

**Effects of *Thinopyrum ponticum* chromosome segments transferred into durum wheat on yield components and related morpho-physiological traits in Mediterranean rain-fed conditions**

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**Abstract**

To verify previous results obtained on spaced plants regarding positive effects on yield-related traits of *Thinopyrum-Th. ponticum* chromosomal segments transferred into durum wheat, field plot trials were carried out over two years in Central Italy. Three durum wheat *-Th.-Th.-Thinopyrum T. ponticum* near isogenic recombinant lines (NIRLs), possessing 23%, 28% and 40% of their distal 7AL arm replaced by alien 7AgL chromatin, respectively, were included in the study. These lines underwent detailed phenotyping of more than 30 agronomic, physiological and qualitative traits, involving several novel traits at different developmental stages. Significant positive effects of defined 7AgL sub-regions on grain and tiller number and flag leaf dimensions, were confirmed over the two seasons, but also positive impacts on chlorophyll content and length of some phenological phases were identified. Overall, in spite of rather different weather conditions in both the two seasons analysed, grain and biomass yield of all three NIRLs were 3–11% higher than their respective controls, while maintaining grain protein content higher than 15% across years in the presence of all 7AgL segments. Comparative analysis supported previous evidence that the NIRL with 28% 7AgL is the most promising for breeding exploitation, as in its 5% most proximal 7AgL portion gene(s)/QTL(s) enhancing various yield-contributing traits were located, including productive tiller number (+20%), flag leaf width and area (+8%), chlorophyll content at late grain filling (+15%) and booting-to-anthesis phases (+22%). These results suggest that mechanism(s) underlying the positive effects on yield are likely to be associated with the establishment of photosynthetically active canopy in interaction with effective biomass partitioning and, to some extent, phenology.

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**Keywords:** Chromosome engineering; Productivity; **Tiller number**; **Grain number**; **Flag leaf**; **Chlorophyll content**

## 1 Introduction

Due to climate change, the availability of arable land, as well as demographic and related food security issues, the need to increase the yield of major crops represents a primary goal for breeding programs, perhaps more urgent and challenging than ever (FAO, 2013). While yield gains of major cereals (maize, rice, wheat) were significant in the second half of the 20th century, the current scenario indicates increments of no more than 1% per year (Fischer and Edmeades, 2010; Fischer et al., 2009). This figure applies to global wheat production, including both bread wheat (*Triticum aestivum* L.,  $2n = 6x = 42$ , genomes AABBDD) and durum wheat (*Triticum durum* L. var. *durum*,  $2n = 4x = 28$ , genomes AABB). Of the two cultivated *Triticum* species, the former is undoubtedly far more important worldwide in terms of area, production and trade (Curtis and Halford, 2014; FAO 2014). *Durum Durum Durum* (Durum (NO Italics)) wheat, on the other hand, remains of traditional or growing economic importance for European and North African **countries/countries** surrounding the Mediterranean basin, as well as in North America (Canada and USA), Mexico, the Middle East (mainly Turkey and Syria), Central Asia (mainly Kazakhstan) and Australia (see, e.g., Le Lamer and Rousselin, 2011).

Yield increases following the boost due to the mid-20th century Green Revolution, do not appear to be different for durum durum durum than bread wheat. Surveys of Canadian (McCaug and Clarke, 1995), Italian and Spanish (Royo et al., 2008), and also CIMMYT germplasm (Pfeiffer et al., 1996) indicate similar trends. A clear plateauing of yield gains has been observed in the last two decades for both bread and durum durum durum wheat, and an alarming decline of yield stability (Calderini and Slafer, 1999; De Vita et al., 2010; Royo et al., 2008), possibly associated with climate instability.

Progress in yield obtained so far is mainly due to an increase in harvest index (HI), both in bread (Fischer, 2011; Fischer et al., 2009; Reynolds et al., 2009) and durum durum durum (De Vita et al., 2007) wheat. However, although approaching its theoretical maximum of 62% (Austin et al., 1980), HI was not significantly improved beyond 55% in the last two decades (Foulkes et al., 2011; Gaju et al., 2009; Reynolds et al., 2009). The difference between the actual and theoretical levels of HI is larger in the case of durum durum durum as compared to bread wheat, implying that there is more possibility for improvement (Royo et al., 2007). Nevertheless, it is largely recognized that further yield gains will primarily be reached by increasing biomass and its partitioning into spikes, without significant alteration in HI (Bustos et al., 2013; Curtis and Halford, 2014; Czaczylo-Mysza et al., 2013; Fischer and Edmeades, 2010; Fischer et al., 2009; Foulkes et al., 2011; Reynolds et al., 2009). Enhancement of resource use efficiency has been suggested as an effective way to improve biomass (Hawkesford et al., 2013; and reference therein). This could be achieved through an increase in light interception area and/or radiation use efficiency of canopy and spike (e.g. Parry et al., 2011; Reynolds et al., 2005, 2009; Reynolds et al., 2005, 2009; Zhu et al., 2010), especially around flowering time (Fischer and Edmeades, 2010), and through the extension of the active life of leaves (Fischer et al., 2009; Reynolds et al., 2009). Further improvements in yield potential must also involve increases in sink size during grain filling, as wheat appears to be more sink- than source-limited (Miralles and Slafer, 2007; Reynolds et al., 2005, 2009). To reach this goal, either larger grain size or higher grain number (spike<sup>-1</sup> and m<sup>-2</sup>) are necessary, the latter trait being largely recognized as the main contributor to yield increases in bread (Fischer, 2011; Reynolds et al., 2009; Slafer, 2003) and durum durum durum wheat (De Vita et al., 2007; Pedro et al., 2011). The positive correlation of grain number with spike dry weight at anthesis (Fischer, 2008; Gaju et al., 2009; González et al., 2011), duration of stem-elongation phase and related spike growth, including the onset and rate of floret death (Ferrante et al., 2010; Fischer et al., 2009; González et al., 2011; Isidro et al., 2011; Kirby, 1988) are noteworthy, as well as the availability of resources and their effective use during spike growth (Ferrante et al., 2013a). As a whole, a variety of factors contributing to spike fertility and to partitioning of assimilates into the developing spike represent sensible targets for breeding (Reynolds et al., 2009; and references therein). Considering that the complex source-sink interplay is highly conditioned by the environment, elucidation of the genetic and related physiological basis of the above-described interactions is of paramount importance for selection of novel germplasm, adaptable to present and future climatic and socio-economic scenarios (Miralles and Slafer, 2007; Slafer 2003). In fact, current environmental changes in traditional wheat cropping areas are likely to favour gradual displacement of wheat to drier and hotter areas (Ceoloni et al., 2014a; Fischer and Edmeades, 2010). Therefore, field evaluation in different environments is essential for identification of plant ideotypes showing good yield plasticity in space and time.

A wide genetic basis is largely recognized as an essential prerequisite in the search for favourable alleles to enhance an array of traits that either can directly or indirectly contribute to yield improvement of the cultivated germplasm. In durum durum durum wheat, a number of pedigree studies, performed using agronomic data or, more recently, DNA markers, have shown that modern varieties, including several from CIMMYT/ICARDA breeding programs, originate from a restricted number of founders (Royo et al., 2009 and reference therein). To increase the variability and hence the potential of modern varieties, exploitation of natural variation present in crop relatives, including wild, distant species, is a highly advantageous approach (e.g. Ashikari and Matsuoka, 2006; Cavanagh et al., 2013; Maxted et al., 2011; Mba et al., 2012; McCouch, 2004; Reynolds et al., 2009; Tester and Langridge, 2010).

Generally, genes determining resistance/tolerance to biotic and abiotic stresses or quality traits have been targeted so far within the wild gene pool, altogether harnessing a limited number of alien donor species (see, e.g., a review for the durum durum durum wheat case by Ceoloni et al., 2014b). However, a large amount of unadapted and wild germplasm can still be investigated and potentially exploited (Borrelli et al., 2014; Ceoloni et al., 2014b; Nazco et al., 2012). To this aim, an efficient use of alien variation is needed, a problem nowadays amended with relative ease by the integration of traditional selection techniques with modern analytical tools, such as molecular markers, association and genome-wide mapping, new generation sequencing, bioinformatics and cytogenetic techniques (Ceoloni et al., 2014b; Hawkesford et al., 2013; Mba et al., 2012; Tester and Langridge, 2010).

