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Engineered durum wheat germplasm with multiple alien introgressions: agronomic and quality performance

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Abstract: If genetic gains in wheat yield are to be achieved in today's breeding, increasing genetic variability of cultivated genotypes is an essential requisite to meet. To this aim, alien gene transfer through chromosome engineering (CE) is a validated and sound strategy. Attempts to incorporate more than one alien segment into cultivated wheat have been rare, particularly for tetraploid durum wheat. Here we present the agronomic and quality performance of the first successful CE-mediated multiple introgression into the latter species. By assembling into 7AL, 3BS and 1AS arms of a single genotype homoeologous segments of *Thinopyrum ponticum* 7el$_1$L, *Aegilops longissima* 3S^{l}S, and *Triticum aestivum* 1DS arms, respectively, we have stacked several valuable alien genes, comprising *Lr19+Sr25+Yp* (leaf and stem rust resistance and a gene increasing semolina yellowness), *Pm13* (powdery mildew resistance) and *Gli-D1/Glu-D3* (genes affecting gluten properties), respectively. Advanced progenies of single, double and triple recombinants were field-tested across three years in a typical durum wheat growing area of Central Italy. The results showed that not only all recombinants had normal phenotype and fertility, but also that one of the triple recombinants had the highest yield through all seasons compared with all other recombinants and control cultivars. Moreover, the multiple introgressions enhanced quality traits, including gluten characteristics and semolina yellow index. Presence of effective disease resistance genes confers additional breeding value to the novel and functional CE products, which can greatly contribute to crop security and safety.

Keywords: chromosome engineering; wheat breeding; *Aegilops longissima*; *Thinopyrum ponticum*; gluten quality; yield; leaf rust; stem rust; powdery mildew

1. Introduction

Durum wheat (*Triticum durum* var. *durum*, 2n = 4x = 28, AB genomes) is a major staple crop in the Mediterranean Basin, where its cultivation largely replaced that of tetraploid emmer, *T. dicoccum*, by the first millennium B.C. [1]. In keeping with the crop's geographical origin, hence adaptation to mild winters and dry summers, the Mediterranean region represents the largest world's growing area (60%) for durum wheat [2]. Other major cropping regions are in Northern United States of America, Canada and Northern Mexico, besides minor ones in Southern Eurasia, India, South Australia and Argentina [3–5]. Durum wheat is mostly used for pasta making, but it is also the raw material for producing other traditional foods, mostly typical of Mediterranean countries, such as flat breads, couscous and bulgur. Current re-discovery of traditional foods on one hand, and, on the other hand, the new consumption habits of the growing urban populations, particularly in Asian and African developing countries [6–8], are boosting popularity and demand for wheat- and, specifically, durum wheat-derived products, such as pasta and couscous [5,9]. Strong value chains for such products, already in place in traditional durum wheat growing countries and interestingly emerging for unconventional territories and markets [5], lead to forecast an increase in the global durum wheat cropping, over the current 5-6% of total wheat production [10,11]. Moreover, with respect to the more worldwide spread bread wheat (*T. aestivum*, 2n = 6x = 42, ABD), durum wheat exhibits an exceptional adaptation to most booming and threatening climatic stresses, notably heat and drought. In a future perspective, this feature will be able to make durum wheat, a strategic crop and commodity for marginal land farmers in the Mediterranean environment [5], together with other few drought tolerant species, [e.g. 12,13]. Therefore, interventions aimed at maintaining and broadening the durum wheat genetic basis are highly required and beneficial to sustainably cope with current and forthcoming limitations to secure and safe yields [14].

Among advanced plant breeding strategies, chromosome engineering (CE) represents an effective approach to achieve genetic gains in wheat by resorting to its related gene pools, including those of the wild Triticeae species (reviewed in [14–18]). Through CE, chromosomal segments harbouring useful genes can be transferred from related (e.g. wild) genomes into those of cultivated wheats with high precision [19–22]. In most cases, CE-based transfers rely on the promoting effect on pairing and recombination between corresponding, albeit not fully homologous (i.e. homoeologous), chromosomes of different Triticeae species exerted by mutations for wheat *Ph* (Pairing homoeologous) genes, mainly *Ph1* [23,24]. Whatever the intergenomic/interspecific cytogenetic relatedness, CE is inherently based on sexual gene transfer, hence representing an excellent non-GMO, yet non-conventional breeding option. The CE approach, in particular, offers a sustainable way to effectively use the still little exploited exotic genes from secondary and tertiary gene pools, and make them relevant for agriculture. Many genes have been so far transferred in wheat by means of CE, mainly disease resistance genes, but also genes for abiotic stress tolerance, grain quality and yield-related traits (reviewed in [14,16,25]). Nevertheless, major impact on breeding of genotypes created through CE have been rare so far, and mainly regarded hexaploid bread wheat, due to its bigger economic importance and better tolerance to chromosome manipulations with respect to tetraploid durum wheat. In the latter, phenomena such as sterility, reduced seed germination, segregation distortion, and anomalies of plant habit are more often observed than in the former upon alien transfers, with linkage drag more dramatically worsening with increasing segment size (e.g. [15,25–31]).

Transfer of alien chromatin in wheat through CE generally involved single segments deriving form one donor species, and targeted single wheat chromosomes. However, the possibility of combining, i.e. pyramiding, useful genes from different alien sources in a single genotype represents an appealing target, potentially enabling simultaneous enrichment of crop genotypes with a variety of novel, valuable features. This can be achieved either by "nesting" chromosome portions of different but closely related alien sources in a single alien segment of a given wheat recipient chromosome, or by stacking multiple alien segments on different wheat chromosomes. The former approach implies that the alien chromatin of primary and subsequent transfers share homologous or homoeologous relationships, hence being capable of recombination. It was through homologous

recombination between the 6RL arms of different rye (*Secale cereale* L.) cultivars, each inserted in wheat genetic backgrounds, that the *Pm20* powdery mildew resistance gene of one rye source was combined with residing genes of the other rye accession [32]. Several examples of recombination-based wheat-alien gene pyramiding involve group 7 chromosomes of perennial grass species belonging to the *Thinopyrum* genus [15,16]. In bread wheat, "composite" alien segments, including chromatin from hexaploid *Th. intermedium* and decaploid *Th. ponticum* were generated on the 7DL arm, contributing the *Bdv2* BYDW resistance gene and the leaf rust (*Lr19*) and stem rust (*Sr25*) resistance genes, respectively [33,34]. Furthermore, two effective Fusarium head blight (FHB) resistance QTL, one from *Th. ponticum* accession "el$_2$" (*Fhb-7el$_2$* or *Fhb7*) and the other from diploid *Th. elongatum* (*Fhb-7EL*), were combined with *Lr19* and other valuable genes of the "el$_1$" accession of *Th. ponticum* onto the 7DL arm [35–38]. The same pyramiding of *Fhb-7el$_2$* or *Fhb-7EL* with *Lr19* and other 7el$_1$ genes was also realised in durum wheat within a single *Thinopyrum* segment, distally located on the 7AL arm [35,39]. Since the size of the alien block introgressed in all the quoted cases remains within tolerable amounts by the recipient genome, such recombinant genotypes represent promising materials for use in breeding and cultivation.

On the other hand, stacking multiple alien segments from more than one alien species within a single wheat genotype has more unpredictable outcomes in view of practical exploitation. Examples are limited to a few even for the more amenable hexaploid bread wheat genome. One such case is that of Singh et al. [40], who combined the widely exploited whole-arm 1RS·1BL translocation (1RS from rye, *S. cereale*; [41]) with the 7AgL (= 7el$_1$L) sizable translocation from *Th. ponticum* (named T4 or Agatha, reviewed in [16]) on the 7DL arm. In addition to the *Lr19+Sr25* rust resistance genes and yield-contributing genes of 7AgL derivation (see [16]), the 1RS arm was known to carry multiple disease resistance genes [42] and to determine positive effects on yield, both in bread wheat [41,43] and durum wheat [31]. Apart from the lateness defect and some grain yield penalty associated with the double translocation, its breeding potential, as of any 1RS·1BL translocation, was limited by the presence on 1RS of the secalin *Sec-1* locus, which negatively affects dough quality [44]. Another example of multiple alien segment stacking was reported by Ali et al. [45], who identified bread wheat lines with enhanced resistance to wheat streak mosaic virus (WSMV), due to the contemporary presence on wheat 4D and 1B chromosomes, respectively, of two short arm centric translocations, one from *Th. intermedium* (4Ai#2S), bearing the *Wsm1* gene, and the other from rye (1RS), probably carrying an enhancer of the WSMV resistance. Good field performance of the isolated recombinant and the absence of any meiotic instability associated with the alien chromatin presence, gave hope for their use in breeding programs [45].

A remarkable example of multiple alien introgressions is represented by the Chinese cultivar Xiaoyan 6, widely cultivated in the 1980s-'90s and later used as a core parent for bread wheat breeding in China. In Xiaoyan 6, at least two wheat chromosomes (2A and 7D) carry chromosomal segments from *Th. ponticum*, with genes contributing tolerance to diseases and stressful environmental conditions, as well as good quality and yield stability [46]. Interestingly, Xiaoyan 6 derivatives, in which the rye 1RS arm or even the entire 2R chromosome were introduced in place of wheat 1BS and 2D, respectively, are cytogenetically stable, have additional disease resistances and beneficial agronomic attributes, including high seed-set, making them readily usable in production [47].

