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

Ljiljana Kuzmanović [a*], Roberto Ruggeri [a], Jason A. Able [b], Filippo M. Bassi [c], Marco Maccaferri [d], Roberto Tuberosa [d], Pasquale De Vita [e], Francesco Rossini [a], Carla Ceoloni [a*]

[a] Department of Agricultural and Forest Sciences (DAFNE), University of Tuscia, Viterbo, Italy

[b] School of Agriculture, Food and Wine, Waite Research Institute, The University of Adelaide, Glen Osmond, Australia

[c] ICARDA, Biodiversity and Integrated Gene Management, Rabat Institutes, Rabat, Morocco

[d] Department of Agricultural Sciences, University of Bologna, Bologna, Italy

[e] CREA-CI Cereal Research Centre for Cereal and Industrial Crops, Foggia, Italy

[*] Corresponding authors:

ljiljanakuzmanovic@gmail.com (L. Kuzmanović)
ceoloni@unitus.it (C. Ceoloni)

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 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 25 yield-related traits. The results from 11 different trials across contrasting environments of three countries and five years revealed that the presence of 7AgL chromatin had the strongest positive effect on spike (+11%) and grain number (+9%) m^{-2} , which represented the main contributors to the observed increases in grain yield and biomass (2-6%). In particular, grain yield was significantly increased (+18%) in 7AgL-carrier NIRLs in the water-limited South Australian environment. NIRLs carrying 23% and 28% 7AgL (named R5 and R112, respectively) displayed 2-30% higher grain yield with respect to the site's mean in most of the tested environments. Thus, also for other positive 7AgL genes they carry (*Lr19*, *Sr25*, *Yp*), they could be readily moved into site-directed breeding pipelines. Across all environments evaluated, the NIRL with a 40%-long 7AgL segment (named R23) resulted in increasing effects on grain number m^{-2} and $spike^{-1}$ (averaging 47% and 14%, respectively) when compared with its control (no-7AgL). From comparison with the other NIRLs, this major gene/QTL can be located within the most proximal 28-40% 7AgL region, exclusive to the R23 recombinant.

The present study represents an important validation of the use of chromosomally engineered genetic stocks for targeted durum wheat improvement.

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

Introduction

Durum wheat (*Triticum durum* var. *durum*, 2n = 4x = 28, genomes AB) is cultivated on approximately 8% of the world's wheat area, and is economically important in the Mediterranean basin, North America's Great Plains and Mexico, as well as Australia, Russia, Kazakhstan, India, Ethiopia and Argentina (Bassi and Sanchez-Garcia, 2017; Royo et al., 2014). Due to the socio-economic challenges emerging from the rapid human population increase across the world, breeding for continual improvement in yield potential (among other traits) is a priority for durum wheat breeders. Given that wheat provides calories for about 20% of human nutrition (FAO, 2013), current yield increases of not more than 1% year^{-1} for both durum and bread wheat, will be insufficient to meet the food demand in the imminent future (Fischer and Edmaedas, 2010; Ray et al., 2013). Further, with ongoing climate change responsible for a progressive displacement of the areas where durum wheat is traditionally grown (Ceoloni et al., 2014a; Habash et al., 2009) the goal of significantly increasing yield potential is even more challenging. Environmental factors are, indeed, those that have a major impact on wheat productivity, with temperature extremes and water shortages being the most detrimental (Dodig et al., 2012; Fleury et al., 2010). Recent assessment of the effect of increasing temperature on wheat yield, including the top producing countries, predicts an approximate 6% loss in global yield for each degree-Celsius increase in temperature (Liu et al., 2016; Zhao et al., 2017). These yield reductions will depend on the interaction of temperature with other limiting factors, such as rainfall, CO_2 emissions, and nitrogen supply, which impose the need for environment-specific breeding strategies to select for highly adapted genotypes (Elía et al., 2018; Luo et al., 2005; Tricker et al., 2018; Zhao et al., 2017). Consequently, for a particularly complex trait such as yield, understanding and dissecting the multi-layered genotype × environment interaction in multi-environment trials is a crucial step towards closing the gap between the actual and attainable yields, particularly when several different constraints are present (Araus et al., 2003a; Bassi and Sanchez-Garcia, 2017; Maccaferri et al., 2011; Marti and Slafer, 2014; Parent et al., 2017; Slafer et al., 2014; Tardieu and Tuberosa, 2010; Zaim et al. 2017).

To develop durum genotypes with improved yield and adaptability to more frequent incidence of drought and heat stress and/or altered rainfall distribution (Habash et al., 2009; Tadesse et al.,

2016), enhancing the genetic background through targeted introgressions is a powerful strategy, given that cultivated germplasm represents only a very small fraction of the variability present in nature (Royo et al., 2009 and references therein; Zaim et al. 2017). Wheat-alien introgression experiments conducted in the past proved to be a valid approach to harness the genetic diversity of alien, mostly wild, segments of wheat-related gene pools (Ceoloni et al., 2014b, 2017a; Dempewolf et al., 2017; Prohens et al., 2017; Zhang et al., 2017). To achieve this, addition/substitution/translocation/recombinant lines, harbouring parts of alien genomes, can be used to facilitate the introduction of desired genes into stable and adapted genotypes (e.g. Ceoloni et al., 2015). Targeted and precise exploitation of useful genes from these materials is readily possible through efficient sexual means, foremost the cytogenetic methodologies of "chromosome engineering" (Ceoloni et al., 2005, 2014a, 2014b). 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.

The use of wild relatives for yield improvement in wheat has so far 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). Noteworthy examples regard mostly the hexaploid bread wheat, more widely cultivated, and benefiting from a higher ploidy level with respect to durum wheat, hence a higher buffering ability toward chromosome manipulations (reviewed in Ceoloni et al., 2014a, 2015; Mondal et al., 2016). One of the most notable and documented cases of alien introgression with positive effects on wheat yield, is 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 and 7AL arm of bread and durum wheat, respectively. In bread wheat, the sizeable 7AgL translocation named T4 (~70% of the recipient 7DL arm, harbouring *Lr19+Sr25+Yp* genes) led to increased grain yield, biomass and grain number (10-35%) 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). Under water stress, however, yields for CIMMYT T4 derivatives turned out to be lower than control lines (Monneveux et al., 2003; Singh et al., 1998), as was the case for T4 derivatives developed in Australian adapted genetic backgrounds, when tested in high- and low-yielding environments (Rosewarne et al., 2015).

In durum wheat, three fractions of the same 7AgL chromatin, spanning 23%, 28% and 40% of the recipient 7AL arm of cv. Simeto, and all containing the *Lr19+Sr25+Yp* genes (Ceoloni et al.,

2005), were separately introgressed into near-isogenic recombinant lines (NIRLs), and observed across four years in one rain-fed locality of Central Italy (Kuzmanović et al., 2014; 2016). The range of increases in grain yield, biomass and grain number was 3-39%, depending on the recombinant type, season and experimental procedure (spaced plants in Kuzmanović et al., 2014, plot trials in Kuzmanović et al., 2016). In addition, and in contrast to the bread wheat (T4) studies, characterization of the three durum wheat-*Th. ponticum* recombinants comprised more traits, including detailed phenology, spike fertility and flag leaf attributes, and revealed 19 enhanced traits in association with the presence of specific 7AgL portions. This enabled a structural-functional dissection of the 7AgL chromatin incorporated onto the durum 7AL, with consequent assignment of yield-contributing genes (previously associated to the entire T4 segment) to defined 7AgL sub-regions (Kuzmanović et al., 2014, 2016). The increase of several yield-related traits was recorded in each of the three durum wheat-*Th. ponticum* NIRLs. However, the one carrying the 28%-long 7AgL segment was identified as the best performing line, with a high number of yield-related traits (tiller/spike number, flag leaf dimensions and chlorophyll content, grain yield, biomass, duration of stem elongation phase) being evidently enhanced by genetic factor(s) located within the 23-28% chromosomal stretch of its 7AgL segment.

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* recombinants across an array of rain-fed environments located in three different countries, and to evaluate possible environment/segment-specific associations in view of using these recombinants across environments or in site-directed breeding programs.

Materials and methods

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 five years and three countries. Simeto (pedigree: selection from Capeiti 8 x Valnova) is an early flowering variety, released in 1988, and 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 *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_{5-9} (R5 and R112) and BC_4F_{5-9} (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.

Field experiments

A total of 11 rain-fed field trials were carried out over five years and five locations where durum is typically cultivated (three in Italy, one in Morocco and in 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 11 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 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, with plot size variation across trials (referring in particular to VT11, FG11 and AUS13), due to seed availability (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 10 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 (Fig. 1) 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 (– NIRLs), hence to eliminate the indirect yield-contributing factor of *Lr19*-carriers (+ NIRLs).