Targeting wild relatives as a potential path to improvement of yield-related traits, may not appear a smart approach, given the poor yielding potential that these species usually display, and the infrequent, positive effects of wild introgressions into the crop genome (Ceoloni et al., 2014a). Few cases have been reported so far, and mainly in bread wheat rather than durum durum durum wheat, the latter being less tolerant to chromosome manipulations because of its tetraploidy (Ceoloni et al., 2005, 2014b). One notable example is the introgression of the viridescence gene (*Vir*) from *Triticum dicoccoides* ( $2n = 4x = 28$ , genomes AABB) into bread wheat, associated with prolonged productivity at grain filling (Simmonds et al., 2008). Similarly, the translocation of a segment of *Thinopyrum bessarabicum* (Savul. & Rayss) Löve ( $2n = 2x = 14$ , genome JJ or E<sup>b</sup>E<sup>b</sup>) onto chromosome arm 2JS of bread wheat 2BS, increased the number of spikes per plant and grains per spike (Qi et al., 2010), while the presence of defined portions of *Agropyron cristatum* (L.) Beauv. ( $2n = 4x = 28$ , genome PPPP) chromosome 6P determined an increased number of florets and kernels per spike and of fertile tillers (Ye et al., 2015).

Of particular importance for both bread and durum durum durum wheat yield is the decaploid wheatgrass *Thinopyrum ponticum* (Popd.) Barkworth & D.R. Dewey ( $2n = 10x = 70$ , genomes E<sup>b</sup>E<sup>b</sup>E<sup>b</sup>StSt or JJJJ<sup>s</sup>J<sup>s</sup>, see Ceoloni et al., 2014b), well known and utilized since the mid-20th century as an abundant source of useful genes for wheat (e.g. Li and Wang, 2009). Among them, various resistance and also grain quality genes located on the long arm of *7h*. *ponticum* chromosome 7(=7AgL or 7(=7AgL or 7-eL) were extensively exploited in wheat breeding (reviewed in Ceoloni et al., 2014b). As to yield-related genes/quantitative trait loci (QTL), the first evidence of the positive effects associated with the alien 7AgL were reported in bread wheat, in the near-isogenic lines (NILs) of the T4 translocation line (Dvorak and Knott, 1977) into different recurrent backgrounds. Such lines, developed by CIMMYT, were all characterized by the presence of a sizeable portion

of 7AgL (70% of the 7DL recipient arm), carrying the *Lr19*, *Sr25* and *Yp* genes. Evaluation of T4 NILs across different environments, revealed a 10–15% increase in biomass, yield and grain number to be consistently associated with the presence of 7AgL chromatin (Monneveux et al., 2003; Reynolds et al., 2001; Singh et al., 1998). In order to better explore the effects on yield so far altogether ascribed to the conspicuous 7AgL portion of the T4 donor, we report here the use of three durum durum wheat–*Th. ponticum* near-isogenic recombinant lines (NIRLs), developed through chromosome engineering by Ceoloni et al. (2005), and characterised by having their distal 7AL portions replaced by 23, 28 and 40% of 7AgL chromatin of the same origin as that of T4 translocation. Field assessment of yield-contributing traits on single plants had previously confirmed positive effects of 7AgL on durum AgL-on durum wheat yield parameters (Kuzmanović et al., 2014). In the latter study, enhanced effects were detected on a wide range of traits, and the increases were higher than observed in bread wheat (+25% of tiller number/plant, +28% of biomass, +36% of grain yield and number, and +11% of flag leaf width and area). At the same time, comparative analysis of NIRLs harbouring 7AgL segments of various size, corresponding to fractions of the 70%-long 7AgL segment of T4 line, for the first time, allowed allocation of loci controlling 10 of the analysed traits to defined 7AgL portions, hence dissection of the variety of yield effects so far loosely attributed to the T4 segment.

The study by Kuzmanović et al. (2014) was based on the assessment of traits exhibited by spaced plants; therefore, there was no certainty that the conclusions could be reproduced at normal wheat sowing rates. Besides this, there was no information on possible physiological factors involved in the observed increase of yield-related traits in durum durum wheat, associated with the presence of 7AgL segments. Therefore, a multi-year field plot experiment was undertaken in the same environment, representative of typical Mediterranean conditions, using the same three durum durum wheat–*Th. ponticum* NIRLs and their corresponding null controls. Besides enabling verification of 7AgL effects on relevant yield traits under conditions of normal cropping density, the plot trials described here have been enriched by analysis of additional parameters (e.g. fertility and biomass at anthesis, duration of phenological phases, chlorophyll content at grain filling), altogether allowing a preliminary assessment of the physiological mechanisms associated with the expression of the novel yield-related QTL introgressed into the durum durum durum wheat background, as well as of the possible interactions among the various yield components.

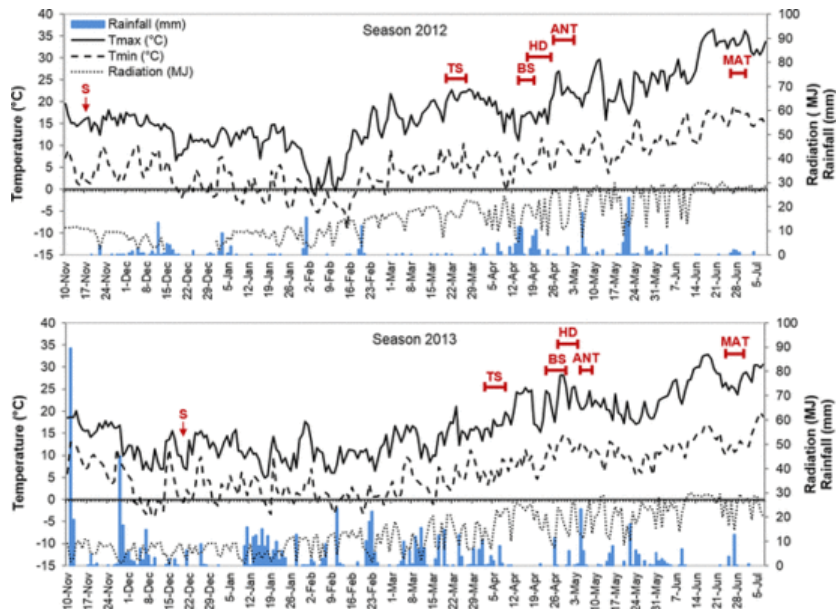
## 2 Materials and methods

### 2.1 Plant materials

Three durum durum wheat–*Th. ponticum* near isogenic recombinant lines (NIRLs), named R5-2-10, R112-4 and R23-1 (hereafter referred to as R5, R112 and R23, respectively), developed by backcrossing the original recombinant lines (Ceoloni et al., 2005) to the recurrent cv. Simeto, were used. The NIRLs are characterized by having 23%, 28% and 40%, respectively, of the distal portion of their 7AL arm pairs replaced by *Th. ponticum* 7AgL chromatin, including the *Lr19 + Yp + Sr25* genes in the subtelomeric portion common to all lines. Each of the genotypes, corresponding to BC<sub>5</sub>F<sub>6,7</sub> (R5 and R112) and BC<sub>4</sub>F<sub>6,7</sub> (R23) progenies, was represented by homozygous carrier (HOM<sup>+</sup>) and non-carrier (HOM<sup>-</sup>) plants of the given 7AgL segment. Each HOM<sup>+</sup> and HOM<sup>-</sup> genotype included two families originating from sister lines.

### 2.2 Location, experimental design and plant growth

Field experiments were carried out during two consecutive growing seasons, 2011–12 and 2012–13 (hereafter referred to as 2012 and 2013 respectively), at the experimental farm of the University of Tuscia, Viterbo, Italy (Lat N 42.426418° Lon E 12.080573°). The growing area is characterized by a Mediterranean climate, with mean annual air temperature of about 14.5 °C (–4 °C min –4 °C min –35 °C max) and precipitation of 790 mm. For both years, daily weather data were retrieved from the meteorological station located within 200 m from the experimental site and they are reported in Fig. 1. A randomized complete block design with 3 replicates for each sister family was applied, giving a total of 36 plots (6 per each HOM<sup>+</sup>/HOM<sup>-</sup> genotype). The size of each 10 row-plot was 1.5 × 1.5 m. Plants were machine-sown at 350 seeds m<sup>-2</sup> rate on 17 November 2012 and 20 December 2013. Nitrogen fertilization (180 kg ha<sup>-1</sup>) 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; Zadoks et al., 1974) as urea (50% of total N), and the third 15 days later as ammonium nitrate (30% of total N). Weed control was performed during tillering by a single distribution of the commercial broadleaf herbicide Ariane II<sup>®</sup> according to manufacturer's instructions. The fungicide Folicur<sup>®</sup> was applied once at the mid-grain filling period in order to avoid leaf rust spreading on HOM<sup>-</sup> plants (*Lr19* non-carriers), hence eliminating this indirect yield-contributing factor of *Lr19*-carriers (=HOM<sup>+</sup>) vs. their controls (=HOM<sup>-</sup>). After anthesis, plot trials were protected from possible bird damage by elevating a 2 m high net structure.