To our knowledge, only one case of multiple segment stacking can be recorded for durum wheat. This resulted from a successful attempt to combine in a single tetraploid genotype three different individual transfers, involving the 7AL, 3BS and 1AS wheat arms, each bearing homoeologous portions of *Th. ponticum* 7el$_1$L, *Aegilops longissima* (2n = 2x =14) 3S^{l}S, and *T. aestivum* 1DS arms, respectively [48,49]. The individual transfer lines were selected among an array of wheat-alien recombinant types, obtained by *ph1*-induced homoeologous recombination, as bearing the respective target genes associated with alien segments of minimal length and exhibiting satisfactory agronomic performance. With all three alien segments being inserted at the most distal ends of the respective wheat arms, the *Th. ponticum* 7el$_1$L portion spans 23% of the recombinant 7AL and harbours the *Lr19+Sr25* resistance genes, but also the *Yp* gene, increasing endosperm and semolina yellow pigmentation [49,50], along with several QTL enhancing yield-related traits [28,29,51]. The *Ae.*

longissima 3S^{l}S segment contains *Pm13*, a highly effective resistance gene to powdery mildew, and replaces around 20% of the 3BS arm [26,52,53]. Finally, the 1DS chromosome segment derived from *T. aestivum*, harbours the *Gli-D1*/*Glu-D3* storage protein genes, and replaces 17% of durum wheat 1AS arm, containing the *Gli-A1*/*Glu-A3* homoeoloci [26,54,55]. Introduction of the *Gli-D1*/*Glu-D3* genes into the tetraploid context resulted in improved SDS, gluten index and dough strength (W) values, as well as a good dough tenacity-to-elasticity (P/L) ratio, potentially suitable for both pasta and bread making [49,56]. Meiotic stability of the triple introgression line was shown not to be upset, and the simultaneous transmission of the three alien segments to be normal through both germlines [25,48]. Notwithstanding this, potential use in breeding and cultivation of any type of wheat-alien recombinant line depends upon its overall agronomic performance validated under field conditions. Some preliminary small-scale evaluation of the original durum wheat-triple alien recombinant, developed in the background of the Italian cv. Simeto, was previously carried out, and gave promising results for both yielding capacity and grain quality [49]. In our durum wheat pre-breeding program, we have transferred the three alien segments in different varietal backgrounds. Here we report the results of the first field-scale comparative evaluation, run over a 3-year period, of the agronomic performance of durum wheat recombinants with one, two or all three of the described alien segments, in the prevailing background of the French cv. Karur, well adapted to Central-Northern Italy conditions. Semolina quality attributes of genotypes, with the most promising productive features, will also be illustrated.

2. Materials and Methods

2.1. Plant material

Six durum wheat recombinant lines (RLs) containing one, two or three chromosome segments from different alien Triticeae species were employed for the yield assessment in the field (Table 1). Each exotic segment harbours the respective gene(s) of interest, namely *Lr19+Sr25+Yp* (7el$_1$L), *Gli-D1*/*Glu-D3* (1DS) and *Pm13* (3S^{l}S, see Introduction), and is present within each recombinant line in homozygosis. The RLs were isolated in the F_2 generation after three backcrosses (BC_3) to the French cv. Karur of the initial triple recombinant (= durum wheat line, homozygous for all three alien introgressions selected in BC_1 progeny to the Italian cv. Simeto). The newly isolated recombinants possess the LMW-2 allelic form of low-molecular-weight glutenin subunits (LMW-GS) at the *Glu-B3* locus on 1BS, associated with best end-use quality of durum wheat (reviewed in [57]), and the cv. Karur alleles at the *Glu-B1* locus on 1BL, coding for the '6+8' high-molecular-weight glutenin subunits (HMW-GS) (unpublished). On 1AS, the *Gli-D1*/*Glu-D3* alleles transferred from bread wheat (see Introduction) replace the 1AS homoeoalleles. The recombinants analysed here represented F_{4-6} progenies after BC_3 to Karur and were assessed together with six durum wheat cultivars: Karur and Simeto, the prevailing genotypes in the pedigree of the RLs, and cvs. Kanakis, Ramirez, Achille and Dylan, chosen as good yielders and widely cultivated in Italy, with similar heading date to that of cv. Karur and the RLs [58,59].

Table 1. Description of alien introgressions and recombinant lines used in the present study.

Recombinant chromosome	Donor species	Alien segment size (% arm)	Alien genes	Recombinant line					
				R11-20	R9-11	R9-71	R9-59	R2-21	R11-8
7AS·7AL-7el$_1$L	*Th. ponticum*	23	*Lr19+Sr25+Yp*	+	+	+	+	+	+
1AL·1AS-1DS	*T. aestivum*	17	*Gli-D1/Glu-D3*	+	+	+	+	+	–
3BL·3BS-3S'S	*Ae. longissima*	< 20	*Pm13*	+	+	+	–	–	–

2.2. Plot trials and growing seasons

Three rain-fed field trials were carried out in Viterbo (42° 25' N, 12° 4' E, Central Italy) in the 2014-15, 2015-16 and 2016-17 seasons (from this point onward referred to as 2015, 2016 and 2017, respectively), and used for yield assessment of the novel chromosomally engineered genotypes. Details on each experimental year are reported in Table 2. In the first two seasons, all six recombinants were tested together with the two recurrent cultivars Karur and Simeto in small plots (1.5 m x 1.5 m). Based on the results from the first two years, only the three most promising recombinants, i.e. R11-20, R9-11 and R2-21, were tested in regular plots (1.5 m x 7 m) in the 2017 season, along with six control cultivars, namely Karur, Simeto, Kanakis, Achille, Ramirez and Dylan. In all field experiments, a complete randomized block design with three replicates for each genotype was used, resulting in 24 total plots in 2015 and 2016 seasons, and 27 plots in 2017. During the growing seasons, data for daily temperatures (minimum, mean and maximum) and rainfall (Table 2) were retrieved from the meteorological station located at the experimental farm of the University of Tuscia, where all trials were carried out. In all seasons, nitrogen fertilization (208 kg ha^{-1}) was split into three applications: the first was given at sowing as di-ammonium phosphate (22% of total N applied), the second when the first node was detectable above ground (Zadoks 31 phase, [60]) as urea (38% of total N), and the third at heading (Zadoks 57) as ammonium nitrate (40%). Weed control was performed during tillering by a single distribution of commercial herbicide Atlantis®Pro at a rate of 1.5 L ha^{-1}. The commercial fungicide Folicur®WG was applied once during grain filling period in order to evaluate the impact of alien introgressions on yield performance, independently of the possible advantage provided by the *Lr19*, *Sr25* and *Pm13* genes against leaf rust, stem rust and powdery mildew attacks. To monitor the efficacy of the alien resistance genes, 5-10 rows of corresponding near-isogenic lines were separately grown in each season without any fungicide treatment.

Table 2. Description of experimental seasons analysed in the present study.

Season	2014-15	2015-16	2016-17
Sowing date	15 Jan 2015	17 Nov 2015	6 Dec 2016
Harvest date	30 Jun 2015	30 Jun 2016	28 Jun 2017
Crop cycle length (days)	166	226	204
Sowing density (seed/m^2)	350	350	350
Plot size (m^2)	2.25	2.25	10.5
Total rainfall (mm)	238	383	176
Mean temperature at heading (°C)	18.6	13.2	12.1
Sowing to heading			
Rainfall (mm)	215	257	122
T_{min} (°C)	4.8	4.2	3.4
T_{mean} (°C)	9.6	9.3	8.7
T_{max} (°C)	15.1	14.9	14.6
Heading to harvest			
Rainfall (mm)	22	126	54
T_{min} (°C)	12.9	12.2	12.5
T_{mean} (°C)	19.7	17.9	19.7
T_{max} (°C)	26.8	24.3	27.0

2.3. Measurement of agronomic traits

In the 2015 and 2016 seasons, heading date (HD) was recorded as number of days from April 1st to the plant stage when 50% of culms in a plot had reached the Zadoks 55 phase. At maturity and post-harvest, the following traits were recorded: plant height (PH), grain yield m^{-2} (GYM2), biomass m^{-2} (BM2), harvest index (HI; as GYM2/BM2 ratio), grain number m^{-2} (GNM2), spike number m^{-2} (SNM2), thousand grain weight (TGW), grain yield $spike^{-1}$ (GYS), grain number $spike^{-1}$ (GNS), grain number $spikelet^{-1}$ (GNSP), spikelet number $spike^{-1}$ (SPN), spike length (SL), spike chaff dry weight

(CHAFF), and spike fertility index (SFI; as GNS/CHAFF ratio). PH was measured on eight culms per plot just before harvest. Four 1 m-long rows were harvested per each plot to obtain yield traits per unit area (GYM2, BM2, HI, SNM2, GNM2) and TGW. TGW was obtained from weighing two 200-seed samples plot^{-1} and then used to calculate GNM2 as GYM2×1000/TGW. From each harvested sample, eight randomly selected spikes were taken for measurements of single spike traits (GYS, GNS, GNSP, SPN, CHAFF and SFI). All dry weights of spikes, chaff and total aboveground biomass were recorded after 48 h oven-drying at 65 °C. Narrow sense heritability (h^2) was calculated for each trait across the 2015 and 2016 seasons as follows [61]:

$$h^2 = \sigma^2_G/(\sigma^2_G + \sigma^2_{G\times Y}/n)$$

where n is number of years/environments tested, G and Y are the genotype and year effects, respectively and:

$$\sigma^2_G = (MS_G - MS_{G\times Y}) / n$$
$$\sigma^2_{G\times Y} = MS_{G\times Y}$$

where MS is the mean square.

The 2015 trial alone was partly described in [25] by a subset of yield-related traits, included also in the present multi-year assessment (SNM2, BM2, GYM2, GNM2, HI). Furthermore, traits such as TGW, GYS, GNS and GNSP were re-analysed here with respect to [25], on a bigger number of samples per plot (8 vs. 5), and in the case of HD, by adopting April 1st as more common starting date for day count instead of the sowing date.

In the 2017 season, two lateral rows were trimmed from each 10.5 m^2 plot, and the remaining 7 m^2 of the plot area harvested and used for measurement of GY (grain yield ha^{-1}), SNM2, TGW, GNM2, and test weight (TW). HD and PH were determined in the same way as in the first two seasons. TW was assessed on a grain sample for each plot by using a Schopper Chondrometer equipped with a 250 ml cylindrical metal container.

2.4. Grain and semolina quality tests

Quality traits were determined on seeds harvested in the 2017 season for RLs R11-20, R9-11 and R2-21 and cv. Karur. The same amount of seeds from three replicated plots of each genotype was combined in a single 1.2 kg sample to be used for all analyses. All tests were performed on technical duplicates, derived from the same grain sample. Grain protein and moisture were assessed by Inframatic 9500 NIR grain analyser (Perten Instruments, Hägersten, Sweden). Ash content was determined in the furnace according to the ISO 2171/1993 method. Analyses of semolina and gluten parameters were carried out on semolina samples obtained with a laboratory mill Labormill 4RB (Bona, Monza, Italy), after the grains were tempered for 18h to 17% moisture. Colour analysis was performed using the reflectance colorimeter Chroma meter CR-200 (Konica Minolta Inc., Tokyo, Japan) and absolute values of brightness (L^*) and yellow-blue chromacity (b^*) measured by manufacturer's instructions. The b^* value represents the variation in semolina yellow index and is highly correlated with yellow pigment content of whole meal flour extracts [62,63]. The brightness reading was used to determine brownness i.e. brown index as (100 - L^*). Gluten parameters (wet gluten, dry gluten, gluten index) were measured according to the ISO 10275 - 01/1994 method by using Glutomatic 2200 instrument (Perten Instruments, Hägersten, Sweden). Brabender farinograph (Brabender GmbH & Co. KG, Germany) was used to assess farinograph quality number (FQN), water absorption (14% moisture), peak time, stability and softening at 12 minutes after the peak time (E_{icc}), according to the "Italiana B" method, which corresponds to a modification of the ICC115/1 standard method for using the Brabender farinograph. Specifically, while according to the ICC115/1 method the peak time represents the point of farinogram curve just before the first signs of dough softening, the "Italiana B" method measures the peak time as a time lapse from the start of the test until the first highest value on the farinogram curve is reached. Moreover, differently from the ICC115/1 method, "Italiana B" implies use of a constant amount of flour/semolina (300 g), regardless of its humidity, and a variable amount of water to reach the optimal ratio between the two components (500 FU).

