Yield of chromosomally engineered durum wheat-*Thinopyrum ponticum* recombinant lines in a range of contrasting rain-fed environments

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Abstract

Introgressions of *Thinopyrum ponticum* 7AgL chromosome segments, spanning 23%, 28% and 40% of the distal end of durum wheat 7AL arm, were previously shown to contain multiple beneficial gene(s)/QTL for yield-related traits, in addition to effective disease resistance (*Lr19, Sr25*) and quality (*Yp*) genes. In the present study, durum wheat near isogenic recombinant lines (NIRLs), harbouring each of the three introgressions, were included for the first time in multi-location field trials, to evaluate general and environment-specific effects of the alien chromatin on 26 yield-related traits. Results from nine different trials across contrasting environments of Italy, Morocco and South Australia over four years revealed that the overall impact of 7AgL introgressions into the tetraploid wheat background did not incur, except in one environment, a major yield penalty. The effect of the

three 7AgL segments on individual yield-contributing traits, resulted in significant increases of biomass m^{-2} (+9%), spike number m^{-2} (+13%), grain number m^{-2} (+11%) and spikelet^{-1} (+8%), as well as a significant decrease in grain weight (−8%). When the separate NIRLs were analysed, each of the three 7AgL segments were associated with specific yield component variation. The effects of the 40%-long segment proved to be the most stably expressed across environments and involved significant increases of spike and grain number m^{-2} (13% and 15%, respectively), grain number spike^{-1} (10%) and spike fertility index (46%), though accompanied by a significant decrease in thousand grain weight (−23%). In spite of this trade-off between grain number and grain weight, their interplay was such that in four trials, including drier environments, a grain yield advantage was observed. This evidence, and comparison with the two other NIRLs, substantiates the hypothesized existence of major gene(s)/QTL for grain number in the most proximal 28–40% 7AgL region, exclusive to the 40%-long 7AgL introgression. The present study represents an important validation of the use of chromosomally engineered genetic stocks for durum wheat improvement, targeting not only disease resistance and quality traits but also relevant yield components.

Abbreviations: NIRL, near isogenic recombinant line; HI, harvest index; SNM2, spike No. m^{-2}; BM2, biomass m^{-2}; GYM2, grain yield m^{-2}; GNM2, grain No. m^{-2}; TGW, 1000 grain weight; GYS, grain yield spike^{-1}; GNS, grain No. spike^{-1}; GNSP, grain No. spikelet^{-1}; SPN, spikelet No. spike^{-1}; PH, plant height; HD, days to heading; SDWA, spike dry weight at anthesis; TDWA, tiller dry weight at anthesis; SIA, spike index at anthesis; SDW, spike dry weight at harvest; TDW, tiller dry weight at harvest; CHAFF, chaff dry weight at harvest; SL, spike length; SFI, spike fertility index; FLW, flag leaf width; FLL, flag leaf length; FLA, flag leaf area; CHLZ75, chlorophyll content at Zadoks 75; CHLZ77, chlorophyll content at Zadoks 77; CHLZ79, chlorophyll content at Zadoks 79; GLM, general linear model

Keywords: Alien introgression; Chromosome engineering; Grain number; Tiller number; Wheat breeding

1 Introduction

Due to the continuing need to enhance crop productivity to feed a burgeoning world population, amidst climate changes that will further add to the pressure of availability of land and water, the goal of significantly increasing yield of staple crops such as wheat is becoming more challenging than ever (Ray et al., 2013; Liu et al., 2016; Zhao et al., 2017). To mitigate against these ongoing challenges, crop species are already undergoing a 'migration' from their conventional growing regions (Chapman et al., 2012). Durum wheat (*Triticum durum* var. *durum*, 2n = 4x = 28, genomes AB), cultivated on approximately 8% of the world's wheat area (Mediterranean basin, North America's Great Plains, Mexico, Australia, Russia, Kazakhstan, India, Ethiopia and Argentina; Bassi and Sanchez-Garcia, 2017; Royo et al., 2014), exemplifies this migration phenomenon, with its progressive displacement from traditional cultivation areas (Ceoloni et al., 2014a). To develop durum wheats with improved yield and adaptation, understanding and dissecting the multi-layered genotype × environment interaction is crucial if we are to close the gap between actual and attainable yields (Bassi and Sanchez-Garcia, 2017; Dodig et al., 2012; Maccaferri et al., 2011; Marti and Slafer, 2014; Parent et al., 2017; Slafer et al., 2014; Tardieu and Tuberosa, 2010; Zaïm et al., 2017).

A powerful strategy to enhance the genetic potential and plasticity of cultivated germplasm resides in the exploitation of the ample and still largely untapped variability present in wild wheat relatives (Zaïm et al., 2017; Ceoloni et al., 2014b, 2017a; Dempewolf et al., 2017; Prohens et al., 2017; Zhang et al., 2017). Targeted and precise introgression of useful alien genes is readily possible through efficient sexual means, foremost the cytogenetic methodologies of "chromosome engineering" (Ceoloni et al., 2005, 2014a, 2014b, 2015). This approach, integrated with continuously developing techniques of genome and chromosome analysis (e.g. marker-assisted selection, association mapping, next generation sequencing, *in situ* hybridization), represents a unique platform for creation of novel and breeder-friendly genetic stocks.

In the majority of cases, alien genes transferred into cultivated *Triticum* species have enhanced biotic and abiotic stress tolerance, thus indirectly contributing to yield improvement (Ceoloni et al., 2014a). Use of wild relatives for direct improvement of wheat yield components has instead been sporadic, as their productivity is poor, and conspicuous effects on wheat yield rarely observed (Ceoloni et al., 2015; Dempewolf et al., 2017; Zhang et al., 2017). Nonetheless, noteworthy examples do exist in the widely cultivated bread wheat, where there appears to be a higher buffering ability when chromosomes are manipulated vs. tetraploid durum wheat (reviewed in Ceoloni et al., 2014a, 2015; Mondal et al., 2016). One such example concerned the transfer of a portion from the group 7 chromosome arm (7AgL or $7el_1L$) of the decaploid perennial species *Thinopyrum ponticum* (Popd.) Barkworth & D. R. Dewey (2n = 10x = 70, genomes $E^eE^eE^xStSt$, see Ceoloni et al., 2014b) onto the 7DL arm of bread wheat. The sizeable 7AgL translocation, named T4 (~70% of the recipient 7DL arm, harbouring the *Lr19*+*Sr25*+*Yp* genes), led to increased grain yield (5–35%), biomass (6–31%) and grain number (10–18%) across a number of non-moisture stress environments, and in different backgrounds of CIMMYT germplasm (Monneveux et al., 2003; Reynolds et al., 2001; Singh et al., 1998; Tripathi et al., 2005; Miralles et al., 2007).