**Fig. 1** Weather conditions during 2012 and 2013 seasons, as retrieved from the meteorological station of the experimental farm of the University of Tuscia, Viterbo (Italy), and time of completion (red bars) of phenological phases of the three durum wheat-Triticum wheat-F.7h. ponticum NIRLs (S, sowing date; TS, terminal spikelet; BS, booting; HD, heading; ANT, anthesis; MAT, harvest maturity). [\[For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.\]](#)

### 2.3 Measurements of yield-related, morpho-physiological and quality traits

During vegetative growth, duration of phenological phases was calculated from the sowing date in thermal time units (GDD = growing degree days), as described by [Isidro et al. \(2011\)](#). Terminal spikelet (TS) phase (Zadoks 30) was determined on the basis of observation of main culms, at the time when the 1st swelling node was detectable at 1 cm above ground. Booting (BS; Zadoks 45), heading (HD; Zadoks 55), anthesis (ANT; Zadoks 65), and harvest ripe (MAT; Zadoks 92) phases were recorded when 50% of main culm spikes in a plot reached the given phase. Values of TS, BS, ANT and MAT were then used to obtain duration of the following phases: stem elongation (SE; from TS to ANT), booting-to-anthesis (BS-ANT; from BS to ANT) and grain filling (GF; from ANT to MAT).

Details on all other traits assessed are given in [Table 1](#). Samples of culms and flag leaves were randomly chosen within each plot. All dry weights of culms, spikes, chaff and total aboveground biomass were recorded after 48 h oven drying at 65 °C. FF (see [Table 1](#) for acronyms) was determined at W95 or W10 stages of the Waddington scale ([González et al., 2011](#)); SI calculated as SDWA/CDWA ratio, SFI as ratio of GNS and CHAFF ([Isidro et al., 2011](#)), while FLA was determined using the formula  $FLW \times \frac{FLLx}{FLL} \times 0.75$  ([Dodig et al., 2010](#)). During the GF period, Chl was measured by using a hand-held meter SPAD 502 (Konica-Minolta, Japan), while Flav and NBI were assessed by a hand-held meter Dualex 4 Scientific (FORCE-A, Orsay, France). HI determined on 25-culm sample plot<sup>-1</sup> was used for BM2 estimation, once the total plot area was trashed and weighed (=GYM2/HI). TGW was obtained from weighing two 100-seed samples plot<sup>-1</sup> and then used to evaluate GNM2 (=GYM2 × 1000/TGW). HCW was determined on cleaned grain samples by a Shopper chondrometer equipped with a 250-mL cylinder according to the method UNI n. 10281, while PROT and GLU were determined by using the near infrared whole grain analyser Mininfra 2000T (Infracont Kft, Slovakia), calibrated for durum durum durum wheat, according to a standardized procedure for the instrument.

**Table 1** Timetable and sample details of traits assessed during the 2012 and 2013 growing seasons (IC, individual culms; FL, individual flag leaves; 25C, 25 culms; TH, total plot harvest; G, grain sample; Z, Zadoks; 70–71, watery ripe; 73, early milk; 75, medium milk; 77, late milk; 79, very late milk; 81, very early dough).

Trait	Acronym	No. samples plot <sup>-1</sup> <sup>a</sup>	Sample type	Growth stage		
				Anthesis	Grain filling (Zadoks stages)	Maturity
Spike dry weight (g)	SDWA	6	IC	x		
	SDW	6	IC			x

Culm dry weight (g)	CDWA	6	IC	x		
	CDW	6	IC			x
Spike index	SI	6	IC	x		
No. fertile florets	FF	6	IC	x		
Spike length (cm)	SL	6	IC			x
Chaff dry weight (g)	CHAFF	6	IC			x
Spikelet No.	SPN	6	IC			x
Grain No. spike <sup>-1</sup>	GNS	6	IC			x
Grain No. spikelet <sup>-1</sup>	GNSP	6	IC			x
Grain yield spike <sup>-1</sup> (g)	GYS	6	IC			x
Spike fertility index	SFI	6	IC			x
Flag leaf width (cm)	FLW	10	FL		Z77	
Flag leaf length (cm)	FLL	10	FL		Z77	
Flag leaf area (cm <sup>2</sup> )	FLA	10	FL		Z77	
Flag leaf chlorophyll content	Chl	10	FL		Z70-71, Z73, Z75, Z77, Z79 <sup>b</sup> , Z81 <sup>b</sup>	
Flag leaf flavonol content	Flav	10	FL		Z70-71 <sup>b</sup> , Z73 <sup>b</sup> , Z75 <sup>b</sup> , Z77 <sup>b</sup> , Z79 <sup>b</sup> , Z81 <sup>b</sup>	
Nitrogen balance index	NBI	10	FL		Z70-71 <sup>b</sup> , Z73 <sup>b</sup> , Z75 <sup>b</sup> , Z77 <sup>b</sup> , Z79 <sup>b</sup> , Z81 <sup>b</sup>	
Plant height (cm)	PH	10	IC			x
Harvest index	HI	1	25C			x
Spike No. m <sup>-2</sup>	SNM2	1	TH			x
Grain yield m <sup>-2</sup> (g)	GYM2	1	TH			x
Biomass m <sup>-2</sup> (g)	BM2	1	TH			x
1000 grain weight (g)	TGW	2	G			x
Grain number m <sup>-2</sup>	GNM2	1	TH			x
Hectolitre weight (g)	HCW	1	G			x
Protein content (%)	PROT	1	G			x
Gluten content (%)	GLU	1	G			x

<sup>a</sup> For total sample number per genotype, see [experimental design \(§ 2.2 Section \(§ 2.2\)\)](#).

<sup>b</sup> Recorded in 2013 only.

## 2.4 Statistical analyses

All statistical analyses were performed by SYSTAT12 software (Systat Software Incorporated, San Jose, CA, USA). To investigate the effects of genetic and/or environmental factors and interactions between them on recorded variables, an analysis of variance (ANOVA) was performed applying to the [2-year](#) dataset a general linear model (ANOVA-GLM) as a mixed effect model. Two such models were employed: ANOVA-GLM1, for the analysis of the overall effect of presence/absence of alien segments, and

ANOVA-GLM2, for the analysis of individual 7 AgL segment effects. Each variable (i.e. trait measured) was entered as a 'dependent' factor against 'independent' factors. The latter were: genotype background (G), i.e. background genetic information from the recurrent variety, year (Y), presence/absence of the 7AgL segment [7-AgL segment] 7-AgL alone in ANOVA-GLM1 or 7-AgL(G), i.e. nested in the background, in ANOVA-GLM2], and year-nested replica [R(Y)], used in the model as the error. Year was considered a random effect factor because the field trials were replicated over two years under the same experimental conditions and the same design. First order [ $Y \times G$  and  $R(Y)$  in both models;  $Y \times 7\text{AgL}$  and  $7\text{AgL}$  and  $7\text{AgL} \times G$  in ANOVA-GLM1;  $7\text{AgL}(G)$  in ANOVA-GLM2], and second order [ $Y \times 7\text{AgL}(G)$  in ANOVA-GLM2] interactions between the above factors were analysed as well. For the traits measured in the 2013 season only, 'independent' factors did not comprise Y, nor the first or second order interactions involving Y. In all analyses three levels of significance were considered, corresponding to  $P < 0.05$ ,  $F_{<0.01 \text{ and } P_{\leq 0.01 \text{ and } P_{\leq 0.001}}$ . When significant factors and/or interactions between them ( $F$  values) were observed, a pairwise analysis was carried out by the Tukey Honestly-Significant-Difference (HSD) test at the 0.95 confidence level.

A simple correlation matrix was constructed for a subset of traits recorded in both experimental years. Each pair of variables was correlated by calculating Pearson's correlation coefficients ( $r$  value), while the significance levels were obtained using the Bonferroni method. Simple linear regression analysis was carried out by applying the least squares method for data fitting, at 0.95 confidence level.