2.5. Statistical analysis

To assess differences between genotypes, an analysis of covariance (ANCOVA) was applied. Genotype (G) was entered as a fixed factor against replicate (R) as the covariate. For the analysis of year effect in interaction with genotype across the 2015 and 2016 years (all recombinants tested, see above), analysis of variance (ANOVA) was used by applying a general linear model (GLM) on the 2-year dataset. Each variable (i.e. trait measured) was entered as a 'dependent' factor against 'independent' factors, i.e. genotype (G), year (Y), and replicate (R). Replicate was used in all models as the error, and analysed as a year-nested, first-order interaction [R(Y)]. The first-order interaction G × Y was also examined. In all analyses three levels of significance were considered, corresponding to $P<0.05$, $P<0.01$ and $P<0.001$. When significant factors and/or interactions between them (F values) were observed, a pairwise analysis was carried out by the Tukey Honestly Significant Difference test at the 0.95 confidence level. Normality of data was assessed for each variable by Kolmogorov-Smirnov (K-S) test. In addition, for the 2-year dataset from the 2015 and 2016 seasons, a principal component analysis (PCA) was used to examine the nature of relationships between key productivity traits and genotypes. PCA was performed and graphed in the R Environment (R Project for Statistical Computing 3.5.2, The R Foundation for Statistical Computing, Vienna, Austria, 2018), by functions *prcomp()* and *ggbiplot()*. All other analyses were made by SYSTAT12 software (Systat Software Incorporated, San Jose, CA, USA).

3. Results

3.1. Environmental conditions

The three experimental seasons were characterized by similar temperature conditions throughout the growth cycle (Table 2), with higher values (particularly the minimum temperatures) with respect to the site's multi-year means [64–66]. Winters in all three seasons were unusually mild for the area. The 2015 and 2017 seasons were typically very hot and dry in the period from heading to harvest; yet in these years, events of sudden temperature falls were observed around anthesis (from mid-April to beginning of May), which could have had some negative impact on spike fertility [64–66]. Rainfall amount and distribution were more unstable and variable between the seasons (Table 2). Anomalous heavy rains were recorded from October to December in 2015 and 2017 seasons, which determined a full soil moisture profile and a significant delay of the sowing date (Table 2). On the other hand, in 2016 season unusually abundant rainfall occurred in the period from heading onwards and during grain filling. However, the total precipitations in all three seasons resulted to be lower than the 30-year site's mean, which could be associated with the observed general increase in temperatures.

3.2. Yield assessment

3.2.1. Small-scale plots

In the first two seasons (2015 and 2016), when all six recombinant lines were tested in small-scale trials, a GLM model was applied to examine the genotype and year effects on the expression of recorded traits (Table 3). Irrespective of the number of alien segments contained, all RLs showed good performance for all traits, being in most cases superior to at least one of the control cultivars, Karur and Simeto. The range and distribution of values for all traits were different to a variable extent (Figure S1) and, overall, RLs showed to be more similar to Karur than to Simeto, as expected from their pedigree (see Materials and Methods). The two control varieties were significantly different for a number of traits, with Karur heading about one week later and showing superior values (13-66%) for the majority of other traits (except TGW, GYS and GNSP, Table 4) compared with Simeto. Although most traits showed normal distribution (K-S test, Table 4), positive transgressive segregation was observed in the case of spike traits, such as yield, biomass and grain number (GYS, CHAFF, GNS), for which all RLs displayed higher values with respect to both control varieties

(Figure S1 and Table 4). Narrow-sense heritability was high for GNM2, SNM2, HD, TGW and essentially for all spike traits, and medium for GYM2, BM2, HI and PH (Table 4), indicating the presence of strong genetic factors influencing the expression of yield potential of the recombinants.

Across the first two years, the genotype effect (G) was statistically significant for all parameters, with three of them, i.e. GYM2, GNM2 and SL, being independent of the year (Y) effect (Table 3). The two RLs with the highest grain yield m^{-2} across the two seasons were the triple recombinants R11-20 and R9-11 (Table 4). Interestingly, R11-20 (though not R9-11) yielded significantly more (+52% GYM2) than a third triple recombinant, R9-71. With respect to the latter, R11-20 had also significantly higher biomass (+33% BM2), harvest index (+15% HI), thousand grain weight (+10% TGW), grain yield $spike^{-1}$ (+18% GYS) and spikelet fertility (+10% GNSP). As a whole, R9-71 was the least productive of all RLs and showed reduced performance for most yield traits unit $area^{-1}$ vs. both controls (Table 4).

As revealed by the Tukey test analysis of significant G × Y interactions (see Table 3), genotype-dependent differences emerged more clearly under the 2016 environmental conditions, rather than in 2015 (Figure 1). In 2016, the triple recombinants R11-20 and R9-11 had the highest values for harvest index (HI), grain yield (GYS) and grain number (GNS) $spike^{-1}$, with differences being significant vs. more than one of the other genotypes (Figure 1, Table S1). On the other hand, the 2015 environment was probably at a disadvantage for R9-11, since its yield (GYM2, GYS), 1000-grain weight (TGW), and spike fertility (GNS, SFI) were medium-to-low vs. those of other genotypes (Table S1). In the same year, no yield penalty affected the triple recombinant R11-20, which resulted significantly superior for spike fertility traits (GYS, GNSP) when compared with R9-59 (double) and R9-11 (triple) recombinants, and with Karur (Figure 1, Table S1). Therefore, being the only recombinant that in both years confirmed higher grain yield with respect to both Karur and Simeto, and higher values for all other traits vs. at least one control cultivar (Table S1), R11-20 can be considered as the durum wheat recombinant with the most promising yield potential.

Table 3. Mean squares from the GLM for yield and yield-related traits of the 8 genotypes across the 2015 and 2016 seasons [df, degrees of freedom; GYM2, grain yield m^{-2} (g); BM2, biomass m^{-2} (g); HI, harvest index; GNM2, grain number m^{-2}; SNM2, spike number m^{-2}; PH, plant height, (cm); HD, heading date (days); TGW, thousand grain weight (g); GYS, grain yield $spike^{-1}$ (g); GNS, grain number $spike^{-1}$; GNSP, grain number $spikelet^{-1}$; SFI, spike fertility index; SL, spike length (cm); SPN, spikelet number; CHAFF, spike biomass without seeds(g)]. *, **, and *** indicate significance at $P<0.05$, $P<0.01$ and $P<0.001$ levels, respectively.

Factor	G		Y		G x Y		R(Y)		Error
df	7		1		7		4		28
GYM2	12704.4	*	8991.0		7932.3		1341.9		3871.5
BM2	53432.8	*	1197633.8	**	31304.2		17905.1		16021.7
HI	0.003	***	0.128	***	0.002	**	0.002	*	0.000
GNM2	5963918.1	**	470143.0		2046131.3		242809.3		1357093.7
SNM2	1993.7	*	3700.1	*	550.7		252.9		776.3
PH	98.3	***	297.8	***	56.1	**	31.0		12.2
HD	59.4	***	2227.7	***	5.0	*	0.792		2.1
TGW	120.9	***	103.2	***	9.4		8.7		5.4
df	7		1		7		4		364
GYS	2.1	***	2.5	**	1.9	***	0.3		0.2
GNS	1470.5	***	3260.0	***	341.2	***	106.2		60.2
GNSP	1.1	***	37.6	***	0.3	**	0.1		0.1
SFI	926.3	***	1457.7	***	220.5	**	433.5	***	76.5
SL	59.9	***	0.004		0.504		1.381	*	0.465
SPN	222.5	***	1047.7	***	26.1	***	5.7		2.629
CHAFF	0.3	***	1.7	***	0.1		0.2	***	0.035

Table 4. Mean values of yield-related traits and narrow-sense heritability of the analysed genotypes across 2015 and 2016 seasons (GYM2, grain yield m^{-2}; BM2, biomass m^{-2}; HI, harvest index; GNM2, grain number m^{-2}; SNM2, spike number m^{-2}; PH, plant height; HD, heading date; TGW, thousand grain weight; GYS, grain yield $spike^{-1}$; GNS, grain number $spike^{-1}$; GNSP, grain number $spikelet^{-1}$; SFI, spike fertility index; SL, spike length; SPN, spikelet number; CHAFF, spike chaff; h^2, narrow-sense heritability; K-S, Kolmogorov-Smirnov normality test). Letters in each row correspond to the ranking of the Tukey test at $P<0.05$ level following the GLM analysis.