The *Lr19*+*Sr25*+*Yp* gene package is considered particularly valuable for durum wheat breeding, due to the high efficacy of *Lr19* and *Sr25* resistance genes towards main rust diseases of the crop (Gennaro et al., 2009; Ceoloni et al., 2014a), and the contribution to increase carotenoid pigmentation and semolina colour of the *Yp* gene (Gennaro et al., 2007). Of several durum wheat-*Th. ponticum* recombinants obtained through chromosome engineering (Ceoloni et al., 2005), three, carrying 23%, 28% and 40% of the recipient 7AL arm replaced by homoeologous 7AgL segments (corresponding to distal portions of the T4 introgression), were employed in development of near-isogenic recombinant lines (NIRLs) into the background of cv. Simeto. The three NIRLs, evaluated for yield performance across four years in one rain-fed locality of Central Italy, showed increases in grain yield (1–36%), biomass (2–38%) and

grain number (3–39%) associated with presence of 7AgL chromatin (Kuzmanović et al., 2014, 2016). Furthermore, detailed phenotyping of the NIRLs for several yield-contributing traits enabled the structural-functional dissection of 7AgL chromatin incorporated onto 7AL, with assignment of yield-contributing *Th. ponticum* genes/alleles (previously associated to the entire T4 segment) to specific 7AgL sub-regions (Kuzmanović et al., 2014, 2016).

With no information from other environments on the expression of 7AgL and its effects on yield in durum wheat, the aim of the present work was to assess the yield performance of the same three durum wheat-*Th. ponticum* NIRLs across an array of rain-fed environments, and to evaluate possible environment/7AgL segment-specific associations with final yield and individual yield-related traits, ultimately in view of using these recombinants across environments or in site-directed breeding programs.

2 Materials and methods

2.1 Plant materials

Three durum wheat-*Th. ponticum* NIRLs, named R5-2-10, R112-4 and R23-1 (hereafter referred to as R5, R112 and R23, respectively), developed in the background of cv. Simeto by repeated backcrossing (BC) (Ceoloni et al., 2005), were used across four years and three countries. Simeto (pedigree: selection from Capeiti 8 x Valnova) is a variety released in 1988, well adapted to the Italian growing conditions. The NIRLs have portions of *Th. ponticum* 7AgL chromosome arm replacing 23%, 28% and 40% of their distal 7AL arm, respectively, and all three lines include the (insert the word : "alien", i.e. "....include the alien *Lr19+Sr25+Yp* genes...")*Lr19+Yp+Sr25* genes in the sub-telomeric region. Similarly to the plant material described in Kuzmanović et al. (2016), each of the genotypes analysed, corresponding here to $BC_5F_{5\text{-}9}$ (R5 and R112) and $BC_4F_{5\text{-}9}$ (R23) progenies, was represented by either being a homozygous carrier ("+") or non-carrier ("–") of the given 7AgL segment. Each "+" and "− "NIRL included two families originating from sister lines.

2.2 Field experiments

A total of nine rain-fed field trials were carried out over four years and four locations where durum is typically cultivated (two in Italy, one in Morocco and in one Australia) and used for the multi-environment yield assessment. Details on all trials are reported in Table 1. Years and locations were combined and hereafter referred to as environments, with a specific acronym assigned in Table 1. Two of the nine trials have been described previously in Kuzmanović et al. (2016) (VT12 and VT13), from where a subset of traits was considered in the present analysis. In ~~ten~~eight environments, all three NIRLs with respective controls were used, while in AUS14, only R5 and R112 were analysed. Sowing densities applied were those commonly used in each of the experimental locations (Table 1). In all field experiments, complete randomized block designs with three replicates for each sister line was used, resulting in a total of 24 plots in AUS14 and 36 plots in the other eight environments (2 per each sister line, i.e. 6 per each +/– NIRL). Meteorological data during the growing seasons for daily temperatures (minimum, mean and maximum) and rainfall (Table 2) were retrieved from meteorological stations at experimental sites, except for MOR14, for which the data were downloaded from NASA's (National Aeronautics and Space Administration, USA) site for Prediction of Worldwide Energy Resource (http://power.larc.nasa.gov). All trials were managed according to standard local practices including fertilization, weed, pest and disease control, in order to avoid, in particular, leaf rust spreading on *Lr19* non-carrier plants (– (Please reduce the space between "-" and the following word, NIRLs (leave some small distance from but not that much))NIRLs), hence to eliminate the indirect yield-contributing factor of *Lr19*-carriers (+ NIRLs).

Table 1 Description of locations and field experiments analysed in this study (NIRL, Near Isogenic Recombinant Line).

alt-text: Table 1

Environment acronym	Location	Latitude	Longitude	Altitude (m)	Season	Total rainfall (mm)	Mean temperature at heading (C°)	NIRLs tested	Sowing date	Crop cycle length (days)	Sowing density (seed/m²)	Plot size (m²)
VT12	Experimental farm of the University of Tuscia, Viterbo (Central Italy)	42° 25' N	12° 4' E	301	2011/12	248	13.6	R5, R112, R23	17/11/2011	226	350	2.3
VT13	"	"	"	"	2012/13	534	17.2	"	20/12/2012	192	350	2.3
VT14	"	"	"	"	2013/14	676	12.7	"	29/11/2013	213	350	4.5
VT15	"	"	"	"	2014/15	337	16.5	"	15/12/2014	197	350	4.5
BO14	Experimental farm of the University of Bologna, Cadriano (North Italy)	44° 33' N	11° 24' E	33	2013/14	598	13.1	"	07/11/2013	236	350	4.2
MOR14	Marchouch (Morocco)	33° 36' N	-6° 43' W	440	2013/14	229	13.0	"	03/12/2013	189	300	6

MOR15	"	"	"	"	2014/15	349	14.1	"	18/11/2014	204	300	6
AUS13	Waite campus, University of Adelaide, Urrbrae (South Australia)	34° 58' S	138° 38' E	48	2013	305	16.9	"	19/06/2013	179	150	1.6
AUS14	"	"	"	"	2014	296	14.3	R5, R112	15/05/2014	193	150	5

Table 2 Weather conditions for growing seasons in nine environments analysed, as retrieved from meteorological stations at experimental sites, or in the case of MOR14 only, downloaded from NASA's site for Prediction of Worldwide Energy Resource (http://power.larc.nasa.gov). Environment acronyms are as described in Table 1.

alt-text: Table 2

Environment	Sowing to heading				Heading to harvest			
	Rainfall (mm)	T_{min} (°C)	T_{mean} (°C)	T_{max} (°C)	Rainfall (mm)	T_{min} (°C)	T_{mean} (°C)	T_{max} (°C)
VT12	155	2.7	7.9	13.8	93	11.4	18.6	25.8
VT13	413	3.8	8.4	13.7	121	11.4	17.3	23.7
VT14	486	4.1	9.0	14.3	190	11.3	17.5	24.0
VT15	312	4.0	8.4	13.5	25	12.0	18.8	25.8
BO14	422	4.0	8.5	12.9	176	13.3	19.5	25.8
MOR14	180	5.8	10.7	17.5	49	11.7	18.6	27.2
MOR15	342	6.6	10.9	16.4	7	13.2	19.5	27.2
AUS13	266	10.0	14.2	18.4	39	12.7	19.6	24.3
AUS14	260	9.1	13.3	17.5	36	12.8	18.9	25.1

2.3 Measurements of yield-related traits

A list of traits and details on environments in which the materials were analysed, as well as on the sample type and number for each replicated plot, are reported in Table 3. Measurements of all traits were performed as described in Kuzmanović et al. (2016) with some modifications. HD was considered as number of days from sowing to heading. 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. SIA was calculated as SDWA/TDWA ratio, and SFI as the ratio between GNS and CHAFF at maturity (Isidro et al., 2011). SFI~~,~~ is a spike fertility parameter ~~that is~~ positively correlated with fruiting efficiency (Abbate et al., 2013; Martino et al., 2015), that is calculated as the ratio between GNS and SDWA (e.g., Slafer et al., 2015; Terrile et al., 2017). FLA was determined using the formula FLW × FLL × 0.75 (Dodig et al., 2010). Chlorophyll content was measured by using a hand-held meter SPAD 502 (Konica-Minolta, Japan) at medium milk (Zadoks 75; Zadoks et al., 1974), late milk (Zadoks 77) and very late milk (Zadoks 79) developmental stages. HI was determined as GYM2/BM2 in MOR14, MOR15, AUS13 and AUS14, where GYM2 and BM2 were obtained directly from the total harvested area. In other environments, HI was determined on 25-culm samples plot^{-1}, and at harvest 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^{-1} and then used to calculate GNM2 (=GYM2 × 1000/TGW).