## 3 Results

### 3.1 Environmental conditions

The two experimental seasons were somewhat different, with 2012 exhibiting more typical conditions for durum durum wheat growth, as shown by lower average temperatures and higher radiation levels in the pre-anthesis period (Reynolds et al., 2005; Villegas et al., 2015), and higher average temperatures in the post-anthesis period as compared to the 2013 season (Fig. 1). Around anthesis (typically in May), maximum temperatures ranged between 16 °C and 30 °C in 2012, while they were between 17 °C and 26 °C in 2013. A similar trend was maintained during the grain filling period, with average maximum temperatures of 26 °C in 2012 and 23 °C in 2013. The most important difference between the seasons was in rainfall amount and distribution. The 2013 season was characterized by a 2.2 fold higher rainfall over the entire crop cycle compared to 2012 (534 mm vs. 242 mm), with remarkable differences during the vegetative growth up to terminal spikelet stage (400 mm vs. 93 mm), as well as during the stem elongation phase (90 mm vs. 69 mm), and, in particular, at the time just preceding anthesis (30 mm vs. 4 mm; HD to ANT, Fig. 1).

### 3.2 Yield and quality traits

#### 3.2.1 Yield per unit area and grain quality

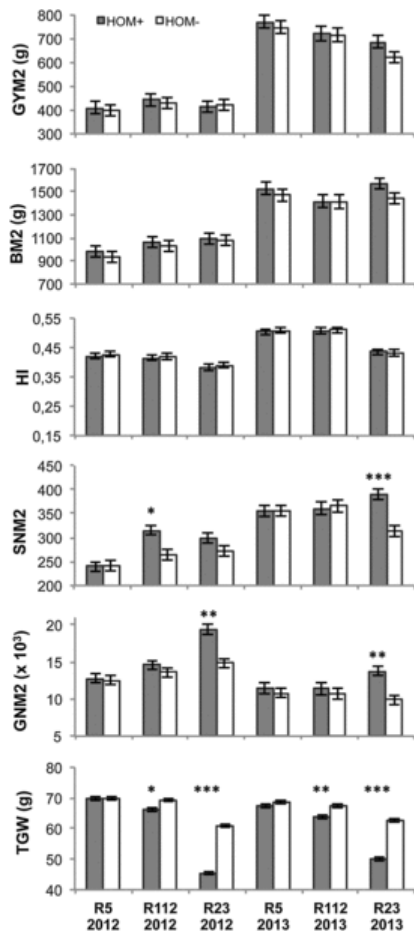
Across the two years, taken altogether the 7-AgL segments of the three recombinants (ANOVA-GLM1, see Materials and Methods Section 2), yield and yield components  $\text{m}^{-2}$  increased in the 7-AgL+ set of lines vs. the 7-AgL- AgL- control set, though to a variable extent and level of significance (Table 2 and Supplementary Table 1). The incremental effect of the alien segment was highly significant for SNM2 (+9%) and GNM2 (+15%); however, due to a concomitant decrease in TGW (-9%), values of GYM2 and BM2 did not result significant higher, though exhibiting an average 4% increase. For both traits the increase was mostly apparent in 2013, whereas SNM2 showed a more conspicuous increment in 2012. HI remained unaffected by alien segment presence through the two years.

**Table 2** Main yield-related traits per unit area of 7-AgL-carrier vs. 7-AgL-non carrier durum AgL-carrier vs. 7-AgL-non carrier durum wheat NIRLs over the two experimental years (GYM2, grain yield  $\text{m}^{-2}$ ; BM2, biomass  $\text{m}^{-2}$ ; HI, harvest index; SNM2; spike number  $\text{m}^{-2}$ ; GNM2, grain number  $\text{m}^{-2}$ ; TGW, thousand grain weight; 2y-years, two years).

Trait	Data set	7AgL+	7AgL-	ANOVA p-value	7AgL effect (%)
GYM2	2y	574.0	554.9	0.207 <sup>ns</sup>	3.5
	2012	421.5	416.4	0.761 <sup>ns</sup>	1.2
	2013	724.9	694.9	0.273 <sup>ns</sup>	4.3
BM2	2y	1274.8	1224.7	0.088 <sup>ns</sup>	4.1
	2012	1041.8	1012.5	0.447 <sup>ns</sup>	2.9
	2013	1504.9	1439.8	0.159 <sup>ns</sup>	4.5
HI	2y	0.44	0.45	0.478 <sup>ns</sup>	-0.9
	2012	0.41	0.41	0.424 <sup>ns</sup>	-1.5

	2013	0.48	0.48	0.891 <sup>ns</sup>	-0.2
SNM2	2y	328.0	301.2	0.000 <sup>***</sup>	8.9
	2012	284.9	259.2	0.005 <sup>**</sup>	9.9
	2013	369.1	345.2	0.030 <sup>*</sup>	6.9
GNM2	2y	13872.5	12056.7	0.000 <sup>***</sup>	15.1
	2012	15589.9	13635.0	0.003 <sup>**</sup>	14.3
	2013	12159.5	10473.9	0.001 <sup>**</sup>	16.1
TGW	2y	60.4	66.6	0.000 <sup>***</sup>	-9.2
	2012	60.5	66.8	0.000 <sup>***</sup>	-9.5
	2013	60.3	66.3	0.000 <sup>***</sup>	-9.0

In order to evaluate the contribution of individual 7-AgL segments to the observed overall variations in yield and yield-related traits, a dedicated ANOVA-GLM2 analysis (see Materials and Methods) was applied. This revealed a significant effect on all yield components of the Y factor, as well as of the Y  $\times$  G, [7AgL(G)] and Y  $\times$  G, [7-AgL(G)] and Y  $\times$  7AgL(G) interactions for several traits (see below and Supplementary Table 2a). Fig. 2 reports mean values of main yield components for each experimental year, and shows that environmental conditions in the two seasons influenced to a different extent the expression of yield traits in the NIRLs.



**Fig. 2** Year  $\times$  7AgL(G) interaction for yield components per unit area of the three *durum durum wheat-Th. durum wheat-T. Th. ponticum* NIRLs in 2012 and 2013 (GYM2, grain yield  $m^{-2}$ ; BM2, biomass  $m^{-2}$ ; HI, harvest index; SNM2, spike number  $m^{-2}$ ; GNM2, grain number  $m^{-2}$ ; TGW, thousand-grain weigh).

\* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$  after Tukey HSD test. HOM+ and HOM- represent homozygous carriers and non-carriers of the given 7AgL segment, respectively.

As to the specific alien segment effect [7AgL(G)], increases in GNM2 and SNM2 were clearly ascribable to the 40%- and 28%-long 7AgL segments of R23 and R112. In particular, the former determined increments across years of 34% and 18% for GNM2 and SNM2, respectively, over the values of its HOM- control. The amount of GNM2 increase was remarkably constant through seasons, reaching 30% in 2012 and 39% in 2013 (Fig. 2). However, it was always paralleled by a significant decrease in TGW (average -23%, Table 3 and Fig. 2). R112, in turn, had a significant 20% higher SNM2 in 2012 (Fig. 2; +9% across years, non-significant, Table 3), and an average 7% increase, albeit non-significant, in GNM2 across years (Table 3). R112 also exhibited a TGW reduction, but of much smaller magnitude (average 5%) than that of R23 (Table 3 and Fig. 2). However, as a result of compensation between the primary yield components and of the different environmental conditions characterizing the two seasons, none of the three recombinants exhibited significantly enhanced GYM2. Nonetheless, no yield penalty was observed compared to the respective HOM- controls (Table 3 and Fig. 2), but higher GYM2 (in the range of 3 to 11%, Fig. 2). With a similar increase of BM2 values (from 4 to 9%, Fig. 2), results from ANOVA-GLM2 analysis confirmed the absence of any alteration in HI in each of the recombinant types.

**Table 3** Mean values and coefficient of variation (CV) of main yield traits per unit area and quality traits of the three *durum durum wheat-Th. durum wheat-T. Th. ponticum* NIRLs (HOM+, 7AgL carriers; HOM-, 7AgL carriers; HOM-, 7AgL non-carriers) across 2012 and 2013 seasons (GYM2, grain yield  $m^{-2}$ ; BM2, biomass  $m^{-2}$ ; HI, harvest index; SNM2, spike number  $m^{-2}$ ; GNM2, grain number  $m^{-2}$ ; TGW, thousand grain weight; HCW, hectolitre weight; PROT, grain protein content; GLU, gluten content;). Letters in each row correspond to the ranking of the Tukey test at  $P \leq 0.01$  (capital) and  $P < 0.05$  (lower case) levels.