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/m^2)	Plot size (m^2)
FG11	Beccarini station, Manfredonia (South Italy)	41° 29' N	15° 50' E	6	2010/11	335	14.0	R5, R112, R23	20/12/2010	179	350	0.8
VT11	Experimental farm of the University of Tuscia, Viterbo (Central Italy)	42° 25' N	12° 4' E	301	2010/11	420	15.4	"	13/12/2010	199	350	0.8
VT12	"	"	"	"	2011/12	248	13.6	"	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	0.6
AUS14	"	"	"	"	2014	296	14.3	R5, R112	15/05/2014	193	150	5

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

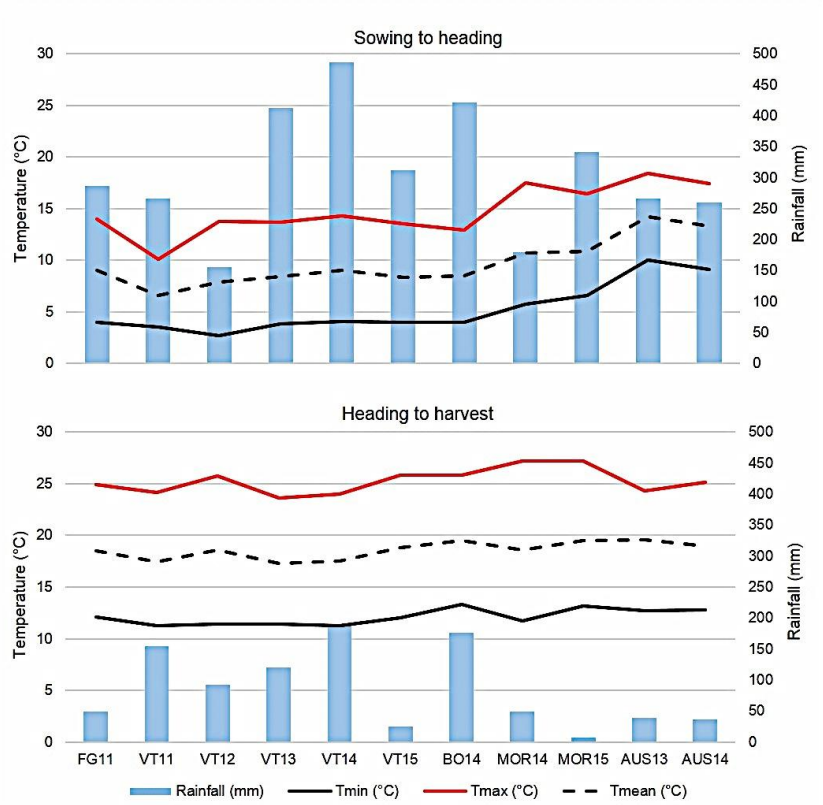

Fig. 1. Weather conditions for growing seasons in 11 environments analysed, as retrieved from meteorological stations at experimental sites, or in the case of MOR14 only, downloaded from NASA's site for Prediction of Worldwide Energy Resource (http://power.larc.nasa.gov). Environment acronyms are as listed in Table 1.

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 for each replicated plot, are reported in Table 2. Measurements of all traits were performed as described in Kuzmanović et al. (2016) with the following modifications: HD was considered as number of days from sowing to heading; HI was calculated as GYM2/BM2 of the total harvested area in FG11, VT11, MOR14, MOR15, AUS13 and AUS14, and of 25-culm samples in other environments; chlorophyll content (CHL) was measured by using a hand-held meter SPAD 502 (Konica Minolta, Japan) only; CHL was recorded at medium milk (Zadoks 75; Zadoks et al., 1974), late milk (Zadoks 77) and very late milk (Zadoks 79) developmental stages.

Trait		Acronym	Environment											Sample type/plot
			FG11	VT11	VT12	VT13	VT14	VT15	BO14	MOR14	MOR15	AUS13	AUS14	
1	Harvest index	HI	x	x	x	x	x	x	x	x	x	x	x	TH/25C
2	Spike No. m^{-2}	SNM2	x	x	x	x	x	x	x	x	x	x	x	TH
3	Biomass m^{-2} (g)	BM2	x	x	x	x	x	x	x	x	x	x	x	TH
4	Grain yield m^{-2} (g)	GYM2	x	x	x	x	x	x	x	x	x	x	x	TH
5	Grain No. m^{-2}	GNM2	x	x	x	x	x	x	x	x	x	x	x	TH
6	1000 grain weight (g)	TGW	x	x	x	x	x	x	x	x	x	x	x	G
7	Grain yield $spike^{-1}$ (g)	GYS	x	x	x	x	x	x	x	x	x	x	x	IC
8	Grain No. $spike^{-1}$	GNS	x	x	x	x	x	x	x	x	x	x	x	IC
9	Grain No. $spikelet^{-1}$	GNSP	x	x	x	x	x	x	x	x	x	x	x	IC
10	Spikelet No. $spike^{-1}$	SPN	x	x	x	x	x	x	x	x	x	x	x	IC
11	Plant height (cm)	PH	x	x	x	x	x	x	x	x	x	x	x	IC
12	Days to heading	HD	x	x	x	x	x	x	x	x	x	x	x	day count
13	Spike dry weight at anthesis (g)	SDWA			x	x	x	x	x			x	x	IC
14	Tiller dry weight at anthesis (g)	TDWA			x	x	x	x	x			x	x	IC
15	Spike index at anthesis	SIA			x	x	x	x	x			x	x	IC
16	Spike dry weight at harvest (g)	SDW			x	x	x	x	x			x	x	IC
17	Tiller dry weight at harvest (g)	TDW			x	x	x	x	x			x	x	IC
18	Chaff dry weight at harvest (g)	CHAFF			x	x	x	x	x			x	x	IC
19	Spike length (cm)	SL			x	x	x	x	x			x	x	IC
20	Spike fertility index	SFI			x	x	x	x	x			x	x	IC
21	Flag leaf width (cm)	FLW			x	x	x	x	x			x	x	FL
22	Flag leaf length (cm)	FLL			x	x	x	x	x			x	x	FL
23	Flag leaf area (cm^2)	FLA			x	x	x	x	x			x	x	FL
24	Chlorophyll content at Zadoks 75	CHLZ75			x	x	x	x	x				x	FL
25	Chlorophyll content at Zadoks 77	CHLZ77			x	x	x	x	x			x		FL

Table 2. Traits and sample details assessed across 11 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 listed in Table 1.

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 25 traits and not all of the three NIRLs were analysed in all 11 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. 1-8 listed in Table 2, GLM2 traits No. 1-25, while GLM3 comprised traits No. 1-24. 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 GLMs], and replicate [R alone in GLM3 and environment-nested, R(E), in other GLMs]. The latter factor 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).

Results

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 (Fig. 1), with a little more variation between them in the period from sowing to heading (7.7°C on average) than from heading to maturity (2.6°C on average). By contrast, the environments were considerably different for the rainfall amount received (Table 1) and its distribution during the growing season (Fig. 1). 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 southeast 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 (Fig. 1). As for the Italian trial sites, seasons 2011 and 2012 were more typical and very favourable for durum wheat cultivation when compared to the 2013 to 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). VT11, FG11 and VT12 had rainfall distribution and temperature trends that were conducive for good crop growth during the entire life cycle (Fig. 1; 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 rainy 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.

Yield response across environments

According to the observed highly significant R^2 values of linear regression (Fig. 2a), all NIRLs (both + and –) positively responded to better thermo-pluviometric patterns and higher environmental indices across environments. The b coefficient values around 1 for R5 and R112 genotypes were

indicative of an average yield stability (Finlay and Wilkinson, 1963), and the consistently higher grain yield with respect to the site's mean indicated their general adaptability. In contrast, the R23 NIRL pair, despite b coefficient values around 1 (i.e. average stability indicator), had poorer grain yield than the site's mean in all environments, probably due to the lower adaptability of the genetic background of its representative families, less isogenic compared to the recurrent cv. Simeto parent than the two other NIRLs (see § 2.1).

With an average GYM2 across environments between 1.03 and 7.05 t/ha (Fig. 2b), 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). The productivity of R5 and R112 NIRLs was typically higher than the environmental means, as shown from their yield gains ranging 3-30% and 2-27%, respectively. Yield of R23 NIRLs (both "+" and "–") was always under the site's mean (-1 to -47%). Yet, in 5 out of 10 environments, the presence of the R23 7AgL segment evidently had a positive effect, reducing the background-dependent yield disadvantage (Fig. 2b). The two more productive 7AgL-carrier lines (R5+ and R112+), displayed yield gains with respect to their control NIRLs in 6 and 4, respectively, out of the 11 environments. The highest gain of R5+ (and of all 7AgL+ lines) was observed in low-yielding AUS13 (+30%), a similar value to that recorded in medium-yielding MOR15 (+26%). In the same MOR15, R112+ showed the highest gain (+21%). By contrast, a notable yield penalty was observed for R112+ in low-yielding AUS14 (-18%), and for R23+ in medium-yielding MOR15 (-47%), VT11 (-28%) and FG11 (-19%).

