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Assessing the Ability of Durum Wheat-*Thinopyrum ponticum* Recombinant Lines to Suppress Naturally Occurring Weeds under Different Sowing Densities

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Abstract: The use of synthetic chemicals in cropping systems is becoming more controversial and highly debated worldwide, owing to its impacts on the environment, food safety, and human health. For this reason, sustainable crop management strategies are gaining increasing interest. In this perspective, agronomic practices and use of disease-resistant and competitive genotypes represent valuable tools in the hands of farmers. The competitive ability of two durum wheat-*Thinopyrum ponticum* recombinant lines (named R5 and R112), carrying effective resistance genes towards main rust diseases and enhanced yield-related traits in their alien chromosome segments, was investigated in comparison with that of a widely grown commercial cultivar (Tirex), under two sowing densities (250 and 350 seeds m⁻²), in the presence or absence of weeds. Yield-related traits and specific attributes that confer competitive ability were recorded in two subsequent seasons. R5 was the most weed-suppressive genotype, whereas Tirex was the least competitive. R112 was the best yield performer under favorable weather conditions (5.6 t ha⁻¹), while it suffered the presence of weeds in the drier year (−38% grain yield). Although 350 seeds m⁻² appeared to be the most effective sowing density for suppression of weeds (−16% weed biomass), adoption of the lower density can optimize grain yield and limit weed infestation in dry seasons. A suitable combination of sowing density and genotype choice can improve yield performance. R112 required the higher sowing rate to maximize grain yield (+43% as compared to the lower sowing rate), while R5 proved to be a density-neutral genotype.

Keywords: durum wheat; flag leaf; sowing density; weed-competitive genotype; weed suppression

1. Introduction

Since the global population is expected to grow from 7 billion to 9 billion by 2050, crop production needs to double to meet future food demand [1]. At the same time, there is an increasing interest in low-input agriculture, and the concept that yield gain must be achieved by approaches that reduce environmental impact is receiving more support and attention also from the scientific community [1–3]. This has important implications on agronomic strategies to be adopted for cereals (especially maize, rice, and wheat) that are (and will remain) the most important source of total food consumption [4].

Even though the most widely grown wheat in the world is common wheat (*Triticum aestivum* L.), durum wheat (*Triticum turgidum* L. subsp. *durum* (Desf.) Husn.) is a staple food for millions of people especially in the Mediterranean Basin. Flour and semolina are used to make many traditional products, such as pasta, couscous, bulgur, and different types of bread [5,6]. Therefore, reducing chemical inputs

without negatively affecting yield performance, represents a crucial goal to increase the environmental and economic sustainability of durum wheat production systems and also food safety for millions of people.

Unlike conventional cropping systems, there are limited options for pest management in low-input and organic production systems. Thus, weed and pathogen pressure often reduce crop and yield quality dramatically in low-input and organic production systems. The repeated use of herbicides for weed control has resulted in increased cases of herbicide resistance and represents an additional difficulty for today's cropping, particularly in smallholder systems (e.g., [7]). In response to these issues, integrated control strategies are gaining increased attention from stakeholders (e.g., farmers, seed companies, agronomists, breeders, consumers). Besides the use of mechanical tools, many cultural control methods may be applied to address the problem of weeds, including the management of sowing date and rate, the employment of crop rotations and diversification, and the use of plant water extracts as well as the selection of competitive cultivars [8–11]. The latter seems to be a highly attractive option when coupled with disease resistance attributes [7,12]. In fact, the tendency of EU policy to limit the use of synthetic plant protection products has led to a significant reduction in the number of herbicides and fungicides, thus urgently requiring a new plant protection paradigm for future farming [13]. Crop breeding, together with agronomic strategies, can be a valuable tool to face this challenge [14]. Although the nature of the relationship between yielding potential and weed suppressing ability is not completely understood (reviewed in [7]), screening for genotypes expressing traits related to competitive ability is typically accompanied by negative implications on yield potential and stress tolerance [15]. Work in this area has been limited by the difficulty to reach an acceptable trade-off among all these aspects (disease resistance, competitive ability, and yield potential) and the polygenic control of competitiveness trait, as well as by a lower priority given to weed tolerance over time (e.g., [7]). One possible strategy would be the identification of traits that confer competitive ability in genotypes that have already displayed a good trade-off between yield performance and resistance to pathogens. To do this, exploring genetic variability should not be limited to cultivated wheats, but include the underutilized and rich exotic germplasm (wild relatives, landraces) and its derivatives (wheat-alien substitution/translocation/recombinant lines). This rationale was followed in this work to verify the potential weed-competitive ability of two durum wheat-*Thinopyrum ponticum* recombinant lines that were recently found to have good yield performance in a range of contrasting environments, i.e., Italy, Morocco, and Australia [16]. These genotypes are also carriers of the *Lr19+Sr25+Yp* gene package that is considered particularly valuable for durum wheat breeding, due to the high efficacy of *Lr19* and *Sr25* resistance genes towards main rust diseases of the crop, and the contribution to carotenoid pigmentation and semolina color of the *Yp* gene [17–19]. Additionally, results from previous studies [16,18] revealed such durum wheat-*Th. ponticum* recombinant lines to possess yield-related traits that are frequently associated with weed-competitive ability, including a significant increase of biomass production, enhanced flag leaf area, and large seed size [14,20,21]. Whereas the ability to successfully compete with naturally occurring weeds was not directly assessed in the novel durum wheat-*Th. ponticum* genotypes, investigation of this trait is prompted by knowledge of the high capacity of perennial wheatgrass species, including *Th. ponticum*, to suppress annual weeds in noncrop areas [22,23]. Therefore, assessing weed-competitive ability and yield performance of lines possessing *Th. ponticum* introgressions is not only warranted but also highly desired for use in sustainable breeding and cultivation.

As previously stated, different agronomic tactics can be associated with cultivar selection to improve weed control efficacy. Among them, the optimization of seed rate has been repeatedly emphasized as a valid option for weed management of winter cereals, both in the organic and integrated systems [24–27]. However, the optimal seeding rate for adequate trade-off between grain yield performance and weed control is extremely variable since it largely depends on compensatory effects, weed species, and environmental conditions [14,28–30]. In the Mediterranean environment, a sowing rate of 350 seeds m⁻² is normally used for winter durum wheat plantings [31–33]. Individual

yield-contributing traits, such as tillering capacity as well as leaf dimensions and architecture, also play an important role in determining the sowing rate, possibly allowing a reduction of seed number per unit area [34]. Thus, site-specific studies are constantly needed to increase our knowledge on the complex interrelationships of different wheat genotypes, naturally occurring weed flora, plant density, and environment [35,36].

This study was planned to (1) assess the competitive ability of two stable and well-yielding durum wheat-*Th. ponticum* recombinant lines against naturally occurring weed flora and (2) evaluate the performance of these new genotypes at normal and reduced sowing rate.