Trait	R5_HOM+	R5_HOM- R112HOM+ R112HOM- R23HOM+ R23HOM-	R112HOM+ R112HOM-	R112HOM- (R112 HOM-)	R23HOM+ (R23 HOM+)	R23HOM- (R23 HOM-)
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	Mean		CV	Mean		CV	Mean		CV	Mean		CV	Mean		CV	Mean		CV
<b>Yield traits</b>																		
GYM2 (g)	571.7	ns	0.34	557.4	ns	0.34	551.2	ns	0.29	543.2	ns	0.29	550.3	ns	0.27	521.6	ns	0.23
BM2 (g)	1222.2	ns	0.26	1177.0	ns	0.25	1204.6	ns	0.20	1179.6	ns	0.20	1328.7	ns	0.20	1260.8	ns	0.16
HI	0.46	ns	0.10	0.47	ns	0.09	0.46	ns	0.11	0.46	ns	0.11	0.41	ns	0.07	0.41	ns	0.12
SNM2	292.0	BC	0.23	294.6	BC	0.23	333.7	AB	0.12	304.8	ABC	0.19	344.8	A	0.16	292.4	C	0.11
GNM2	12133.7	B	0.13	11746.0	B	0.10	13317.8	B	0.18	12418.8	B	0.15	16536.5	A	0.20	12368.7	B	0.26
TGW (g)	68.5	A	0.03	69.3	A	0.02	64.9	B	0.03	68.4	A	0.03	47.8	D	0.06	61.9	C	0.03
<b>Grain quality traits</b>																		
HCW (g)	83.3	ab	0.01	82.7	bc	0.01	83.0	b	0.01	82.2	c	0.01	83.8	a	0.01	82.1	c	0.01
PROT (%)	15.7	ns	0.08	15.9	ns	0.06	15.8	ns	0.08	15.9	ns	0.06	15.4	ns	0.05	15.2	ns	0.06
GLU (%)	35.1	ns	0.09	35.4	ns	0.07	35.2	ns	0.10	35.5	ns	0.07	34.0	ns	0.07	33.5	ns	0.07

Hectolitre weight (HCW) was similar between NIRLs, and slightly higher when compared to their controls, with the 2% difference being significant for R112 and R23 recombinants (Table 3). Two-year average protein and gluten content (PROT, GLU) was independent of the presence of any of 7-AgL segments, as shown by a non-significant 7-AgL(G) interaction (Supplementary Table 2a). Absolute values were similar between the analysed genotypes, being above 15% for protein content, and around 34% for gluten content (Table 3).

### 3.2.2 Yield-related traits of individual culms

Presence of the alien segment [7-AgL(G)] had a highly significant effect on various traits recorded on individual culms in R23 and R5 NIRLs vs. their controls, while in R112 NIRL produced notable variation only for PH (Supplementary Table 2a and Table 4). At anthesis, the 23%-long 7-AgL segment of R5 significantly decreased FF and CDWA by 8%, but in turn increased SI by 13%. The R23 NIRL had a significantly decreased dry weight of both SDWA (-21%) and CDWA (-21%) and CDWA (-16%) in the presence of its 7-AgL segment. The negative effect of this segment on dry weight was also detected at maturity (see SDW, CDW, and CHAFF in Table 4), together with significantly lowered GYS (-14%). However, similar to what was observed for grain number per unit area (GNM2), the 7-AgL segment present in R23 significantly increased grain number at the single spike level (13% and 8% for GNS and GNSP, respectively), which was accompanied by a remarkably increased SFI (+37%). The Y factor had a highly significant effect on single culm traits when considered alone; however, no significant interaction of Y and presence of 7-AgL segments was observed for any of the traits [Y × 7-AgL(G)] (Supplementary Table 2a).

**Table 4** Mean values and coefficient of variation (CV) of individual culm traits of the three durum durum wheat-Th. ponticum NIRLs (HOM+, 7-AgL carriers; HOM-, 7-AgL carriers; HOM-, 7-AgL non-carriers) across 2012 and 2013 seasons (FF, fertile floret number at anthesis; SDWA, spike dry weight at anthesis; CDWA, culm dry weight at anthesis; SI, spike index at anthesis; SDW, spike dry weight at maturity; CDW, culm dry weight at maturity; SL, spike length; SPN, spikelet number spike<sup>-1</sup>; GNS, grain number spike<sup>-1</sup>; GNSP, grain number spikelet<sup>-1</sup>; GYS, grain yield spike<sup>-1</sup>; CHAFF, chaff dry weight; SFI, spike fertility index; PH, plant height). Letters in each row correspond to the ranking of the Tukey test at P ≤ 0.01 (capital) and P < 0.05 (lower case) levels.

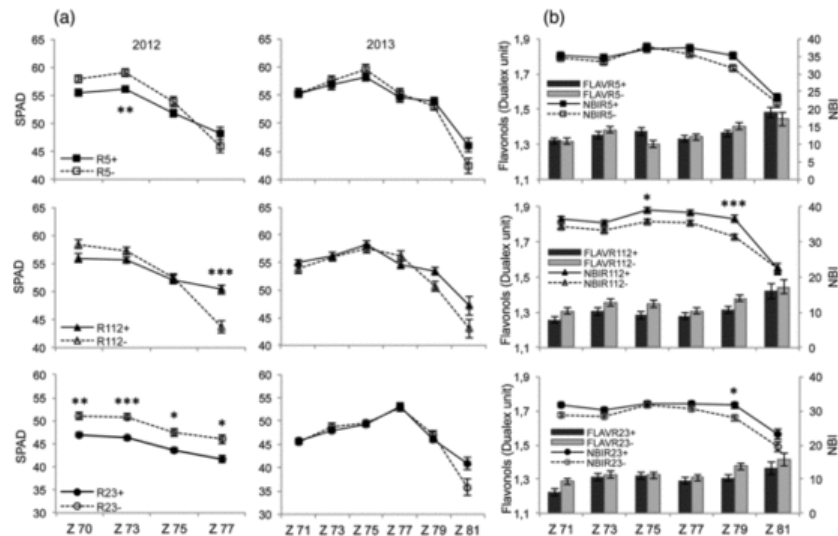
Trait	R5_HOM+			R5_HOM-R112HOM+R112HOM-R23HOM+R23HOM-			R112HOM+(R112_HOM+)		R112HOM-(R112_HOM-)		R23HOM+(R23_HOM+)		R23HOM-(R23_HOM-)					
	Mean		CV	Mean		CV	Mean	CV	Mean	CV	Mean	CV	Mean	CV				
FF	32.8	B	0.18	35.7	A	0.16	34.4	AB	0.18	33.3	AB	0.19	31.7	B	0.19	33.2	AB	0.20
SDWA (g)	0.59	A	0.20	0.60	A	0.16	0.59	A	0.19	0.60	A	0.20	0.46	B	0.19	0.58	A	0.20
CDWA (g)	3.62	b	0.19	3.93	A	0.17	3.64	b	0.19	3.68	ab	0.20	3.29	c	0.20	3.90	ab	0.17
SI	0.17	A	0.14	0.15	BC	0.15	0.16	AB	0.13	0.16	A	0.16	0.14	D	0.15	0.15	CD	0.12
SDW (g)	3.73	A	0.17	3.55	A	0.17	3.56	A	0.12	3.57	A	0.18	3.07	B	0.18	3.58	A	0.27

CDW (g)	6.33	AB	0.17	6.06	B	0.15	6.14	B	0.16	6.00	B	0.16	5.91	B	0.19	6.78	A	0.22
SL (cm)	5.9	B	0.06	5.9	BC	0.08	5.7	BC	0.07	5.70	C	0.08	6.6	A	0.08	6.5	A	0.09
SPN	16.2	B	0.08	16.0	B	0.08	15.9	B	0.07	15.7	B	0.10	17.6	A	0.09	17.4	A	0.13
GNS	42.9	B	0.20	41.0	B	0.17	43.4	B	0.14	41.9	B	0.20	50.3	A	0.15	44.7	B	0.28
GNSP	2.7	AB	0.19	2.6	B	0.15	2.7	AB	0.14	2.7	AB	0.18	2.8	A	0.12	2.6	B	0.23
GYS (g)	2.9	A	0.18	2.8	A	0.18	2.8	A	0.12	2.8	A	0.20	2.4	B	0.18	2.8	A	0.29
CHAFF (g)	0.79	ab	0.24	0.70	Cd	0.25	0.75	abc	0.28	0.71	bcd	0.32	0.65	d	0.24	0.81	a	0.32
SFI	57.1	B	0.28	62.0	B	0.26	61.8	B	0.30	64.5	B	0.50	80.8	A	0.19	59.1	B	0.32
PH (cm)	79.3	B	0.06	79.0	BC	0.07	77.4	C	0.06	74.8	D	0.07	97.6	A	0.05	98.7	A	0.05

### 3.3 Flag leaf traits

ANOVA-GLM2 analysis demonstrated a significant involvement of G, Y and 7-AgL(G) in the control of flag leaf dimensions in the two experimental years (Supplementary Table 2a). As to the alien segment presence [7-AgL(G)], the 28%-long segment of R112 was associated with significant increase (8%) of FLW and FLA (Supplementary Table 3), and the positive effect on the latter trait was particularly evident in 2013 (+10%), as revealed by Tukey's HSD test with a significant Y x Z-AgL(G) interaction (Supplementary Table 2a). In contrast, the 7-AgL segment of R23 was associated with 4%, 10% and 13% decrease in FLW, FLL and FLA, respectively, across years.

