109.00	3149.88	3129.43 ± 20.43	2793.00	3614.00	3131.37 ± 172.73	18.65
	PH	80.00	100.00	87.42 ± 1.426	85.63	86.88	86.25 ± 0.625	76.25	100.00	86.87 ± 5.06	5.67
	DtH	111.25	118.00	116.00 ± 0.43	112.13	112.88	112.50 ± 0.37	108.25	115.00	112.81 ± 1.59	3.68
	PC	9.88	13.13	11.73 ± 0.19	12.90	14.10	13.50 ± 0.60	10.70	13.93	12.18 ± 0.663	14.23
	WGC	23.18	34.67	29.54 ± 0.68	30.00	32.00	31.00 ± 1.00	26.21	37.25	31.22 ± 2.27	13.88
	YPC	3.75	11.99	8.16 ± 0.50	5.68	6.01	5.84 ± 0.16	3.75	6.72	5.21 ± 0.73	46.14
	GS	39.60	53.20	46.48 ± 0.91	27.70	32.20	29.95 ± 2.25	31.40	48.60	43.15 ± 4.06	40.20
MCH	GY	2552.10	4290.08	3755.06 ± 105.45	3877.21	4252.34	4064.77 ± 187.56	1.00	3615.95	2605.60 ± 870.63	16.58
	PH	86.25	97.50	91.71 ± 0.88	95.63	101.88	98.75 ± 3.12	92.5	96.25	93.75 ± 0.88	7.12
	DtH	102.00	113.00	106.56 ± 0.97	106.00	106.50	106.25 ± 0.25	102.00	113.00	107.25 ± 2.39	3.36
	PC	10.56	12.84	11.90 ± 0.16	11.50	12.00	11.75 ± 0.25	11.31	13.19	12.20 ± 0.42	5.74
	WGC	20.75	28.42	25.08 ± 0.56	24.00	26.00	25.00 ± 1.00	23.18	28.83	25.97 ± 1.22	7.33
	YPC	5.99	11.51	8.12 ± 0.40	6.51	7.06	6.78 ± 0.27	3.42	8.56	5.87 ± 1.05	31.32
	GS	41.40	57.60	48.02 ± 1.04	30.40	34.00	32.20 ± 1.80	25.00	46.80	37.25 ± 4.53	34.98

GY: grain yield; PH: plant height; DtH: days to heading; PC: protein content; WGC: wet gluten content; YPC: yellow pigment content; GS: gluten strength (SDS-test); MCH: Marchouch; TST: Tassaoute.

From the agro-phenological perspectives, as shown in Table 6, plant height and days to heading indicated a low variation in both growth environments. At the Marchouch site, plant height ranged from 86.25 to 101.88 cm, and the tallest genotypes were those of susceptible group with an average of (98.75 cm ± 3.12). At the Tassaoute site, it varied between 76.25 and 100.00 cm, with the susceptible group showing the highest value (87.42 cm ± 1.426) compared to other group of genotypes. In the same case, days to heading varied between 102.00 and 113.00 days at the Marchouch site and between 108.25 and 118.00 days at the Tassaoute site, where the susceptible group presented a short time of spike appearance with mean values of (112.50 days ± 0.37) and (106.25 days ± 0.25) at the Tassaoute and Marchouch sites, respectively. The range of variation across all 22 tested durum wheat genotypes for grain yield was limited in both growth conditions; it ranged from 2552.10 to 4290.08 g and from 2793.00 to 4144.75 g at the Marchouch and Tassaoute sites, respectively. The highest mean values for yield were recorded for the susceptible group (4064.77 g ± 187.56) and for the escaped group (3552.09 g ± 77.44) at the Marchouch and Tassaoute sites, respectively.