2. Materials and Methods

2.1. Plant Materials

Two durum wheat-*Th. ponticum* recombinant lines, named R5-2-10 and R112-4 (hereafter referred to as R5 and R112, respectively), developed in the near-isogenic background of cultivar Simeto by repeated backcrossing [37], were used. Simeto (pedigree: Selection from Capeiti 8 × Valnova) is a variety released in 1988 and largely cultivated in Southern Europe. It is well adapted to the Italian growing conditions and included in the pedigree of several Italian durum wheat cultivars. Simeto is a medium size (74–81 cm) and medium-early maturing commercial variety. The two recombinant lines have portions of *Th. ponticum* 7e1L chromosome arm replacing 23% (R5) and 28% (R112) of the respective distal 7AL arm, and both lines include the alien *Lr19+Sr25+Yp* genes in the subtelomeric 7e1L region. The lines tested here were assessed together with the modern durum cultivar Tirez (pedigree: Svevo × Nefer, released in Italy in 2007), widely grown in Italy. Tirez is a medium size (77–85 cm) and medium-early maturing commercial variety, characterized by high and constant stable productivity under both conventional and organic farming systems [38]. In particular, with respect to other modern durum wheat varieties and the older cv. Simeto, Tirez repeatedly showed constantly higher tillering ability (data from the Italian network for the evaluation of durum wheat cultivars), which is commonly related to a superior competitive ability against weeds. The tillering capability of Tirez also emerged in a field plot trial carried out in 2015 in Viterbo (central Italy), in which the agronomic performance of different durum wheat-*Th. ponticum* recombinant lines was compared with that of some modern cultivars [39].

2.2. Location and Experimental Design

Field experiments were conducted during two consecutive growing seasons, 2014–2015 and 2015–2016 (hereafter referred to as 2015 and 2016, respectively), at the experimental farm of the University of Tuscia, Viterbo, Italy (42°25' N, 12°08' E, 310 m above sea level). The growing area is characterized by a Mediterranean climate, with mean annual air temperature of about 14.5 °C and precipitation of 790 mm. Total rainfall and average temperature of durum wheat growing season (from mid-November to early July) in this area are 468 mm and 11 °C, respectively. Weather data were retrieved from the meteorological station located 200 m from the experimental site and are reported in Figure 1. In both years, the preceding crop was chickpea (*Cicer arietinum* L.). The soil type was silty clay, pH 6.9, and organic matter content 2.0%. Soil was ploughed at 40 cm depth in September and sowing was performed on 9 January 2015 and 13 November 2015. Delayed sowing in 2015 (about 1 month) was due to heavy and persistent rains during fall (194 mm in November and 97 in December).

Experiments were set up in a split-split-plot design with three replications. In both years, two sowing rates were applied in each main plot (4.5 m × 14 m): Low (250 seeds m⁻²) and normal (350 seeds m⁻²). Within each main plot, the three durum wheat genotypes described above were sown. Subplots (1.5 m × 14 m) were divided into two sub-subplots (1.5 m × 7 m), with or without naturally occurring weeds (hereafter referred to as weedy and weeded, respectively). In weeded plots, weeds were chemically controlled by a single distribution of commercial herbicide Ariane™ II at a rate of 3.5 L ha⁻¹. Each sub-subplot consisted of 10 rows spaced at 0.15 m. Furthermore, four 0.9 m × 0.9 m

quadrats were randomly allocated into the experimental design to screen the naturally occurring weeds and to allow the calculation of the competitive balance index (Cb, see ahead). Nitrogen fertilization (180 kg ha^{-1}) was split into three applications: The first was given before sowing as di-ammonium phosphate (20% of total N applied), the second when the first node was detectable above ground (Zadoks 31, [40]) as urea (50% of total N), and the third 25 days later as ammonium nitrate (30% of total N). Harvesting was performed on 3 July 2015 in the first year (crop cycle length: 176 days) and 5 July 2016 in the second year (crop cycle length: 236 days).

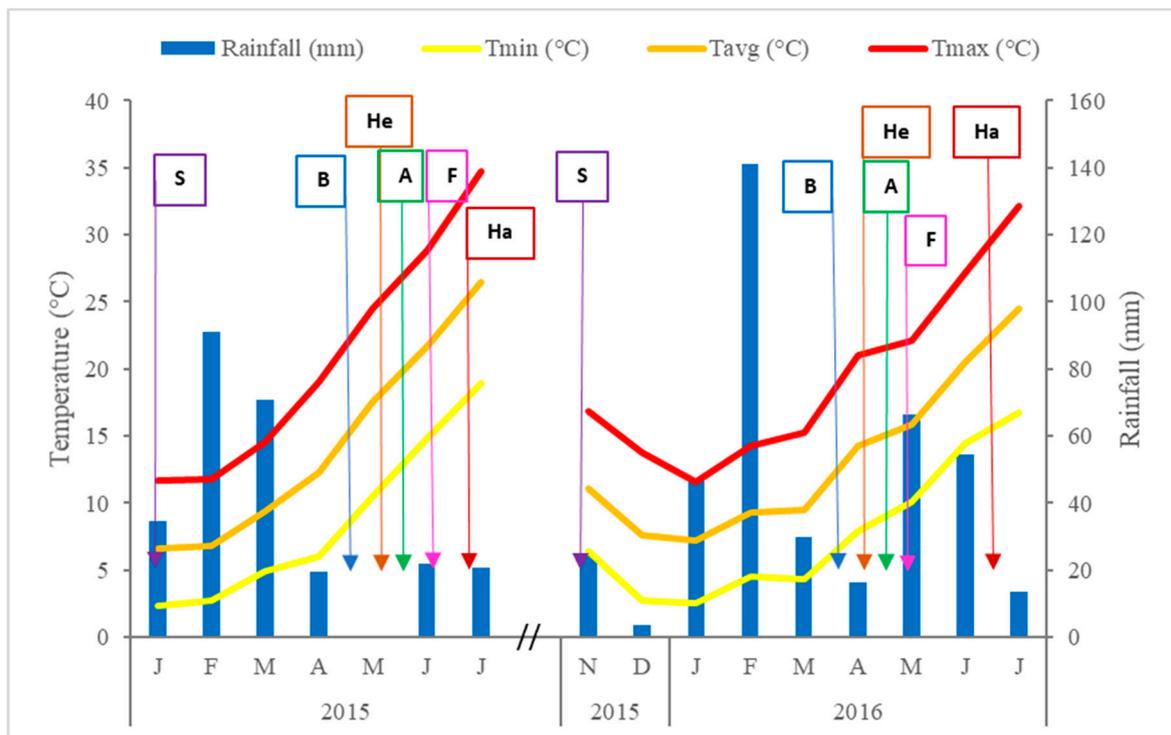


Figure 1. Weather conditions during 2015 and 2016 seasons, as retrieved from the meteorological station of the experimental farm of the University of Tuscia, Viterbo (Italy), and indication of sowing date (S) and growth stages of durum wheat (B, booting; He, heading; A, anthesis; F, grain filling; Ha, harvest maturity). Tmin: mean minimum air temperature; Tavg: average air temperature; Tmax: mean maximum air temperature.