Analysis of chlorophyll content of flag leaves during grain filling similarly showed significant variations among the recombinant lines, in relation to the presence of some of the 7-AgL segments and to the year (Fig. 3; Supplementary Table 2a). In 2012, R5 and R112 NIRLs exhibited slightly lower Chl up to Z75 stage as compared to their HOM- controls; yet, at Z77 the trend changed in favour of HOM+ plants, conferring them with prolonged greenness (Fig. 3a). At this stage, the difference in Chl between HOM+ and HOM- plants (+15%) was highly significant only for the R112 NIRL. In the same year, the R23 NIRL had on average 10% significantly lower Chl during the entire GF phase when compared to its control. No differences in the Chl trend during GF were observed in 2013 between any of the NIRLs and their controls. Nevertheless, starting from Z79 stage, a higher, although not significant Chl in all HOM+ plants was displayed, being most evident in the R112 NIRL. The observed tendency of R112 to maintain greenness for a longer period during GF was supported by the results obtained for Flav and NBI in 2013 (Fig. 3b; Supplementary Table 2b). Generally, at all GF times the content of flavonols was lower and NBI higher in all HOM+ vs. HOM- genotypes, although this was particularly notable in the R112 NIRL at advanced Zadoks 75 and Zadoks 79 stages, when significantly increased NBI (+9% and +16%, respectively) was observed compared to its control, which indicates a higher photosynthetic activity.



**Fig. 3** Flag leaf chlorophyll and flavonol contents of the three durum wheat *Triticum durum* NIRLs recorded during the grain filling period: (a) chlorophyll content in 2012 and 2013, (b) flavonol content (FLAV) and nitrogen balance index (NBI) in 2013 (Z, Zadoks). \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ . The symbols '+' and '-' represent homozygous carriers and non-carriers of the given 7-AgL segment, respectively.

### 3.4 Phenology

Although the duration of developmental phases did not vary much across the two years, as shown by the coefficients of variation (CV, Supplementary Table 4), some significant differences were identified between the 7AgL-carrier NIRLs and their controls. The plant growth cycle was, on average, 29 days longer in the 2012 season, mainly because of the earlier sowing date (over 1 month) and partly due to meteorological conditions, with the time from sowing to TS notably lengthened, and BS, HD, and ANT periods being, on average, 2 days longer in 2012 vs. 2013 (Fig. 1). Significant effects of genotype background (G) and of 7AgL-segments [7-AgL-segments [7-AgL(G)] were observed on duration of almost all phases (Supplementary Table 2a). The same analysis (ANOVA-GLM2, see Materials and methods) also showed a significant effect of the year, both alone (Y) and in interaction with the presence of 7AgL-segments [Y x 7AgL-segments [Y x 7AgL(G)], on length of most developmental phases. Yet, only for BS-ANT and SE duration the Tukey HSD test revealed significant differences among recombinants, with 22% longer BS-ANT in R112 and R5 and 5% longer SE in R112 as compared to their controls (data not shown).

### 3.5 Correlations among yield components and yield-contributing traits

Correlation coefficients between the traits analyzed in this study are given in Supplementary Table 5, and scatter plots and regression lines of GYM2 and related traits are shown in Fig. 4. GYM2, and the yield components BM2, SNM2, and HI, all showed highly positive correlation with each other across the years ( $r$  from 0.54 to 0.94), while no correlation was observed between them and GNM2. GYM2, BM2, SNM2 and HI, were highly positively correlated with spike fertility traits GNSP and SFI ( $r$  from 0.56 to 0.72), and negatively correlated with CDWA ( $r$  from -0.51 to -0.51 to -0.72). GNM2 was the only trait positively correlated with the duration of SE phase ( $r = 0.52$ ), and, as often observed, negatively correlated with TGW ( $r = -0.59$ ). GNM2 was negatively correlated with flag leaf traits ( $r$  from -0.50 to -0.50 to -0.63), which showed instead a positive relationship with GYM2, BM2, SNM2 and HI ( $r = 0.49-0.90$ ). Highly significant regression values validated the positive relationship of GYM2 with its key components SNM2 and BM2, flag leaf traits FLA and ChI77, as well as with GNSP and SI (Fig. 4). The relationship of GYM2 with TGW and GNM2 lacked any significant correlation across the years, yet in the case of GNM2, regression analysis showed a significant  $R^2$  for the separate years. This was probably due to different environmental conditions in the two seasons, which caused marked differences in the grain yield and number of all the three genotypes tested. The same was true for the relationship between GNM2 and SNM2 (Fig. 4). These results indicate a more significant involvement of grain number than TGW in the formation of the final yield in the tested *durumdurumdurum* wheat NIRLs.

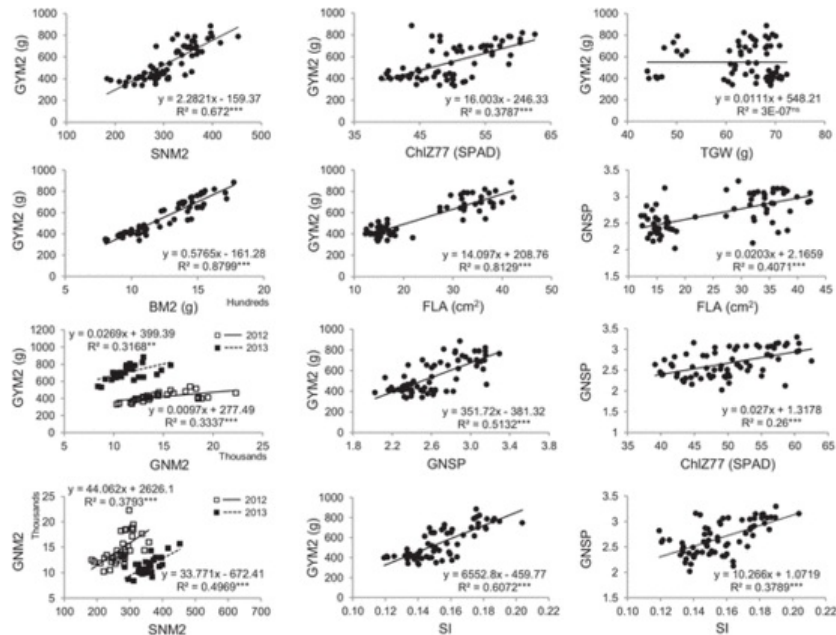


Fig. 4 Scatterplots of the means of main grain yield components vs. other yield-related traits from the three *durumdurum-wheat-Tdurum-wheat-T-Th. ponticum* NIRLs evaluated across two growing seasons (GYM2, grain yield m<sup>-2</sup>; SNM2, spike number m<sup>-2</sup>; BM2, biomass m<sup>-2</sup>; GNM2, grain number m<sup>-2</sup>; TGW, thousand-grain weight; ChI77, flag leaf chlorophyll content at late milk stage of grain filling; FLA, flag leaf area; GNSP, grain number spikelet<sup>-1</sup>; SI, spike index at anthesis).

Similarly to what was shown by the GYM2-GNM2 relationship, yield-related traits measured on individual culms, specifically GNS and GNSP, were not significantly correlated with GYS. Given the strong dependency of GYM2 on GNSP described above, the absence of any GNS/GNSP-GYS correlation was rather unexpected. Positive involvement of GNSP in grain yield formation could be however validated by significant  $R^2$  in relationships with SI, FLA or ChI77 (Fig. 4), all traits positively correlated with GYM2

(see above).