In terms of the technological traits, the data on yellow pigment contents and gluten strength (SDS-test) indicated the highest variation in the testing sites. The highest mean values for yellow pigment trait were obtained for the escape group, ranging from 3.42 to 11.51 ppm at the Marchouch site and from 3.42 to 11.51 ppm at the Tassaoute site. In a similar case, the gluten strength trait was between 25.00 and 57.60 ppm at the Marchouch site and between 27.7 and 53.20 ppm at the Tassaoute site, with the escape group presenting the highest mean values for both sites. Regarding wet gluten content, the highest values were obtained for the resistant group in both environments. It ranged from (23.18 to 37.25%)

and from (20.75 to 28.83%) at the Tassaoute and Marchouch sites, respectively. As for the protein content trait, it was between 9.88% and 14.10% for the Tassaoute site and between 10.56% and 13.19% for the Marchouch site. The highest value of this trait was seen for the susceptible and resistant groups at the Tassaoute and Marchouch sites, respectively.

3.4. Correlation, Analysis of Principal Component and Genotypic Classification According to Agro-Phenological and Grain Quality Performance

3.4.1. Correlation among Traits

Pearson's correlation coefficients for the agro-phenological and grain quality traits of the studied materials in both environments are presented in Tables 7 and 8. Highly significant positive correlations were found between wet gluten content and protein content in both environments: Marchouch (r = 0.805 **) and Tassaoute (r = 0.872 **). However, significant (−0.500 *) and negative correlations were detected between protein content and days to heading at the Tassaoute site. Yellow pigment content showed a highly significant positive correlation with gluten strength (SDS-test) (r = 0.872 **) only at the Marchouch site. No significant correlations between other quantitative traits were observed.

Table 7. Correlation coefficients among the quantitative traits evaluated at the Marchouch site.

	GY	PH	DtH	PC	WGC	YPC	GS
GY		0.234	−0.006	−0.318	−0.308	0.252	−0.128
PH			−0.092	0.210	−0.015	−0.035	−0.259
DtH				−0.202	−0.359	0.153	−0.264
PC					0.805 **	−0.035	0.333
WGC						−0.076	0.273
YPC							0.437 *
GS							

Note: * significant at the 0.05 probability level, ** highly significant at 0.01 probability level, GY: grain yield; PH: plant height; DtH: days to heading; PC: protein content; WGC: wet gluten content; YPC: yellow pigment content; GS: gluten strength (SDS-test).

Table 8. Correlation coefficients among the quantitative traits evaluated at the Tassaoute site.

	GY	PH	DtH	PC	WGC	YPC	GS
GY		−0.098	0.120	0.012	0.173	0.172	0.190
PH			0.212	−0.008	0.077	−0.219	−0.074
DtH				−0.500 *	−0.339	0.398	0.300
PC					0.872 **	−0.320	−0.334
WGC						−0.296	−0.120
YPC							0.339
GS							

Note: * significant at 0.05 probability level, ** highly significant at 0.01 probability level, GY: grain yield; PH: plant height; DtH: days to heading; PC: protein content; WGC: wet gluten content; YPC: yellow pigment content; GS: gluten strength (SDS-test).

3.4.2. Principal Component Analysis (PCA)

Principal component analysis (PCA) was performed on all attributes (Table 9), and genotypes were subjected to biplot analysis for assessing the relationships between all of attributes at once and their reaction to Hessian fly infestation in testing environments. Principal component analysis was performed for seven traits recorded for all genotypes in both study sites: Marchouch and Tassaoute. Out of seven traits, only first three principal components (PCs) exhibited an eigen value greater than 1.00 (Table 9) and explained 34.26% and 37.15% of the variation in the Tassaoute and Marchouch sites, respectively.

Table 9. Principal component, eigen values, and percentages of total variation and cumulative percentages of the first four principal components for the seven characteristics of the twenty-two durum wheat genotypes.