2.3. Sampling and Measurements

2.3.1. Yield and Yield-Related Traits

At ripening (Zadoks 90), wheat plants from a $0.9 \text{ m} \times 0.9 \text{ m}$ quadrat per sub-subplot were manually cut at ground level and weighed. The number of spikes was counted and then plants were oven dried at $65 \text{ }^\circ\text{C}$ to determine dry weight. A subsample of 15 spikes was used to count the number of grains per spike, while thousand grain weight (TGW) was determined by counting 60-g seed samples and then applying the appropriate proportion. Grain yield was determined at 13% moisture content, harvesting 8.4 m^2 sampling areas per sub-subplot. Grain yield loss (GYL) due to weed presence was calculated according to the following formula:

$$\text{GYL} = 100 \times [(Y_0 - Y)/Y_0] \quad (1)$$

where Y_0 is the grain yield in weeded plots and Y is the grain yield in weedy plots [41].

2.3.2. Competitive Ability against Weeds

In weedy plots, weed aboveground biomass was also collected from the same quadrats used for durum wheat biomass determination, weighed, identified, and then oven dried at 65 °C to determine dry weight. The same procedure was followed to determine dry weight in pure stand weed quadrats.

The competitive balance index (Cb) was used to assess the competitive ability of durum wheat genotypes against weeds. Cb was calculated as follows:

$$Cb = \ln [(C_w/C)/(W_c/W)] \quad (2)$$

where C_w is the aboveground biomass per unit area of the crop in weedy conditions, W_c is the aboveground biomass per unit area of the weeds in the crop, C is the aboveground biomass per unit area of the crop in weed-free conditions, and W is the aboveground biomass per unit area of the weeds in pure stand, respectively. Cb index is used to quantify the competitive ability of the crop against the weeds in crop-weed systems. Crop with Cb higher than, equal to, or lower than 0 is considered more, equally, or less competitive, respectively, vs. weeds [42].

2.3.3. Light Interception

The photosynthetically active radiation flux (PAR, mol m⁻² s⁻¹) was measured with a linear ceptometer (SS1-UM-2.0, DELTA-T Devices LDT, Cambridge, UK) at the following growth stages: Booting (Zadoks 47), heading (Zadoks 57), anthesis (Zadoks 65), and grain filling (Zadoks 75). Measurements were taken in three different points: In the upper, middle, and lower part of each sub-subplot. In each point, the three following measurements were registered: (1) above-canopy PAR, (2) canopy reflectance (ceptometer turned upside down), and (3) total canopy light interception (below canopy PAR). Light interception (IPAR) was then calculated according to the following formula:

$$\text{Light interception (\%)} = ((A - B) - C)/(A - B) \times 100 \quad (3)$$

where A = above-canopy PAR, B = reflected PAR, and C = below canopy PAR [43].

2.3.4. Optical Measurement of Physiological Traits

Chlorophyll content (CHL), flavonol content (FLAV) and nitrogen balance index (NBI) were assessed by a portable leaf-clip device "Dualox 4 Scientific" (FORCE-A, Orsay, France). Dualox readings were taken on five randomly selected flag leaves per sub-subplot, at the same growth stages described above for PAR measurements (from booting to grain filling). NBI combines chlorophyll and flavonol contents and it represents an indicator of leaf nitrogen status [44].

2.3.5. Morphological Traits

In both seasons, 10 flag leaves were randomly selected in each sub-subplot and their length (FLL) and width (FLW) were measured by a ruler. Flag leaf area (FLA) was then calculated using the following formula [45]:

$$FLA = FLW \times FLL \times 0.75 \quad (4)$$

Plant height was recorded at harvest and measured from the ground to the tip of the spikes, excluding awns in both years.

2.4. Statistical Analyses

Data were statistically analyzed by ANOVA, in order to test the main effects of year, genotype, seeding rate, weeds, and their interactions. Significantly different means were separated at the 0.05 probability level ($p = 0.05$) by the least significant difference (lsd) test. Pearson's correlation coefficient

was used to assess the relationship among grain yield loss, grain yield in weedy and weeded conditions, and competition balance index. Statistical analyses were carried out using R 3.5.2 software [46].

3. Results

3.1. Yield and Yield-Related Traits

Treatments differently affected all the measured traits (Table 1). Total rainfall during growing season differed between years. It was 238 mm in 2015 and 382 mm in 2016, with a large difference especially in the period from heading to harvest (22 mm in 2015 and 136 mm in 2016, Figure 1). Differences in rainfall between the two growing seasons are likely to account for the significant year \times treatment interaction effects. In fact, in both seasons, air temperatures during crop cycle were within the range of the ideal temperatures for wheat growth [47]. Grain yield was significantly higher in 2016 than 2015 for all the genotypes in both weeded and weedy conditions ($p = 0.027$, $lsd = 0.47$, Figure 2). In the drier and hotter 2015 season, competition from weeds significantly reduced grain yield in R112 and Tirex (-38% and -29% , respectively) while it had no significant effect on R5 production (-9.8%). In the more favorable year (2016), grain yield did not vary moving from weeded to weedy plots for R112 (-3%), while it was significantly reduced for R5 and Tirex in weedy conditions (-17.9 and -18.5% , respectively).

Table 1. Results of the ANOVA for yield and yield-related traits.

Source of Variation	Grain Yield	Spikes m^{-2}	Kernels $Spike^{-1}$	Thousand Grain Weight
Year (Y)	***	**	n.s.	*
Sowing density (D)	**	***	n.s.	n.s.
Genotype (G)	n.s.	***	***	***
Weed presence (W)	***	***	*	*
Y \times D	**	**	n.s.	n.s.
Y \times G	**	***	n.s.	**
D \times G	**	***	n.s.	n.s.
Y \times W	n.s.	**	n.s.	n.s.
G \times W	n.s.	***	n.s.	n.s.
Y \times G \times W	*	*	n.s.	n.s.

Level of significance: 0 '***', 0.001 '**' and 0.01 '*' 0.05; n.s.: not significant. Other interactions are not reported since they were not significantly different at the 0.05 probability level.

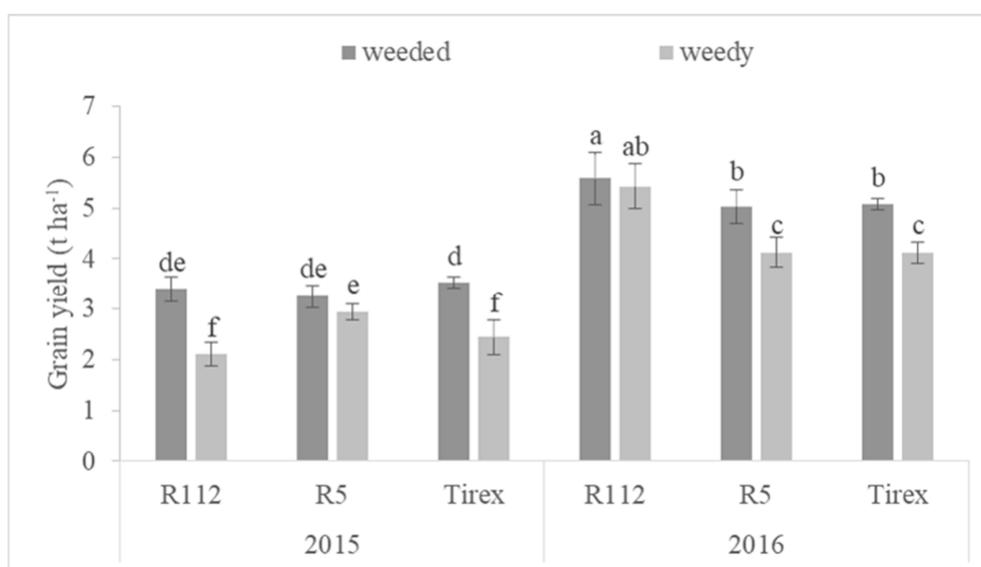


Figure 2. Grain yield: Year \times genotype \times weed presence interaction. Bars sharing the same letter are not significantly different at the 0.05 probability level.