Table 3 Traits and sample details assessed across nine environments analysed in this study [TH, total plot harvest; 25C, 25 culms; G, grain sample (1 or 2); IC, individual culms (5–10); FL, individual flag leaves (5–10)]. Environment acronyms are as described in Table 1.

alt-text: Table 3 (As we see Table 3 layout in the Proof file, some "Trait" descriptions have been arranged in 2 rows (e.g. 13 Spike dry weight at anthesis (g)). We think that in order to compact the Table content, it would be better to move all remaining columns (from the "Acronym" column onward) to the right. You should have enough spece to leave on 1 row only each "Trait" description.)

Trait	Acronym	Environment									Sample type/plot	No. samples plot^{-1} ~~replica~~ replica^{-1}
		VT12	VT13	VT14	VT15	BO14	MOR14	MOR15	AUS13	AUS14		

1	Harvest index	HI	X	x	x	x	x	x	x	x	x	TH/25C	1
2	Spike No. m^{-2}	SNM2	x	x	x	x	x	x	x	x	x	TH	1
3	Biomass m^{-2} (g)	BM2	x	x	x	x	x	x	x	x	x	TH	1
4	Grain yield m^{-2} (g)	GYM2	x	x	x	x	x	x	x	x	x	TH	1
5	Grain No. m^{-2}	GNM2	x	x	x	x	x	x	x	x	x	TH	1
6	1000 grain weight (g)	TGW	x	x	x	x	x	x	x	x	x	G	2
7	Grain yield $spike^{-1}$ (g)	GYS	x	x	x	x	x	x	x	x	x	IC	6
8	Grain No. $spike^{-1}$	GNS	x	x	x	x	x	x	x	x	x	IC	6
9	Grain No. $spikelet^{-1}$	GNSP	x	x	x	x	x	x	x	x	x	IC	6
10	Spikelet No. $spike^{-1}$	SPN	x	x	x	x	x	x	x	x	x	IC	6
11	Plant height (cm)	PH	x	x	x	x	x	x	x	x	x	IC	10
12	Days to heading	HD	x	x	x	x	x	x	x	x	x	day count	1
13	Spike dry weight at anthesis (g)	SDWA	x	x	x	x	x			x	x	IC	6
14	Tiller dry weight at anthesis (g)	TDWA	x	x	x	x	x			x	x	IC	6
15	Spike index at anthesis	SIA	x	x	x	x	x			x	x	IC	6
16	Spike dry weight at harvest (g)	SDW	x	x	x	x	x			x	x	IC	6
17	Tiller dry weight at harvest (g)	TDW	x	x	x	x	x			x	x	IC	6
18	Chaff dry weight at harvest (g)	CHAFF	x	x	x	x	x			x	x	IC	6
19	Spike length (cm)	SL	x	x	x	x	x			x	x	IC	6
20	Spike fertility index	SFI	x	x	x	x	x			x	x	IC	6
21	Flag leaf width (cm)	FLW	x	x	x	x	x			x	x	FL	10
22	Flag leaf length (cm)	FLL	x	x	x	x	x			x	x	FL	10
23	Flag leaf area (cm^2)	FLA	x	x	x	x	x			x	x	FL	10
24	Chlorophyll content at Zadoks 75	CHLZ75	x	x	x	x	x				x	FL	10
25	Chlorophyll content at Zadoks 77	CHLZ77	x	x	x	x	x			x		FL	10
26	Chlorophyll content at Zadoks 79	CHLZ79									x	FL	10

2.4 Statistical analyses

All analyses were performed by SYSTAT12 software (Systat Software Incorporated, San Jose, CA, USA). To investigate the effects of genetic and environmental factors and interactions between them on recorded traits, an analysis of variance (ANOVA) was performed, applying to datasets a general linear model (GLM) as a mixed effect model. Three such models were employed (= GLM1-3), depending on the data subset taken into consideration, as not all of the 26 traits and not all of the three NIRLs were analysed in all nine environments. In GLM1 and GLM2, datasets comprised environments where all three NIRLs were tested, while in GLM3, only the AUS14 dataset was analysed. GLM1 included traits No. 1–12 listed in Table 3, GLM2 traits No. 1-25, while GLM3 comprised traits No. 1-24 and 26. GLM1 was applied for the analysis of the overall effect of presence/absence of alien segments on main yield-related traits across environments, while GLM2 and GLM3 were applied for the analysis of individual 7AgL 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, environment (E), presence/absence of the 7AgL segment [7AgL alone in GLM1 or 7AgL(G), i.e. nested in the background, in other GLMs], and

replicate [R alone in GLM3 and environment-nested, R(E), in other GLMs]. Replicate was used in the models as the error. First order [E × G; R(E); E × 7AgL; 7AgL(G)], and second order [E × 7AgL(G)] interactions between the above factors were analysed as well. 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.

A correlation matrix was built for a subset of traits recorded in all environments. 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 a 0.95 confidence level.

In order to analyse any genotype-specific response for grain yield (GYM2) across environments, the environmental index was calculated (= average value of all participating genotypes) in each environment and combined with the environmental mean of each genotype in a simple linear regression (b coefficient statistics). GYM2 values were transformed in logarithmic to attain linearity and homogeneity of errors (Finlay and Wilkinson, 1963) and to observe the relative ("intrinsic") variability of interest (Becker and Leon, 1988).