		F1	F2	F3	F4
Marchouch	Eigen value	2.39	1.52	1.29	0.85
	Total variation (%)	34.26	21.78	18.49	12.17
	Cumulative percentage (%)	34.26	56.04	74.53	86.70
Tassaoute	Eigen value	2.60	1.34	1.17	0.73
	Total variation (%)	37.15	19.22	16.71	10.51
	Cumulative percentage (%)	37.15	56.37	73.08	83.60

Selection based on a combination of indices may provide a more useful criterion for improving durum wheat, although correlation coefficients are useful to find the degree of overall linear association between any two attributes [32]. Thus, a better approach than a correlation analysis such as a biplot is needed to identify superior genotypes for tested environments. In the present study, principal component analysis (PCA) revealed that the first three principal components (PCs) represent the maximum variability in testing environments (Table 10). Hence, the traits falling to these three PCs may be given due importance in durum wheat improvement programs. For the Marchouch site, the first component was mostly associated with traits such as the number of days to heading, protein content, and wet gluten content. Yellow pigment content and gluten strength (SDS-test) had positive loading in the second component, whereas the third component consisted mainly of two agro-phenological traits, plant height and grain yield. However, for the Tassaoute site, the number of days to heading, protein content, wet gluten content, yellow pigment content, and gluten strength (SDS-test) had highest loading in the first component. The grain yield and gluten strength (SDS-test) traits had positive loading in the second component, while plant height had positive loading in the third component.

Table 10. Principal component analysis for seven traits in twenty-two durum wheat genotypes across the two study sites.

Site	Trait	F1	F2	F3
Marchouch	GY	−0.414	0.299	0.677
	PH	−0.109	−0.338	0.784
	DtH	−0.529	−0.009	−0.333
	PC	0.904	−0.148	0.161
	WGC	0.933	−0.157	0.118
	YPC	−0.003	0.856	0.231
	GS	0.496	0.736	−0.130
Tassaoute	GY	−0.079	0.757	0.195
	PH	0.083	−0.374	0.865
	DtH	−0.691	0.000	0.510
	PC	0.895	0.264	0.118
	WGC	0.821	0.414	0.251
	YPC	−0.620	0.391	−0.118
	GS	−0.499	0.489	0.179

GY: grain yield; PH: plant height; DtH: days to heading; PC: protein content; WGC: wet gluten content; YPC: yellow pigment content; GS: gluten strength (SDS-test).

The first two components accounted for 56.04% and 56.37% of the total variation for the Marchouch and Tassaoute sites, respectively (Figures 2 and 3). A high principal component (PC) score for a particular genotype in a particular component denotes high values for the variables in that particular genotype. Based on PC1 and PC2 in the first component genotypes, G12 had the highest PC score followed by G8, G14, G1, G15, G13, G9, G18, G7, G21, G10, G16, G6, G11, G17, and G5, whose excellent protein quality and precocity was contributed to mostly by their high protein content and wet gluten content as well as low values for number of days to heading, whereas in the second component, genotype G2 had the maximum score, followed by G20, G19, G22, G3, and G4 for yellow pigment content, gluten strength (SDS-test), and plant height in the Marchouch site (Figure 2, Table 10).

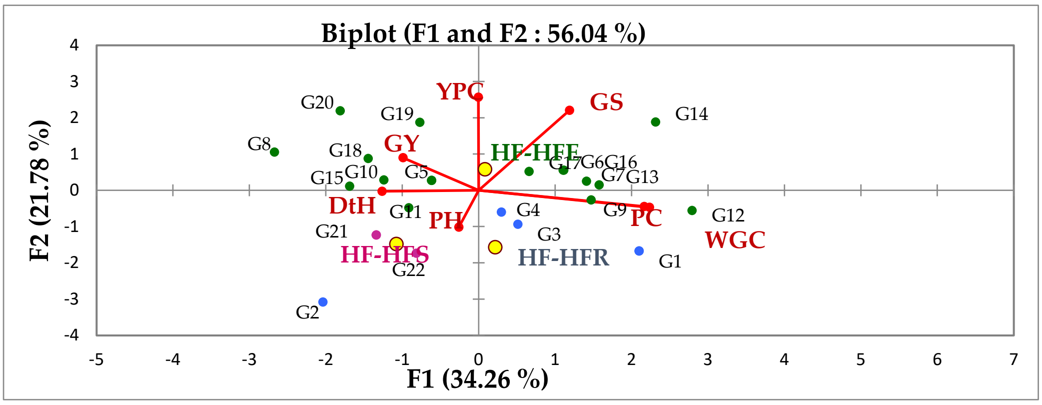

Figure 2. Principal component analysis (PCA) based on seven traits of durum wheat genotypes collected in the Marchouch site. GY: grain yield; PH: plant height; DtH: days to heading; PC: protein content; WGC: wet gluten content; YPC: yellow pigment content; GS: gluten strength (SDS-test), HF-HFR: resistant genotypes to H fly larval attack; HF-HFE: susceptible with escape to Hessian fly larval attack; HF-HFS: susceptible with no escape to Hessian fly larval attack; G: genotype.