Reducing sowing rate from 350 to 250 seeds m^{-2} did not affect grain yield in 2015 (the drier year), while in 2016 it resulted in a significantly negative response (-20.7% , $p = 0.0048$, $lsd = 0.31$, Figure 3A). Sowing rate did not affect the yield of R5 or Tirex, but reducing sowing rate resulted in a significant yield decrease of R112 (-44% , $p = 0.0034$, $lsd = 0.52$, Figure 3B).

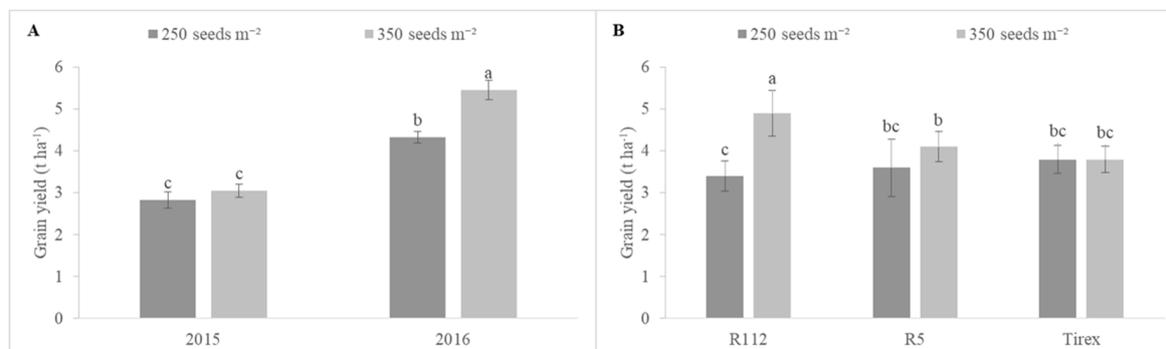


Figure 3. Grain yield: (A) Year \times sowing density interaction, (B) genotype \times sowing density interaction. Bars sharing the same letter are not significantly different at the 0.05 probability level.

Except for kernels per spike, yield components were influenced by weather conditions between years and varied with different patterns according to genotypes and weed presence (Table 1). The number of spikes per unit area was significantly affected by the year \times genotype \times weed presence interaction, with results very similar to those described for grain yield ($p = 0.049$, $lsd = 17.31$, Supplementary Figure S1). This yield component was also affected by genotype \times sowing density interaction, showing an outcome close to that reported for grain yield in Figure 3B ($p < 0.001$, $lsd = 14.38$, Supplementary Figure S2A). Moreover, the number of spikes per unit area was influenced by the year \times sowing density interaction ($p = 0.002$, $lsd = 8.73$). The values were similar in the drier year, while in the wetter season, normal density produced a number of spikes per unit area significantly higher than that recorded in the plots seeded with a lower rate (Supplementary Figure S2B). Thousand grain weight (TGW) was influenced by the year \times genotype interaction ($p = 0.004$, $lsd = 2.34$), with R5 having a significantly higher TGW than the other genotypes in both years (Supplementary Figure S3). Kernels per spike were separately affected only by weed presence and genotype. Specifically, weeded plots had 35.5 kernels per spike vs. 33.4 of weedy ones ($p = 0.021$, Table 2), while R112 produced significantly more kernels per spike (37.7) than R5 (34.2) and this latter significantly more than Tirex (31.4, $p < 0.001$, $lsd = 2.64$).

Table 2. Yield-related traits as affected by genotype, weeds, sowing density, and year.

Treatments	Genotype			Weed Presence		Sowing Density (seeds m^{-2})		Year	
	R112	R5	Tirex	Weeded	Weedy	250	350	2015	2016
Spikes m^{-2}	212.6 b	196.3 c	239.4 a	229.5 a	202.7 b	205.3 b	226.9 a	194.6 b	237.6 a
Kernels spike $^{-1}$	37.7 a	34.2 b	31.4 c	35.5 a	33.4 b	36.0 n.s.	32.8 n.s.	33.1 ns	35.7 n.s.
TGW (g)	51.8 b	56.8 a	47.1 c	52.9 a	50.9 b	52.1 n.s.	51.7 n.s.	46.8 b	57.1 a

For each treatment, means sharing the same letter were not significantly different at the 0.05 probability level, n.s.: not significant.

3.2. Competitive Ability against Weeds

In 2015, naturally occurring weeds were *Fumaria officinalis* L. (34%), *Polygonum aviculare* L. (28%), *Papaver rhoeas* L. (20%), *Convolvulus arvensis* L. (6%), and other species (12%), while in 2016 the following weeds were found: *Lamium amplexicaule* L. (29%), *Fumaria officinalis* L. (29%), *Papaver rhoeas* L. (17%), *Veronica spp* (11%), *Picris hieracioides* L. (9%), and other species (5%). The competitive balance index (Cb) and the aboveground biomass of weeds and durum wheat in both weedy and weeded conditions were

influenced by the year \times genotype interaction (Table 3). The aboveground biomass was significantly higher in 2016 than 2015 for all durum wheat genotypes (Figure 4B,C). R112 aboveground biomass was higher than that of the other genotypes in 2016 (+16%), while it was not significantly different from the others in 2015, both in weedy ($p = 0.038$, $lsd = 1.64$) and weeded ($p = 0.022$, $lsd = 1.61$) conditions (Figure 4B,C). Weed biomass was significantly higher in 2015 than 2016 for R112 and Tirex, while it was similar between years for R5 ($p < 0.001$, $lsd = 0.22$, Figure 4D). Weed aboveground biomass was also influenced by the year \times sowing density interaction ($p = 0.011$, $lsd = 0.18$, Table 3). Specifically, no difference was detected in weed biomass between low and normal sowing density (1.9 vs. 1.8 t ha⁻¹ dry matter (DM), respectively) when limiting weather conditions occurred (2015), while in the most favorable year (2016) a significantly higher weed biomass was found for the lowest sowing density (1.5 vs. 1.0 t ha⁻¹ DM, Supplementary Figure S4).

Table 3. Results of the ANOVA for durum wheat (DW) biomass in weedy and weeded condition, weed biomass, and competition index (Cb, competitive balance index).