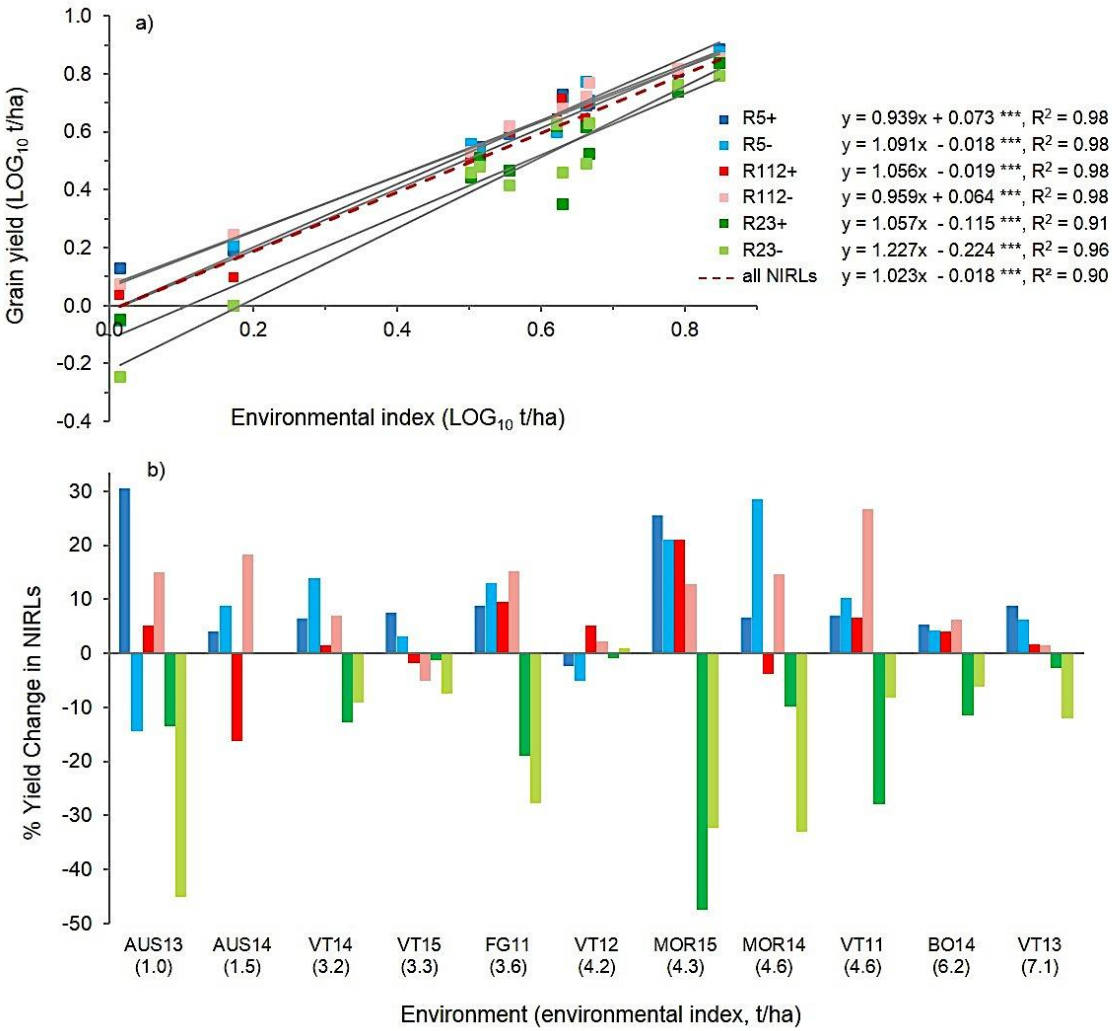

Fig. 2. Regression lines showing the relationship of individual grain yields (GYM2) of the six NIRLs and environments analysed (a), and the percentage yield change of the six NIRLs with respect to the site's mean (b). Environment acronyms are as listed in Table 1.

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

Across all 10 environments where all three NIRLs were tested (Table 1), when data from the three "+" recombinant or three "-" control lines were pooled, yield parameters m^{-2} turned out to be increased to a variable extent in 7AgL+ vs. 7AgL- genotypes (Tables 3 and Supplementary Table 1). The most significant positive 7AgL effects were observed for SNM2 (+11%) and GNM2 (+9%), accompanied though by a significant decrease in TGW and GYS. However, HI of 7AgL+ lines remained unchanged (Table 3). Although the environment factor alone (E, Supplementary Table 1) was highly significant for all traits, confirming that the environments tested were different, the only significant association between E and 7AgL presence was observed for the increase of GNM2 in 7AgL+ vs. 7AgL- lines in VT12 (Tukey test, not shown). ANOVA performed for each of the 10 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 3). SNM2, GNM2 and GNS were significantly increased in 7AgL+ vs. 7AgL- NIRLs in several environments, particularly in the lowest- (AUS13) and highest-yielding (BO14, VT13), as well as in the medium-yielding VT12. AUS13 turned out to be the only environment in which GYM2 together with BM2 were significantly increased (+18% and +14%, respectively) in 7AgL+ vs. 7AgL- NIRLs. This may indicate good potential for adaptation to highly stressed conditions conferred by the 7AgL introgressions.

Trait	NIRLs	Environments											
		ALL	AUS13	AUS14	VT14	VT15	FG11	VT12	MOR15	MOR14	VT11	BO14	VT13
HI	7AgL+	0.353	0.234	0.375	0.424	0.453	0.312	0.406	0.262	0.297	0.330	0.442	0.482
	7AgL-	0.354	0.224	0.420	0.410	0.466	0.305	0.412	0.289	0.306	0.345	0.447	0.483
	ANOVA *P*-value	0.30	0.420	0.056	0.351	0.578	0.795	0.484	0.533	0.666	0.358	0.790	0.943
	7AgL effect (%)	0	4	-11	3	-3	2	-1	-9	-3	-4	-1	0
SNM2	7AgL+	265.6	121.3	54.9	188.6	179.8	240.8	284.9	371.8	338.5	299.1	372.4	370.5
	7AgL-	239.9	91.6	52.9	187.0	164.9	221.7	259.2	356.9	354.0	317.4	343.0	342.2
	ANOVA *P*-value	0.004**	0.000***	0.865	0.880	0.265	0.254	0.036*	0.684	0.643	0.228	0.041*	0.028*
	7AgL effect (%)	11	32	4	1	9	9	10	4	-4	-6	9	8
BM2	7AgL+	1102.1	474.1	376.3	726.3	739.1	1152.5	1041.8	1681.3	1570.7	1324.4	1397.0	1512.2
	7AgL-	1044.5	416.2	402.5	794.3	679.4	1199.7	1012.5	1487.5	1543.0	1468.0	1399.6	1443.2
	ANOVA *P*-value	0.273	0.001**	0.583	0.218	0.254	0.405	0.471	0.065	0.636	0.066	0.973	0.120
	7AgL effect (%)	6	14	-7	-9	9	-4	3	13	2	-10	0	5
GYM2	7AgL+	390.4	110.9	136.8	311.1	332.6	359.4	421.5	424.9	467.0	442.2	612.2	721.2
	7AgL-	383.8	94.3	169.0	330.2	316.8	360.5	416.4	428.3	475.4	508.6	625.4	688.6
	ANOVA *P*-value	0.597	0.029*	0.115	0.433	0.304	0.972	0.752	0.959	0.844	0.105	0.685	0.281
	7AgL effect (%)	2	18	-19	-6	5	0	1	-1	-2	-13	-2	5
GNM2	7AgL+	7779.8	1132.5	3058.1	5681.6	5703.0	7646.1	15589.9	9349.4	10297.3	7253.1	10234.4	12297.9
	7AgL-	7151.3	813.1	3323.2	5544.7	5101.9	6972.0	13635.0	8488.2	9188.1	7788.1	9526.1	10425.8
	ANOVA *P*-value	0.000***	0.000***	0.510	0.903	0.172	0.222	0.038*	0.353	0.110	0.290	0.172	0.001**
	7AgL effect (%)	9	39	-8	2	12	10	14	10	12	-7	7	18
TGW	7AgL+	51.8	34.8	46.6	55.4	59.2	47.1	60.5	46.4	45.6	60.5	60.1	60.3
	7AgL-	55.8	38.1	51.0	59.9	62.3	51.6	66.8	49.7	51.1	65.0	65.9	66.3
	ANOVA *P*-value	0.000***	0.006**	0.148	0.097	0.417	0.120	0.032*	0.274	0.048*	0.059	0.021*	0.005**
	7AgL effect (%)	-7	-9	-9	-8	-5	-9	-9	-7	-11	-7	-9	-9
GNS	7AgL+	43.4	24.6	59.4	54.8	49.8	57.1	43.4	52.6	46.9	38.7	42.3	47.7
	7AgL-	41.3	21.8	66.3	47.7	51.3	55.9	41.4	56.6	44.9	41.2	43.9	43.7
	ANOVA *P*-value	0.076	0.028*	0.023*	0.004**	0.460	0.718	0.280	0.142	0.358	0.107	0.532	0.006**
	7AgL effect (%)	5	13	-10	15	-3	2	5	-7	5	-6	-3	9
GYS	7AgL+	2.4	1.0	2.7	3.1	2.9	2.7	2.6	3.1	2.2	2.3	2.3	2.9
	7AgL-	2.5	1.1	3.2	3.0	3.2	2.9	2.8	3.5	2.3	2.6	2.6	3.0
	ANOVA *P*-value	0.000***	0.168	0.022*	0.595	0.130	0.441	0.041*	0.071	0.368	0.029*	0.124	0.269
	7AgL effect (%)	-5	-8	-18	3	-9	-5	-6	-13	-6	-12	-11	-4

Table 3. Main yield-related traits unit area^{-1} and spike^{-1} of 7AgL-carrier vs. 7AgL-non carrier durum wheat-*Th. ponticum* NIRLs across environments (HI, harvest index; SNM2, spike number m^{-2}; BM2, biomass m^{-2}; GYM2, grain yield m^{-2}; GNM2, grain number m^{-2}; TGW, thousand grain weight; GNS, grain number spike^{-1}; GYS, grain yield spike^{-1}). In 10 out of the 11 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 <0.001.

The more extensive and detailed GLM2 model (Supplementary Table 2), used to examine individual effects of each of the three 7AgL segments (Table 4), 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 2] was different, as revealed by analysis of the three "+" vs. "–" NIRL pairs. Taken the 10 environments as a whole, the R5+ recombinant did not show any significant trait change due to its 7AgL segment, while R112+ differed from its R112– control for HD (+3%), HI (-3%), TGW (-3%) and FLL (-9%) (Table 4). On the other hand, R23+ was the recombinant for which the highest number of significant differences with respect to its R23– control were observed. Although 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 (FLW, FLL, FLA)], the same 7AgL portion was clearly associated with significant increases of several other parameters directly contributing to grain yield, i.e. SNM2 (+10%), GNM2 (+5%), GNS (+6%), GNSP (+9%) and SFI (+46%) (Table 4).