3 Results

3.1 Environments

The field trials described here cover a wide range of environmental conditions, as usually observed in areas of durum wheat cultivation. Overall, environments were comparable for mean temperatures (Table 2), with a little more variation between them in the period from sowing to heading (6.3 °C) than from heading to maturity (2.3 °C). By contrast, the environments were considerably different for the rainfall amount received (Table 1) and its distribution during the growing season (Table 2). Large differences in rainfall input were recorded, particularly from heading to maturity, being in the range from 7 to 190 mm. The two seasons in South Australia were virtually identical for the precipitation received, nearly approaching the average values of the agricultural south-east areas of the State, while being the warmest years on record (since 1910), particularly through the second half of the season (www.bom.gov.au). The two trials in Morocco were also characterized by very warm seasons, with average daily maximum temperatures from May until harvest, often exceeding the site's average daily maximum values (22–26 °C) by up to 16 °C (www.weatherspark.com). Although less abundant, precipitation events were better distributed in MOR14 than in MOR15 (Table 2). As for the Italian trial sites, season 2012 was more typical and very favourable for durum wheat cultivation when compared to the 2013–2015 seasons. The latter three seasons were characterized by exceptional and numerous precipitation events and an overall increase in temperature during the entire crop cycle (www.informatoreagrario.it). VT12 had rainfall distribution and temperature trends that were conducive for good crop growth during the entire life cycle (Table 2; Kuzmanović et al., 2016). Still, in VT12, drought stress was present with precipitation amounts significantly below the site's mean, and higher maximum temperatures during the second part of the growth cycle (www.informatoreagrario.it). Conversely, seasons 2013 to 2015 in Italy had unusually wet and mild winters, with a full soil moisture profile. Particularly heavy and prolonged rain periods prior to sowing and during the grain filling period occurred in VT13, VT14 and BO14, while VT15 resulted in very dry and hot conditions from heading onwards with respect to the former three environments and to the site's mean values.

3.2 Yield response across environments

According to the observed highly significant R^2 values of linear regression (Fig. 1a), all NIRLs (both + and –) positively responded to better thermo-pluviometric patterns and higher environmental indexes across environments. The b coefficient values around 1 for all NIRLs were indicative of their average yield stability (Finlay and Wilkinson, 1963). On the other hand, the consistently higher grain yield with respect to the site's mean of R5 and R112 genotypes indicated their general adaptability, while the yield of the R23 NIRL pair, being poorer than the site's mean in all environments, indicated lower adaptation. This was probably due to the genetic background of R23 representative families, which were less isogenic compared to the recurrent cv. Simeto parent than the two other NIRLs (see § 2.1).

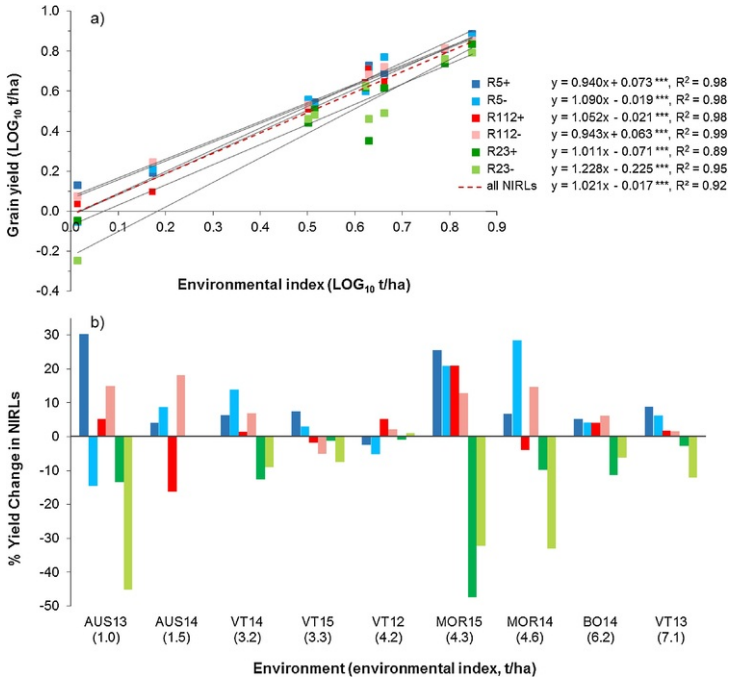

Fig. 1 Regression lines showing the relationship of individual grain yields of the six NIRLs and nine environments analysed (a), and the percentage yield change of the six NIRLs with respect to the site's mean (b). Environment acronyms are as described in Table 1.

alt-text: Fig. 1

With an average grain yield across environments between 1.03 and 7.05 t/ha (Fig. 1b), environments were arbitrarily classified as low-yielding (grain yield lower than 2 t/ha), medium-yielding (grain yield between 2 and 5 t/ha), and high-yielding (grain yield higher than 5 t/ha). Typically higher productivity of R5 and R112 NIRLs was shown from their yield gains vs. the environmental means ranging from 3 to 30% and from 2 to 27%, respectively. Yield of R23 NIRLs was always under the site's mean (−1 to −47%). Still, in four out of eight environments (all but AUS14, Table 1), the presence of the R23 7AgL segment evidently had a positive effect, reducing the background-dependent yield disadvantage (Fig. 1b). The two highest gains of all 7AgL (Please do not leave space between 7AgL and + (in some places in the Proof, they have been separated in two text lines, which should be avoided))+ lines was observed for R5+ in low-yielding AUS13 (+30%) and medium-yielding MOR15 (+26%). In MOR15, R112+ also showed a prominent gain of +21%. By contrast, a notable yield penalty was observed for R112+ in the low-yielding environment AUS14 (-18%), and for R23+ in the medium-yielding MOR15 (-47%).

3.3 7AgL-associated effects on yield and yield-related traits

Across all eight environments where all three NIRLs were tested (all but AUS14, Table 1), and with data from the three "+" recombinant or three "-" (Text should be: ... three "-" control lines...)control lines pooled, yield parameters were increased to a variable extent in 7AgL (Please do not leave space between 7AgL and + (in some places in the Proof, they have been separated in two text lines, which should be avoided))+ vs. 7AgL- genotypes (Table 4, Supplementary Tables S1 and S2). The most significant positive 7AgL effects were observed for SNM2 (+13%), BM2 (+9%), GNM2 (+11%), and GNSP (+8%) accompanied though by a significant decrease in TGW and GYS. However, HI of 7AgL + lines remained unchanged. Although the environment factor alone (E, Supplementary Table S1) was highly significant for all traits, confirming that the environments tested were different, the association between E and 7AgL turned out to be not significant for any of the traits (as observed from the Tukey test for E × 7AgL, data not shown). ANOVA performed for each of the nine environments separately, in view of highlighting the 7AgL effects in the various contrasting conditions, revealed specific associations between positive 7AgL-linked effects and sites (Table 4 and Supplementary Table S2). SNM2, GNM2 and GNS were significantly increased in 7AgL (Please do not leave space between 7AgL and + (in some places in the Proof, they have been separated in two text lines, which should be avoided))+ vs. 7AgL- NIRLs in several environments, particularly in the lowest- (AUS13) and the highest-yielding (BO14, VT13), as well as in the medium-yielding VT12. AUS13 was the only environment in which GYM2 together with BM2 were significantly increased (+18% and +14%, respectively) in 7AgL (Please do not leave space between 7AgL and + (in some places in the Proof, they have been separated in two text lines, which should be avoided))+ vs. 7AgL- NIRLs. This may indicate potential for adaptation to stressed conditions conferred by the 7AgL introgressions.

Table 4 Main yield-related traits of 7AgL-carrier vs. 7AgL-non carrier durum wheat-*Th. ponticum* NIRLs across environments (GYM2, grain yield m~~-2, BM2, biomass 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).

