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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

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Abstract

Key message

A major locus for resistance to different Fusarium diseases was mapped to the most distal end of *Th. elongatum* 7EL and pyramided with *Th. ponticum* beneficial genes onto wheat 7DL.

Abstract

Perennial Triticeae species of the *Thinopyrum* genus are among the richest sources of valuable genes/QTL for wheat improvement. One notable and yet unexploited attribute is the exceptionally effective resistance to a major wheat disease worldwide, Fusarium head blight, associated with the long arm of *Thinopyrum elongatum* chromosome 7E (7EL). We targeted the transfer of the temporarily designated *Fhb-7EL* locus into bread wheat, pyramiding it with a *Th. ponticum* $7el_1L$ segment stably inserted into the 7DL arm of wheat line T4. Desirable genes/QTL mapped along the T4 $7el_1L$ segment determine resistance to wheat rusts (*Lr19*, *Sr25*) and enhancement of yield-related traits. Mapping of the *Fhb-7EL* QTL, prerequisite for successful pyramiding, was established here on the basis of a bioassay with *Fusarium graminearum* of different 7EL-$7el_1L$ bread wheat recombinant lines.

These were obtained without resorting to any genetic pairing promotion, but relying on the close 7EL-7el$_1$L homoeology, resulting in 20% pairing frequency between the two arms. *Fhb-7EL* resided in the telomeric portion and resistant recombinants could be isolated with useful combinations of more proximally located 7el$_1$L genes/QTL. The transferred *Fhb-7EL* locus was shown to reduce disease severity and fungal biomass in grains of infected recombinants by over 95%. The same *Fhb-7EL* was, for the first time, proved to be effective also against *F. culmorum* and *F. pseudograminearum*, predominant agents of crown rot. Prebreeding lines possessing a suitable 7EL-7el$_1$L gene/QTL assembly showed very promising yield performance in preliminary field tests.

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Electronic supplementary material

The online version of this article (doi:10.1007/s00122-017-2939-8) contains supplementary material, which is available to authorized users.

Introduction

Wheat is the world's most widely cultivated cereal (over 220 million hectares in 2014, FAO 2017), and ranks second only to maize (and before rice) in world grain production (about 750 million tonnes in 2016, FAO 2016). Wheat production is vital in both emerging and growing economies, with more than two billion people worldwide relying on it as a staple food. In parallel with increasing concern about the rising world population (Evans 2009), current and projected climate changes pose an additional threat to global wheat production, causing a variety of abiotic constraints and these, in turn, influencing plant disease epidemics (Barzman et al. 2015). As part of the global

warming-driven disease and pest movements (Bebber et al. 2013), various modifications in emergence, or re-emergence, and spread are being detected or foreseen for wheat diseases. Focusing on fungal pathogens, which are responsible for most of the yield losses in wheat at the global scale (Oerke 2006), several surveys already indicate increased aggressiveness of yellow rust pathotypes adapted to warmer temperatures in India (Solh 2010), Europe (Mboup et al. 2012) and in USA (Gautam et al. 2013). Moreover, mid- to long-term simulation studies forecast that temperature and humidity ~~which~~ increases above normal will also predispose the wheat crop to more severe epidemics of the other rust diseases, such as leaf rust in Germany (Juroszek and von Tiedemann 2013), as well as leaf and stem rusts in India (Gautam et al. 2013). Likewise, favoured by warm, wet weather at anthesis, Fusarium head blight (FHB) has re-emerged in USA (Chakraborty and Newton 2011). In perspective, higher spring temperatures are expected to anticipate anthesis date, creating a more favourable timing for flower-infecting fungi, like *Fusarium* species, whose incidence is predicted to increase substantially in many countries worldwide, from South America, India, and China to northern Europe (Bebber et al. 2013; Gautam et al. 2013; Juroszek and von Tiedemann 2013; Zhang et al. 2014).