Trait	R5+			R5-			R112+			R112-			R23+			R23-		
	Mean	SE		Mean	SE		Mean	SE		Mean	SE		Mean	SE		Mean	SE	
HI	0.39	0.01	a	0.37	0.02	a	0.37	0.01	b	0.38	0.01	a	0.31	0.01	c	0.31	0.02	c
SNM2	254.2	13.5	b	234.1	16.6	b	268.3	13.3	ab	237.5	16.1	ab	274.5	13.1	a	249.1	15.5	b
BM2	1089.6	54.2	ns	989.9	66.2	ns	1115.7	61.7	ns	1017.5	68.3	ns	1101.1	54.4	ns	1134.7	64.6	ns
GYM2	426.5	24.8	ns	395.9	33.3	ns	408.3	23.8	ns	395.8	31.4	ns	337.1	22.4	ns	357.1	28.5	ns
GNM2	7378.3	487.6	B	7058.7	642.7	B	7710.6	536.0	AB	7170.6	672.9	AB	8250.6	661.4	A	7233.9	689.3	C
TGW	58.4	1.3	A	56.7	1.8	A	54.2	1.5	B	56.5	1.7	A	42.6	1.0	C	54.1	1.7	B
GYS	2.6	0.1	AB	2.4	0.1	AB	2.5	0.1	AB	2.5	0.1	A	2.0	0.1	C	2.5	0.1	B
GNS	43.1	1.2	ab	40.6	1.8	ab	43.0	1.4	ab	41.6	1.7	ab	44.0	1.6	a	41.6	2.3	b
GNSP	2.5	0.1	A	2.3	0.1	A	2.4	0.1	A	2.4	0.1	A	2.4	0.1	A	2.2	0.1	B
SPN	17.9	0.3	C	18.1	0.3	C	18.2	0.3	BC	17.9	0.3	C	18.8	0.3	B	19.8	0.5	A
PH	78.4	0.8	C	76.3	1.0	CD	77.0	0.7	CD	74.5	1.0	D	93.8	1.1	B	97.9	1.2	A
HD	126.2	2.6	C	123.6	3.4	C	127.7	2.7	B	123.8	3.3	C	131.4	2.6	A	133.2	3.2	A
SDWA	0.76	0.03	AB	0.79	0.04	AB	0.74	0.03	AB	0.78	0.03	A	0.55	0.02	C	0.66	0.02	B
TDWA	4.46	0.14	A	4.82	0.20	A	4.39	0.14	A	4.51	0.15	A	3.83	0.14	B	4.65	0.22	A
SIA	0.17	0.01	ns	0.16	0.01	ns	0.17	0.00	ns	0.17	0.00	ns	0.15	0.00	ns	0.15	0.01	ns
SDW	3.26	0.12	A	3.17	0.12	A	3.20	0.12	A	3.17	0.12	A	2.57	0.11	B	3.02	0.16	A
TDW	5.26	0.21	b	4.99	0.23	b	5.09	0.23	b	4.92	0.25	b	4.75	0.20	c	6.19	0.18	a
CHAFF	0.87	0.04	ab	1.02	0.10	ab	0.94	0.05	a	0.93	0.05	ab	0.74	0.04	b	0.90	0.04	a
SL	6.5	0.1	ns	6.7	0.2	ns	6.4	0.1	ns	6.3	0.1	ns	7.2	0.1	ns	7.3	0.2	ns
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
FLW	1.8	0.03	A	1.7	0.04	AB	1.9	0.03	AB	1.8	0.03	B	1.7	0.02	B	1.7	0.04	C
FLL	20.3	0.59	ab	21.7	0.90	ab	19.9	0.59	bc	21.8	0.90	a	18.8	0.57	c	20.9	0.79	a
FLA	31.1	1.67	A	30.0	1.80	A	31.1	1.70	A	32.0	1.80	A	26.3	1.39	B	30.0	2.11	A
CHLZ75	49.8	1.6	ns	53.1	1.6	ns	49.5	1.5	ns	51.0	2.0	ns	41.8	1.3	ns	45.1	1.4	ns
CHLZ77	42.8	1.2	A	43.1	1.5	A	44.4	1.3	A	42.6	1.5	AB	39.3	1.2	B	43.4	1.4	AB

Table 4. Mean values and standard errors (SE) of yield-related traits of the three durum wheat-*Th. ponticum* NIRLs (+, 7AgL carriers; −, 7AgL non-carriers) across 10 environments where all three recombinants and their respective controls were analysed (df, degrees of freedom; HI, harvest index; SNM2, spike number m^{-2}; BM2, biomass m^{-2}; GYM2, grain yield m^{-2}; GNM2, grain number m^{-2}; TGW, thousand grain weight; GYS, grain yield $spike^{-1}$; GNS, grain number $spike^{-1}$; GNSP, grin number $spikelet^{-1}$; SPN, spikelet number $spike^{-1}$; PH, plant height; HD, heading date; SDWA, spike dry weight at anthesis; TDWA, tiller dry weight at anthesis; SIA, spike index at anthesis; SDW, spike dry weight at maturity; TDW, tiller dry weight at maturity; CHAFF, chaff dry weight; SL, spike length; SFI, spike fertility index; FLW, flag leaf width; FLL, flag leaf length; FLA, flag leaf area; CHLZ75, flag leaf chlorophyll content at Zadoks 75; CHLZ77, flag leaf chlorophyll content at Zadoks 77). Letters in each row correspond to the ranking of the Tukey test at $P < 0.01$ (capital) and $P < 0.05$ (lower case) levels.

7AgL × E interaction

Individual alien segment effects were significant to a variable extent in particular environments, and for specific traits, as indicated by the Tukey test for significant E × 7AgL(G) interactions (GLM2, Supplementary Tables 2 and 3). Environment-specific differences between R5+ and R112+ NIRLs and their respective "–" controls were observed in the low-yielding AUS13 only. At this site, positive 7AgL effects were highlighted for HI (+27%) and FLW (15%) of R5+, while negative effects were observed for flag leaf dimensions (-13% on average for FLL of both NIRLs, -17% for FLA of R112+) and CHAFF (-38% of R5+). Data from AUS14, in which R5 and R112 NIRLs only were grown, revealed that presence of both 7AgL segments was not particularly advantageous for the main yield traits (traits No 1-8 from Table 2; Supplementary Table 3). The GLM3 model identified only a few significant differences associated with the presence of the 7AgL segment ([7Ag(G)]; Supplementary Table 4): incremental, for FLW of both recombinants (+37% for R5+ and +25% for R112+), FLA of R5+ (+42%) and HD of R112+ (+6%); detrimental, for GNSP of R112+ (-14%) and for CHL75 of R5+ (-7%) (Supplementary Table 3).

On the other hand, the R23+ NIRL was highly responsive to different environments for several traits. The enhancing effect of its 7AgL segment for GNM2 and SFI was particularly pronounced in VT12-13 (+35% on average), MOR14 (+56%) and AUS13 (>300%) for the former trait, while in VT13-14 (+47% on average), BO14 (+95%) as well as in AUS13 (+53%) for the latter trait (Supplementary Table 3). Also, some of the identified depressing effects of the 40%-long 7AgL segment were significantly associated with certain, even contrasting environments: TGW in VT11-15, BO14 and AUS13 (ranging -18 to -27%); GYS in MOR15 (-43%); dry weight at maturity (SDW, TDW) in BO14 (-37% and -28%, respectively); FLA in BO14 and VT14 (-22% and -33%, respectively).

Correlations of yield traits

Scatter plots of regression analysis (R^2) for the pairs of main yield traits are shown in Fig. 3, and coefficients of correlation (*r*) are reported in Table 5. For all environments, final grain yield per area (GYM2) was mostly dependent on SNM2, BM2 and GNM2 (R^2 = 0.80, 0.70, and 0.62, 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 GYM2 was observed with grain number and biomass, shown by a significant *r* value in 10 and 9, respectively, out of the total 11 environments tested (58-93% for GNM2 and 61-95% for BM2). In

four low- and medium-yielding environments, GYM2 was positively influenced also by SNM2, GYS and GNSP (*r* = 50-79%). TGW confirmed to be less important for final grain yield, the two parameters being significantly correlated in only three medium-to-high yielding environments (*r* = 60-82%). Finally, GNM2, the key trait for wheat grain yield, increases, as expected, and is primarily influenced by SNM2 (*r* = 62-96%, significant in six environments), grain number spike^{-1} (GNS) and spikelet^{-1} (GNSP) (*r* = 54-96%, significant in five environments), particularly in low- and medium-to-low environments.

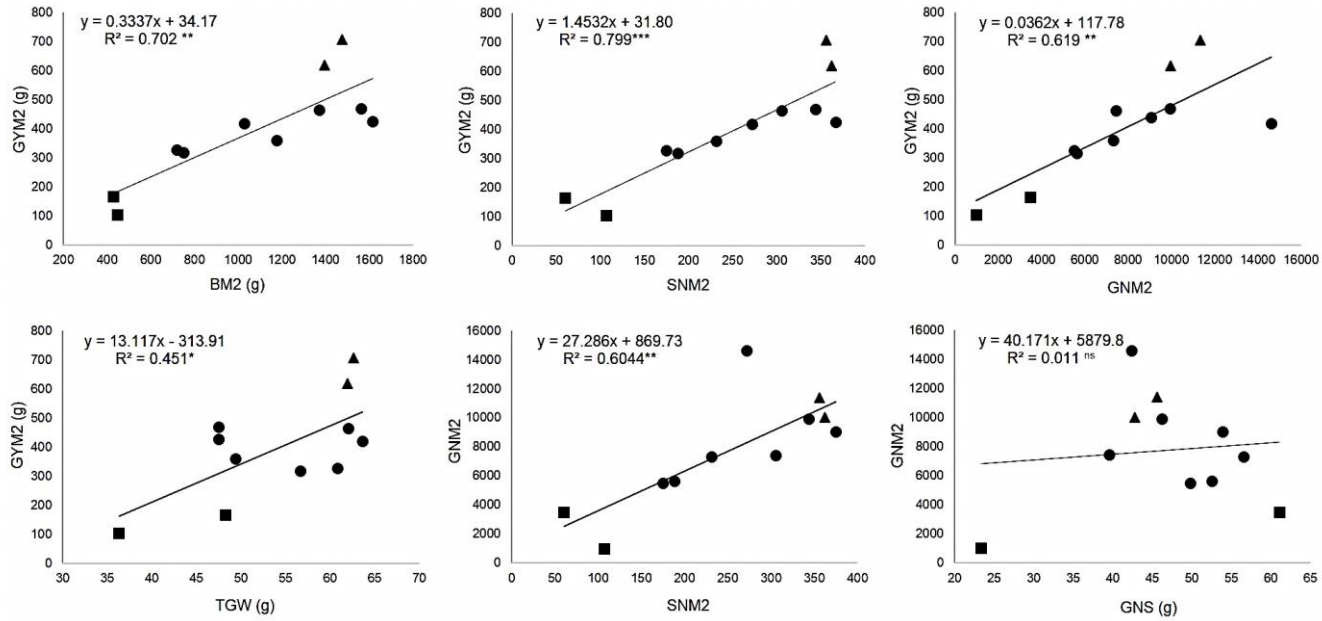

Fig. 3. Scatterplots of the means of grain yield and grain number m^{-2} vs. main grain yield components from the three durum wheat-*Th. ponticum* NIRLs evaluated across 11 environments analysed (GYM2, grain yield m^{-2}; BM2, biomass m^{-2}; SNM2, spike number m^{-2}; GNM2, grain number m^{-2}; TGW, thousand-grain weight; GNS, grain number spike^{-1}).