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Trait	NIRLs	Environment									
		ALL	AUS13	AUS14	VT14	VT15	VT12	MOR15	MOR14	BO14	VT13
GYM2	7AgL+	384.8	110.9	136.8	311.1	332.6	421.5	424.9	467.0	612.2	721.2
	7AgL-	372.6	94.3	169.0	330.2	316.8	416.4	428.3	475.4	625.4	688.6
	P-value	0.782	0.029*	0.115	0.433	0.304	0.752	0.959	0.844	0.685	0.281
	7AgL effect (%)	3	18	-19	-6	5	1	-1	-2	-2	5
BM2	7AgL+	1064.3	474.1	376.3	726.3	739.1	1041.8	1681.3	1570.7	1397.0	1512.2
	7AgL-	979.6	416.2	402.5	794.3	679.4	1012.5	1487.5	1543.0	1399.6	1443.2
	P-value	0.018*	0.001**	0.583	0.218	0.254	0.471	0.065	0.636	0.973	0.120
	7AgL effect (%)	9	14	-7	-9	9	3	13	2	0	5
HI	7AgL+	0.359	0.234	0.375	0.424	0.453	0.406	0.262	0.297	0.442	0.482
	7AgL-	0.360	0.224	0.420	0.410	0.466	0.412	0.289	0.306	0.447	0.483
	P-value	0.516	0.420	0.056	0.351	0.578	0.484	0.533	0.666	0.790	0.943
	7AgL effect (%)	0	4	-11	3	-3	-1	-9	-3	-1	0
GNM2	7AgL+	7871.2	1132.5	3058.1	5681.6	5703.0	15589.9	9349.4	10297.3	10234.4	12297.9
	7AgL-	7100.5	813.1	3323.2	5544.7	5101.9	13635.0	8488.2	9188.1	9526.1	10425.8
	P-value	0.000***	0.000***	0.510	0.903	0.172	0.038*	0.353	0.110	0.172	0.001**
	7AgL effect (%)	11	39	-8	2	12	14	10	12	7	18
SNM2	7AgL+	262.6	121.3	54.9	188.6	179.8	284.9	371.8	338.5	372.4	370.5
	7AgL-	233.3	91.6	52.9	187.0	164.9	259.2	356.9	354.0	343.0	342.2
	P-value	0.009**	0.000***	0.865	0.880	0.265	0.036*	0.684	0.643	0.041*	0.028*
	7AgL effect (%)	13	32	4	1	9	10	4	-4	9	8
PH	7AgL+	82.4	74.7	82.6	91.8	75.1	87.1	85.0	84.7	86.2	82.4
	7AgL-	81.4	72.3	81.7	90.9	76.2	87.0	89.4	83.9	86.4	81.3
	P-value	0.965	0.214	0.490	0.862	0.777	0.984	0.357	0.776	0.960	0.773
	7AgL effect (%)	1	3	1	1	-1	0	-5	1	0	1
HD	7AgL+	130.0	99.4	129.5	142.2	139.3	153.6	131.7	105.8	166.4	132.0
	7AgL-	127.0	95.3	126.6	141.0	138.7	153.2	130.4	105.7	165.6	130.6
	P-value	0.011*	0.000***	0.295	0.499	0.334	0.575	0.609	0.904	0.562	0.011*
	7AgL effect (%)	2	4	2	1	0	0	1	0	1	1
TGW	7AgL+	50.8	34.8	46.6	55.4	59.2	60.5	46.4	45.6	60.1	60.3
	7AgL-	55.3	38.1	51.0	59.9	62.3	66.8	49.7	51.1	65.9	66.3
	P-value	0.000***	0.006**	0.148	0.097	0.417	0.032*	0.274	0.048*	0.021*	0.005**
	7AgL effect (%)	-8	-9	-9	-8	-5	-9	-7	-11	-9	-9

In 8 out of the 9 environments, all three recombinants and their respective controls were analysed (ALL); in AUS14, only R5 and R112 NIRLs were included. Positive and negative 7AgL effects are highlighted in *green* and *red*, respectively. **P* <0.05, ***P*< 0.01, ****P* <0.001. Environments are ordered from the lowest- to the highest- yielding one, and their acronyms are as described in Table 1 (for interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).(This part of the legend to Table 4 (from "In 8 out of the 9 environments" to...the web version of this article)." must follow the text above, so to make a single set of lines, not interrupted by the Table, but preceding it.)

The more extensive and detailed GLM2 model (Supplementary Table S3), used to examine individual effects of each of the three 7AgL segments (Table 5 and Supplementary Table S4), showed that E alone was highly significant for all traits, but also the majority of interactions including it. Across all environments, the influence of the individual 7AgL segments on the various traits [7AgL(G), Supplementary Table S3] was different, as revealed by analysis of the three "+" vs. "–" NIRL pairs. Across the eight environments (all but AUS14, Table 1) where all three recombinants were analysed, the R5+ recombinant did not show any significant trait change due to its 7AgL segment, although in the AUS13 environment alone several significant differences emerged (see § 3.3.1). Also the comparison between R112+ and its R112– control across all environments showed only minor significant differences for HI (-3%), TGW (-4%), HD (+4%) and FLL (−9%).

Table 5 Mean values and standard errors (SE) of main yield-related traits unit area~~$^{-1}$ and spike^{-1} of the three durum wheat-*Th. ponticum* NIRLs (+, 7AgL carriers; −, 7AgL non-carriers) across 8 environments where all three recombinants and their respective controls were analysed (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}; SFI, spike fertility index). Letters in each row correspond to the ranking of the Tukey test at *P*< 0.01 (capital) and *P*< 0.05 (lower case) levels.

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Trait	R5+			R5-			R112+			R112-			R23+			R23-		
	Mean	SE		Mean	SE		Mean	SE		Mean	SE		Mean	SE		Mean	SE	
GYM2	418.3	29.4	ns	382.6	39.7	ns	396.0	28.7	ns	373.0	35.9	ns	340.9	27.1	ns	360.5	34.3	ns
BM2	1041.3	62.1	ns	932.1	76.4	ns	1067.1	73.7	ns	938.5	73.8	ns	1084.1	65.8	ns	1079.1	76.9	ns

HI	0.39	0.01	a	0.38	0.02	a	0.37	0.01	b	0.38	0.02	a	0.31	0.02	c	0.32	0.02	c
GNM2	7376.0	591.9	B	6929.3	775.9	B	7660.7	656.7	B	6956.7	807.2	B	8576.8	803.4	A	7458.8	849.2	C
SNM2	251.1	16.0	AB	228.8	19.6	AB	263.0	15.9	AB	230.4	18.9	AB	273.5	15.8	A	241.8	18.6	B
PH	77.9	0.9	c	75.8	1.0	cd	76.1	0.8	d	73.2	0.9	d	93.2	1.2	b	97.8	1.4	a
HD	127.2	3.1	C	123.6	4.0	C	129.2	3.2	B	123.7	3.9	C	133.6	3.1	A	135.0	3.7	A
TGW	57.5	1.5	A	56.0	2.1	A	53.2	1.7	B	55.6	2.0	A	41.6	1.1	C	54.2	2.0	B
GYS	2.6	0.1	A	2.3	0.2	A	2.4	0.1	A	2.4	0.2	A	2.0	0.1	B	2.5	0.2	A
GNS	42.5	1.4	ab	38.8	1.9	b	42.5	1.6	ab	40.1	2.0	ab	44.1	1.9	a	40.2	2.6	b
SFI	50.5	2.7	B	48.1	4.3	B	51.9	3.1	B	48.3	4.0	B	68.5	4.6	A	46.9	3.9	B

On the other hand, R23+ was the recombinant for which the highest number of significant differences with respect to its R23– control were observed irrespective of the E factor (Table 5 and Supplementary Table S4). While a number of yield-related parameters were depressed by the presence of the 40%-long 7AgL segment [TGW, GYS, SPN, PH, dry weight at anthesis and maturity (SDWA, TDWA, SDW, TDW, CHAFF), flag leaf dimensions (FLL, FLA)], the same 7AgL portion was clearly associated with significant increases of several other parameters directly contributing to grain yield, i.e. SNM2 (+13%), GNM2 (+15%), GNS (+10%), GNSP (+10%) and SFI (+46%).