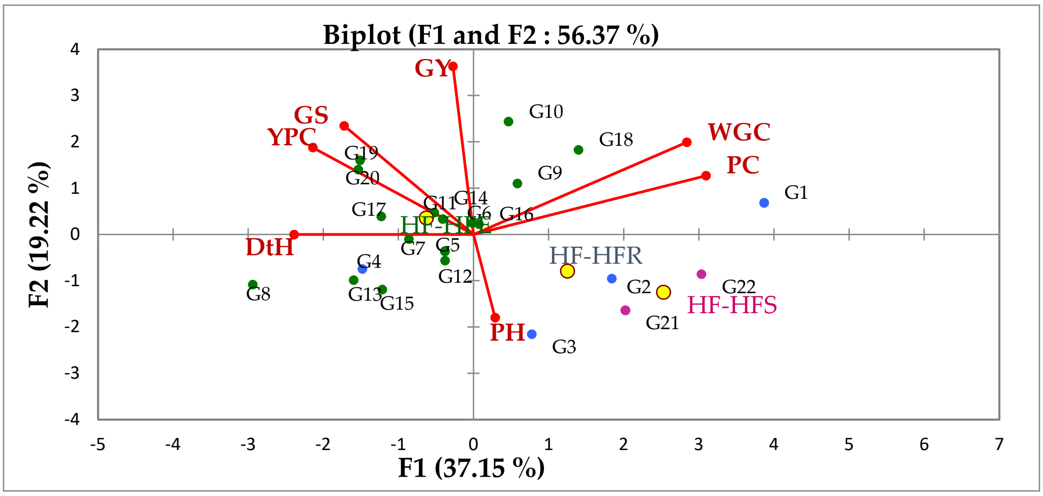

Figure 3. Principal component analysis (PCA) based on seven traits of durum wheat genotypes collected in the Tassaoute site. GY: grain yield; PH: plant height; DtH: days to heading; PC: protein content; WGC: wet gluten content; YPC: yellow pigment content; GS: gluten strength (SDS-test); HF-HFR: resistant genotypes to Hessian fly larval attack; HF-HFE: susceptible with escape to Hessian fly larval attack; HF-HFS: susceptible with no escape to Hessian fly larval attack; G: genotype.

For the Tassaoute site, the majority of the genotypes were scattered on the side of the first principal component, with genotypes such as G1, G2, G4, G5, G7, G8, G11, G13, G14, G15, G17, G20, G21, and G22 excelling in technological and precocity, which were contributed to mostly by high their protein content, wet gluten content, and yellow pigment content as well low values for number of days to heading (Figure 3, Table 10). Genotypes such as G1, G2, G21, and G22 were dispersed in the positive side of the main traits such as high values for protein content and wet gluten content and low values for number of days to heading. In the negative side of the PC1, the advanced durum wheat lines G4, G5, G7, G8, G11, G13, G14, G15, G17, and and G20 were characterized by high values in yellow pigment content and gluten strength (SDS-test). Selection of genotypes that have high PC1 and low PC2 are suitable for both studied environments. The advanced lines G1, G5, G7, G8, G11, G13, G14, G15, and G17 presented the best protein content and wet gluten content, and low number of days to heading and grain yield, and were identified as the superior genotypes for both environments since the Hessian fly larvae attacks were not severe in the field.

3.4.3. Genotypic Classification According to Agro-Phenological and Grain Quality Performances

The analysis assigned the twenty-two durum wheat genotypes into three main groups for the Marchouch site and four main groups for the Tassaoute site (Figures 4 and 5).