Pairwise traits	Environment																							
	AUS13		AUS14		VT14		VT15		FG11		VT12		MOR15		MOR14		VT11		BO14		VT13	
GYM2 - GNM2	0.85	***	0.87	**	0.90	***	0.63	*	0.75	**	0.58	*	0.93	***	0.72	**	0.93	***	0.63	*	0.56	
GYM2 - TGW	0.35		0.36		0.26		0.03		0.56		0.02		0.82	***	0.60	*	0.66	**	0.41		0.26	
GYM2 - SNM2	0.50	**	0.74		0.83	***	0.22		0.00		0.54	*	0.55		-0.29		0.70	***	-0.21		0.53	
GYM2 - BM2	0.68	***	0.87	**	0.95	***	0.64	*	0.13		0.81	***	-0.23		0.64	*	0.89	***	0.61	*	0.64	**
GYM2 - GYS	0.62	***	0.17		0.73	**	0.19		0.55		0.09		0.22		0.73	**	0.78	***	0.45		0.16	
GYM2 - GNS	0.82	***	-0.09		0.58		0.25		0.05		0.07		-0.14		0.49		0.50		0.37		0.23	
GYM2 - GNSP	0.79	***	0.19		0.65	*	0.26		0.53		0.24		0.04		0.73	**	0.54	*	0.47		0.55	
GYM2 - PH	-0.48	**	0.07		0.05		0.10		-0.53		0.01		-0.62	*	-0.56		-0.37		-0.49		-0.45	
GYM2 - HD	-0.33		-0.38		-0.35		-0.49		-0.62		-0.26		-0.75	**	-0.61	*	0.06		-0.46		-0.44	
GNM2 - SNM2	0.81	***	0.96	***	0.76	***	0.61		0.44		0.62	**	0.56		-0.07		0.73	***	-0.11		0.71	**
GNM2 - GNS	0.76	***	0.96	***	0.70	**	0.35		0.23		0.66	**	-0.22		0.49		0.54	*	0.29		0.54	
GNM2 - GNSP	0.75	***	-0.20		0.70	**	0.13		0.52		0.75	***	0.02		0.73	**	0.56	*	0.33		0.50	

Table 5. Pearson's correlation coefficients between pairs of main yield-related traits involved in coarse and fine regulation of final grain yield across 11 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}; GYS, grain yield $spike^{-1}$; GNS, grain number $spike^{-1}$; GNSP, grin number $spikelet^{-1}$; PH, plant height; HD, heading date). 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. Environment acronyms are as listed in Table 1.

Discussion

To evaluate, for the first time, the effect of different environmental conditions on the final yield of three durum wheat-*Th. ponticum* recombinant lines, having 23%, 28% and 40% of 7AgL chromatin on their 7AL distal end, 11 rain-fed field trials in five locations worldwide over five years were undertaken. The present multi-location analysis not only confirmed significant yield-related 7AgL effects previously observed in one location only (Kuzmanović et al., 2016), but also revealed their stable expression across a range of contrasting environments.

The overall effect of the three 7AgL segments taken together (all "+" vs. all "–" NIRLs comparison) in the multi-environment analysis (Table 3) was incremental for total grain yield and biomass m^{-2} (2-6%), mainly due to significant increases of spike/tiller number m^{-2} (SNM2), and related grain number m^{-2} (GNM2). Thus, the 7AgL case supports the consolidated evidence that in both bread and durum wheat, coarse regulation of yield is based primarily on grain number m^{-2} and biomass (Marti et al., 2016; Pedro et al., 2011; Slafer et al., 2014). In absolute terms, all NIRLs displayed their highest yield potential in high-yielding environments, where higher rainfall, particularly from heading to maturity, was recorded (Central and Northern Italy; Table 1, Fig. 1), while showed lower yields in more water- and heat-stressed locations, such as South Australia (Fig 2a, Supplementary Table 3). The trend we observed may reflect a background effect, with best performances of the prevailing cv. Simeto background in the environments for which it was originally bred, and confirms the positive correlation of yield and total amount of rainfall, particularly from heading onwards, characterizing durum wheat growth under Mediterranean rain-fed conditions (Araus et al., 2003b). As to the 7AgL-carrier lines; previous studies indicate bread wheat T4 derivatives, whose sizeable 7AgL segment includes those of the durum wheat recombinants described here, benefit from higher water availability (Singh et al., 1998; Monneveaux et al., 2003; Rosewarne et al., 2015). Nonetheless, the results from the environment-by-environment ANOVA (Table 3) revealed potential of the durum wheat 7AgL+ lines for grain yield (+18%) and biomass (+14%) increase also under heavy heat and drought stress conditions, such as those of AUS13. Somewhat unexpectedly, the same was not observed in the similarly stressed AUS14 trial, characterized by a yield penalty of 7AgL+ lines (R5 and R112 only, see § 2.2) vs. their controls. A possible reason for the contrasting results could be the length of the growth cycle, which in AUS13 was shorter than in AUS14, due to the much later sowing date in the former (Table 1). The association between shorter growth cycle and yield increase was already detected in bread wheat T4 derivatives grown under drought stress (Singh et al., 1998; Monneveux et al., 2003), and its dependence on varietal background was hypothesized (Sibikeev et al., 2000). Our results suggest that the same may be true

for durum wheat genotypes harbouring 7AgL introgressions, although further investigation in a range of additional environments would be needed to demonstrate conclusively.

Comparison among the individual recombinant genotypes (7AgL+ NIRLs) across the wide range of environments tested revealed the R5 recombinant to be associated with grain yield increases (GYM2) vs. its control 7AgL– line (+1-52%) in the highest number (6 out of 11) of trials (Supplementary Table 4), as well as the highest grain yield gains with respect to the sites' mean in 10 such trials (Fig. 2b). Interestingly enough, while the 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 totalled 52%, paralleled by a significant increase in HI (+27%) and FLW (+15%) (Supplementary Table 4). This evidence suggests a high level of adaptation of R5+ to drought-prone environments (see also MOR15, Fig. 2b), which has greatly contributed to the overall significant increase in GYM2 observed for all three 7AgL+ lines in AUS13 (Table 3; Supplementary Table 3). In contrast, R5+ yield was somewhat reduced (-4%) vs. 7AgL– controls in AUS14, although meteorological conditions were very similar (Table 1, Fig. 1) and flag leaf size confirmed to be significantly increased (+36% FLW, +42% FLA; Supplementary Table 3). Flag leaf size is known to be correlated with photosynthetic activity and yield in wheat under drought (Foulkes et al., 2007; Habash et al., 2007; Quarrie et al., 2006); yet, this was not advantageous for R5+ in AUS14 under a longer growth cycle with respect to AUS13, which could account for the contrasting yield performance of the R5+ genotype.

Contrary to the good plasticity of the R5+ genotype, the R112+ recombinant, previously identified as the most promising for its yield potential in Central Italy environments (Kuzmanović et al., 2016), showed to be less stable across the tested environments (Supplementary Table 3). Nonetheless, average values of all trials showed increased spike (SNM2, +13%) and grain number (GNM2, +2%), as well as 3% higher grain yield (GYM2) and a consistent tendency for wider flag leaf (though significant in AUS14 only) and chlorophyll content for R112+ vs. R112– plants (Supplementary Table 3). This confirmed that yield formation in R112+ depends mostly on tiller number development and potentially increased photosynthetic activity of leaves, both contributing to grain number formation. Tiller number is greatly influenced by the environment and determines wheat 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 sites, where more favourable conditions for tillering were evidently met (VT12, VT15, MOR15, Supplementary Table 3). At high-yielding sites, R112+ produced essentially the same as the controls (BO14, VT13), while a major yield penalty was recorded in low-yielding AUS14 (-29% GYM2, Supplementary Table 3), concomitantly with a significant, environment-specific decrease in spike

fertility (-14% of GNSP). The significantly larger flag leaf size (+25% FLW) and higher chlorophyll content (+23% CHL79) of R112+ recorded in AUS14 was unfavourable for grain yield (Supplementary Table 3). Capacity of a wheat plant to maintain flag leaf greenness generally enhances photoassimilation (Foulkes et al., 2007; Peremarti et al., 2014); yet, in environments such as South Australia, where high irradiance often comes along with drought stress, increased temperatures and high wind (Izaloo et al., 2008; Fleury et al., 2010), high chlorophyll content could provoke oxidative damage and thus reduce photosynthetic capacity (Long et al., 2015; Parry et al., 2011; Tricker et al., 2018; Zhu et al., 2010). Another possible reason for the reduced yield of R112+ in the Australian environment might reside in the interaction of its root system with the soil features at this site. Previous simulations determined that a shallow root system is more favourable in this type of environment (reviewed in Izanloo et al., 2008), which contrasts with the root system architecture (RSA) of R112+, found to be 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 were found to be crucial adaptive mechanisms associated to yield increases in rice (Uga et al., 2013; 2015; Ahmadi et al., 2014) and wheat (Lopes and Reynolds, 2010; Manchadi et al., 2006; Slack et al., 2018). However, the shallow, clay-limestone soils, typical of most Adelaide Plain's and of our testing site, might have hindered the potential of the R112+ root system, limiting its access to water and nutrients at deeper layers. In fact, it was reported that root impedance reduces leaf elongation and the number of tillers in wheat (Jin et al., 2015). Therefore, the R112+ recombinant looks to be unsuitable for dry environments with combined stress factors, such as heat, drought and hostile soil structure. Instead, to exploit at best its 7AgL-linked positive effects on tiller/spike number, flag leaf photosynthetic activity (see also Kuzmanović et al., 2014, 2016), and RSA characteristics (Virili et al., 2015), it could be profitably employed in breeding directed to environments with optimal thermo-pluviometric patterns and soil characteristics (as VT12, VT15 and MOR15).