Genotype	**R11-20**		**R9-11**		**R9-71**		**R9-59**		**R2-21**		**R11-8**		**Simeto**		**Karur**		**h^2**	**K-S test**
7el$_1$/1D/3S^{l} [a)]	**+ + +**		**+ + +**		**+ + +**		**+ + −**		**+ + −**		**+ − −**		**− − −**		**− − −**			**(*P*-value)**
GYM2 (g)	354.9	a	339.8	ab	233.2	b	261.2	ab	261.7	ab	276.6	ab	249.0	ab	327.9	ab	0.38	0.196
BM2 (g)	954.2	a	907.8	ab	714.8	b	793.0	ab	752.0	ab	793.1	ab	830.1	ab	969.6	a	0.41	0.058
HI	0.38	a	0.38	a	0.33	bc	0.34	abc	0.36	ab	0.35	abc	0.31	c	0.35	abc	0.33	0.084
GNM2	6691.9	a	6663.0	a	4827.8	ab	5145.1	ab	5269.2	ab	5372.6	ab	3992.8	b	6645.2	a	0.66	0.188
SNM2	203.5	ab	190.8	ab	161.5	b	172.3	ab	177.8	ab	189.8	ab	186.7	ab	219.9	a	0.72	0.226
PH (cm)	79.1	a	69.8	bcd	67.5	d	68.8	cd	74.8	abc	75.0	abc	74.5	abc	76.0	ab	0.43	0.052
HD (days)	32.3	c	36.8	a	35.7	ab	34.8	abc	35.2	ab	34.3	abc	26.7	d	33.0	bc	0.92	0.369
TGW (g)	53.0	b	50.7	bc	48.3	c	50.6	bc	49.7	bc	51.6	b	62.4	a	49.0	bc	0.92	0.442
GYS (g)	3.02	a	2.81	ab	2.55	bcd	2.55	bcd	2.75	abc	2.71	bc	2.47	cd	2.39	d	0.06	0.001
GNS	57.1	a	55.9	a	53.3	abc	50.1	bc	54.0	ab	52.7	abc	39.6	d	48.7	c	0.77	0.000
GNSP	2.69	a	2.45	bc	2.45	bc	2.30	cd	2.52	ab	2.55	ab	2.47	bc	2.18	d	0.69	0.053
SFI	64.6	abc	60.9	bcd	65.3	ab	59.8	cd	67.0	a	67.0	a	55.9	d	68.7	a	0.76	0.101
SL (cm)	8.7	abc	9.2	a	8.6	bc	9.0	ab	9.2	a	8.0	d	5.8	e	8.3	cd	0.99	0.219
SPN	21.3	bc	22.7	a	21.7	abc	21.8	abc	21.9	abc	21.0	c	15.9	d	22.2	ab	0.88	0.233
CHAFF (g)	0.90	ab	0.94	a	0.82	bcd	0.84	ab	0.82	bc	0.81	bcd	0.70	d	0.71	cd	0.79	0.000

a) Symbols of alien chromosomes involved in the transfers into A or B genome chromosomes of durum wheat

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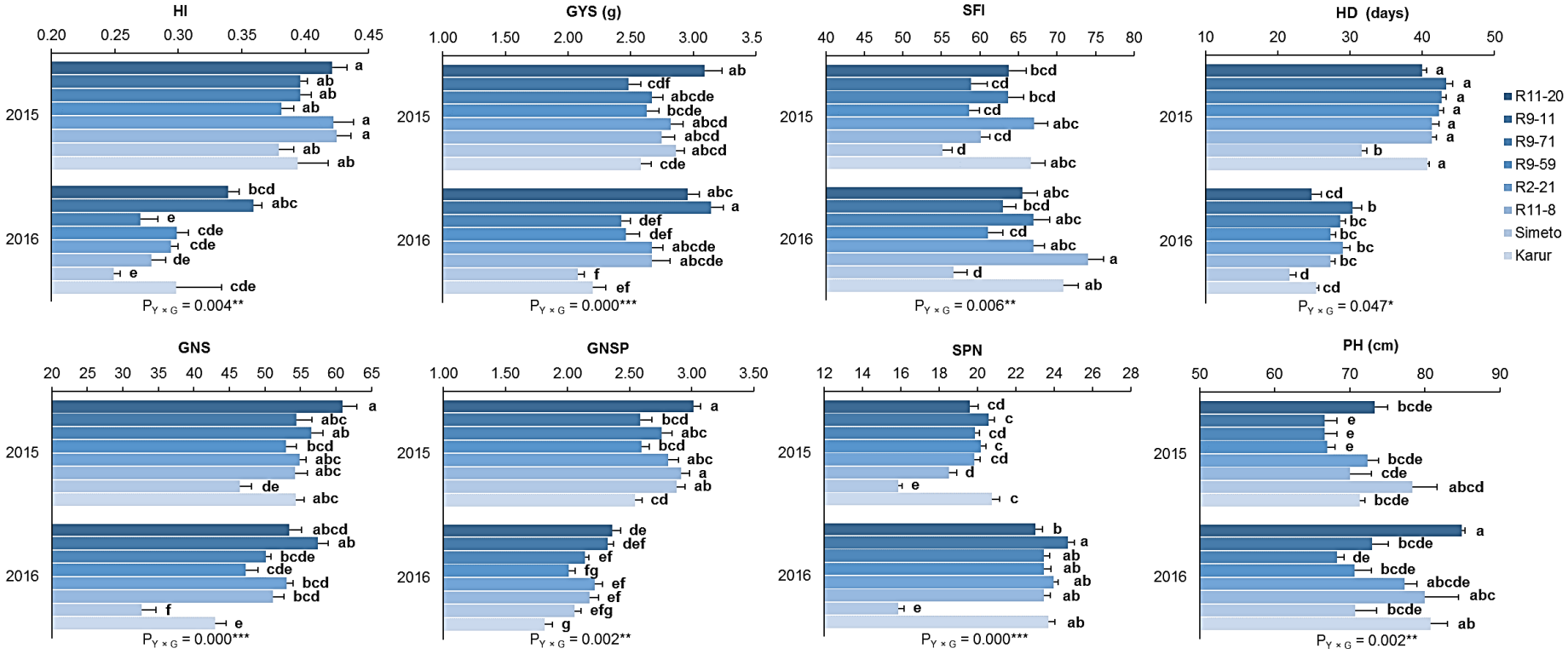

Figure 1. Significant G × Y interactions of yield-related traits recorded across 2015 and 2016 seasons for the eight tested genotypes and analysed by the GLM model (HI, harvest index; GYS, grain yield spike^{-1}; SFI, spike fertility index; HD, heading date; GNS, grain number spike^{-1}; GNSP, grain number spikelet^{-1}; SPN, spikelet number spike^{-1}; PH, plant height). Letters for each trait represent ranking of the Tukey test at P<0.05 level. ** and *** indicate significance at P<0.01 and P<0.001 level, respectively.

3.2.2. Relationship between main yield components

To identify the grain yield (GYM2) components that mainly contributed to its final expression in 2015 and 2016 trials, a principal component analyses (PCA) was conducted (Table S2). The first two axes accounted for 74.7% of the total variance (axis 1: 45%, axis 2: 29.7%), as visualised by a bi-plot graph (Figure 2). Principal component 1 (PC1) was largely and positively related to grain yield, biomass and spike number m^{-2} (the angle between the latter two showing strong interrelatedness), and to a lesser extent to thousand-grain weight (TGW). At the same time, PC1 was negatively influenced by heading date. Increases on PC2 were related mainly to grain number and yield $spike^{-1}$ and less to heading date, while the TGW weighted moderately in negative direction. These results are in line with those of GLM analysis, showing that higher yield of the RLs was always accompanied by higher biomass production, harvest index and spike fertility parameters (see above).

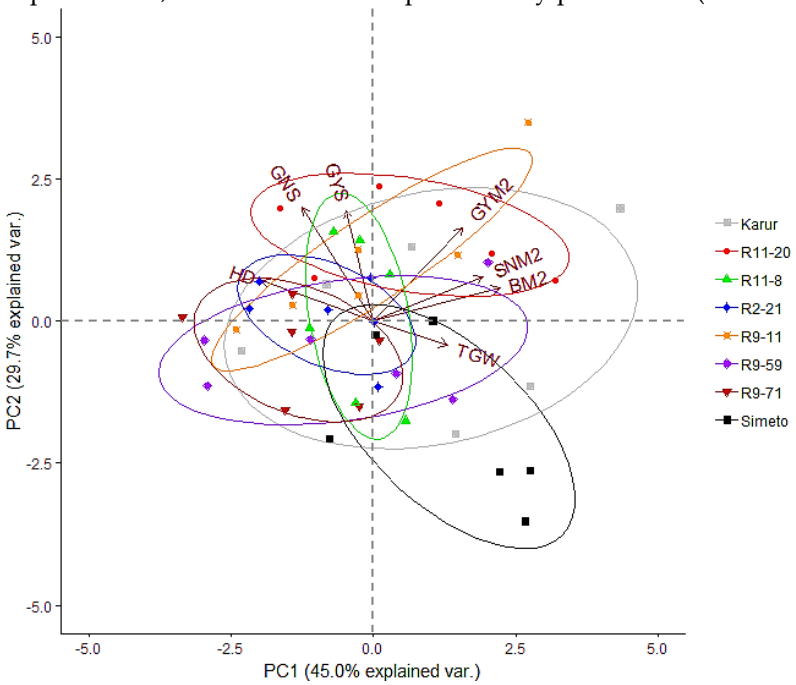

Figure 2. Bi-plot of the first two axes of principal component analysis for the main yield components of the eight genotypes analysed in 2015 and 2016 (GYM2, grain yield m^{-2}; BM2, biomass m^{-2}; SNM2, spike number m^{-2}; TGW, thousand grain weight; GNS, grain number $spike^{-1}$; GYS, grain yield $spike^{-1}$; HD, heading date).

3.2.3. Large-scale plots and grain quality of the most promising recombinants

The two best performing triple recombinants in 2015 and 2016 seasons, R11-20 and R9-11, and one of the two (similarly performing) double recombinants, namely R2-21, were further assayed in large plot trials in 2017. Along with the two recurrent varieties Karur and Simeto, the RLs were also compared with four of the most productive and largely cultivated varieties in Italy in recent years (Table 5). ANCOVA showed differences between genotypes to be significant for six out of seven traits (Table S3). All RLs and cvs. Karur and Achille had about one week later heading date than the other lines (Table 5). Plant height of all genotypes showed to be somewhat lower with respect to previous years, probably due to reduced rainfall in 2017. The environmental conditions in 2017 were comparable to those of 2015 (Table 2), in both years apparently favouring the R11-20 yield potential. By contrast, R9-11 and R2-21 yielded on average 23% less than R11-20 (GY, Table 5), the difference being significant in the case of R9-11. The results from 2017 showed that also under larger scale cultivation, R11-20 displayed significantly higher GY (+33%) vs. the other triple recombinant, i.e. R9-11. At the same time, R11-20 yield was the second highest in 2017 (Table 5), inferior (but not

significantly) only to that of Kanakis, the most productive cultivar in the Tyrrhenian coastal area in the same year [66]. Grain number m^{-2} was the trait which mostly contributed to GY increase of R11-20, as shown by the Pearson correlation (Table S4). Its thousand-grain weight, on the other hand, was better than that of Karur, yet lower than that of most other genotypes, not having a significant effect on final yield (Table 5).

Table 5. Mean values of yield related traits recorded in 2017 season (HD, heading date; PH, plant height; GY, grain yield; SNM2, spike number m^{-2}; GNM2, grain number m^{-2}; TGW, thousand grain weight; TW, hectolitre/test weight). Letters in each row correspond to the ranking of the Tukey test at $P<0.05$ level.