Source of Variation	DW Biomass (Weedy)	DW Biomass (Weeded)	Weed Biomass	Cb
Year (Y)	**	**	**	*
Sowing density (D)	n.s.	n.s.	**	*
Genotype (G)	n.s.	n.s.	***	***
Y \times D	n.s.	n.s.	*	n.s.
Y \times G	*	*	***	**

Level of significance: 0, **** 0.001, *** 0.01 and ** 0.05; n.s.: not significant. Other interactions are not reported since they were not significantly different at the 0.05 probability level.

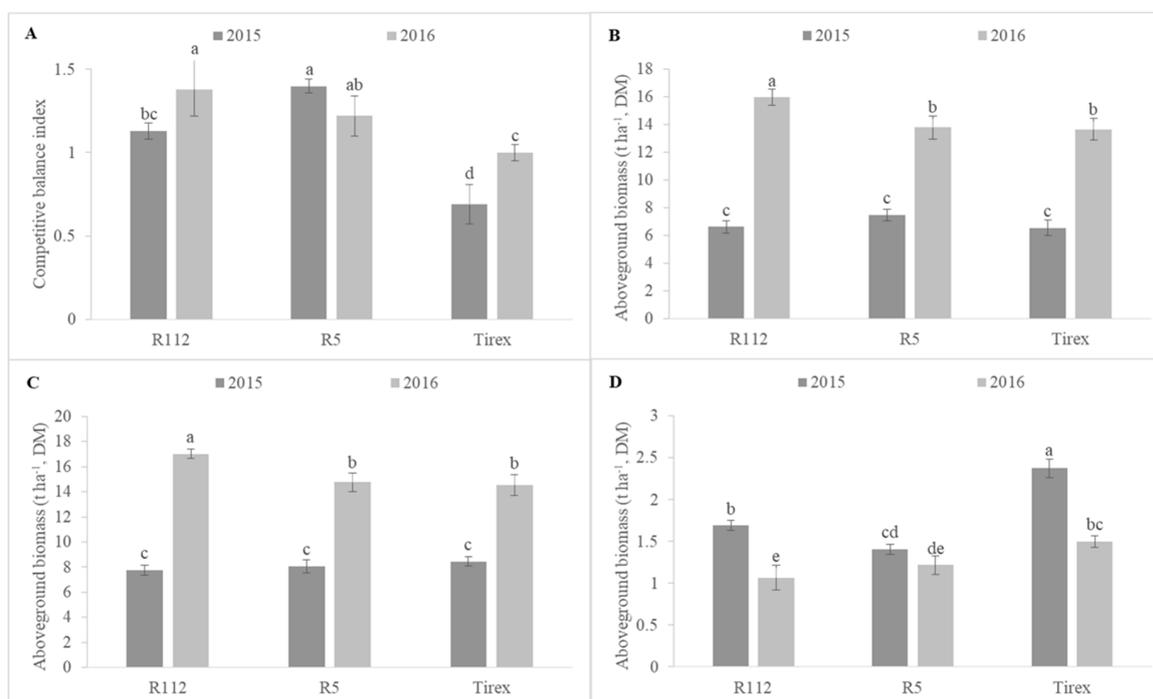


Figure 4. Competitive balance index (A) and aboveground biomass of: (B) Durum wheat in weedy condition, (C) durum wheat in weeded condition, (D) weeds. Genotype \times year interaction. Bars sharing the same letter are not significantly different at the 0.05 probability level.

The Cb index was always higher than 0 but it significantly varied between years and genotypes ($p = 0.003$, $lsd = 0.19$, Figure 4A). In 2015, R5 showed the highest Cb value (1.4) followed by R112 (1.1) and Tirex (0.7). In 2016, R112 and R5 obtained similar results (1.4 and 1.2, respectively) while Tirex showed a significantly lower Cb value (1.0). Sowing density also significantly affected the competitive

ability of durum wheat ($p = 0.029$), with a normal rate having higher Cb value than a lower one (1.3 vs. 1.0, data not shown).

Cb showed a significant positive correlation with grain yield in both weedy and weeded condition (Table 4), while it was negatively correlated with grain yield loss ($r = -0.53$).

Table 4. Pearson's correlation coefficients among competitive index (Cb), grain yield loss (GYL), grain yield in weedy condition (GYw), and grain yield in weeded condition (GYwf).

	GYL	Cb	GYw	GYwf
GYL	1			
Cb	-0.53 *			
GYw	-0.69 *	0.57 *		
GYwf	-0.21	0.36 *	0.84 *	1

* Significant at the 0.05 probability level.

3.3. Light Interception and Flag Leaf Chlorophyll Content

Intercepted radiation was significantly influenced by year \times genotype \times weed presence interaction at each phenological phase (Table 5). Intercepted PAR in 2016 was always higher than that detected in 2015 for all genotypes and weed presence at each phenological phase (Figure 5). In 2015, weedy plots of R112 and Tirex intercepted an amount of radiation significantly higher than that of weeded ones at all phenological phases, while weedy and weeded R5 plots intercepted a similar amount of radiation at both booting and heading stage. Overall, R5 weeded plants showed a radiation interception significantly higher than that of the other weeded genotypes in 2015. Conversely, in 2016 when weather conditions became favorable, R112 intercepted more radiation than R5 and Tirex.

Table 5. Results of the ANOVA for intercepted radiation (IPAR), chlorophyll content (CHL), flavonol content (FLAV), and nitrogen balance index (NBI) in different growing stages (B, booting; H, heading; A, anthesis; F, grain filling).

Source of Variation	IPAR				CHL				FLAV				NBI			
	B	H	A	F	B	H	A	F	B	H	A	F	B	H	A	F
Year (Y)	*	*	**	**	n.s.	n.s.	n.s.	n.s.	*	n.s.						
Sowing density (D)	n.s.															
Genotype (G)	n.s.	n.s.	**	n.s.	**	*	**	***	*	n.s.	*	***	*	n.s.	n.s.	n.s.
Weed presence (W)	***	***	***	***	n.s.	n.s.	n.s.	n.s.	*	n.s.	*	n.s.	*	*	*	n.s.
Y \times D	n.s.	*	n.s.	n.s.	n.s.	n.s.										
Y \times G	***	**	***	**	n.s.											
Y \times W	*	**	n.s.	*	n.s.											
D \times G	n.s.	n.s.	***	n.s.	*	n.s.	n.s.	n.s.	n.s.							
D \times W	n.s.	*	n.s.	**	n.s.											
G \times W	n.s.	n.s.	n.s.	**	n.s.											
Y \times G \times W	**	*	**	*	n.s.											

Level of significance: 0, '****' 0.001, '***' 0.01 and '*' 0.05; n.s.: not significant. Other interactions are not reported since they were not significantly different at the 0.05 probability level.