Finally, yield performance of the R23+ NIRL represents an intriguing case. In fact, while the background genotype, common to the R23– NIRL, was probably responsible for generally lower adaptability and consequent productivity throughout environments compared to the other two NIRL pairs (see § 3.2 and Supplementary Table 3), the specific, positive and negative effects of its 40%-long 7AgL segment, were consistently expressed in the multi-environment analysis (Table 4). The most important incremental effects validated throughout all trials were on SFI, GNM2 as well as SNM2 (Table 4 and Supplementary Table 3). 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 (no. 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+, and so in the shared 23-28% 7AgL segment, harbours a gene/QTL for tiller/spike number m^{-2}, evidently a major factor of its yield potential (Supplementary Table 3 ; see also Kuzmanović et al., 2014; 2016). However, in contrast to the R112+ case, in which 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 probably a more prominent effect on the genotype's sink (i.e. grain number m^{-2}). Confirming a different yield formation from that hypothesized for R112+, flag-leaf related traits were instead depressed in R23+. Moreover, the same genotype also had reduced biomass at anthesis and maturity when compared to its R23– control (about -20%, Table 4). Both traits appear to be highly correlated with grain number (e.g. Fischer, 2008; González et al., 2011; Slafer et al., 2015); hence, it was unexpected that R23+ could consistently sustain such a large gain in grain number (+47% on average across the 10 environments; Supplementary Table 3). 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). Recent work by Marti et al. (2016) established that growth and N partitioning in the two weeks before anthesis are key factors in determining differences in wheat yield performance, particularly for grain number. The same authors suggest that this growth period is not relevant for total biomass production at anthesis, but is crucial for determining the relative grain number. In line with this, Terrile et al. (2017) also reported a negative correlation between the fruiting efficiency and spike dry matter at anthesis in bread wheat. As the nature of this relationship 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. Guo et al. (2017) suggested a critical role of carbohydrate metabolism and phytohormones in regulating the floret primordia survival (FPS) and final grain number in wheat. Floret primordia formation (FPF) and their survival are known to be proportional to the availability of assimilates allocated for their development during spike growth before anthesis (Ferrante et al., 2013; González et al., 2011; Slafer et al., 2015). In R23+ plants, the number of fertile florets at anthesis (mirroring the FPF) showed to be similar to its R23- control (Kuzmanovic et al., 2016), while the number of seeds at maturity was enhanced, as a consequence of higher FPS (Table 4 and Supplementary Table 3; see also Kuzmanovic et al., 2014, 2016). Therefore, translocation of assimilates to grains is likely to be affected rather than their accumulation in spike tissues. 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, where these favourable conditions were not met (Table 1, Fig. 1;

Supplementary Tables 2 and 3). This was not, however, paralleled by higher GYM2 vs. R23- controls in all environments, due to the lower TGW of R23+ plants (Table 4 and Supplementary Table 3). Due to this trade-off, increased fruiting efficiency is considered to be generally irrelevant for actual yield increases (Slafer et al., 2015). Nonetheless, this negative relationship may not be "constitutive", i.e. the factor(s) increasing fruiting efficiency may be independent of the size of florets (i.e. potential grains); also, resource partitioning to developing florets may be increased and mortality of distal florets reduced, thus ensuring yield gains (Slafer et al., 2015, and references therein). Similar mechanisms may apply to the R23+ case, as it showed higher grain yield vs. R23– controls in five out of 10 trials (+7-58%, Supplementary Table 3), always coinciding with conspicuous increases in SFI, GNM2 and also SNM2. When even just one of these traits was decreased in R23+ plants, the overall yield was up to 22% lower than that of the 7AgL– controls (e.g. VT11, MOR15, Supplementary Table 3), indicating their combined contribution to R23+ productivity. Interestingly, the highest grain yield increases of R23+ genotype vs. R23– control were observed in dry environments of AUS13 and MOR14 (+58% and +34%, respectively), indicating R23+ maintains its capacity for fruiting efficiency even under drought and/or heat stress conditions. Recently, spike fertility was shown to be the key trait contributing to yield performance of durum wheat grown under severe and protracted heat stress (Sall et al., 2018). In the same AUS13 and MOR14 stressed environments, an increment in spike number (SNM2) was also exhibited by R23+ vs. R23– genotype, particularly in the former trial (+127%), while the same was not consistently detected in the R112+ vs. R112– comparison (Supplementary Table 3), confirming the lower trait stability of the latter recombinant type across contrasting environments. The possibility of fully unlocking the R23+ yield potential for practical exploitation remains, however, somewhat challenging, because of the linkage between the genes/QTL for grain number and spike fertility, and a gene(s) causing segregation distortion (*Sd*) and possibly correlated depression of a number of morpho-physiological traits (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 at least countered by transferring the R23 7AgL segment to different recipient cultivars. In fact, similar to the bread wheat T4 case, also the interaction of the R23 40%-long 7AgL segment with varying genetic backgrounds resulted in improved transmission of the recombinant chromosome, with little or no effect on plant phenotype (Ceoloni et al., 2014b).

Conclusions

Analysis of the three 7AgL introgressions into durum wheat across a range of variable environments has validated their potential for yield improvement and has shown that the three analysed recombinant lines have benefited from the desirable gene/QTL content originating from the wild *Th. ponticum* donor. By being developed in a cultivar best adapted to Italian growing conditions (Simeto), the three recombinants exhibited variable performances in stressed environments, like South Australia and, to some extent, Morocco. In general, under no significant water stress, all lines responded well, mainly through regulation of spike/tiller number, the most important adaptive trait in both bread and durum wheat under rain-fed conditions (Elhani et al., 2007; Slafer et al., 2014). However, the observed significant increases in grain yield and number for 7AgL+ vs. 7AgL- NIRLs in heat and drought-stressed AUS13 (Table 3), indicate that the 7AgL yield-related gene/QTL content may also be beneficial under adverse growing conditions. The analysed set of NIRLs represent a valuable toolkit for deciphering physiological mechanisms and identifying genes involved in grain yield regulation, as they display significantly different phenotypes for a number of traits associated with specific 7AgL segments. Apart from genotype-dependent "stabilization" that the R23 7AgL segment may require (see above), all segments can be readily moved into more site-directed (R112) or across environment (mainly R5 and R23) breeding pipelines for exploitation of their *Lr19+Sr25+Yp* genes, as well as for additional traits contributing to yield increase and stability. 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* head blight 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). 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).

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References

Ahmadi, N., Audebert, A., Bennett, M.J., Bishopp, A., de Oliveira, A.C., Courtois, B., Diedhiou, A., Diévart, A., Gantet, P., Ghesquière, A., Guiderdoni, E., Henry, A., Inukai, Y., Kochian, L., Laplaze, L., Lucas, M, Luu, D.T., Manneh, B., Mo, X., Muthurajan, R., Périn, C., Price, A., Robin, S., Sentenac, H., Sine, B., Uga, Y., Véry, A.A., Wissuwa, M., Wu, P., Xu, J. 2014. The roots of future rice harvests. Rice, 7, 29.

Araus, J.L., Bort, J., Steduto, P., Villegas, D., Royo, C. 2003a. Breeding cereals for Mediterranean conditions: ecophysiological clues for biotechnology application. Ann. Appl. Biol. 142, 129-141.

Araus, J.L., Villegas, D., Aparicio, N., García del Moral, L.F., El Hani, S., Rharrabti, Y., Ferrio, J.P., Royo, C. 2003b. Environmental factors determining carbon isotope discrimination and yield in durum wheat under Mediterranean conditions. Crop Sci. 43, 170-180.

Bassi, F.M., Sanchez-Garcia, M. 2017. Adaptation and stability analysis of ICARDA durum wheat elites across 18 Countries. Crop Sci. 57, 2419–2430, doi: 10.2135/cropsci2016.11.0916.

Becker, H.C., and Leon, J. 1988. Stability analysis in plant breeding. Plant breeding, 101, 1-23.

Ceoloni, C., Forte, P., Gennaro, A., Micali, S., Carozza, R., Bitti, A. 2005. Recent developments in durum wheat chromosome engineering. Cytogenet. Genome Res. 109, 328–334, http://dx.doi.org/10.1159/000082416.

Ceoloni, C., Kuzmanović, L., Forte, P., Gennaro, A., Bitti, A., 2014a. Targeted exploitation of gene pools of alien Triticeae species for sustainable and multi-faceted improvement of the durum wheat crop. Crop Pasture Sci. 65, 96–111.

Ceoloni, C., Kuzmanović, L., Gennaro, A., Forte, P., Giorgi, D., Grossi, M.R., Bitti, A. 2014b. Genomes, chromosomes and genes of perennial Triticeae of the genus *Thinopyrum*: the value of their transfer into wheat for gains in cytogenomic knowledge and 'precision' breeding. In: Tuberosa, R., Graner, A., Frison, E. (Eds.), Advances in Genomics of Plant Genetic Resources. Springer, Dordrecht, The Netherlands, pp. 333–358.

Ceoloni, C., Kuzmanović, L., Forte, P., Virili, M.E., Bitti, A. 2015. Wheat-perennial Triticeae introgressions: major achievements and prospects. In: Molnár-Láng M, Ceoloni C, Doležel J, eds. Alien introgression in wheat - Cytogenetics, Molecular Biology, and Genomics. pp 273-313; Springer. ISBN: 978-3-319-23493-9, doi: 10.1007/978-3-319-23494-6.