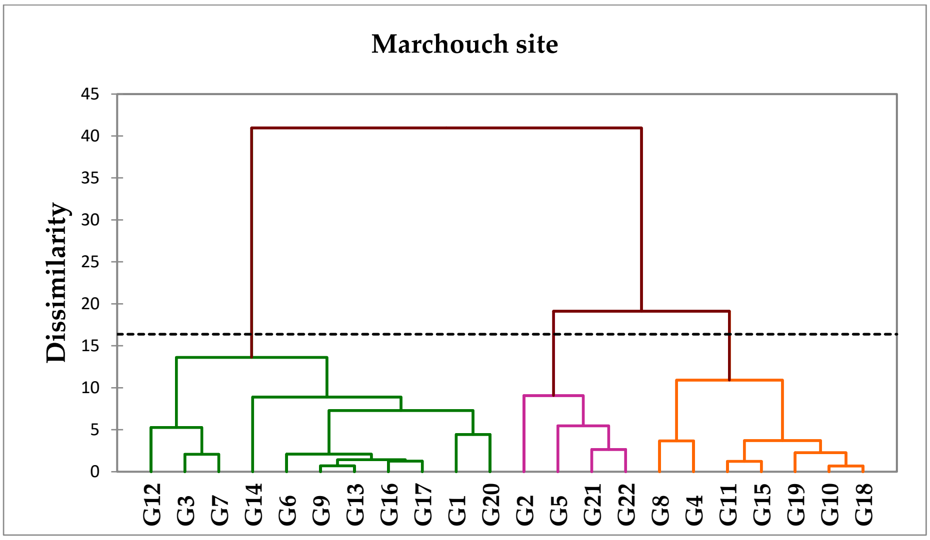

Figure 4. Tree diagrams of twenty-two durum wheat genotypes for seven studied variables recorded for the Marchouch site using hierarchical cluster analysis (Ward's method and squared Euclidean distance). G: genotype.

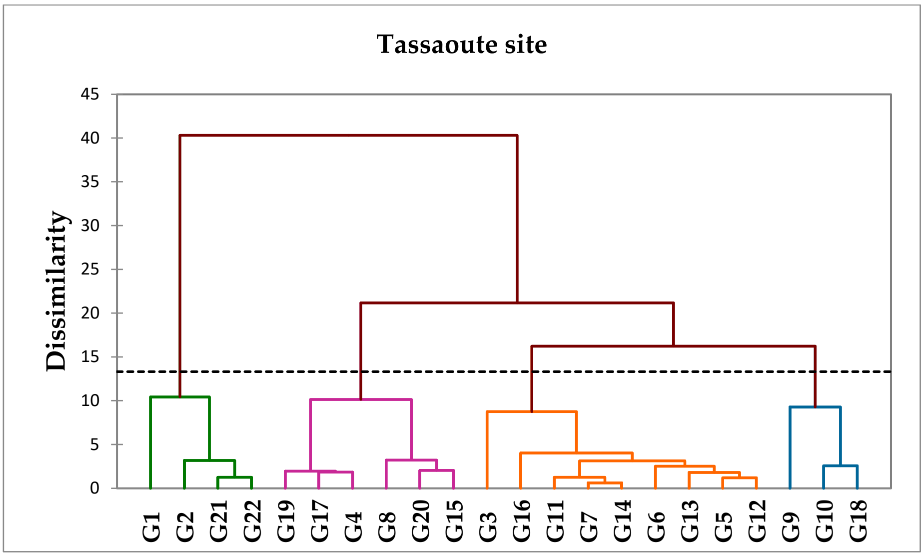

Figure 5. Tree diagrams of twenty-two durum wheat genotypes for seven studied variables recorded for the Tassaoute site using hierarchical cluster analysis (Ward's method and squared Euclidean distance). G: genotype.