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The lesson from such scenarios is that robust crop protection represents a much greater challenge than ever, and substantial progress needs to be made soon to secure food production sustainability (Beddington 2010; Boonekamp 2012). Thus, while crop improvement through breeding has been key to the past successes of agriculture, to meet the current and emerging challenges, unconventional approaches must be integrated with the traditional procedures, maximizing the use of recently developed technologies. It is widely recognized that enlargement of the crop genetic base with unexploited allelic variants of valuable genes is crucial to counter genetic erosion of cultivated genepools, and create novel germplasm with more diversified adaptive traits (McCouch 2004; Mujeeb-Kazi et al. 2013; Ceoloni et al. 2014a). For the cultivated wheat species, both bread wheat, *Triticum aestivum* L. ($2n = 6x = 42$), and durum wheat, *T. durum* Desf. ($2n = 4x = 28$), there appears to be a wealth of natural genetic resources of alien Triticeae, and

also a unique richness of cytogenetic materials that have greatly facilitated knowledge of the wheat and other Triticeae genomes and targeted alien gene introgression via chromosome engineering strategies (reviewed in Ceoloni and Jauhar 2006; Qi et al. 2007; Molnár-Láng et al. 2015).

Among the genepools of wheat relatives that can be accessed with relative ease is that of perennial Triticeae. Several wheat–alien combinations have been developed with members of this vast group of grasses, the majority involving the genus *Thinopyrum* Löve (reviewed in Ceoloni et al. 2015). Of the many species attributed to the genus, both the diploids (2*n* = 2*x* = 14), *Th. elongatum* (Host) D.R. Dewey (syn. *Agropyron elongatum*, *Lophopyrum elongatum*) and *Th. bessarabicum* (Savul. and Rayss) Löve (syn. *Agropyron bessarabicum*), and many of the polyploids, notably the hexaploid (2*n* = 6*x* = 42) *Th. intermedium* (Host) Barkworth and D.R. Dewey (syn. *Agropyron intermedium*, *Elytrigia intermedia*), and the decaploid (2*n* = 10*x* = 70) *Th. ponticum* (Popd.) Barkworth and D.R. Dewey (syn. *Agropyron elongatum*, *Lophopyrum ponticum*, *Elytrigia pontica*), are probably the most extensively exploited alien species in wheat breeding. In fact, given the relative close affinity between their basic genomes and those of wheat, mainly the D genome of *T. aestivum* (Liu et al. 2007), they have worked as highly valuable sources of a multitude of genes/quantitative trait loci (QTL) to improve resistance to biotic and abiotic stresses, as well as quality and yield-related traits (Ceoloni et al. 2015). A remarkable case is that of the *Th. ponticum* chromosome named $7el_1$ (Sharma and Knott 1966; Knott et al. 1977) or 7Ag (Sears 1973) because of its homoeology to wheat group 7, which was variously engineered to produce several wheat translocation and recombinant lines, both in bread and in durum wheat (reviewed in Ceoloni et al. 2014b, 2015). Development of such materials was primarily prompted by the presence on the long arm of $7el_1$ ($7el_1L$) of the highly effective leaf rust resistance gene *Lr19* (Gennaro et al. 2009 and references therein). Its relatively close linkage with the more distal *Sr25* stem rust resistance gene, proved to be effective against Ug99 races in recent years (Li and Wang 2009; Liu et al. 2010), has enhanced the validity of $7el_1L$ transfers. The even closer linkage of *Lr19* with a *Yp* gene for yellow pigment content (YPC), considered somewhat detrimental for bread wheat transfers, turned out to be particularly attractive for durum wheat breeding (Ceoloni et al. 2005,

2014a).

Among the bread wheat transfer lines, the best characterized and significantly impacting on breeding is the radiation-induced translocation named T4 (=Agatha), bearing a 70% long $7el_1L$ segment distally inserted onto the wheat 7DL arm (Dvorak and Knott 1977; Friebe et al. 1996). This sizable $7el_1L$ segment not only showed good compensation ability for the replaced 7DL portion (Friebe et al. 1994) but also provided considerable yield advantage when present in the background of several bread wheat cultivars and in different world areas, including Mexico, India, and Australia (Singh and Rajaram 1991; Singh et al. 1998; Reynolds et al. 2001; Monneveaux et al. 2003; Sivasamy et al. 2010; Rosewarne et al. 2015). These results on bread wheat were further corroborated by similar evidence from the analyses of various 7AL-$7el_1L$ durum wheat recombinant lines (Ceoloni et al. 2005), which were also useful to attribute to defined sub-regions of the $7el_1L$ arm several QTL for specific yield-contributing traits, only loosely assigned to the large T4 segment in the previous studies (Kuzmanović et al. 2014, 2016). Thus, a unique panel of genes/QTL along the distal $7el_1L$ arm contributes to either directly or indirectly (rust resistance genes) to enhance the yield performance of recipient wheats. However, all $7el_1L$ wheat transfers showed complete susceptibility to major diseases caused by fungi of *Fusarium* genus, primarily FHB (see, e.g., Shen et al. 2004; Oliver et al. 2005; Forte et al. 2014).