Ceoloni, C., Kuzmanović, L., Ruggeri, R., Rossini, F., Forte, P., Cuccurullo, A., Bitti, A. 2017a. Harnessing genetic diversity of wild gene pools to enhance wheat crop production and sustainability: challenges and opportunities. Diversity, 9, 55, doi:10.3390/d9040055.

Ceoloni, C., Forte, P., Kuzmanović, L., Tundo, S., Moscetti, I., De Vita, P., Virili, M.E., D'Ovidio, R. 2017b. Cytogenetic mapping of a major locus for resistance to Fusarium head blight and crown rot of wheat on *Thinopyrum elongatum* 7EL and its pyramiding with valuable genes from a *Th. ponticum* homoeologous arm onto bread wheat 7DL. Theor. Appl. Genet. 130, 2005-2024, doi:10.1007/s00122-017-2939-8.

Dempewolf, H., Baute, G., Anderson, J., Kilian, B., Smith, C., Guarino, L. 2017. Past and future use of wild relatives in crop breeding. Crop Sci. 57, 1070–1082, doi: 10.2135/cropsci2016.10.0885.

Dodig, D., Zoric, M., Kobiljski, B., Savic, J., Kandic, V., Quarrie, S., Barnes, J. 2012. Genetic and association mapping study of wheat agronomic traits under contrasting water regimes. Int. J. Mol. Sci. 13, 6167-6188, doi:10.3390/ijms13056167.

Elía, M., Slafer, G.A., Savin, R. 2018. Yield and grain weight responses to post-anthesis increases in maximum temperature under field grown wheat as modified by nitrogen supply. Field Crops Res. 221, 228–237, https://doi.org/10.1016/j.fcr.2018.02.030.

Elhani, S., Martos, V., Rharrabti, Y., Royo, C., García del Moral, L.F., 2007. Contribution of main stem and tillers to durum wheat (*Triticum turgidum* L.var. durum) grain yield and its components grown in Mediterranean environments. Field Crops Res. 103, 25–35.

FAO. 2013. FAOSTAT Statistics Database, available at http://www.fao.org/faostat/en/#home. Food and Agriculture Organization of the United Nations, Rome, Italy. (verified on 2/11/2017).

Ferrante, A., Savin, R., Slafer, G.A. 2013. Floret development and grain setting differences between modern durum wheats under contrasting nitrogen availability. J. Exp. Bot. 64, 169–184.

Finlay, K.W., Wilkinson, G.N. 1963. The analysis of adaptation in a plant-breeding programme. Aust. J. Agric. Res. 14, 742-754.

Fischer, R.A., 2008. The importance of grain or kernel number in wheat: a reply to Sinclair and Jamieson. Field Crops Res. 105, 15–21.

Fischer, R.A., Edmeades, G.O. 2010. Breeding and cereal yield progress. Crop Sci. 50, S85–S98.

Fleury, D., Jefferies, S., Kuchel, H., Langridge, P. 2010. Genetic and genomic tools to improve drought tolerance in wheat. J. Exp. Bot. 61, 3211–3222, doi:10.1093/jxb/erq152.

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

Foulkes, M.J., Sylvester-Bradley, R., Weightman, R., Snape, J. W. 2007. Identifying physiological traits associated with improved drought resistance in winter wheat. Field Crops Res. 103, 11-24.

Giunta, F., De Vita, P., Mastrangelo, A. M., Sanna, G., Motzo, R. 2018. Environmental and genetic variation for yield-related traits of durum wheat as affected by development. Front. Plant Sci., 9, 8, doi: 10.3389/fpls.2018.00008.

González, F.G., Miralles, D.J., Slafer, G.A. 2011. Wheat floret survival as related to pre-anthesis spike growth. J. Exp. Bot. 62, 4889–4901.

Grossi, M.R., Gennaro, A., Forte, P., Bitti, A., Ceoloni, C. 2009. Segregation distortion following introgression of *Thinopyrum ponticum* DNA into durum wheat. Proceedings of the 53rd Italian Society of Agricultural Genetics Annual Congress, Torino, Italy, 16-19 September 2009, http://www.geneticagraria.it/attachment/SIGA_2009/7_20.pdf

Guo Z., Chen, D., Alqudah, A.M., Röder, M.S., Ganal, M.W., Schnurbusch, T. 2017. Genome-wide association analyses of 54 traits identified multiple loci for the determination of floret fertility in wheat. New Phytologist 214, 257–270.

Habash, D.Z., Bernard, S., Schondelmaier, J., Weyen, J., Quarrie, S.A., 2007. The genetics of nitrogen use in hexaploid wheat: N utilization, development and yield. Theor. Appl. Genet. 114, 403–419.

Habash, D.Z., Kehel, Z., Nachit, M. 2009. Genomic approaches for designing durum wheat ready for climate change with a focus on drought. J. Exp. Bot. 60, 2805–2815, doi:10.1093/jxb/erp211.

Izanloo, A., Condon, A.G., Langridge, P., Tester, M., Schnurbusch, T. 2008. Different mechanisms of adaptation to cyclic water stress in two South Australian bread wheat cultivars. J. Exp. Bot 59, 3327–3346, doi:10.1093/jxb/ern199.

Jin K., Shen, J., Ashton, R.W., White, R.P., Dodd, I.C., Phillips, A.L., Parry, M.A.J., Whalley, W.R. 2015. The effect of impedance to root growth on plant architecture in wheat. Plant Soil 392, 323–332, doi: 10.1007/s11104-015-2462-0.

Kuzmanović, L., Gennaro, A., Benedettelli, S., Dodd, I.C., Quarrie, S.A., Ceoloni. C. 2014. Structural-functional dissection and characterization of yield-contributing traits originating from a group 7 chromosome of the wheatgrass species *Thinopyrum ponticum* after transfer into durum wheat. J. Exp. Bot. 65, 509-525. doi:10.1093/jxb/ert393.

Kuzmanović, L., Ruggeri, R., Virili, M.E., Rossini, F., Ceoloni, C. 2016. Effects of *Thinopyrum ponticum* chromosome segments transferred into durum wheat on yield components and related morpho-physiological traits in Mediterranean rain-fed conditions. Field Crops Res. 186, 86–98, http://dx.doi.org/10.1016/j.fcr.2015.11.007.

Liu, B., Asseng, S., Müller, C., Ewert, F., Elliott, J., Lobell, D.B., Martre, P., Ruane, A.C., Wallach, D., Jones, J.W., Rosenzweig, C., Aggarwal, P.K., Alderman, P.D., Anothai, J., Basso, B., Biernath, C., Cammarano, D., Challinor, A., Deryng, D., De Sanctis, G., Doltra, J., Fereres, E., Folberth, C., Garcia-Vila, M., Gayler, S., Hoogenboom, G., Hunt, L.A., Izaurralde, R.C., Jabloun, M., Jones, C.D., Kersebaum, K.C., Kimball, B.A., Koehler, A.-K., Kumar, S.N., Nendel, C., O'Leary, G.J., Olesen, J.E., Ottman, M.J., Palosuo, T., Prasad, P.V.V., Priesack, E., Pugh, T.A.M., Reynolds, M., Rezaei, E.E., Rötter, R.P., Schmid, E., Semenov, M.A., Shcherbak, I., Stehfest, E., Stöckle, C.O., Stratonovitch, P., Streck, T., Supit, I., Tao, F., Thorburn, P., Waha, K., Wall, G.W., Wang, E., White, J.W., Wolf, J., Zhao, Z., Zhu, Y. 2016. Similar estimates of temperature impacts on global wheat yield by three independent methods. Nat. Clim. Change, 6, 1130-1136.

Long, S.P., Marshall-Colon, A., Zhu, X.-G. 2015. Meeting the global food demand of the future by engineering crop photosynthesis and yield potential. Cell 161, 56-66, http://dx.doi.org/10.1016/j.cell.2015.03.019.

Lopes, M.S., Reynolds, M.P. 2010. Partitioning of assimilates to deeper roots is associated with cooler canopies and increased yield under drought in wheat. Funct. Plant Biol. 37, 147–156.

Luo, Q., Bellotti, W., Williams, M., Bryan, B. 2005. Potential impact of climate change on wheat yield in South Australia. Agric. For. Meteorol. 132, 273-285.

Maccaferri, M., Sanguineti, M.C., Demontis, A., El-Ahmed, A., del Moral, L.G., Maalouf, F., Nachit, M., Nserallah, N., Ouabbou, H., Rhouma, S., Royo, C., Villegas,D., Tuberosa, R. 2011. Association mapping in durum wheat grown across a broad range of water regimes, J. Exp. Bot. 62, 409–438, https://doi.org/10.1093/jxb/erq287.

Manschadi, A.M., Christopher, J., de Voil, P., Hammer, G.L. 2006. The role of root architectural traits in adaptation of wheat to water-limited environments. Funct. Plant Biol. 33, 823-837, https://doi.org/10.1071/FP06055.

Marti, J., Slafer, G.A. 2014. Bread and durum wheat yields under a wide range of environmental conditions. Field Crops Res. 156, 258-271.

Marti, J., Araus, J.L., Slafer. G.A. 2016. Sink-strength determines differences in performance between breadand durum wheat. Field Crops Res. 198, 101–111, http://dx.doi.org/10.1016/j.fcr.2016.08.020.

Miralles, D.J., Resnicoff, E., Carretero, R. 2007. Yield improvement with *Lr19* translocation in wheat: which plant attributes are modified? In: Spiertz, J.H.J., Struik, P.C., Laar, H.H. van (Eds.), Scale and complexity in plant systems research: gene-plant-crop relations. Frontis, 21, 169-176.