According to the results recorded in the Marchouch site, the first group included eleven advanced durum wheat lines, namely G1, G3, G4, G6, G7, G9, G12, G13, G14, G16, and G17 characterized by high protein content, high wet gluten content, high gluten strength, and low number of days to heading. The second group included four genotypes (G1, G5, G21, and G22) grouped together, which had low yellow pigment content, low gluten strength, and high grain yield and plant height. The third group included seven advanced lines, namely G8, G10, G11, G15, G18, G19, and G20 characterized by high yellow pigment content. However, for the Tassaoute site, the first group included four genotypes, namely G1, G2, G21, and G22, which had low yielding, low yellow pigment content, low gluten strength, low number of days to heading, and high protein content. The second group included nine genotypes: G3, G5, G6, G7, G11, G12, G13, G14, and G16 characterized by low wet gluten content and high plant height and high number of days to heading. The third group included the six advanced lines G4, G8, G15, G17, G19, and G20 grouped together, which had high yellow pigment content, high gluten strength, and short plant height. The fourth group included three genotypes: G9, G10, and G18 characterized by high grain yield, high wet gluten content, and high gluten strength (Figure 4).

4. Discussion

4.1. Genotype, Environment Effects and Their Interaction and Heritability (H)

The genotypic and environmental effects and their interaction are of utmost importance for breeding programs. Our results indicate that the variation due to genotypes, environments and their interactions were significant for all attributes, confirming the finding of previous studies conducted on Moroccan durum wheat cultivars [26,33,34]. Thus, when the same genotype is subjected to different environments, it can produce a wide range of phenotypes. These phenotypic variations are attributable to the effect of the environment on the expression and function of genes influencing the trait [19,26]. In addition, the significant effect of G $\times$ E interaction on the traits implies that durum wheat genotypes behave differently according to variations in both environments, leading to the necessity to identify and select environment-specific genotypes. So, it is important to evaluate the real impact of these factors and their interactions with phenotypic variation in the traits. Additionally, considerable genetic variability occurred within groups of genotypes for all the traits in testing sites, confirming that the variability in crop species is pivotal since it provides the foundation for selection and for successful plant breeding programs [35].

Heritability is a measure of the phenotypic variance attributable to genetic causes and has a predictive function in plant breeding. It is an indicator for the transfer of traits from parents to their progeny. Heritability estimation gives information about the degree of genetic control in the expression of particular traits and phenotypic reliability in predicting their breeding value. High heritability indicates less environmental influence in the observed variation [36,37]. According to [38], the higher the heritability estimates, the simpler selection procedures are. Broad-sense heritability only shows if there is sufficient genetic variation in a population and how the population will respond to selection pressure [39,40]. In our results, the highest heritability was obtained for grain yield, plant height, and all grain quality traits, which is similar to results presented by other authors when more than two environments were used for testing [41,42]. Additionally, many investigations have reported that most grain quality traits in wheat have high heritability values and they can be improved through conventional plant breeding methods, meaning that genetic improvement for technological quality is possible [43]. Furthermore, heritability has been widely used in wheat to evaluate the magnitude of variance in breeding material, to determine appropriate selection procedures, and to predict the effect on breeding advancement upon the improvement of important traits [27,44,45]. In this context, it is important to understand the masking effect of the environment relative to actual heritable traits by determining the genotype according to environment interaction and identifying traits that show stable effects across different environments [46]. Moreover, it is crucial to indicate whether the breeding programs are successful by evaluating their genetic gains [23,47]. Such evaluations can help in identifying the traits that contribute to productivity and grain quality improvement and developing the future breeding approaches.