3.3.1 7AgL × E interaction

Individual alien segment effects were significant to a variable extent in particular environments, and for specific traits, as revealed by the Tukey test (data not shown) for significant E × 7AgL(G) interactions (GLM2, Supplementary Tables S3 and S5). From this analysis across environments, significant differences between R5+ and R5- control were observed in the low-yielding AUS13 environment, the most significant one being a 27% increase of HI. Considering the AUS13 dataset alone, additional significant differences in favour of R5+ could be highlighted, namely a 52% increase in grain yield (GYM2), paralleled by significantly higher values of GYS (+30%), GNM2 (+45%), GNS (+21%), SFI (+77%) and BM2 (+21%) (Supplementary Table S5). As for the R112+ recombinant, the multi-environment dataset showed significant depression of TGW (−13%), and flag leaf size (-15% for FLL, -17% for FLW) when compared to the R112- control in AUS13, while biomass production (BM2) was enhanced in MOR15 (+24%). Data from AUS14, in which only the R5 and R112 NIRLs were grown, revealed that presence of both 7AgL segments was not particularly advantageous for the main yield traits (Supplementary Table S5). The GLM3 model identified some significant differences associated with the presence of the 7AgL segment ([7Ag(G)]; Supplementary Table S6), the most important ones being flag leaf size (+37% and +25% FLW for R5+ and R112+, respectively; +42% FLA for R5+) and spike fertility (-14% GNSP for R112+) (Supplementary Table S5).

On the other hand, the R23 + NIRL was highly responsive to different environments for several traits. The significant enhancing effect of its 7AgL segment for GNM2 and SFI was particularly pronounced in VT12-VT13 (+30% and +39%, respectively) and MOR14 (+56%) for the former trait, while in VT13-VT14 (+35% and +59%, respectively) and BO14 (+95%) for the latter (Supplementary Table S5). Of the identified depressing effects of the 40%-long 7AgL segment, particularly evident even across contrasting environments was TGW, ranging from -18 to -27% in VT12-15, BO14 and AUS13. Similarly to the R5 case, analysis of the AUS13 dataset alone showed significant increases of GYM2 (+58%), GNM2 (over 3 fold), SNM2 (+128%), GNS (+49%), GNSP (+67%) and BM2 (+33%) to be also associated to R23+ vs. R23-, as well as a significant reduction of TGW (-19%), GYS (−0%), TDWA (−19%) and TDW (−43%) (Supplementary Table S5).

3.4 Correlations of yield traits

Scatter plots of regression analysis (R^2) for the pairs of main yield traits are shown in Fig. 2, and coefficients of correlation (r) are reported in Table 6. For all environments, final grain yield per area (GYM2) was mostly dependent on SNM2, BM2 and GNM2 ($R^2 = 0.80$, 0.72, and 0.64, respectively), while the contribution of TGW was lower ($R^2 = 0.45$). Nevertheless, as observed from significant r coefficients, the involvement of given traits to GYM2 formation in each environment was different. In line with linear regression analysis, the strongest and the most transversal positive correlation of grain yield was observed with grain number and biomass, shown by a significant r value in eight out of the total nine environments tested (58–93% for GNM2 and 61–95% for BM2). TGW confirmed to be less important for final grain yield, the two parameters being significantly correlated in only two medium-yielding environments ($r = 60$–82%). Finally, GNM2, the key component of increased grain yield, as expected, was primarily influenced by SNM2 ($r = 62$–96%, significant in five environments), grain number $spike^{-1}$ (GNS) and $spikelet^{-1}$ (GNSP) ($r = 66$–96%, significant in three and four environments, respectively), particularly in low- and medium-to-low environments.

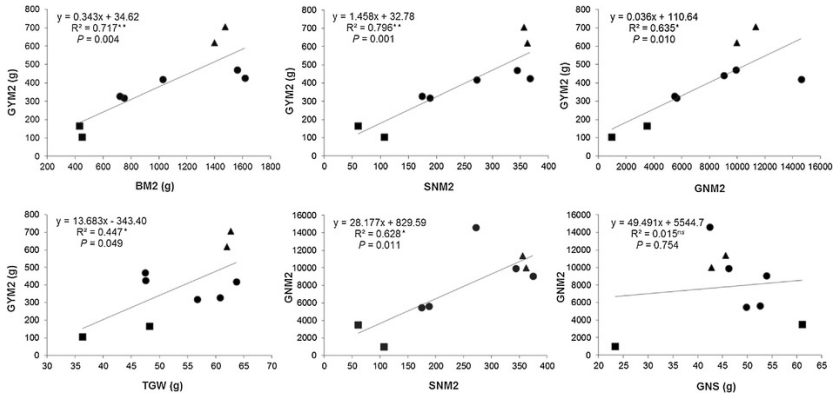

Fig. 2 Scatterplots of the means of grain yield and grain number m^{-2} vs. main grain yield components from the three durum wheat-*Th. ponticum* NIRLs evaluated across nine environments (GYM2, grain yield m~~$^{-2}$, BM2, biomass m^{-2}~~; BM2, biomass m^{-2}; SNM2, spike number m^{-2}; GNM2, grain number m^{-2}; TGW, thousand-grain weight; GNS, grain number spike^{-1}). *P <0.05, **P < 0.01, "ns" not significant. Low- (GY < 2 t ha^{-1}), medium- (GY 2-5 t ha^{-1}) and high- (GY > 5 t ha^{-1}) yielding environments are represented by *square*, *circle* and *triangle* symbols, respectively.

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Table 6 Pearson's correlation coefficients between pairs of main yield-related traits involved in coarse and fine regulation of final grain yield across nine environments tested (GYM2, grain yield m^{-2}; GNM2, grain number m^{-2}; TGW, thousand grain weight; SNM2, spike number m~~$^{-2}$, BM2, biomass m^{-2}~~; BM2, biomass m^{-2}; GYS, grain yield spike^{-1}; GNS, grain number spike^{-1}; GNSP, grin number spikelet^{-1}; PH, plant height; HD, heading date).