Mondal, S., Rutkoski, J.E., Velu, G., Singh, P.K., Crespo-Herrera, L.A., Guzmán, C., Bhavani, S., Lan, C., He X., Singh, R.P. 2016. Harnessing diversity in wheat to enhance grain yield, climate resilience, disease and insect pest resistance and nutrition through conventional and modern breeding approaches. Front. Plant Sci. 7, 991, doi: 10.3389/fpls.2016.00991.

Monneveux, P., Reynolds, M.P., Gonzalez Aguilar, J., Singh, R.P. 2003. Effects of the *7DL.7Ag* translocation from *Lophopyrum elongatum* on wheat yield and related morphophysiological traits under different environments. Plant Breeding 122, 379–384. doi:10.1046/j.1439-0523.2003.00856.x

Parent, B., Bonneau, J., Maphosa, L., Kovalchuk, A., Langridge, P., Fleury, D. 2017. Quantifying wheat sensitivities to environmental constraints to dissect G x E in the field. Plant Physiol., pp 00372.

Parry M.A.J., Reynold, M., Salvucci, M.E., Raines, C., Andralojc, P.J., Zhu, X.-G., Price, G.D., Condon, A.G., Furbank, R.T. 2011. Raising yield potential of wheat. II. Increasing photosynthetic capacity and efficiency. J. Exp. Bot. 62, 453–467, doi:10.1093/jxb/erq304.

Pedro, A., Savin, R., Habash, D.Z., Slafer, G.A., 2011. Physiological attributes associated with yield and stability in selected lines of a durum wheat population. Euphytica 180, 195–208.

Peremarti, A., Marè, C., Aprile, A., Roncaglia, E., Cattivelli, L., Villegas, D., Royo, C. 2014. Transcriptomic and proteomic analyses of a pale-green durum wheat mutant shows variations in photosystem components and metabolic deficiencies under drought stress. BMC Genomics 15, 125.

Prohens, J., Gramazio, P., Plazas, M., Dempewolf, H., Kilian, B., Díez, M.J., Fita, A., Herraiz, F.J., Rodríguez-Burruezo, A., Soler, S., Knapp, S., Vilanova, S. 2017. Introgressiomics: a new approach for using crop wild relatives in breeding for adaptation to climate change. Euphytica 213, 158, https://doi.org/10.1007/s10681-017-1938-9.

Quarrie, S.A., Pekic-Quarrie, S., Radosevic, R., Rancic, D., Kaminska, A., Barnes, J.D.,Leverington, M., Ceoloni, C., Dodig, D., 2006. Dissecting a wheat QTL for yield present in a range of environments: from the QTL to candidate genes. J. Exp.Bot. 57, 2627–2637.

Ray, D.K., Mueller, N.D., West, P.C., Foley, J.A. 2013. Yield Trends Are Insufficient to Double Global Crop Production by 2050. PLOS One 8, e66428.

Reynolds, M.P., Calderini, D.F., Condon, A.G., Rajaram, S. 2001. Physiological basis of yield gains in wheat associated with the LR19 translocation from *Agropyron elongatum*. Euphytica 119, 137–141.

Rosewarne, G., Bonnett, D., Rebetzke, G., Lonergan, P., Larkin, P.J. 2015. The potential of *Lr19* and *Bdv2* translocations to improve yield and disease resistance in the high rainfall wheat zones of Australia. Agronomy 5, 55-70.

Royo, C., Elias, E.M., Manthey, F.A., 2009. Durum wheat breeding. In: Prohens, J.,Nuez, F., Carena, M.J. (Eds.), Handbook of Plant Breeding, Vol. 3. Springer Science+Business Media, LLC, Cereals, pp. 199–226.

Royo, C., Nazco, R., Villegas, D. 2014. The climate of the zone of origin of Mediterranean durum wheat (*Triticum durum* Desf.) landraces affects their agronomic performance. Genet. Resour. Crop Evol. 61, 1345–1358, doi 10.1007/s10722-014-0116-3.

Sibikeev, S.N., Voronina, S.A., Krupnov, V.A. 2000. Effects from 7DL-7Ae# 1 translocation on resistance to environmental factors and grain quality of bread wheat. In: EWAC newsletter: proceedings of the 11th EWAC conference, 24-28 July 2000, Novosibirsk, Russia, pp. 169-170.

Sall, A.T., Cisse, M., Gueye, H., Kabbaj, H., Ndoyel, I., Filali-Maltouf, A., Belkadi, B., El-Mourid, M., Ortiz, R., Bassi, F.M. 2018. Heat tolerance of durum wheat (*Tritcum durum* Desf.) elite germplasm tested along the Senegal River. J Agr Sci. 10, 217-233, https://doi.org/10.5539/jas.v10n2p217.

Singh, R.P., Huerta-Espino, J., Rajaram, S., Crossa, J. 1998. Agronomic effects from chromosome translocations 7DL.7Ag and 1BL.1RS in spring wheat. Crop Sci. 38, 27–33.

Slack, S., York, L.M., Roghazai, Y., Lynch, J., Bennett, M., Foulkes, J. 2018. Wheat shovelomics II: Revealing relationships between root crown traits and crop growth. BioRxiv 280917; doi: https://doi.org/10.1101/280917.

Slafer, G.A., Savin, R., Sadras, V.O. 2014. Coarse and fine regulation of wheat yield components in response to genotype and environment. Field Crops Res. 157, 71–83, http://dx.doi.org/10.1016/j.fcr.2013.12.004

Slafer, G.A., Elia, M., Savin, R., García, G.A., Terrile, I.I., Ferrante, A., Miralles, D.J., González, F.G. 2015. Fruiting effi ciency: an alternative trait to further rise wheat yield. Food Energy Secur 4, 92–109, doi: 10.1002/fes3.59.

Tadesse W., Amri, A., Sanchez-Garcia, M., El-Bouhssini, M., Karrou, M., Patil, S., Bassi F., Baum, M., Oweis, T. 2016. Improving wheat production in the Central and West Asia and North Africa (CWANA) region. In: Langridge, P. (Ed.), Achieving sustainable cultivation in wheat, Vol. 2, Burleigh Dodds Science Publishing Limited, Cambridge, UK, http://dx.doi.org/10.19103/AS.2016.0004.39.

Tardieu, F., Tuberosa, R. 2010. Dissection and modelling of abiotic stress tolerance in plants. Curr. Opin. Plant Biol. 13, 206-212.

Terrile, I.I., Miralles, D.J., González, F.G. 2017. Fruiting efficiency in wheat (*Triticum aestivum* L.): Trait response to different growing conditions and its relation to spike dry weight at anthesis and grain weight at harvest. Field Crops Res. 201, 86–96, http://dx.doi.org/10.1016/j.fcr.2016.09.026.

Tricker, P.J., ElHabti, A., Schmidt, J., Fleury, D. 2018. The physiological and genetic basis of combined drought and heat tolerance in wheat. J Exp. Bot., doi.org/10.1093/jxb/ery081.

Tripathi, S.C., Sayre, K.D., Kaul, J.N. 2005. Planting systems on lodging behavior, yield components, and yield of irrigated spring bread wheat. Crop Sci. 45, 1448–1455.

Uga, Y., Sugimoto, K., Ogawa, S., Rane, J., Ishitani, M., Hara, N., Kitomi, Y., Inukai, Y., Ono, K., Kanno, N., Inoue, H., Takehisa, H., Motoyama, R., Nagamura, Y., Wu, J., Matsumoto, T., Takai, T., Okuno, K., Yano, M. 2013. Control of root system architecture by DEEPER ROOTING 1 increases rice yield under drought conditions. Nat. Genet. 45, 1097-1102, doi:10.1038/ng.2725.

Uga, Y., Kitomi, Y., Ishikawa, S., Yano, M. 2015. Genetic improvement for root growth angle to enhance crop production. Breeding Sci. 65, 111–119, doi:10.1270/jsbbs.65.111.

Virili, M.E-, Kuzmanović, L., Bitti, A., Salvi, S., Tuberosa, R., Ceoloni, C. 2015. Analysis of seminal root architecture in durum wheat-*Thinopyrum ponticum* recombinant lines. Proceedings of the

Joint Meeting SIBV-SIGA, Milan, Italy – 8-11th September, 2015. ISBN 978-88-904570-5-0, http://www.geneticagraria.it/attachment/SIGA_2015/2_08.pdf.

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

Zhang, H., Turner, N.C., Simpson, N., Poole, M.L. 2010. Growing-season rainfall, ear number and the water-limited potential yield of wheat in south-western Australia. Crop Pasture Sci. 61, 296–303.

Zhang H., Mittal, N., Leamy, L.J., Barazani, O., Song, B.-H. 2017. Back into the wild—Apply untapped genetic diversity of wild relatives for crop improvement. Evol. Appl. 10, 5-24, doi: 10.1111/eva.12434.

Zaïm, M., El Hassouni, K., Gamba, F., Filali-Maltouf, A., Belkadi, B., Sourour, A., Amri, A., Nachit, M., Taghouti, M., Bassi, F. M. 2017. Wide crosses of durum wheat (*Triticum durum* Desf.) reveal good disease resistance, yield stability, and industrial quality across Mediterranean sites. Field Crops Res. 214, 219-227, http://dx.doi.org/10.1016/j.fcr.2017.09.007.

Zhao, C., Liu, B., Piao, S., Wang, X., Lobell, D.B., Huang, Y., Huang, M., Yao, Y., Bassu, S., Ciais, P., Durand, J.-L., Elliott, J., Ewert, F., Janssens, I.A., Li, T., Lin, E., Liu, Q., Martre, P., Müller, C., Peng, S., Peñuelas, J., Ruane, A.C., Wallach, D., Wang, T., Wu, D., Liu, Z., Zhu, Y., Zhu, Z., Asseng, S. 2017. Temperature increase reduces global yields of major crops in four independent estimates. PNAS 114, 9326–9331.

Zhu, X.-G., Long, S.P., Ort, D.R. 2010. Improving photosynthetic efficiency for greater yield. Annu. Rev. Plant Biol. 61, 235–61.