4.2. Comparing Mean Values of the Three Groups of Genotypes

At the Marchouch site, the best-performing group was the susceptible group, which is more productive and earlier than the escaped and resistant groups since the genotype's mean values were smaller than other groups' mean values. Unlike the Tassaoute site, the escape group was more productive and moderately earlier than the susceptible and resistant groups. This result confirms a previous finding on durum wheat that showed the effect of the environment on plant height, grain yield, and days to heading in wheat plants [13,48]. In our investigation, the Hessian fly larvae attacks were insufficient in causing significant losses to the plants; therefore, the agro-phenological behavior of these groups of genotypes can be attributed to the genetic differences as much as the environments in which they were tested. In terms of grain quality, the highest values for yellow pigment content and gluten strength were found for the escaped group in both environments. This is probably because this group of lines benefitted specifically from selection for this criterion, while the parental material had a high level of yellow color. However, it recorded the lowest values for protein and wet gluten content, as these traits are highly heritable and dependent on environmental conditions [49]. Although environmental factors play important roles in the carotenoid concentrations of cereals, the genetic component is predominant [49–51]. Contrary to the resistant genotype, it recorded the greatest value for wet gluten content and the lowest value for yellow pigment content at the two experiment sites, and it recorded the highest value for protein content at the Marchouch site and strong wet gluten content at the Tassaoute site. These results can be explained by the fact that the incorporation of Hessian fly resistance genes probably was accompanied by lower coloration due to genes carried by the resistant parents, which are genetically similar (DON-MTD14 and ICAMOR are sister lines). For all advanced lines, protein content differences were small and should be attributed to environmental conditions. Although the protein content is the main factor in determining the final use of wheat, there is often a need to measure the properties that are indicative of the protein quality [52,53]. This also depends on variety [54–56] and cultural practices during growth [57–61]. Moreover, durum wheat's protein content is important for both nutritional and functional reasons and is a fundamental factor influencing the mixing, processing, and cooking characteristics [62,63]. As a result, improving the protein content of durum varieties remains a primary goal of durum wheat breeders. Unlike the susceptible group, it showed low technological aspects in both environments, such as gluten strength, yellow pigment content, and wet gluten and protein content. On the one hand, this low quality seems obvious because of the objectives that were set during the development of these materials, which essentially aimed at improving adaptation and grain productivity. On the other hand, according to [13,64,65], ancient durum wheat cultivars also differ from modern ones concerning grain quality, particularly in terms of protein content and gluten strength. Regardless of the nitrogen fertilization rate, old durum wheat varieties are generally characterized by higher protein percentages compared with modern ones because of their lower grain yields. Nevertheless, they have not benefited from breeding programs focused on improving pasta quality and are therefore characterized by a lower gluten strength compared with modern varieties [26,49,66].

4.3. Correlation and Principal Component and Hierarchical Classification Analyses According to Agro-Phenological and Technological Performances

Highly significant correlation coefficients are scattered throughout the matrix. Some of them are obvious, such as those among quality parameters, while others are more or less fortuitous. Protein content was highly correlated with wet gluten content in both environments, which is consistent with previous studies on durum wheat by [67–69]. Therefore, the positive correlation of protein content with this trait indicates the possibility of simultaneous improvement of both traits via effective selection. However, a negative and significant correlation was observed between protein content and days to heading only at the Tassaoute site. This negative relationship seems to be derived from the fact that these traits are genetically independent, and, as a result, they can be selected separately.

In addition, an significant and positive correlation was observed between gluten strength and yellow pigment content. The results of [70] corroborate this observation. In our investigation, the differences between correlation coefficients indicated that environmental and genetic factors could influence the relationship between the traits. Because of this, wheat breeders have consistently worked to incorporate these traits into early generation lines in their breeding programs to minimize the effect of environmental conditions on the expression of these traits.

Various techniques have been successfully used to contribute to genetic diversity and thereby create diverse genotypes. Among these methods, the most frequent and successful are the use of agro-phenological and technological traits, which are commonly used for estimation of genetic variation in many breeding programs [71]. The primary goal of evaluating collected germplasm for various quantitative traits is to classify these genotypes for different breeding programs. Among these techniques, cluster and principal component analysis are commonly used in the genotype evaluation process.