Trait	Genotype																	
	R11-20		**R9-11**		**R2_21**		**Kanakis**		**Achille**		**Ramirez**		**Karur**		**Dylan**		**Simeto**	
$7el_1/1D/3S^{l}$ a)	+ + +		+ + +		+ + –		– – –		– – –		– – –		– – –		– – –		– – –	
HD (days)	29	ab	31	a	31	a	25	de	28	bc	26	cd	30	ab	23	f	23	ef
PH (cm)	72.7	ab	60.7	c	72.7	ab	77.7	a	75.0	ab	74.7	ab	72.0	ab	69.3	abc	66.3	bc
GY (t ha^{-1})	4.63	ab	3.49	c	3.67	bc	5.22	a	4.36	abc	4.17	bc	3.97	bc	3.95	bc	3.93	bc
SNM2	263.3		284.4		254.4		262.2		230.0		254.4		252.2		247.8		240.0	ns
GNM2	9775.0	a	7570.0	abc	7609.1	abc	9426.0	ab	7893.5	abc	8374.5	abc	9059.6	ab	7327.4	bc	6223.9	c
TGW (g)	47.5	bcd	46.1	cd	48.3	bcd	55.2	abc	55.3	ab	49.8	bcd	43.8	d	54.1	bc	63.5	a
TW (kg hL^{-1})	81.3	cd	80.5	d	82.0	c	85.4	a	86.2	a	86.1	a	82.1	c	83.7	b	83.8	b

a) Symbols of alien chromosomes involved in the transfers into A or B genome chromosomes of durum wheat

Grain, semolina and gluten quality parameters were assessed in three of the multiple recombinant lines and the good-quality Karur parent (Table 6). As a whole, no negative effect on quality traits was found to be associated with contemporary presence of the alien segments into the durum background. Semolina milling yield was high (around 64%) and comparable in all four genotypes (Table 6). This result was in line with the observed high values (> 80 kg hL^{-1}) of the correlated test weight (Table 5). Similar among the sampled genotypes was also protein content, with values not inferior to 15%. On the other hand, recombinant lines, all carriers of the $7el_1L$-linked *Yp* trait and the associated *Psy1* gene controlling carotenoid biosynthesis (see, e.g. [36]), had up to +9% higher yellow index, a measure of carotenoid pigment content, as compared to Karur. Combined with lower ash content (-10% in R11-20), this contributes to the increase of semolina brightness and to carotenoid stability during semolina processing into pasta [67]. Gluten characteristics were all indicative of an increased strength of gluten extracted from recombinant lines, which incorporates the *Glu-D3*-encoded LMW-GS as main differential protein components vs. Karur. As a result, in the three recombinant lines carrying the 1DS segment in place of the most distal 1AS portion, gluten index (GI) was significantly enhanced of over 20% (GI = 98.5) in R11-20 (and similarly in R2-21 and R9-11) with respect to the already good value of Karur (GI = 81.8). The higher gluten strength of recombinant lines compared with Karur was consistent with significantly different values of farinograph parameters, including a longer mixing time to bring their dough to optimum development (peak time), an almost doubled stability and largely reduced subsequent softening (E_{icc}, FQN) (Table 6). This outcome is altogether indicative of a strong dough tolerance to fermentation and mechanical stress, as required for bread making [68].

Table 6. Grain and semolina quality traits of the selected genotypes in the 2017 season (E_{icc}, softening after 12 min; FQN, farinograph quality number). Letters in each row correspond to the ranking of the Tukey test at $P<0.05$ level. *, ** and *** indicate significance at $P<0.05$, $P<0.01$ and $P<0.001$ level, respectively.

Trait	Recombinant line								ANCOVA
	R11-20		R9-11		R2-21		Karur		*P* value
$7el_1/1D/3S^{l}$ a)	+ + +		+ + +		+ + –		– – –		
Grain protein content (%)	15.4		16.4		15.0		16.1		0.072
Grain moisture (%)	11.1		11.2		11.5		11.4		0.151
Yellow index (*b**)	31.6		30.1		31.7		29.1		0.202
Brown index (100 - *L**)	10.4	b	10.0	b	11.6	a	10.2	b	0.002**
Semolina yield (%)	64.2		64.1		64.3		64.5		0.932
Ash content (%)	0.82	d	0.85	c	0.87	b	0.91	a	0.000***
Gluten index (%)	98.5	a	95.1	a	98.2	a	81.8	b	0.001**
Water absorption (14%)	60.8		61.7		59.7		62.1		0.526
Peak time (min)	5.0	a	5.1	a	5.3	a	4.2	b	0.002**
Stability (min)	18.0	b	16.8	b	20.7	a	10.5	c	0.001**
E_{icc} (FU)	30.0	c	37.0	b	23.0	d	45.0	a	0.000***
FQN	170.0	b	150.0	c	200.0	a	100.0	d	0.000***

a) Symbols of alien chromosomes involved in the transfers into A or B genome chromosomes of durum wheat

4. Discussion

In the present study, the effects on agronomic and quality traits of the first successful multiple alien segment introgression into durum wheat were evaluated in rain-fed plot trials, carried out in Central Italy over three growing seasons. The durum wheat-alien recombinants, some of them simultaneously harbouring chromosome segments from *Th. ponticum*, *Ae. longissima* and *T. aestivum*, showed an excellent tolerance to the presence of one, two and even three alien segments (each one occupying not more than 23% of the recipient chromosome arm), as indicated by the absence of any adverse effects on plant fitness, productivity and grain quality. This result is highly significant, and not always readily expected in durum wheat breeding involving alien introgressions. In fact, negative impact of even single alien segments on morpho-physiological, yield and grain quality traits were often observed in durum wheat background as a consequence of linkage drag or excessive size of alien segments, particularly of wild origin (see Introduction). In the present study, not only all recombinants had normal phenotype and fertility, but also two of the triple recombinants, namely R11-20 and R9-11, had the highest yield (GYM2, Table 4) compared with control cultivars and all other recombinants. In particular, differences in the expression of yield traits between R11-20 and other genotypes were often significant across the three seasons, indicating very good productive potential of this genotype.

Nonetheless, comparisons for yield traits between specific chromosomal makeups, such as those of single vs. double or triple recombinants and vs. control varieties (absence of any alien segment), revealed considerable variation within each of them, irrespective of presence/absence of alien introgression(s). This prevents from directly associating any trait enhancement with presence *per se* of a given alien segment. Indeed, considering the residual background heterogeneity of the recombinant materials analysed here, the consistent yield advantage of some of them, notably of R11-20, over other recombinant types and most of high yielding checks (Table 5), is likely ascribable to the positive interaction of the alien alleles with those of the specific wheat background. In addition to a genetic basis (see, e.g. [69]), such line-specific phenotypes may well also have an epigenetic underpinning, which contributes to further diversity of crop-alien species combinations (e.g. [70,71]). Only in the case of the GNS trait, presence of a genetic determinant(s) within the 23%-long 7el$_1$L *Th. ponticum* segment might be hypothesised, as all six recombinants carry this segment and had significantly higher GNS vs. Karur and Simeto in the 2016 trial (Table S1). Improved values of spike-related traits (including grain number, spike fertility and also harvest index) were previously associated with presence of this segment [28,29,51], particularly when the corresponding durum wheat-*Th. ponticum* recombinant line R5 in the near-isogenic background of Simeto was tested in hot and dry environments [51]. Thus, a specific linkage of the R5-type segment with sink (i.e. spike) traits cannot be excluded. Still, both in previous trials of R5 alone [51] and in the present study on R5-containing genotypes, yield increase was also found to be associated with higher biomass and spike number m^{-2} (see R11-20 in all three years, R11-8 in 2015, R9-11 in 2016 in Table 4, Table S1, Figure 2). This is indicative of an equally important involvement of the source (leaves, tillers) in final yield formation of the best performing recombinants. Similar trends were observed also for spike traits, i.e. GYS and GNS (sink) and CHAFF (source), for which all recombinants displayed positive transgressive segregation with respect to background varieties Karur and Simeto (Table 4, Figure S1). Therefore, the genetic potential of the recently isolated multiple recombinants seems to be encouraging for future breeding, especially because GYM2 and GNM2 expression proved to be independent of the year effect (Table 3).

Whereas more trials in time and locations are needed to confirm yield stability and adaptability of the novel genotypes, it seems noteworthy the excellent performance of R11-20, especially in the dry and warm 2017 season, when it had an average 14% higher yield vs. widely cultivated and productive cultivars (Table 5). The consistently expressed increase of grain number and biomass in R11-20, especially in hot conditions, is of particular relevance for breeding for heat- and drought-prone environments, where such traits are essential for reaching significant yield gains (e.g. [72,73]).

In addition to sink and source traits that directly contribute to productivity, the presence of leaf rust (*Lr19*), stem rust (*Sr25*) and powdery mildew (*Pm13*) resistance genes represents an

additional breeding value of R11-20 in seasons when disease incidence is high. While leaf rust is a common and recurrent wheat disease in whole Europe and other wheat growing areas, stem rust, not observed since the 1950s [74], is a worryingly re-emerging disease. In fact, new highly virulent races of the stem rust pathogen are posing serious threat to the wheat crop all over the Mediterranean Basin and beyond. In the particularly severe outbreak occurred in Southern and mainland Italy in 2016, which destroyed thousands of hectares of wheat, mostly durum wheat, a new stem rust race, named TTTTF, was found to be almost exclusively present in the rust samples analysed [75]. To the complex virulence pattern of this race, not related to the Ug99 group, only a few *Sr* genes showed to provide effective resistance, namely *Sr31* (from *Secale cereale*), *Sr24* and *Sr25* (both from *Th. ponticum*). The durum wheat variety Cincinnato, a derivative of the R5 recombinant and hence carrier of *Sr25*, was completely unaffected by stem as well as leaf rust attacks in Southern Italy (Biagio Randazzo, pers. comm.). Similarly in Viterbo (Central Italy), where even heavy leaf rust attacks constantly occur and stem rust was detected in the last 4 years, all recombinants incorporating distal 7el$_1$L segments showed complete efficacy of the *Lr19* and *Sr25* genes against the respective rust disease (see, e.g., [36,39], and unpublished). Equally effective proved to be the *Ae. longissima Pm13* gene [76], not yet overcome by any powdery mildew race in various diseased areas worldwide (e.g. [77,78]). Considering the estimated 4.3 to 5 billion US dollars cost of global annual losses due to wheat rust diseases [79], the economic benefit of deploying efficient resistance genes, not to speak of food safety and security, is remarkable. Moreover, the rapid occurrence of new pathogen races, also favoured by climate changes [14,80–82] corroborates the use of resistance genes as the best disease management strategy. In this view, R11-20 recombinant represents an outstanding CE product, with great promise of efficiently facing current and future challenges, ready to be registered for cultivation and to be included in breeding pipelines in several environmental contexts.