Analysis of chlorophyll content of flag leaves during different phenological phases showed significant variations among the genotypes (Table 5). R5 and R112 exhibited a higher Chl content up to Z75 stage as compared to Tirex (+12% and +8% as average, respectively); yet, at Z65 the difference between R112 and Tirex was not significant (Figure 6). R5 chlorophyll content constantly increased until anthesis and then decreased during the grain filling period. Chlorophyll content of Tirex increased from booting to heading stage, remained constant between heading and anthesis, and finally declined during grain filling. R112 chlorophyll content showed a similar trend to that of Tirex but it remained

substantially unchanged from heading to grain filling. This tendency of R112 to maintain greenness during grain filling was confirmed by the results obtained for nitrogen balance index (NBI) between anthesis (Z65) and grain filling stage (Z75). While in this period, NBI of R5 and Tirex decreased by 12.4% and 13%, respectively, and R112 decreased by 7.9% only.

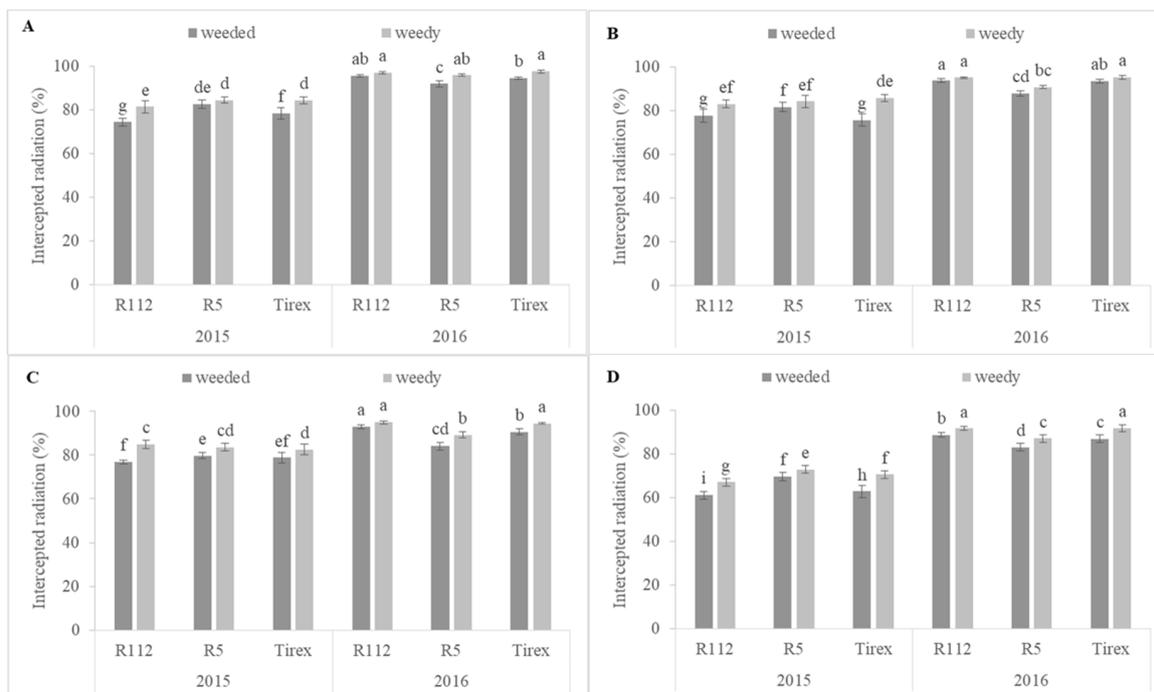


Figure 5. PAR intercepted from durum wheat during: (A) Booting (Z47), (B) heading (Z57), (C) anthesis (Z65), and (D) grain filling (Z75). Year × genotype × weed presence interaction. Bars sharing the same letter are not significantly different at the 0.05 probability level.

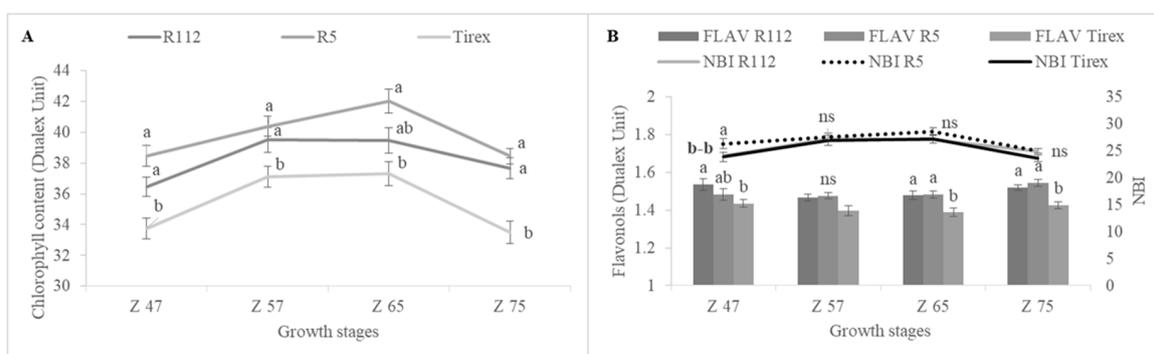


Figure 6. Flag leaf chlorophyll and flavonol contents of the three durum wheat genotypes recorded during booting (Z47), heading (Z57), anthesis (Z65), and grain filling periods (Z75): (A) Chlorophyll content, (B) flavonol content, and nitrogen balance index (NBI) (Z, Zadoks). For each growth stage, means sharing the same letter are not significantly different at the 0.05 probability level.

No statistical differences were observed among genotypes for NBI over time, except for booting stage in which R5 showed a significant NBI increase (9–10%).

3.4. Morphological Traits: Flag Leaf and Plant Height

A significant involvement of genotype in the control of flag leaf area (FLA) was found ($p < 0.001$, $lsd = 2.06$), while sowing density and weed presence did not have significant effect on flag leaf traits

(Table 6). This was due to differences in flag leaf width (FLW) more than flag leaf length (FLL). R5 and R112 showed a FLW similar between each other and significantly higher (15.3% and 13.3%, respectively) as compared with Tirex ($p < 0.001$, $lsd = 0.066$, Table 7). This led to a significant increase in the FLA: 20.6% for R5 and 12.5% for R112. Flag leaf morphology was also significantly affected by the growing season, with all traits showing higher values in 2016 than 2015 (+15% for FLW, +5% for FLL, and +22% for FLA, data not shown).

Table 6. Results of the ANOVA for morphological traits.

Source of Variation	Flag Leaf Width	Flag Leaf Length	Flag Leaf Area	Plant Height
Year (Y)	**	**	*	**
Sowing density (D)	n.s.	n.s.	n.s.	n.s.
Genotype (G)	***	*	***	*
Weed presence (W)	n.s.	n.s.	n.s.	n.s.

Level of significance: 0, '****' 0.001, '***' 0.01 and '*' 0.05; n.s.: not significant. Interactions are not reported since they were not significantly different at the 0.05 probability level.

Table 7. Mean values \pm standard error of the flag leaf dimensions and plant height of the three durum wheat genotypes across the 2015 and 2016 seasons.