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Pairwise traits	Environment								
	AUS13	AUS14	VT14	VT15	VT12	MOR15	MOR14	BO14	VT13
GYM2 - GNM2	0.85 ***	0.87 **	0.90 ***	0.63 *	0.58 *	0.93 ***	0.72 **	0.63 *	0.56
GYM2 - TGW	0.35	0.36	0.26	0.03	0.02	0.82 ***	0.60 *	0.41	0.26
GYM2 - SNM2	0.50 **	0.74	0.83 ***	0.22	0.54 *	0.55	-0.29	-0.21	0.53
GYM2 - BM2	0.68 ***	0.87 **	0.95 ***	0.64 *	0.81 ***	-0.23	0.64 *	0.61 *	0.64 **
GYM2 - GYS	0.62 ***	0.17	0.73 **	0.19	0.09	0.22	0.73 **	0.45	0.16
GYM2 - GNS	0.82 ***	-0.09	0.58	0.25	0.07	-0.14	0.49	0.37	0.23
GYM2 - GNSP	0.79 ***	0.19	0.65 *	0.26	0.24	0.04	0.73 **	0.47	0.55
GYM2 - PH	-0.48 **	0.07	0.05	0.10	0.01	-0.62 *	-0.56	-0.49	-0.45
GYM2 - HD	-0.33	-0.38	-0.35	-0.49	-0.26	-0.75 **	-0.61 *	-0.46	-0.44
GNM2 - SNM2	0.81 ***	0.96 ***	0.76 ***	0.61	0.62 **	0.56	-0.07	-0.11	0.71 **
GNM2 - GNS	0.76 ***	0.96 ***	0.70 **	0.35	0.66 **	-0.22	0.49	0.29	0.54
GNM2 - GNSP	0.75 ***	-0.20	0.70 **	0.13	0.75 ***	0.02	0.73 **	0.33	0.50

Correlations are underlined using a heat colour map of green (positive correlations; dark green P < 0.001, medium green P < 0.01, light green P < 0.05) and red (negative correlations; medium red P < 0.01, light red P < 0.05) shades. Environments are ordered from the lowest- to the highest-yielding, and their acronyms are as described in Table 1 (for interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).(This part of the legend to Table 6 (from "Correlations...." to "the web version of this article)." must follow the text above, so to make a single set of lines, not interrupted by the Table, but preceding it.)

4 Discussion

To evaluate, for the first time, the effect of different environmental conditions on yield and yield-contributing traits of three durum wheat-*Th. ponticum* recombinant lines, having 23%, 28% and 40% of 7AgL chromatin on their 7AL distal end, nine rain-fed field trials in four locations worldwide were undertaken. The analysis showed that the overall impact of the 7AgL introgressions into the tetraploid wheat background on the trade-off between the various yield components, did not result, except for one environment (AUS14, see ahead), in a major yield penalty, nor in any alteration of harvest index. In fact, taking all three 7AgL segments (all + NIRLs) and the nine environments together, an average, ~~*albeit*~~ albeit not significant, 3% increase in grain yield m^{-2} was detected with respect to – (Please reduce the space between - and the following word, i.e. NIRLs (leave some small distance, but not that much))NIRLs (Table 4). This minor difference resulted from four incremental cases (ranging from +1% to 18%) and five others exhibiting a yield reduction (1–2% in three of them). The observed yield performance, associated with the presence of *Th. ponticum* chromatin, together with the *Lr19+Sr25+Yp* set of beneficial genes of the same origin, substantiate the value of the tested genetic stocks for their potential exploitation in durum wheat breeding. Furthermore, the analysis highlighted that yield performances of the 7AgL (Please do not leave space between 7AgL and + (in some places in the Proof, they have been separated in two text lines, which should be avoided))+ lines were mainly due to significant increases of biomass m^{-}

^{2}, spike/tiller number m^{-2}, grain number m^{-2} and spikelet^{-1}, and decreased grain weight (Table 4 and Supplementary Table S2). This evidence confirms previous observations in one location only (Kuzmanović et al., 2014, 2016) and indicates stable expression of such traits across environments. The 7AgL case thus appears to support the consolidated evidence that in both bread and durum wheat, coarse regulation of yield is based primarily on grain number and biomass (Marti et al., 2016; Pedro et al., 2011; Slafer et al., 2014).

Considering the separate NIRL pairs across the range of environments, a number of 7AgL segment-associated effects emerged. R5+ exhibited an average 9% increase (~~albeit~~albeit not significant) in grain yield (GYM2) vs. its control R5– line (Table 5), as well as higher grain yield gains with respect to the sites' mean in eight out of the nine environments (Fig. 1b). While grain yield increase of R5+ vs. R5– plants was slight in high- and medium-yielding environments (1–4% in VT12, VT13, VT15, BO14, MOR15), in low-yielding AUS13 it amounted to a significant 52%, paralleled by a significant increase in several other yield parameters (Supplementary Table S5). These figures, however, were not mimicked by the AUS14 environment, which was characterized by similar meteorological conditions to AUS13 (Tables 1 and 2) but a longer growth cycle (earlier sowing) and a remarkable increase in flag leaf size of R5+ vs. R5- plants (Supplementary Table S5). Flag leaf size is known to be positively correlated with photosynthetic activity and yield in wheat under drought (Foulkes et al., 2007; Habash et al., 2007; Quarrie et al., 2006). The longer AUS14 growing season likely favoured vegetative plant growth, as also evidenced by greater biomass at anthesis besides that bigger flag leaf area (Supplementary Table S5). However, this was not advantageous for R5+, likely leading to higher transpiration and dehydration rates, both negatively affecting final yield (e.g., Izanloo et al., 2008; Tardieu, 2005; Tricker et al., 2018).

As to the R112+ recombinant, average values were increased (when compared to R112-) across all environments for spike (+14%) and grain number (+10%) m^{-2}, as well as 6% higher grain yield (Table 5), in addition to a consistent tendency for wider flag leaf and higher chlorophyll content at late grain filling stages (Supplementary Table S5). These observations confirm that yield formation in R112+ depends mostly on tiller number development and potentially increased photosynthetic activity of leaves, both contributing to grain number formation (see also Kuzmanović et al., 2014, 2016). Tiller number is greatly influenced by the environment and determines wheat's adaptive ability under rain-fed conditions (Elhani et al., 2007; Zhang et al., 2010); thus, it is not surprising that higher grain yield of R112+ vs. control plants (+3-7%) was observed in only three of the experimental environments (VT12, VT15, MOR15, Supplementary Table 5). At high-yielding environments, R112+ produced essentially the same as the controls (BO14, VT13), while a major yield penalty was observed in the low-yielding AUS14 (−29%, Supplementary Table S5), apparently correlated with a significant, environment-specific decrease in spike fertility (−14% of grains spikelet^{-1}). Similarly to the R5+ case (see above), the significantly larger flag leaf size, accompanied by higher chlorophyll content (though not significant) at late grain filling in the R112+ case, seemed to be a disadvantage for R112+ grain yield in the water and heat stressed AUS14 environment (Supplementary Table S5). Another reason for the reduced yield of R112+ in the Australian environment might reside in the interaction of its root system with the soil features. A shallow root system was identified as more favourable in this type of environment (reviewed in Izanloo et al., 2008), which contrasts with the root system architecture (RSA) of R112+, characterized by increased seminal root angle, total root length and root dry weight (Virili et al., 2015). Under drought conditions, a widened root angle and deeper roots are crucial adaptive mechanisms associated with yield increases in rice (Uga et al., 2013, 2015; Ahmadi et al., 2014) and wheat (Lopes and Reynolds, 2010; Manschadi et al., 2006; Slack et al., 2018). However, the shallow, clay-limestone soils, typical of most Adelaide Plain's and of our testing environment, might have hindered the potential of the R112+ root system, limiting its access to water and nutrients at deeper layers. Therefore, to exploit the 7AgL-linked positive effects of R112+ on tiller/spike number, flag leaf photosynthetic activity and RSA characteristics, breeding with these lines could be directed to environments with optimal thermo-pluviometric patterns and soil characteristics.