Since high quality standards are also required for a commercially valuable variety [67,83], quality parameters have also been a major concern in the course of selection of the best candidate(s) for grain marketing among the multiple recombinant lines. The engineered lines produced first-grade commercial grain and semolina, comparable to that of the high-quality cv. Karur, as to important factors for the milling and processing industry, like protein content, semolina yield, ash content and grain moisture (Table 6). Furthermore, the 7el$_1$L and 1DS introgressions confirmed their positive contribution to specific quality attributes. The former conferred a moderate but consistent increase to yellow index of RLs vs. Karur, due to the presence in it of a *Phytoene synthase* allele (*Psy1-7el$_1$*), likely responsible for the associated *Yp* phenotype [36,84]. Since Karur itself is characterized by a high yellow index [85], and the trait has a typically additive expression, the increase in Karur background was of lower magnitude than that observed in the prevailing background of cv. Simeto [39,49,86]. Moreover, besides phytoene synthase, majorly responsible for carotenoid (= yellow colour) accumulation in the grain, other enzymes contribute to modulate semolina yellowness through carotenoid degradation, such as lipoxygenases, peroxidases and polyphenol oxydases [49,63,67], but their contribution to the final semolina colour was not assessed here. As for the effect of 1DS alleles in place of 1AS resident alleles, all multiple recombinants tested confirmed a prominent increase of parameters of gluten quality, as previously observed in single or multiple durum wheat recombinants having the same *T. aestivum* 1DS segment with *Gli-D1*/*Glu-D3* genes [49,56]. In all cases, irrespective of the variation for HMW-GS coded at the *Glu-B1* locus (see Materials and methods and [56]), LMW-GS encoded by *Glu-D3* alleles determined a considerable increase of gluten strength vs. Karur, as indicated by almost 20% higher gluten index (GI). Whereas no clear relationship seems to exist between gluten strength and pasta quality except for definitely undesirable weak gluten, a strong gluten is undoubtedly at the basis of bread making potential of durum wheat dough ([83] and references therein). In fact, GI showed a strong positive correlation with loaf volume of bread made from durum wheat, and so did dough rheological properties, particularly its stability (e.g. [68]). Besides strength, for the overall bread-making ability of durum wheat, additional dough attributes are known to be required, such as elasticity and extensibility. Optimal values for these attributes are typical of bread wheat flour and are mainly associated to HMW- and LMW-GS genes located on chromosome 1D [87]. Thus, as one approach to improve durum wheat bread-making performance,

various chromosome engineering exercises were undertaken, aimed at transferring 1D segments containing different *T. aestivum* 1D-encoded glutenin subunits ([55] and references therein). Among them, the early incorporation of *Glu-D3*-encoded LMW-GS in the 1AL·1AS-1DS recombinant chromosome, subsequently pyramided in the multiple recombinant types described here, resulted in more balanced alveograph values, particularly the tenacity-to-extensibility (P/L) ratio, than those associated with *Glu-D1*-encoded HMW-GS '5+10' ([56] and unpublished). Therefore, as in early work on 1AS/1DS transfer lines carrying *Gli-D1*/*Glu-D3* loci [56,88], the novel multiple recombinants (e.g. R11-20) seem to have good prospects for exploitation as dual-purpose durum wheat. To verify this potential, further quality tests, including alveograph analyses, as well as small-scale pasta and bread (loaf) preparations, are planned.

5. Conclusions

The use of alien genetic resources confirms to be a valid approach to widen the genetic basis of cultivated wheats, particularly through CE. Although CE strategies require adequate time and professional cytogenetic competences in the initial phases of the transfer work, thus being so far underutilised in a breeding perspective, the number of novel breeding lines and the even readily exploitable CE outcomes have been rapidly increasing in recent years (see [89] for a review). The multiple recombinants illustrated in the present work add a relevant contribution to this trend. Whether the CE strategy is designed to improve specific wheat traits, weakened or unavailable in the current germplasm (e.g. [36,39]; this work), or to capture genes/traits from the alien source in a genome-wide, "untargeted" fashion [90], no doubt it is a powerful platform through which the needed genetic variation can be infused into the crop genome to develop more stress-resilient, productive and high-quality durum wheat.

Supplementary Materials: The following are available online at www.mdpi.com/xxx/s1, Figure S1: Boxplots of the yield-related traits recorded through the seasons 2015 and 2016, Table S1: Mean values of yield-related traits of the analysed genotypes in each 2015 and 2016 seasons, Table S2: Correlations between four principal component variants and main yield-related traits of the eight genotypes analysed in the 2015 and 2016 seasons, Table S3: Mean squares from the ANCOVA of yield-related traits in the 2017 season, Table S4: Pearson's correlation coefficients between pairs of yield-related traits recorded for the four tested genotypes in the 2017 season

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References

1. Feldman, M. Historical aspects and significance of the discovery of wild wheats: (origin of wheat, evolution, gene pools. In Proceedings of the Stadler Genetics Symposium; Columbia, MO, USA,, 1977; Vol. 9, pp. 121–145.
2. Royo, C.; Soriano, J.M.; Alvaro, F. Wheat: A crop in the bottom of the Mediterranean diet pyramid. In *Mediterranean Identities - Environment, Society, Culture*; Fuerst-Bjelis, B., Ed.; Intechopen, London, 2017; pp. 381–399.
3. Ranieri, R. Geography of the durum wheat crop. *Pastaria Int.* 2015, pp. 24–36.
4. Bassi, F.M.; Sanchez-Garcia, M. Adaptation and stability analysis of ICARDA durum wheat elites across 18 countries. *Crop Sci.* **2017**, *57*, 2419–2430.
5. Sall, A.T.; Chiari, T.; Legesse, W.; Seid-Ahmed, K.; Ortiz, R.; van Ginkel, M.; Bassi, F.M. Durum wheat (*Triticum durum* Desf.): origin, cultivation and potential expansion in Sub-Saharan Africa. *Agronomy* **2019**, *9*, 1–20.
6. Kearney, J. Food consumption trends and drivers. *Philos. Trans. R. Soc. B Biol. Sci.* **2010**, *365*, 2793–2807.
7. Reardon, T.; Tschirley, D.; Dolislager, M.; Snyder, J.; Hu, C.; White, S. *Urbanization, diet change, and transformation of food supply chains in Asia*; 2014. Available online: http://www.fao.org/fileadmin/templates/ags/docs/MUFN/DOCUMENTS/MUS_Reardon_2014.pdf.
8. Reardon, T.; Echeverria, R.; Berdegué, J.; Minten, B.; Liverpool-Tasie, S.; Tschirley, D.; Zilberman, D. Rapid transformation of food systems in developing regions: Highlighting the role of agricultural research & innovations. *Agric. Syst.* **2019**, *172*, 47–59.
9. Yüksel, A.N.; Oner, M.D.; Bayram, M. Rediscovery of couscous in the world. *Glob. J. Med. Res. – Nutr. Food Sci.* **2018**, *18*, 25–30.
10. Dahl, C. Global durum outlook. Available online: http://www.italmopa.com/wp-content/uploads/2017/05/144_all_1.pdf (accessed on May 27, 2019).
11. International Grains Council. Available online: http://www.igc.int/en/default.aspx (accessed on May 27, 2019).
12. Danieli, P.P.; Primi, R.; Ronchi, B.; Ruggeri, R.; Rossini, F.; del Puglia, S.; Cereti, C.F. The potential role of spineless safflower (*Carthamus tinctorius* L. var. *inermis*) as fodder crop in central Italy. *Ital. J. Agron.* **2011**, *6*, 19–22.
13. Rossini, F.; Provenzano, M.E.; Kuzmanović, L.; Ruggeri, R. Jerusalem Artichoke (*Helianthus tuberosus* L.): A versatile and sustainable crop for renewable energy production in Europe. *Agronomy* **2019**, *9*.
14. Ceoloni, C.; Kuzmanović, L.; Forte, P.; Gennaro, A.; Bitti, A. Targeted exploitation of gene pools of alien Triticeae species for sustainable and multi-faceted improvement of the durum wheat crop. *Crop Pasture Sci.* **2014**, *65*, 96–111.
15. Ceoloni, C.; Kuzmanović, L.; Gennaro, A.; Forte, P.; Giorgi, D.; Grossi, M.R.; Bitti, A. Genomes, chromosomes and genes of perennial triticeae of the genus *Thinopyrum*: the value of their transfer into wheat for gains in cytogenomic knowledge and 'precision' breeding. In *Advances in Genomics of Plant Genetic Resources*; Tuberosa, R., Graner, A., Frison, E., Eds.; Springer, Dordrecht, The Netherlands, 2014; pp. 333–358 ISBN 9789400775756.
16. Ceoloni, C.; Kuzmanović, L.; Forte, P.; Virili, M.E.; Bitti, A. Wheat-perennial Triticeae introgressions: major achievements and prospects. In *Alien Introgression in Wheat - Cytogenetics, Molecular Biology, and Genomics*; Molnár-Láng, M., Ceoloni, C., Doležel, J., Eds.; Springer, 2015; pp. 273–313.
17. Chaudhary, H.K.; Kaila, V.; Rather, S.A.; Badiyal, A.; Hussain, W.; Jamwal, N.S.; Mahato, A. Wheat. In *Alien Gene Transfer in Crop Plants, Volume 2: Achievements and impacts*; Pratap, A., Kumar, J., Eds.; Springer, New York, 2014; pp. 1–26.
18. Feuillet, C.; Langridge, P.; Waugh, R. Cereal breeding takes a walk on the wild side. *Trends Genet.* **2008**, *24*, 24–32.
19. Sears, E.R. Chromosome engineering in wheat. In Proceedings of the Stadler Genetics Symp. 4; Kimber, G., Rédei, G.R., Eds.; Columbia, MO, USA, 1972; pp. 23–38.
20. Sears, E.R. Transfer of alien genetic material to wheat. In *Wheat Science: Today and Tomorrow*; Evans, L.T., Peacock, W.J., Eds.; Cambridge University Press, Cambridge, U.K., 1981; pp. 75–89.

21. Ceoloni, C.; Jauhar, P. Chromosome Engineering of the Durum Wheat Genome. In *Genetic resources, chromosome engineering, and crop improvement: cereals.*; Singh, R.J., Jauhar, P.P., Eds.; CRC Press, 2006; pp. 27–59. ISBN 9780203489260.