Of numerous QTL reported as associated with FHB resistance in wheat, only a few with relatively large effect have been validated across studies and used in breeding, most efforts so far having been centred on *Fhb1*, a major QTL originating from the Chinese cultivar Sumai 3 (reviewed in Buerstmayr et al. 2009; Liu et al. 2009; Gilbert and Haber 2013). Fortunately, however, a wider choice of germplasm providing resistance to FHB can be found in wheat relatives, including *Thinopyrum* species (see for review Ceoloni et al. 2015; Guo et al. 2015). To enrich that $7el_1L$ transfers with this relevant attribute, a primary attempt consisted of pyramiding into $7el_1L$ introgressions portions of a nearly homologous arm, originating from a different *Th. ponticum* accession, and hence named $7el_2L$, containing a

major FHB resistance QTL at its distal end. Facilitated by the close $7el_1L$–$7el_2L$ relationship, leading to nearly complete pairing in hexaploid contexts (Dvorak 1975; Shen and Ohm 2007; Forte et al. 2014), and the relative location of the various target genes/QTL along the two arms (Zhang et al. 2011; Forte et al. 2014), the pyramiding goal has been successfully accomplished in bread wheat (Shen and Ohm 2007; Zhang et al. 2011; Forte et al. 2014) and also durum wheat (Forte et al. 2014). The effect of the QTL on $7el_2L$, recently named *Fhb7* (Guo et al. 2015), is noteworthy, as it is able to reduce FHB severity of spike infection by up to 85% compared to susceptible sibs, and also to substantially decrease the quantity of *Fusarium* biomass in blighted kernels, hence minimizing trichothecene mycotoxin (such as deoxynivalenol, DON) contamination (Forte et al. 2014), a well-known associated risk with *Fusarium* spp. attacks (Gilbert and Haber 2013; Matny 2015).

Nevertheless, in view of the expected increase in the incidence and spreading of *Fusarium* diseases, coupled with the relatively scarcity of available resistance sources in wheat besides *Fhb1* (reviewed in Gilbert and Haber 2013), an additional alien source was taken into consideration in the present research, with the final target of pyramiding it into $7el_1L$ transfers. It consists of an extremely potent FHB resistance locus associated with the genetically long arm of *Th. elongatum* 7E chromosome (7EL), conferring a “Type II” resistance, i.e., resistance to fungal spread within host tissues (Mesterházy et al. 1999). In fact, minimal spreading of *Fusarium graminearum*, the main causal agent of FHB, was consistently found in spikes of 7E substitution lines into bread wheat Chinese Spring and other aneuploid lines containing 7E or 7EL beyond the inoculated floret (Fedak et al. 2003, 2015; Shen et al. 2004; Shen and Ohm 2006; Wang et al. 2010; Miller et al. 2011; Fu et al. 2012; Gou et al. 2016).

Because of its exceptional efficacy, transfer of the 7EL FHB resistance locus (for now referred to as *Fhb-7EL*) has become a highly desired goal in recent years. Apart from the case of some 7E translocations of unknown origin, consisting of sizable (60–80% of the wheat arm) and, in one instance, multiple rearrangements (Fu et al. 2012), an ongoing attempt, based on the use of the wheat *ph1b* mutant as a means of inducing pairing and recombination

between 7E and its wheat homoeologous counterparts, was recently reported (Gou et al. 2016).

In the present research, we adopted an alternative strategy, aimed at the simultaneous exploitation of a panel of useful genes/QTL from different *Thinopyrum* species, including *Fhb-7EL*. A similar effort, directed to build a gene combination from *Th. ponticum* and *Th. intermedium* into wheat, was accomplished by *ph1b*-mediated recombination between a mutant T4 chromosome (T4m) with low YPC, and 7D chromosomes carrying individual *Th. intermedium* translocations with the *Bdv2* gene for resistance to barley yellow dwarf virus (Ayala-Navarrete et al. 2007, 2013). The homoeologous alien portions of the two parental 7D translocated chromosomes, probably belonging to different *Thinopyrum* genomes, had shown no pairing under normal *Ph1* conditions (Ayala-Navarrete et al. 2007).