Finally, the yield performance of the R23 (Please do not leave space between R23 and + (see the line below))+ NIRL represents an interesting case. In fact, while the common background genotype to both R23+ and R23– was most likely responsible for their generally lower productivity throughout environments (Fig. 1 and Supplementary Table S5), specific, both positive and negative, effects linked to its 40%-long 7AgL segment were consistently expressed in the multi-environment analysis (Table 5 and Supplementary Table S4). The most important incremental effects validated throughout all trials were on spike fertility index, grain and spike number m^{-2} (Table 5, Supplementary Tables S4 and S5). Enhancement of the first two traits strongly supports the suggested existence of a large effect QTL for grain number within the 28–40% 7AgL portion specific to the R23+ introgression (Kuzmanović et al., 2014, 2016). Interestingly, a couple of important QTL for spike fertility traits (number of spikelets and grains spikelet^{-1}) were recently identified in the homoeologous 7AL region of durum wheat (Giunta et al., 2018). Furthermore, the results showed that R23+, similarly to R112+, (in the shared 23–28% 7AgL segment), harbours a gene/QTL for tiller/spike number m^{-2} that would contribute as a major factor towards its yield potential, particularly in the drier South Australian (AUS13) and Moroccan (MOR14) environments (Supplementary Table S5; see also Kuzmanović et al., 2014, 2016). However, in contrast to R112+, in which a higher tiller number is likely to have primarily contributed to a more efficient plant source (i.e. leaf area, chlorophyll content), in R23+ the increased tiller number in combination with remarkably higher spike fertility had a more prominent effect on the genotype's sink (i.e. grain number m^{-2}). On the other hand, R23+ had reduced biomass at anthesis (about −18%, Table 5), a trait that appears to be highly correlated with grain number (e.g. Fischer, 2008; González et al., 2011; Slafer et al., 2015). It was therefore unexpected that R23+ could consistently sustain its large gain in grain number m^{-2} and in spike fertility index (+15% and +46%, respectively) across eight environments (all but AUS14; Table 5 and Supplementary Table S4). As the nature of the relationship between the biomass at anthesis and grain number is still uncertain (Slafer et al., 2015; Terrile et al., 2017 and references therein), it would be worthwhile investigating the modes of how assimilates are accumulated and translocated in R23+ during spike development just prior to anthesis. Mechanisms involved in the control of fertile florets survival rate, rather than their absolute number, are likely to be responsible for this (Kuzmanović et al., 2016 and references therein). In fact, R23+ plants had a similar number of fertile florets at anthesis to their R23- controls (Kuzmanović et al., 2016), but a superior number of seeds at maturity (Table 5 and Supplementary Table S5; Kuzmanović et al., 2014, 2016). Particularly noteworthy is the occurrence of increased spike fertility of R23+ vs. R23- not only in environments characterized by profuse rainfall throughout the crop cycle, such as VT14 and BO14, but

also in AUS13 and MOR14, where these favourable conditions were not met (Tables 1 and 2; Supplementary Table S5). Despite the consistently lower thousand-grain weight of R23+ vs. R23- plants, higher grain number of R23+ likely contributed to increments in grain yield (+7 to 58%) observed in four out of the eight environments (Table 5 and Supplementary Table S5). In fact, mechanisms controlling the trade-off between grain number and grain weight may be "non-constitutive", i.e. factor(s) increasing fruiting efficiency may be independent of potential grain size (Slafer et al., 2015; Ferrante et al., 2015), as possibly is the case in R23 + . Interestingly, the highest grain yield increases of R23+ were observed in dry environments of AUS13 and MOR14 (+58% and +34%, respectively), indicating that R23+ maintains its higher seed set even under drought and/or heat stress conditions. This finding is of great relevance, as grain number is recognized as the most susceptible yield component to drought (Richards et al., 2010), and spike fertility was recently shown to be the key trait contributing to yield performance of durum wheat grown under severe and protracted heat stress (Sall et al., 2018).

The possibility of fully unlocking the R23+ yield potential for practical exploitation remains, however, somewhat challenging because of the linkage between the gene(s)/QTL for grain number and spike fertility, and a gene(s) causing segregation distortion (*Sd*) and possibly depression of a number of morpho-physiological traits, including grain development (Ceoloni et al., 2014b; Kuzmanović et al., 2016). Nonetheless, this linkage drag could be broken, e.g. via induced mutations or further homoeologous recombination, or countered by transferring the R23 7AgL segment to different recipient cultivars. In this view, the improved transmission and the limited, if any, downsides of the R23 recombinant chromosome in certain durum wheat genetic backgrounds (Ceoloni et al., 2014b), is encouraging.

Apart from genotype-dependent "stabilization" that the R23 7AgL segment may require, all segments can be readily moved into more site-directed breeding pipelines for exploitation of their *Lr19+Sr25+Yp* genes and traits contributing to yield improvement. Moreover, all three recombinants can be further enriched with other useful alien genes. Recently, several new recombinants in bread and durum wheat have been obtained by chromosome engineering, in which highly effective gene(s)/QTL for resistance to *Fusarium* (NOT ITALIC , i.e. Fusarium) head blight and crown rot, originating from *Thinopyrum* species, were pyramided onto the most telomeric portions of the 7AgL segments described here (Ceoloni et al., 2017b; Forte et al., 2014; Kuzmanović et al., 2017). Preliminary results have shown normal fertility of the new recombinants and even higher yields when compared to control plants. This evidence contributes to strengthen the validity of targeted exploitation of alien variation to enhance yield potential in wheat species (Ceoloni et al., 2014a; Mondal et al., 2016; Zaïm et al., 2017, and references therein).

5 Conclusions

Analysis of the three 7AgL introgression lines into durum wheat across a range of variable environments has validated their potential as donors of *Th. ponticum* 7AgL gene(s)/QTL yield-contributing traits, thus strengthening the already positive effects of the *Lr19+Sr25+Yp* genes. In general, under no significant water stress, 7AgL lines responded well, without significant yield losses, mainly through contribution of grain and spike number, and, to some extent, flag leaf traits. Although the three recombinants exhibited variable performances in stressed environments like South Australia and Morocco, the observed significant increases in grain yield and grain number at these sites, indicate that the 7AgL yield-related gene/QTL content may also be beneficial under adverse growing conditions. Not last, the analysed set of NIRLs represents a valuable toolkit for deciphering physiological mechanisms and identifying genes involved in wheat grain yield regulation.

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Highlights

- Th. ponticum 7AgL introgressions onto durum wheat 7AL improve yield-related traits.
- 7AgL chromatin significantly increases grain number across contrasting environments.
- A major QTL for grain number increase was confirmed in the 28–40% 7AgL interval.
- Results validate the use of chromosome engineering for targeted wheat improvement.

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