22. Qi, L.; Friebe, B.; Zhang, P.; Gill, B.S. Homoeologous recombination, chromosome engineering and crop improvement. *Chromosome Res.* **2007**, *15*, 3–19.

23. Sears, E.R. An induced mutant with homoeologous pairing in common wheat. *Can. J. Genet. Cytol.* **1977**, *19*, 585–593.

24. Giorgi, B. A homoeologous pairing mutant isolated in *Triticum durum* cv. Cappelli. *Mutat. Breed. Newsl.* **1978**, *11*, 4–5.

25. Ceoloni, C.; Kuzmanović, L.; Ruggeri, R.; Rossini, F.; Forte, P.; Cuccurullo, A.; Bitti, A. Harnessing genetic diversity of wild gene pools to enhance wheat crop production and sustainability: Challenges and opportunities. *Diversity* **2017**, *9*.

26. Ceoloni, C.; Biagetti, M.; Ciaffi, M.; Forte, P.; Pasquini, M. Wheat chromosome engineering at the 4x level: the potential of different alien gene transfers into durum wheat. *Euphytica* **1996**, *89*, 87–97.

27. Klindworth, D.L.; Hareland, G.A.; Elias, E.M.; Xu, S.S. Attempted compensation for linkage drag affecting agronomic characteristics of durum wheat 1AS/1DL translocation lines. *Crop Sci.* **2013**, *53*, 422–429.

28. Kuzmanović, L.; Gennaro, A.; Benedettelli, S.; Dodd, I.C.; Quarrie, S.A.; Ceoloni, C. Structural-functional dissection and characterization of yield-contributing traits originating from a group 7 chromosome of the wheatgrass species *Thinopyrum ponticum* after transfer into durum wheat. *J. Exp. Bot.* **2014**, *65*, 509–525.

29. Kuzmanović, L.; Ruggeri, R.; Virili, M.E.; Rossini, F.; Ceoloni, C. Effects of *Thinopyrum ponticum* chromosome segments transferred into durum wheat on yield components and related morpho-physiological traits in Mediterranean rain-fed conditions. *Field Crops Res.* **2016**, *186*.

30. Oak, M.D.; Tamhankar, S.A. 1BL/1RS translocation in durum wheat and its effect on end use quality traits. *J. Plant Biochem. Biotechnol.* **2017**, *26*, 91–96.

31. Zarco-Hernandez, J.A.; Santiveri, F.; Michelena, A.; Javier Peña, R. Durum wheat (*Triticum turgidum*, L.) carrying the 1BL/1RS chromosomal translocation: Agronomic performance and quality characteristics under Mediterranean conditions. *Eur. J. Agron.* **2005**, *22*, 33–43.

32. Friebe, B.; Heun, M.; Tuleen, N.; Zeller, F.J.; Gill, B.S. Cytogenetically monitored transfer of powdery mildew resistance from rye into wheat. *Crop Sci.* **1994**, *34*, 621–625.

33. Ayala-Navarrete, L.; Bariana, H.S.; Singh, R.P.; Gibson, J.M.; Mechanicos, A.A.; Larkin, P.J. Trigenomic chromosomes by recombination of *Thinopyrum intermedium* and *Th. ponticum* translocations in wheat. *Theor. Appl. Genet.* **2007**, *116*, 63–75.

34. Ayala-Navarrete, L.I.; Mechanicos, A.A.; Gibson, J.M.; Singh, D.; Bariana, H.S.; Fletcher, J.; Shorter, S.; Larkin, P.J. The Pontin series of recombinant alien translocations in bread wheat: single translocations integrating combinations of *Bdv2*, *Lr19* and *Sr25* disease-resistance genes from *Thinopyrum intermedium* and *Th. ponticum*. *Theor Appl Genet* **2013**, *126*, 2467–2475.

35. Forte, P.; Virili, M.E.; Kuzmanović, L.; Moscetti, I.; Gennaro, A.; D'Ovidio, R.; Ceoloni, C. A novel assembly of *Thinopyrum ponticum* genes into the durum wheat genome: pyramiding Fusarium head blight resistance onto recombinant lines previously engineered for other beneficial traits from the same alien species. *Mol. Breed.* **2014**, *34*, 1701–1716.

36. Ceoloni, C.; Forte, P.; Kuzmanović, L.; Tundo, S.; Moscetti, I.; De Vita, P.; Virili, M.E.; D'Ovidio, R. Cytogenetic mapping of a major locus for resistance to Fusarium head blight and crown rot of wheat on *Thinopyrum elongatum* 7EL and its pyramiding with valuable genes from a *Th. ponticum* homoeologous arm onto bread wheat 7DL. *Theor. Appl. Genet* **2017**, *130*, 2005–2024.

37. Shen, X.; Ohm, H. Molecular mapping of *Thinopyrum*-derived Fusarium head blight resistance in common wheat. *Mol. Breed.* **2007**, *20*, 131–140.

38. Zhang, X.; Shen, X.; Hao, Y.; Cai, J.; Ohm, H.W.; Kong, L. A genetic map of *Lophopyrum ponticum* chromosome 7E, harboring resistance genes to Fusarium head blight and leaf rust. *Theor. Appl. Genet.* **2011**, *122*, 263–270.

39. Kuzmanović, L.; Mandalà, G.; Tundo, S.; Ciorba, R.; Frangella, M.; Ruggeri, R.; Rossini, F.; Gevi, F.; Rinalducci, S.; Ceoloni, C. Equipping durum wheat—*Thinopyrum ponticum* recombinant lines with a *Thinopyrum elongatum* major QTL for resistance to Fusarium diseases through a cytogenetic strategy.

Front. Plant Sci. **2019**, *10*, 1–17.

40. Singh, R.P.; Huerta-Espino, J.; Rajaram, S.; Crossa, J. Agronomic effects from chromosome translocations 7DL.7Ag and 1BL.1RS in spring wheat. *Crop Sci.* **1998**, *38*, 27–33.
41. Villareal, R.L.; Bañuelos, O.; Mujeeb-Kazi, A.; Rajaram, S. Agronomic performance of chromosomes 1B and T1BL.1RS near-isolines in the spring bread wheat Seri M82. *Euphytica* **1998**, *103*, 195–202.
42. Friebe, B.; Jiang, J.; Raupp, W.J.; McIntosh, R.A.; Gill, B.S. Characterization of wheat-alien translocations conferring resistance to diseases and pests: Current status. *Euphytica* **1996**, *91*, 59–87.
43. Kim, W.; Johnson, J.W.; Baenziger, P.S.; Lukaszewski, A.J.; Gaines, C.S. Agronomic effect of wheat-rye translocation carrying rye chromatin (1R) from different sources. *Crop Sci.* **2004**, *44*, 1254–1258.
44. Graybosch, R.A.; Peterson, C.J.; Hansen, L.E.; Worrall, D.; Shelton, D.R.; Lukaszewski, A. Comparative flour quality and protein characteristics of 1BL/1RS and 1AL/1RS wheat-rye translocation lines. *J. Cereal Sci.* **1993**, *17*, 95–106.
45. Ali, N.; Heslop-Harrison, J.; Ahmad, H.; Graybosch, R.A.; Hein, G.L.; Schwarzacher, T. Introgression of chromosome segments from multiple alien species in wheat breeding lines with wheat streak mosaic virus resistance. *Heredity (Edinb).* **2016**, *117*, 114–123.
46. Li, Z.; Li, B.; Tong, Y. The contribution of distant hybridization with decaploid *Agropyron elongatum* to wheat improvement in China. *J. Genet. Genomics* **2008**, *35*, 451–456.
47. Wang, Z.G.; An, T.G.; Li, J.M.; Molnár-Láng, M.; Ji, J.; Zhong, G.C.; Mu, S.M. Flourescent in situ hybridization analysis of rye chromatin in the background of "Xiaoyan No. 6." *Acta Bot. Sin.* **2004**, *46*, 436–442.
48. Micali, S.; Forte, P.; Bitti, A.; D'Ovidio, R.; Ceoloni, C. Chromosome engineering as a tool for effectively introgressing multiple useful genes from alien Triticeae into durum wheat. In Proceedings of the 10th Int. Wheat Genetics Symposium; Paestum, Italy, 2003; pp. 896–898.
49. Gennaro, A.; Forte, P.; Carozza, R.; Savo Sardaro, M.L.; Ferri, D.; Bitti, A.; Borrelli, G.M.; D'Egidio, M.G.; Ceoloni, C. Pyramiding different alien chromosome segments in durum wheat: Feasibility and breeding potential. *Isr. J. Plant Sci.* **2007**, *55*, 267–276.
50. Ceoloni, C.; Forte, P.; Gennaro, A.; Micali, S.; Carozza, R.; Bitti, A. Recent developments in durum wheat chromosome engineering. *Cytogenet. Genome Res.* **2005**, *109*, 328–334.
51. Kuzmanović, L.; Ruggeri, R.; Able, J.A.; Bassi, F.M.; Maccaferri, M.; Tuberosa, R.; De Vita, P.; Rossini, F.; Ceoloni, C. Yield of chromosomally engineered durum wheat-*Thinopyrum ponticum* recombinant lines in a range of contrasting rain-fed environments. *Field Crops Res.* **2018**, *228*, 147–157.
52. Ceoloni, C.; Del Signore, G.; Ercoli, L.; Donini, P. Locating the alien chromatin segment in common wheat -*Aegilops longissima* mildew resistant transfers. *Hereditas* **1992**, *116*, 239–245.
53. Biagetti, M.; Vitellozzi, F.; Ceoloni, C. Physical mapping of wheat-*Aegilops longissima* breakpoints in mildew-resistant recombinant lines using FISH with highly repeated and low-copy DNA probes. *Genome* **1999**, *42*, 1013–1019.
54. Ceoloni, C.; Ciaffi, M.; Lafiandra, D.; Giorgi, B. Chromosome engineering as a means of transferring 1D storage protein genes from common to durum wheat. In Proceedings of the Proc. 8th Int. Wheat Genet. Symp; Beijing, China, 1995; pp. 159–163.
55. Gennaro, A.; Forte, P.; Panichi, D.; Lafiandra, D.; Pagnotta, M.A.; D'Egidio, M.G.; Ceoloni, C. Stacking small segments of the 1D chromosome of bread wheat containing major gluten quality genes into durum wheat: Transfer strategy and breeding prospects. *Mol. Breed.* **2012**, *30*, 149–167.
56. Ceoloni, C.; Forte, P.; Ciaffi, M.; Nenno, M.; Bitti, A.; De Vita, P.; D'Egidio, M. Chromosomally engineered durum wheat: The potential of alien gene introgressions affecting disease resistance and quality. Durum wheat improvement in the Mediterranean region: new challenges; Royo C, Nachit M, Di Fonzo N, Araus JL (Eds); *Options Méditerranéennes*, Série A: 2000; Séminaires Méditerranéens n. 40, pp. 363–371.
57. D'Ovidio, R.; Masci, S. The low-molecular-weight glutenin subunits of wheat gluten. *J. Cereal Sci.* **2004**, *39*, 321–339.
58. Quaranta, F.; Belocchi, A.; Fornara, M.; Ripa, C.; D'Egidio, M.G. Le varietà di frumento duro in Italia. Risultati della rete nazionale di sperimentazione 1999-2012; Consiglio per la Ricerca e la Sperimentazione in Agricoltura, Rome -Italy: Rome, Italy, 2013; ISBN 978-88-97081-31-9.