This was not the case for the complete 7E and $7el_1$ chromosomes, whose 13.6% pairing frequency in a *Ph1* wheat background (Dvorak 1975) was considered as indicative of close homoeology between the two group 7 chromosomes, belonging to the E genome of *Th. elongatum* and to one of the still debated genomes of *Th. ponticum* (see Ceoloni et al. 2015 for a review), respectively. We reasoned that attempting to rely on 7EL-$7el_1$L pairing in a *Ph1* wheat context would circumvent various *ph1*-associated drawbacks, such as introduction of unwanted chromosomal aberrations into the recipient wheat background, known to cause genomic instability, and hence competitive disadvantage, in recombinant gametes and zygotes (e.g., Ceoloni and Jauhar 2006; Somo et al. 2014). In addition, the need of sequential backcrosses of recombinants to normal wheat, to re-establish a dominant *Ph1* condition and recover stable genotypes, would be allayed, thus speeding the overall pyramiding process.

Given all this, we have crossed the CS7E(7D) substitution line, chosen as donor of *Fhb-7EL*, with the 7D/$7el_1$ translocation line T4, carrier of the valuable genes/QTL described above. As precise genetic and physical location of the *Fhb-7EL* locus was not established so far, we have mapped it along the 7EL arm and established its relative position with respect to the $7el_1$L genes/QTL. We isolated and characterized various 7E/$7el_1$ recombinant types

where target genes/QTL from the two *Thynopyrum* species were effectively pyramided, and evaluated the efficacy of the *Fhb-7EL* locus against *F. graminearum* once inserted into a different chromosomal context than its native one (complete 7E or 7EL). A further aim of the research was then testing the ability of the same locus to respond to infection with additional *Fusarium* species, *F. culmorum* and *F. pseudograminearum*. Besides being involved in FHB, they are the two predominant agents of crown rot (FCR, or foot and root rot), another threatening *Fusarium* disease worldwide (see, e.g., Chakraborty et al. 2006; Ma et al. 2010; Scherm et al. 2013; and references therein). For all these targets, our work has provided important returns, of both basic and potentially applied validity.

Materials and methods

Plant materials

The Chinese Spring (CS) 7E(7D) substitution line ($2n = 42$) was used as donor parent of FHB resistance associated with the 7EL arm (*Fhb-7EL*). Its effectiveness against local *Fusarium* pathotypes was tested prior to the transfer work (see below). To pyramid the *Fhb-7EL* resistance with *Th. ponticum* $7el_1L$-linked valuable traits, the CS7E(7D) substitution line was crossed with the FHB-susceptible T4 (=Agatha) bread wheat $7DS{\cdot}7DL/7el_1L$ radiation-induced translocation line ($2n = 42$), carrying a 70% long $7el_1L$ segment inserted onto the 7DL arm of a prevailingly cv. Thatcher background (Dvorak and Knott 1977; Friebe et al. 1996). To recover $7EL/7el_1L$ recombinants, F_1 hybrid plants, double monosomic for one complete 7E and one $7D/7el_1$ chromosome from T4 line (Fig. 1a), were crossed with normal bread wheat cultivars, including CS and the cv. Blasco (good yielding and high quality, but highly susceptible to both leaf rust and FHB). Heterozygous recombinants identified by means of marker-assisted selection were self-pollinated, and homozygous recombinant types (HOM+), as well as sib plants with normal wheat 7D (HOM−) isolated. Additional genotypes employed in some tests include cv. Thatcher, and the KS24-1 bread wheat $7DS{\cdot}7el_2L$ centric translocation line, whose $7el_2L$ arm is homologous to $7el_1L$ (Kibirige-Sebunya and Knott 1983;

Kim et al. 1993) and carries a *Yp* allele, as well as an effective FHB resistance QTL. Also used were the two Agatha yellow pigment mutants (Knott 1980), one having retained (Agatha 28-4) and the other having apparently lost (Agatha 235-6) the stem rust resistance gene *Sr25* (Knott 1984; Friebe et al. 1994).