In our study, genetic variability as observed through the principal component analysis and clustering method via agro-phenological and technological traits showed relatively similar results. The results of the principal component analysis showed great variability for all attributes. In fact, the high principal component (PC) score for a particular genotype in a particular component denotes high values for the variables in that particular genotype. The genotypes G1, G5, G7, G8, G11, G13, G14, G15, and G17 were the best ones and had the highest values for protein content and wet gluten content, and the lowest values for days to heading and grain yield; they were identified as the superior genotypes for both environments since the Hessian fly larvae attack was not severe in the field. Furthermore, the hierarchical classification made it possible to group all the durum wheat genotypes into distinct groups. Individuals within each group are characterized by well-defined criteria of precocity, adaptation, productivity, and grain quality. The genotypes were clustered into three clusters at the Marchouch site; the genotypes in the first cluster had high protein content, high wet gluten content, high gluten strength, and a low number of days to heading. The genotypes in the second cluster had low yellow pigment content, low gluten strength, high grain yield, and high plant height. The genotypes in the third cluster had high yellow pigment content. However, at the Tassaoute site, the genotypes were clustered into four clusters, in which the genotypes in the first cluster had low yielding, low yellow pigment content, low gluten strength, a low number of days to heading, and a high protein content. The genotypes in the second cluster had low wet gluten content and a high plant height and high number of days to heading. The genotypes in the third cluster had high yellow pigment content, high gluten strength, and short heights. The genotypes in the fourth cluster had high grain yield, high wet gluten content, and high gluten strength. In our investigation, the differentiation of durum genotypes into different clusters in both environments was primarily due to the relatively high contribution of a few traits, rather than a small contribution from each individual trait. According to [72], the most important traits in the first principal component influence the clustering more than the other unimportant traits in the first principal component (PC1). Additionally, the observed variation reflects their genotypic differences and can be attributed to environmental factors. It may also be linked to the origin of durum plant materials, natural selection processes, or breeding efforts, including resistance to diseases and pests. The results indicated that some genotypes close to each other are genetically similar and were grouped in the same cluster in the cluster analyses in both environments (G3, G6, G7, G12, G13, G14, and G16). This can be explained by the fact that the traits used for clustering are subject to both natural and artificial selection, in addition to their high environmental dependence. Therefore, if there is an association between the loci controlling the targeted traits, the influence of environmental factors will be low [73]. According to [74], there may be several reasons for this result. For example, selection based on one environment may not have been performed long enough to determine the various genotypes. Additionally, genes responsible for fitness in one environmental condition are not unique to that environment and may confer fitness in other environments. Furthermore, adaptation to different environments is not based on the accumulation of random genes but rather on a limited set of specific genes. The observed diversity can be utilized in breeding programs to enhance the grain quality and productivity of durum wheat. Agro-phenological and technological traits are expected to offer a general representation of the relationship between durum wheat genotypes and their growing environments. Classifying them into different clusters can aid in predicting the selection of genotypes to design a breeding program. The development of these traits in durum wheat is a highly coordinated process. Therefore, for an effective breeding program, it is essential to consider all the important traits that have an impact on productivity and grain quality performance.

5. Conclusions

This study evaluated the agro-phenological and technological traits in durum wheat to detect the differences between genotypes in terms of their responses to Hessian fly larval attacks and to identify those exhibiting ideal behaviors in terms of both productivity and grain quality. The variation due to genotype, environment, and their interaction was significant for all attributes. Since Hessian fly infestation was not severe in the field, it appears that the measured parameters showed no consistent association with the responses to attacks by this pest. In addition, the agro-phenological and grain quality behavior observed in the three groups of genotypes can probably be attributed to the genetic difference as much as the environments in which they were tested. Furthermore, high levels of heritability were obtained for grain yield, plant height, and all grain quality traits, indicating less environmental influence in the observed variation. These findings should be considered in durum wheat breeding programs. As seen in this study, a positive correlation between protein content and wet gluten content in both environments demonstrated the possibility of simultaneously improving both traits through effective selection. Moreover, principal component and hierarchical classification analysis allowed us to group all the genotypes into distinct clusters. Individuals within each group were characterized by well-defined precocity, adaptation, productivity, and grain quality criteria. The genotypes G1, G5, G7, G8, G11, G13, G14, G15, and G17 were identified as the superior genotypes for both environments, and they could be used to select favorable traits for crosses in genetic improvement programs. Further research based on biochemical or molecular markers is required in the future to better understand the genetic variation in our durum wheat genotypes.

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