59. Rossini, F.; Provenzano, M.E.; Sestili, F.; Ruggeri, R. Synergistic effect of sulfur and nitrogen in the organic and mineral fertilization of durum wheat: Grain yield and quality traits in the Mediterranean environment. *Agronomy* **2018**, *8*, 1–16.

60. Zadoks, J.C.; Chang, T.T.; Konzak, C.F. A decimal code for the growth stages of cereals. *Weed Res.* **1974**, *14*, 415–421.

61. Graziani, M.; Maccaferri, M.; Royo, C.; Salvatorelli, F.; Tuberosa, R. QTL dissection of yield components and morpho-physiological traits in a durum wheat elite population tested in contrasting thermo-pluviometric conditions. *Crop Pasture Sci.* **2014**, *65*, 80–95.

62. Ravel, C.; Dardevet, M.; Leenhardt, F.; Bordes, J.; Joseph, J.L.; Perretant, M.R.; Exbrayat, F.; Poncet, C.; Balfourier, F.; Chanliaud, E.; et al. Improving the yellow pigment content of bread wheat flour by selecting the three homoeologous copies of *Psy1*. *Mol. Breed.* **2013**, *31*, 87–99.

63. Ficco, D.B.M.; Mastrangelo, A.M.; Trono, D.; Borrelli, G.M.; De Vita, P.; Fares, C.; Beleggia, R.; Platani, C.; Papa, R. The colours of durum wheat: A review. *Crop Pasture Sci.* **2014**, *65*, 1–15.

64. Quaranta, F.; Basili, O.; Belocchi, A.; Bottazzi, P.; Cacciatori, P.; Caprara, F.; Ciccoritti, R.; Fabbrini, L.; Mariotti, R.; Mazzon, V.; et al. Risultati della 42ª sperimentazione nazionale 2014-2015. Le varietà di grano duro per le semine 2015. Centro Italia versante tirrenico. *L'informatore Agrario* **2015**, *33 Suppl.*, 21–24.

65. Quaranta, F.; Basili, O.; Belocchi, A.; Bottazzi, P.; Cacciatori, P.; Caprara, F.; Arcangeli, A.; Fabbrini, L.; Locatelli, M.; Mariotti, R.; et al. Risultati della 43ª sperimentazione nazionale 2015-2016. Le varietà di grano duro per le semine 2016. Centro Italia versante tirrenico. *L'informatore Agrario* **2016**, *33 Suppl.*, 20–23.

66. Quaranta, F.; Arcangeli, A.; Basili, O.; Belocchi, A.; Bottazzi, P.; Cacciatori, P.; Fabbrini, L.; Moscaritolo, S.; Mariotti, R.; Mazzon, V.; et al. Speciale Grano Duro. Dettaglio regionale dei risultati: Centro Italia versante tirrenico. *L'informatore Agrario* **2017**, *31*, 49–51.

67. Troccoli, A.; Borrelli, G.M.; De Vita, P.; Fares, C.; Di Fonzo, N. Durum wheat quality: A multidisciplinary concept. *J. Cereal Sci.* **2000**, *32*, 99–113.

68. Yesli, A.; Latati, M.; Tellah, S.; Abdellaoui, Z.; Ounane, G. Physicochemical and rheological properties and bread-making potential of durum flour and semolina. *J. Food, Agric. Environ.* **2017**, *15*, 14–20.

69. Ren, T.H.; Chen, F.; Yan, B.J.; Zhang, H.Q.; Ren, Z.L. Genetic diversity of wheat-rye 1BL.1RS translocation lines derived from different wheat and rye sources. *Euphytica* **2012**, *183*, 133–146.

70. Zhang, Y.; Liu, Z.; Liu, C.; Yang, Z.; Deng, K.; Peng, J.; Zhou, J.; Li, G.; Tang, Z.; Ren, Z. Analysis of DNA methylation variation in wheat genetic background after alien chromatin introduction based on methylation-sensitive amplification polymorphism. *Chinese Sci. Bull.* **2008**, *53*, 58–69.

71. Dong, Z.Y.; Wang, Y.M.; Zhang, Z.J.; Shen, Y.; Lin, X.Y.; Ou, X.F.; Han, F.P.; Liu, B. Extent and pattern of DNA methylation alteration in rice lines derived from introgressive hybridization of rice and *Zizania latifolia* Griseb. *Theor. Appl. Genet.* **2006**, *113*, 196–205.

72. Sall, A.T.; Cisse, M.; Gueye, H.; Kabbaj, H.; Ndoye, I.; Filali-Maltouf, A.; Belkadi, B.; El-Mourid, M.; Ortiz, R.; Bassi, F.M. Heat tolerance of durum wheat (*Tritcum durum* Desf.) elite germplasm tested along the Senegal river. *J. Agric. Sci.* **2018**, *10*, 217–233.

73. Sall, A.T.; Bassi, F.M.; Cisse, M.; Gueye, H.; Ndoye, I.; Filali-Maltouf, A.; Ortiz, R. Durum wheat breeding: In the heat of the Senegal river. *Agric.* **2018**, *8*, 1–12.

74. Bhattacharya, S. Wheat rust back in Europe. *Nature* **2017**, *542*, 145–146.

75. RustTracker.org - A Global Wheat Rust Monitoring System. Available online: https://rusttracker.cimmyt.org/?p=7083 (accessed on December 10, 2019).

76. Ceoloni, C.; Del Signore, G.; Pasquini, M.; Testa, A. Transfer of mildew resistance from *Triticum longissimum* into wheat by induced homoeologous recombination. In Proceedings of the Seventh International Wheat Genetics Symposium; Miller, T., Koebner, R., Eds.; Cambridge, UK, 1988; pp. 221–226.

77. Kwiatek, M.; Belter, J.; Majka, M.; Wiśniewska, H. Allocation of the S-genome chromosomes of *Aegilops variabilis* Eig. carrying powdery mildew resistance in triticale (× *Triticosecale* Wittmack). *Protoplasma* **2016**, *253*, 329–343.

78. Basandrai, A.K.; Basandrai, D. Powdery mildew of wheat and its management. In *Management of wheat and barley diseases*; Singh, D.P., Ed.; Apple Academic Press Inc., chapter 5. 2018 ISBN 978-1-

77188-546-1.

79. Figueroa, M.; Hammond-Kosack, K.E.; Solomon, P.S. A review of wheat diseases - a field perspective. *Mol. Plant Pathol.* **2018**, *19*, 1523–1536.
80. Bebber, D.; Ramotowski, M.; Gurr, S. Crop pests and pathogens move polewards in a warming world. *Nat. Clim Chang.* **2013**, *3*, 985–988.
81. Singh, R.P.; Hodson, D.P.; Huerta-Espino, J.; Jin, Y.; Bhavani, S.; Njau, P.; Herrera-Foessel, S.; Singh, P.K.; Singh, S.; Govindan, V. The emergence of Ug99 races of the stem rust fungus is a threat to world wheat production. *Annu. Rev. Phytopathol.* **2011**, *49*, 465–481.
82. Singh, R.P.; Hodson, D.P.; Jin, Y.; Lagudah, E.S.; Ayliffe, M.A.; Bhavani, S.; Rouse, M.N.; Pretorius, Z.A.; Szabo, L.J.; Huerta-Espino, J.; et al. Emergence and spread of new races of wheat stem rust fungus: Continued threat to food security and prospects of genetic control. *Phytopathology* **2015**, *105*, 872–884.
83. Sissons, M. Role of durum wheat composition on the quality of pasta and bread. *Food* **2008**, *2*, 75–90.
84. Zhang, W.; Dubcovsky, J. Association between allelic variation at the *Phytoene synthase 1* gene and yellow pigment content in the wheat grain. *Theor. Appl. Genet.* **2008**, *116*, 635–645.
85. ARVALIS - Institut du Végétal. Choisir & décider céréales à paille – variétés et interventions d'automne 2018 synthèse nationale. Available online: www.arvalis-infos.fr (accessed on Jul 2, 2019).
86. Gennaro, A.; Borrelli, G.M.; D'Egidio, M.G.; De Vita, P.; Ravaglia, S.; Ceoloni, C. A chromosomally engineered durum wheat-*Thinopyrum ponticum* recombinant line with novel and promising attributes for varietal development. In Proceedings of the 10th Int. Wheat Genetics Symp; Pogna, N.E., Romanò, M., Pogna, E.A., Galterio, G., Eds.; Paestum, Italy, 2003; pp. 881–883.
87. Rogers, W.J.; Rickatson, J.M.; Sayers, E.J.; Law, C.N. Dosage effects of chromosomes of homoeologous groups 1 and 6 upon bread-making quality in hexaploid wheat. *Theor. Appl. Genet.* **1990**, *80*, 281–287.
88. Pogna, N.E.; Mazza, M.; Redaelli, R.; Ng, P.K.W. Gluten quality and storage protein composition of durum wheat lines containing the Gli-D1/Glu-D3 loci. In Proceedings of the 6th Intern. Gluten Workshop; Wrigley, C.W., Ed.; Melbourne, Australia, 1996; pp. 18–22.
89. Molnár-Láng, M.; Ceoloni, C.; Doležel, J. (Eds.) Alien Introgression in Wheat—Cytogenetics, Molecular Biology, and Genomics; Springer: Cham, Switzerland, 2015; pp. 1–385. ISBN 978-3-319-23493-9.
90. Prohens, J.; Gramazio, P.; Plazas, M.; Dempewolf, H.; Kilian, B.; Díez, M.J.; Fita, A.; Herraiz, F.J.; Rodríguez-Burruezo, A.; Soler, S.; et al. Introgressiomics: a new approach for using crop wild relatives in breeding for adaptation to climate change. *Euphytica* **2017**, *213*.