Fig. 1 This **position** for **Fig. 1** is **not appropriate**, as it contains data pertaining to the Results section.
I request to **move it to Results**, in particular immediately after or close to the first paragraph of the section, ending with words: ...critical pairing partners as univalents (Fig. 1d).

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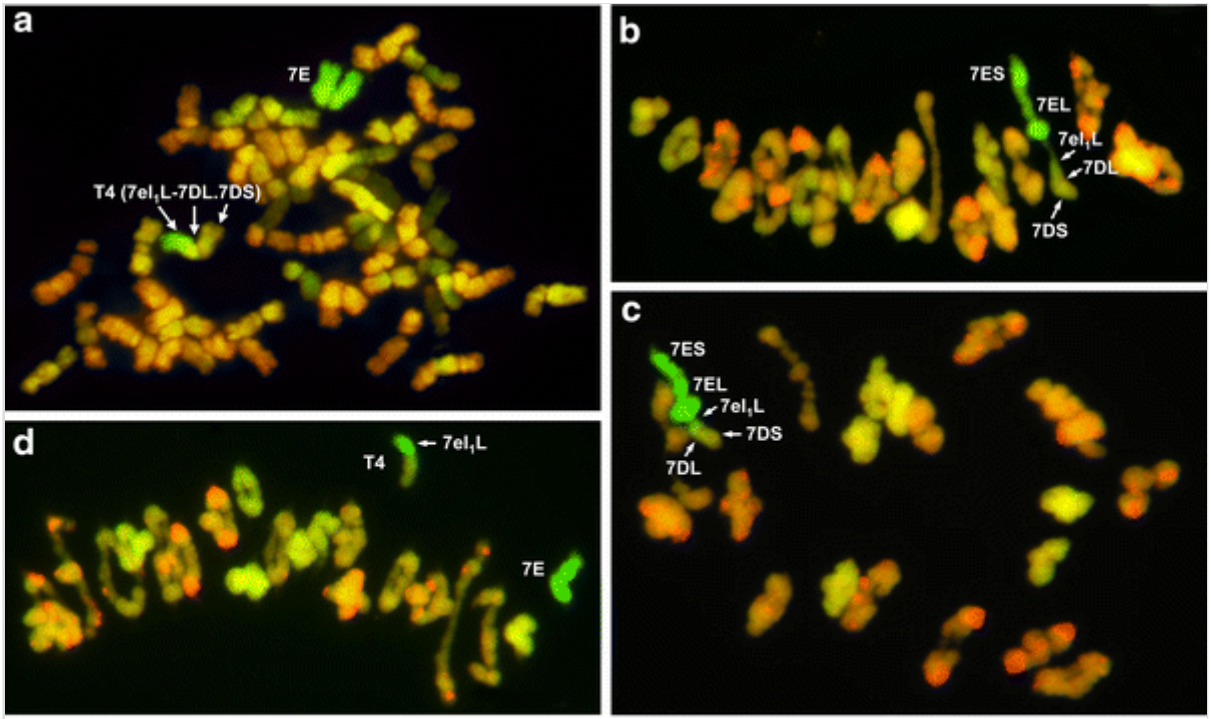
Meiotic pairing and recombination between *Th. elongatum* 7EL and *Th. ponticum* $7el_1L$ segments in [CS7E(7D) × T4] F_1 plants. **a** GISH of a somatic metaphase cell of an F_1 plant, with green fluorescence highlighting the complete 7E and the 70% $7el_1L$ portion of T4 translocated 7D chromosome, with the different chromosomal segments *arrowed*; in **b** and **c,** the complete 7E and the 7D/$7el_1$ (T4) chromosome pair at the level of their L arms, forming a rod bivalent, with relatively distal (**b**) or proximal (**c**) chiasma localization; in **d**, the complete 7E and the 7DS·7DL-$7el_1L$ chromosome of line T4 appear as univalents outside the equatorial plate. Frequency of critical pairing as well as of recombinant and other progeny types from [CS7E(7D) × T4] × bread wheat crosses are given at the *bottom*



Cross combination	7EL - 7el$_1$L pairing		[CS7E(7D) x T4] x bread wheat: % progeny types						
	No. PMCs	% pairing	No. plants	7EL/7el$_1$L recs. (chromos. type)	PT4+7D	7E+7D	7D only	7E+7D+PT4	Misdivision
CS7E(7D) x T4	112	19.6	66	10.6 (7.6 (T4), 3.0 (7E))	24.2	25.8	21.2	9.1	9.1