Results for traits regarding biomass partitioning at anthesis (SDWA, CDWA, SI) also revealed significant contribution of these parameters to some yield components per spike and unit area. SDWA was positively correlated with TGW ( $r = 0.71$ ) and GYS ( $r = 0.53$ ). Similarly, SI was correlated with GYS ( $r = 0.51$ ) and GNSP ( $r = 0.50$ ), and also proved to be positive correlated with yield traits per unit area (SNM2, BM2 and GYM2;  $r$  from 0.53 to 0.77).

## 4 Discussion

To verify previously observed effects on yield-contributing traits of three *Th. ponticum* 7-AgL chromosomal segments transferred into *durum durum durum* wheat, a two-year field experiment was carried out in rainfed Mediterranean conditions of Central Italy. To this aim, *durum durum wheat-Th. ponticum* NIRLs carrying 23%, 28% and 40% of 7-AgL chromatin on their distal 7-AL arm were tested in field plot trials and at common sowing density. With respect to earlier analysis on spaced plants (Kuzmanović et al., 2014), a set of additional physiological and qualitative parameters were assessed at different developmental stages, in order to elucidate the effect of alien chromatin on the trade-off between sink and source traits and final yield expression. In the present study, 7-AgL effects were generally of minor entity compared to those detected on single plants; nonetheless, the positive impact of defined 7-AgL regions on specific yield-contributing traits (e.g. seed number, productive tiller number, flag leaf dimensions) was confirmed. Moreover, effects on some of the latest examined traits (e.g. chlorophyll content, length of some physiological phases) were revealed.

Searching for genes/QTL enhancing yield potential of wheat within gene pools of wild relatives can look rather paradoxical, since wild crop relatives generally have poor agronomic performance. However, evidence of positive effects on relevant yield-related traits is repeatedly emerging when single alien chromosomes or chromosomal segments from various cereal wild germplasm are transferred into the crop background (e.g. Imai et al., 2013; Khush, 2013; Kuzmanović et al., 2014; Mohammed et al., 2014; Song et al., 2013; Wu et al., 2006). Thus, dissection of the involved alien chromosomal region(s) unmasks the desirable trait(s), and also facilitates analysis of the underlying genetic factors and physiological mechanisms. The use of NIRLs characterized by different wheat-alien breakpoints along the critical arm, as in the present example of *durum wheat-Th. ponticum* 7-AL-7-AgL recombinants (Kuzmanović et al., 2014 and this research), represents a valid approach. Analysis of such materials provided fundamental information on genetics of some yield-contributing traits (e.g. tiller and seed number and flag leaf size), including physical mapping of loci involved in their control within the 40% distal 7-AgL. A similar approach applied to bread wheat-rye translocation lines developed from the initial 1RS.1BL centric translocation, enabled identification of QTL for root traits within the distal 15% of 1RS physical length (Sharma et al., 2009, 2011). In both cases, the use of chromosome arm-specific recombinant lines, together with accurate phenotyping and use of molecular markers, have given proof that alien species can be used as a source for improving complex traits such as yield.

### 4.1 Yield performance of the three *durum wheat-Th. ponticum durum wheat-T. ponticum* NIRLs through seasons

Grain yield in wheat is largely determined by environmental factors, particularly under Mediterranean conditions (Villegas et al., 2015). Under our experimental conditions, protracted and intense precipitation during the grain filling period in both seasons (Fig. 1) have likely prevented more marked differences in expression of yield-contributing traits of NIRLs vs. their controls to emerge. Across the two years, significant 7-AgL-linked effects were confirmed, namely increased productive tiller number (R112 and R23 recombinants), flag leaf width and area (R112), and grain number per unit area, per spike and spikelet (R23). Some of the negative 7-AgL effects exhibited by the R23 line were also consistently observed, namely decreased 1000-grain weight, grain yield per spike, as well as flag leaf width and area. Increases in grain yield  $m^{-2}$  (GYM2) of the three recombinants vs. their controls ranged between 3 and 11% through the two years, albeit not being statistically significant.

Considering some year-specific characteristics, in 2013\_Central Italy was particularly disadvantaged by a mild winter and continuous rain during the whole life cycle of the *durum durum durum* wheat crop, whose total production decreased by 11% as compared to the 5-year average in the Tyrrhenian side of Central Italy, where our experimental site was located ([www.informatoreagrario.it](http://www.informatoreagrario.it), 2013). In the present study, although all HOM+ and HOM- genotypes yielded similarly (GYM2), the average yield of the 7-AgL-carrier lines was still 5% superior to their controls. However, it was the R23 recombinant to be particularly favoured by the wetter conditions of 2013, yielding 11% more than its control, predominantly due to increased tiller number (Fig. 2). In contrast, while R23 showed some yield penalty (-2%) in 2012, R112 and R5 NIRLs had a slight, yet stable yield gain (up to 3%) over their controls in both years (Fig. 2).

### 4.2 Main factors contributing to yield associated with 7 AgL introgressions

Overall, 7-AgL-carrier lines and their controls showed a biological cycle of similar length, except for duration of pre-anthesis sub-phases. In particular, significantly longer stem elongation (SE) and booting-to-anthesis (BS-ANT) phases of R112 vs. its control, possibly contributed to its positive yield performance. SE is known to be correlated with the final number of well-developed productive tillers, and was proposed as one of the key traits to target for further yield gains (e.g. Foulkes et al., 2011; Miralles and Slafer, 2007; Reynolds et al., 2009). Likewise, BS-ANT was identified as an essential time-span, whose expansion favours translocation of assimilates to the growing spike, and hence spike fertility (Isidro et al., 2011 and references therein). In line with this view, the largest majority of tillers in the R112 NIRL were seed-bearing at harvest. Recent reports in both bread (Borràs-Geloch et al., 2012; Yu et al., 2015) and *durum durum durum* (Sanna et al., 2014) wheat have identified multiple minor QTL controlling each pre-anthesis sub-phase, which could thus be separately targeted for manipulation. Based on our results, a putative QTL controlling SE and/or BS-ANT duration can be located within the most proximal 5% stretch of 7-AgL (23-AgL (23-28%)) of R112, common to R23 but not to R5. To our knowledge, no QTL controlling such trait(s) has been identified so far on corresponding wheat group 7 chromosome regions.

In the same 7-AgL interval, as already indicated by the analysis of spaced plants (Kuzmanović et al., 2014), and largely confirmed by the present results from plot trials, a genetic factor(s) controlling tiller number is likely to reside. Tiller number is

recognized as direct contributor to the aboveground biomass and grain yield in both bread and *durum durum durum* wheat (Elhani et al., 2007; Zhang et al., 2010), besides showing a positive correlation with other yield-related traits, such as biomass allocation to roots (Allard et al., 2013). Therefore, the involvement of SNM2 in the final biomass, grain yield and number (Table 3, Fig. 2) in the NIRLs analysed here, notably R112 and R23, is not unusual. R112 and R23 recombinants showed different expression (i.e. increases) of SNM2 in the two experimental seasons: in the former, the positive effect of increased SNM2 (+20% in 2012) on yield was clear-cut, as in the absence of any difference in grain yield per spike (GYS) vs. its negative control (Table 4), it was undoubtedly the number of productive tillers that determined higher GYM2 and GNM2. On the other hand, although R23 showed significantly lower GYS (-14%) across years, in 2013 its higher tillering capacity (+25%) allowed the overall yield per unit area to be 11% higher than its control. Such an effect might be attributed to the establishment of a larger sink, which then acted as driving force for assimilate translocation from the source. Although not all tillers become seed-bearing at maturity, tiller number is proportional to the capacity for resource capturing (Sadras and Slafer, 2012), which is particularly important for achieving maximum yield potential in rain-fed conditions (Elhani et al., 2007; García del Moral et al., 2005; Zhang et al., 2010). In the case of the R112 NIRL, higher tillering ability combined with higher leaf area and chlorophyll content would then contribute to the increase of light interception and photosynthesis during grain filling, a combination of traits found to increase yield also in barley (Abeledo et al., 2004; El Soda et al., 2010) and rice (Zhong et al., 2003).