Trait	R112	R5	Tirex
Flag leaf width (cm)	1.84 \pm 0.04 a	1.87 \pm 0.03 a	1.61 \pm 0.02 b
Flag leaf length (cm)	22.75 \pm 0.26 b	23.95 \pm 0.22 a	23.03 \pm 0.20 b
Flag leaf area (cm ²)	31.84 \pm 1.11 a	33.77 \pm 0.92 a	27.72 \pm 0.70 b
Plant height (cm)	72.9 \pm 1.70 b	75.5 \pm 1.51 a	75.0 \pm 1.47 a

In each row, means sharing the same letter are not significantly different at the 0.05 probability level.

Plant height was affected by both genotype and year (Table 6). R5 and Tirex displayed the tallest plants (75.5 cm and 75.0 cm, respectively), while R112, the shortest ones (72.9 cm, $p = 0.014$, $lsd = 1.77$, Table 7). In line with annual amount of precipitations (see paragraph 3.1), the tallest plants were harvested in 2016 (81 cm on average) while the shortest ones in 2015 (67.9 cm, $p = 0.002$, data not shown).

4. Discussion

4.1. Genotypes and Weed Competition

The different response of recombinant lines tested here on grain yield and its components has been illustrated elsewhere [16,18]. However, information must also be provided about the response of these materials to weed competition even in comparison with commercial cultivars.

Besides competition index calculated in this study, a measure of weed tolerance can be determined by comparing yield loss in the presence of weeds relative to weed-free conditions [21,28,36,48]. Outcomes from this two-year experiment indicate that, subject to weed competition, genotypes responded differently according to weather conditions (Figure 2). R5 showed higher competitive ability under the drier conditions that occurred in 2015, while the more favorable (higher rainfall) environment of 2016 favored R112. Conversely, Tirex suffered the presence of weeds in both seasons.

The variation among locations and years in the ability to tolerate weeds was already observed for bread wheat infested by *Lolium rigidum* Gaud., by [49,50] in Australia. This large variability when examining genotype \times year \times weed interactions makes the differences among plant materials hard to point out and data obtained not a reliable support in the decision-making process for farmers [49,50]. Although seasonal variations had a significant influence on tolerance ability of recombinant lines (ability to maintain grain yield in competition with weeds), R5 consistently suppressed weed growth in both years (Figure 4A,D). Competitive ability of a crop consists of two aspects: The ability of the crop (1) to keep yield high when grown in weedy conditions (ability to tolerate competition) and (2) to prevent

weed growth (ability to suppress weeds). The traits contributing to the ability to tolerate competition may differ from those conferring ability to suppress weeds [21,51]. Studies conducted in Australia suggested that weed suppression ability is a more consistent measure of crop competitive ability than crop tolerance [21] and a most desirable agronomic trait, since it has long-term implications for weed management in cropping systems [14]. Among others, canopy height, early vigor, flag leaf dimensions (particularly flag leaf length), and angle were identified as the most important traits contributing to suppressive ability [21,26,52–56]. Averaged over years, R5 showed a significantly higher plant height and flag leaf length than R112 (Table 7). Moreover, in 2015, even though R5 and R112 produced a similar aboveground biomass (Figure 4B,C), the former recombinant line intercepted significantly more radiation than R112 at all growth stages (Figure 6), thus making R5 the best suppressive genotype (Figure 4A,D). This was probably due to a different ‘canopy architecture’ characterizing R5 vs. R112, with R5 having a more planophile leaf inclination than R112. This morphological trait was recently confirmed measuring flag leaf angle of these two recombinant lines grown under controlled conditions (growth chamber experiment, currently ongoing). Results from ANOVA showed that R5 had a flag leaf angle (measured as the angle between the stem and the midrib of the abaxial surface of 10 flag leaves per genotype at heading) significantly higher than that of R112 (62° vs. 15.3° , unpublished data).

Many authors have considered canopy structure as a useful attribute to determine differences in competitive ability among wheat and barley genotypes [15,52]. In the United Kingdom, [57] found that a wheat variety that intercepted a greater percentage of PAR was more effective at suppressing weed growth than that with low light interception ability. Similar results were obtained in Australia by screening a wide range of bread and durum wheat genotypes [21]. In Germany, following a comparison of three bread wheat varieties under organic farming conditions, [58] suggested that higher light interception showed by the most competitive cultivars was principally induced by planophile leaf inclination. Overall, genotypes with more upright leaves were considered less competitive than genotypes with more horizontal leaf orientation [53,59]. Canopy structure could also explain the better performance of R5 in the drier conditions of 2015 as compared to those of 2016. In fact, a spreading leaf habit tends to shade soil since early growing stages, thus limiting evaporation and leaving more water for transpiration and growth in later stages [60,61]. Conversely, when water stress is not a limitation to wheat growth and yield, an erect positioning of the leaves facilitates the penetration of sunlight to bottom leaves, thereby enhancing the total interception of PAR, dry matter accumulation, and grain yield [62,63]. This might well be the case for R112 in the 2016 season, when it proved to be more efficient than R5 and Tirez in intercepting PAR (especially from anthesis to grain filling period, Figure 5), outperforming the other two genotypes on biomass production (Figure 4B,C) and grain yield (Figure 2). In line with these observations, the establishment of photosynthetically active canopy, consistently associated with the R112 alien gene content, was advocated as a likely contributing factor to the positive yield performance of this durum wheat-*Th. ponticum* recombinant line [16,18]. Therefore, an ideal wheat genotype should include a spreading leaf habit at the early growth stages and more vertical leaves during reproductive development [62]. Other factors, such as differential rooting patterns or allelochemical production, could also have influenced the relative abilities of genotypes to suppress weeds [14,21,64,65]. Our results have shown that weed biomass was significantly lower within R5 and R112 vs. Tirez plots with no significant increase in the aboveground biomass of the recombinant lines (Figure 4), which may well suggest allelopathic effects associated with the *Th. ponticum* segments. This is in line with previous studies suggesting that useful allelopathic sources should be sought in *Secale* or wild relatives of the *Triticeae* family, such as *Leymus* and *Thinopyrum* [66,67].

Tirez was consistently less competitive than the recombinant lines in both seasons (Figure 4D). In developing new cultivars, targeting primarily high yield and quality traits, breeders have neglected competitive ability for decades [49,55]. This made the new wheat cultivars totally dependent on chemical weed control [21]. Since there is an increasing interest in sustainable weed management strategies, breeders will need to verify that their new materials are able to tolerate or suppress weeds. In this frame, traits that confer greater competitive ability, without causing a yield penalty, should be

addressed. There is evidence that a satisfactory trade-off between competitiveness and yield potential can be met in oat [68], barley [69–71], and wheat [52,54,72]. By showing a positive and significant correlation between competitive ability (Cb) and grain yield in weed-free condition (Table 4), our study extends this possibility to durum wheat and highlights the possibility to recruit this ability in wild wheat relatives, such as *Th. ponticum* (see [22,23]). Therefore, it is possible to take advantage of stable, chromosomally engineered wheat lines, like R5 and R112, for breeding and cultivation purposes (see, e.g., [16,17,73]). The negative relationship between competitive ability and weed-free yield potential represents a major constraint to breeding for competitive wheat cultivars and was mainly related to the low harvest index of tall varieties with strong suppressive ability [14,15,55]. In organic systems, where weed infestation level is often high and nitrogen availability low, choosing cultivars on the basis of their height rather than yield potential may be a good strategy. However, under conventional regimes, farmers still choose cultivars primarily for their yield potential and disease resistance. In this context, breeding activity should focus on physiological and morphological traits other than plant height. Our study pointed out that flag leaf size and chlorophyll content, amount of intercepted radiation and, probably, canopy architecture all contributed to a higher competitive ability of recombinant lines along with higher or similar yield potential as compared to Tirez.