In contrast to what observed in the *durum durum durum* NIRLs, the increase in tiller number associated with the presence of 7<sup>A</sup>AgL segment in bread wheat T4 NILs was not consistent. While some authors did not observe such a 7<sup>A</sup>AgL-linked effect (Monneveux et al., 2003; Reynolds et al., 2005), others reported 9–15% increases under non-moisture stress conditions, i.e. in irrigated trials (Miralles et al., 2007; Singh et al., 1998; Tripathi et al., 2005). This could well be due to the fact that tiller number is highly dependent on the environment, but also on agricultural practices, i.e. sowing density, nitrogen fertilization rates, and water availability. For this reason, also in the case of our *durum durum wheat-Th durum wheat-T-Th. ponticum* recombinants with enhanced tiller number (R112 and R23), it would be worthwhile studying in more detail the effect of various cultivation practices on their yield potential. Likewise, the possibility of identifying specific 7<sup>A</sup>AgL gene(s) involved in the control of tillering could be explored, although the task may not be easy, given the complex genetic control for this trait in cereals (Hussien et al., 2014; Janssen et al., 2014; Kebrom et al., 2013).

In line with previous observations on T4 bread wheat NILs (Monneveux et al., 2003; Reynolds et al., 2001; Tripathi et al., 2005), all three *durum durum durum* wheat NIRLs showed a larger sink in terms of grain number compared to their controls, both per unit area (GNM2) and at the single spike level (GNS) (Tables 3 and 4, Fig. 2). The difference between HOM+ and HOM- lines was largely significant only in the case of R23 (up to 40%), also characterized by an extremely enhanced spike fruiting efficiency (see SFI). For both traits R23 showed significantly higher values compared with the two other recombinants (Table 4). Thus, in the in the 7<sup>A</sup>AgL portion specific to R23 (28–40% of 7<sup>A</sup>AgL), not only the presence of a QTL for GNS can be confirmed (Kuzmanović et al., 2014), but also positioned a genetic factor with a positive effect on SFI. Indeed, the two traits might correspond to the same locus.

The cause of the increase in GNS of the R23 NIRL lies probably in its increased floret survival rate with respect to its control and to the other recombinants, since the number of fertile florets at anthesis (FF) did not differ between any of HOM+ and HOM- genotypes (Table 4). In fact, it is largely recognized that in wheat the final grain number depends more on floret survival rate rather than on number of developed floral primordia (Ferrante et al., 2010, 2013a, 2013b; González et al., 2011). In the same R23, a severe reduction in TGW was likely responsible for the significantly decreased GYS, in spite of the increased GNS, as well as for the absence of positive GNS-GYS relationship (Supplementary Table 5). Indeed, when the correlation analysis was made on the data set of R5 and R112 genotypes only (data not shown), this relationship showed to be highly significant, confirming an important involvement of grain number in the formation of final yield. The R23 overall picture may also be complicated by a Segregation distortion (*SeSd*) factor(s) uniquely present in the 7<sup>A</sup>AgL segment specific to this NIRL (28–40% of the arm) (Ceoloni et al., 2014b). Either in a direct or indirect manner, the *Sd* gene, or associated factor(s) in the same 7<sup>A</sup>AgL portion, may negatively affect R23 performance, particularly at critical stages such as grain filling, when also flag leaf dimensions and chlorophyll content decrease significantly (Supplementary Table 3; Fig. 3; see also Kuzmanović et al., 2014).

In the present study, flag leaf traits were positively correlated with all main yield components (e.g. GYM2, SNM2, BM2), single spike fertility traits (e.g. GNSP, SFI), as well as spike index at anthesis (Supplementary Table 5). Such a relationship was frequently reported in rice (Wang et al., 2012; Yue et al., 2006), barley (Xue et al., 2008) and wheat (Foulkes et al., 2007; Quarrie et al., 2006; Simpson, 1968). Also, chlorophyll content has frequently been recognized as being correlated with photosynthetic rate in cereals (e.g. Guo et al., 2008; Zhang et al., 2009). In bread wheat, in particular, Habash et al. (2007) provided evidence of positive correlation between flag leaf size and photosynthetic activity during grain filling, assuming a common genetic control for the two traits. Similarly, Hill et al. (2013) showed chlorophyll content, spike number plant<sup>-1</sup> and grain size to be all positively correlated with metabolites involved in photosynthesis. Both findings may apply to the case of R112 recombinant, which, thanks to its higher source (i.e. leaf area, chlorophyll content), and an efficient assimilate partitioning to the spike, did not display any yield penalty, in spite of a higher sink (tiller/grain number) to support. Moreover, R112 exerted a higher NBI at late grain filling, which represents a direct measure of primary metabolism activity, i.e. of synthesis of nitrogen-containing molecules, such as proteins. The present results thus indicate a genetic determinant for chlorophyll content to be located in the same 23–28% 7<sup>A</sup>AgL interval of R112 where a QTL for flag leaf dimensions was previously identified (Kuzmanović et al., 2014). This physical region coincides with the homoeologous region on bread wheat 7<sup>A</sup>AL where a QTL for maximum fluorescence yield of PSII under drought stress was recently identified (Ilyas et al., 2014). Similarly to R112, bread wheat T4 7<sup>A</sup>AgL translocation, showed higher yield associated with increased flag leaf photosynthetic rate during grain filling (Monneveux et al., 2003; Reynolds et al., 2001, 2005). These observations are suggestive of a 'stay-green' mechanism as a possible driving force for biomass and yield increase. 'Stay-green' properties of wheat are known to be key indicators of stress adaptation (Foulkes et al., 2007; Lopes and Reynolds, 2012), associated with the plant's capacity to maintain levels of photosynthesis, especially beneficial in semi-arid and rain-fed growing condition (Adu et al., 2011; Cabrera-Bosquet et al., 2009; Villegas et al., 2000).

## 5 Final remarks and future developments

Agronomic and physiological characterisation of the effects of 7<sup>A</sup>AgL introgressions into *durum durum wheat 7 durum wheat 7*-AL arm have provided important information on novel genetic variability for yield-related traits originating from an alien donor. Significantly positive effects of defined 7<sup>A</sup>AgL regions on traits such as grain and tiller number, flag leaf area, chlorophyll content and duration of stem elongation phase, and on their contribution to productivity, confirm that within the

distal 40% of the alien arm multiple yield-related loci can be exploited without affecting grain quality. However, since the analysis was carried out in a single location and in two rather different growing seasons from the meteorological viewpoint, evaluation of the yield performance of the three *durum durum wheat-Th. durum wheat-T. Th. ponticum recombinant lines* in different environments is certainly worthwhile. To this aim, field trials in different geographical and climatic regions and years are underway. They include additional sites in Italy, representative of contrasting environments (rainfall, temperatures and soil type), as well as in typically drought-prone areas, such as South Australia and North Africa.

No doubt, the R112 recombinant stood out as the one that combines most of the positive effects of 7AgL chromatin on yield components. Within its most proximal 7AgL portion, roughly spanning 5% of the arm, more than one yield-related locus is likely to be located. However, the hypothesis that the observed phenotypes are the result of a single gene/major QTL with pleiotropic effects can also be contemplated. If so, the combined expression of yield-contributing traits in R112 (mainly tiller number, leaf area and chlorophyll content) might be driven by a genetic factor involved in more efficient photosynthesis and assimilate translocation. This appears to be the case for rice, where a single gene (*HYR&9552;HIGHER-YIELD-RICEHYR* = *HIGHER YIELD RICE*) has been identified as a master transcriptional regulator of photosynthesis and related morpho-physiological processes, such as carbon metabolism, root growth and development, and stress tolerance (Ambavaram et al., 2014). In conclusion, the present study shows that increasing grain yield in *durum durum durum* wheat is feasible by making proper use of alien variability, and that the underlying mechanism(s) are likely associated with the establishment of photosynthetically active canopy (tillers, flag leaves, chlorophyll content), in interaction with effective biomass partitioning and, to some extent, phenology.

## Uncited references

Bancal (2009) and Wang and Li (2008).

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.fcr.2015.11.007>.

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## Appendix A. Supplementary data

The following are Supplementary data to this article:

[Multimedia Component 1](#)

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### Highlights

- Durum wheat-*Thinopyron ponticum* recombinant lines were for the first time tested in field plot trials.
  - The 2-year experiment confirmed results from spaced plants as to positive effects of the alien 7-AgL arm on various yield-related traits.
  - The most significant increases concerned spike and grain No./m<sup>2</sup>, flag leaf area, chlorophyll content and length of some phenological phases.
  - Grain yield and biomass showed up to 11% increase, though not significant across variable experimental seasons.
  - QTLs for positive traits were mostly concentrated in a 7-AgL region spanning 23 to 28% of the recipient 7-AL arm.
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