Additionally, a large seed size can be considered a good predictor for competitive ability as well. A study in the United States demonstrated that wheat competitive ability against wild oat increased with increasing seed size [20]. Several studies have shown that wheat plants derived from large seeds resulted in a higher seedling vigor than those coming from small seeds [74–76]. Therefore, the competitive dominance of plants established from large seeds lies in their ability to preempt resource acquisition at the expense of weeds. In our study, we found recombinant lines having a significantly higher TGW than Tirez (Table 2), and this might have also contributed to their competitive ability.

4.2. Sowing Density: Effect on Yield and Weed Competition

Conditions linked to the cultivation site and farming system (e.g., organic vs. conventional) may greatly affect growth of crop and weeds, thus requiring different agronomic tactics. Sowing rate variation is one of the most effective and easy tools by which farmers can simultaneously optimize wheat yield and competitiveness. The positive response in crop competitive ability to the increase of sowing rate is well documented in different wheat-producing countries [14,15]. However, the effect of intraspecific competition on grain yield has to be taken into account, as well as the well-known ability of wheat to compensate for reduced plant density [26,36]. In the drier year of our study (2015), a sowing rate of 250 seeds m^{-2} was optimal, because it provided a similar grain yield to that of higher (350 seeds m^{-2}) sowing density (Figure 3A). Conversely, when weather conditions were favorable throughout the season (2016), the positive effect of normal sowing density (350 seeds m^{-2}) on grain yield was evident (Figure 3A). A similar outcome was found by [77] in a study conducted in a semi-arid region of China: In the 2008 dry season, grain yield decreased linearly with increasing seeding rate from 225 to 340 seeds m^{-2} , while an opposite pattern was observed in the most favorable conditions of 2009. In the present investigation, the marked yield decrease recorded for normal sowing rate in 2015 as compared to 2016 (−44%) can be ascribed to a higher consumption of soil water before stem elongation which, in turn, caused a water shortage and greater competition later when rainfall was scarce or absent. This determined a larger amount of infertile tillers for normal-seeded plots in 2015 than in 2016 (−23% spikes m^{-2} , data not shown).

The sowing rate \times genotype interaction was significant for grain yield, indicating that plant material responded differently to sowing density. Reports from literature highlighted contrasting results on this aspect. Our findings are consistent with those by [78], who tested two winter wheat varieties at different seeding rates in western Canada and those by [26], who compared modern and old cultivars of bread wheat at two sowing densities (250 and 400 seeds m^{-2}) in central Italy. Conversely, [31,79] did not find any significant sowing rate \times genotype interaction working on bread wheat in Nebraska, USA (20 genotypes and 4 seeding rates), and durum wheat in central Italy (3

cultivars and 3 sowing densities), respectively. In our study, Tirex and R5 yielded similarly both at 250 and 350 seeds m^{-2} while R112 performed significantly better at normal than low sowing rate (Figure 3B). This outcome may originate from both a different canopy architecture between R112 and R5, as previously discussed, and a higher plasticity of Tirex and R5 as compared to R112 (Supplementary Figure S2A). It is worth mentioning that in a future climate scenario, where extreme events will be probably more frequent, breeding for density-neutral cultivars will allow crops to be more resilient to climate change. In fact, density-neutral cultivars can achieve yield maximization under both high and limited resources [34,80]. To this respect, the R5 recombinant line, tested both in the present and in previous trials (e.g., [16]) under an array of sowing densities, appears as a highly stable genotype.

The higher sowing density tested in our experiment significantly improved competitive ability of durum wheat as compared to the lower (higher Cb). This was due to a significant reduction in the total weed biomass, whereas no difference was detected in durum wheat aboveground biomass between the two sowing rates (Table 3). However, although total weed biomass was reduced by almost 16% adopting higher sowing density (averaged over seasons and genotypes), a marked difference between the two seeding rates was only recorded in the most favorable season of 2016 (more than 30% of weed biomass reduction, Supplementary Figure S4). Many studies revealed that increases in crop density resulted in a great reduction of weed biomass because of reduced light transmittance [24,81–83]. Since we did not find any significant year \times sowing density interaction for intercepted PAR from booting to grain filling, we hypothesize that the low-light environment hindered weed growth during the early growth stages. Likewise, [36] suggested that crop density is more important to offset competition during vegetative growth rather than during reproductive development.

5. Conclusions

Wheat cultivars developed for low-input or even organic farming systems should combine weed suppressive ability with high yield potential, disease resistance, and quality traits. Thanks to the present study and previous ones involving the same or similar genetic materials [16,18,84], we demonstrated that a suitable trade-off among all the desirable characteristics is feasible, taking advantage of the as-yet poorly exploited alien variation stably inserted in chromosomally engineered wheat lines, such as our durum wheat-*Thinopyrum ponticum* recombinants. Specifically, R5 was consistently the most weed-suppressive genotype across the two years, although its yield potential was lower than R112 in favorable weather conditions. R112 greatly suffered weed competition in the drier season, thus confirming to be better suited to environments with optimal thermo-pluviometric patterns [16]. Compared to both recombinant lines, cv. Tirex showed a relatively poor competitive ability in spite of its good tillering ability. Traits that were found to be associated with crop competitive ability were: the amount of intercepted PAR, flag leaf dimensions, and seed size. Canopy architecture probably also played an important role in determining the significant year \times genotype interactions detected in the present study, but this feature will have to be studied further and more deeply in a specifically designed experiment. Moreover, a hypothesis on the existence of allelopathic effects of recombinant lines on weeds, associated to the presence of *Th. ponticum* chromatin, should not be excluded and deserves more investigation in the future.

Beside morphological traits and environmental conditions, crop competitiveness was determined by sowing density as well. Overall, normal sowing density (350 seeds m^{-2}) proved to be more effective than the lower (250 seeds m^{-2}) for adequate suppression of naturally occurring flora; nonetheless, under dry environments, a reduction of sowing rate was able to optimize grain yield while limiting weed infestation. To this regard, a combination of an appropriate seed rate with a suitable choice of genotype might improve yield performance. In fact, R112 required normal sowing rate to maximize grain yield, while R5 proved to be a density-neutral genotype.

Supplementary Materials: The following are available online at <http://www.mdpi.com/2073-4395/10/5/709/s1>. Figure S1: Number of spikes per unit area: year \times genotype \times weed presence interaction. Figure S2: Number of spikes per unit area: (A) genotype \times sowing density interaction, (B) year \times sowing density interaction.

Figure S3: Thousand grain weight (TGW): Genotype \times year interaction. Figure S4: Weed aboveground biomass: Year \times sowing density interaction.

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