Cytogenetic analyses

Genomic in situ hybridization (GISH)

GISH was conducted on mitotic and meiotic chromosomes of wheat-*Thinopyrum* lines. For chromosome spreads,

fixed root tip cells or pollen mother cells (PMCs) at metaphase I, derived from anthers extracted from freshly collected young spikes at the meiotic stage, were used. Genomic probes consisted of *T. aestivum* and *Thinopyrum* spp. total DNAs, extracted following Tai and Tanksley (1990) and mechanically sheared to 8–10 kb fragments. Given the close relatedness between the *Th. elongatum* and *Th. ponticum* genomes, DNA of either species can be indifferently used to highlight *Thinopyrum* introgressions into wheat, but, for the same reason, discrimination of the specific origin of either introgression is not feasible with standard GISH protocols. Genomic probes were labelled by nick translation including Biotin-11-dUTP (Fermentas) or Digoxigenin 11-dUTP (Roche Diagnostics) in the deoxyribonucleotide (dNTP) mix. Following Anamthawat-Jónsson and Reader (1995), equal quantities (100 ng) of denatured and differently labelled wheat and *Thinopyrum* genomic probes were allowed to preanneal for 30 min at 58 °C, to enrich the hybridization mixture in genome-specific sequences; hybridization was then carried out for 2 h at 63 °C. Following hybridization, digoxigenin- and biotin-labelled probes (see Fig. 1) were correspondingly detected using anti-digoxigenin conjugated with FITC (Roche; green fluorescence) and streptavidin conjugated with Cy3 (Amersham; red fluorescence).

Conventional staining preparations

Standard Feulgen or aceto-carmine staining techniques were applied to assess the chromosome number of some progeny types from [CS7E(7D) × T4] × *T. aestivum* crosses, including, from the molecular marker analyses, putative recombinants, as well as aberrant segregation and misdivision products.

All chromosome preparations were analysed using a Leica DM5000B epifluorescence microscope, equipped with a SPOT-RT3 (Diagnostic Instruments, Inc.) color digital camera and the SPOT™ Advanced Plus imaging software.

Molecular marker analyses

Molecular characterization of the [CS7E(7D) × T4] × *T. aestivum* F_1 progeny and subsequent offspring derived from selfing or BC(s) was performed by use of PCR-based markers, located along homoeologous group 7 wheat and other Triticeae chromosomes. For some of them, information on map position and/or relevant polymorphism was available from ~~the~~ previous studies, while for several others, particularly for the 7E marker alleles, they were established in the course of this work (Table 1; Figs. S1 and S2).

Table 1

Group 7 molecular markers used to characterize $7el_1$-7E recombinant genotypes isolated in the course of the work

Marker (s)	Type	Arm	Allele(s)	References
SDAUK66	EST	L	7D, $7el_1$, 7E[a]	Guo et al. (2015)
BE405003	EST	L	7A, 7B, 7D, $7el_1$[a], 7E[a]	Dilbirligi et al. (2004), Forte et al. (2014)
CFA2240	SSR	L	7A, 7D, $7el_1$	Shen and Ohm (2007), Zhang et al. (2011), Forte et al. (2014)
BE445653	EST	L	7A, 7B, 7D, $7el_1$, 7E[a]	Shen and Ohm (2007), Zhang et al. (2011), Forte et al. (2014)
MAG1932	EST-STS	L	7B, $7el_1$, 7E[a]	Xue et al. (2008), Zhang et al. (2011), Hu et al. (2012b)
[a]Newly established polymorphism and mapping (mapping only for MAG1932, BE474428, BE424364, and M7E markers)				
[b]*PLUG* PCR-based landmark unique gene (EST-PCR)				
[c]*SLAF-seq* specific length amplified fragment sequencing				

Marker (s)	Type	Arm	Allele(s)	References
BE474428	EST-SSR	L	7A, 7B, 7D, 7E[a]	Mullan et al. (2005)
BF145935	EST	L	7A, 7D, $7el_1$, 7E[a]	Shen and Ohm (2007), Zhang et al. (2011), Forte et al. (2014)
STSPsy1	STS-CAPS	L	7A, $7el_1$, 7E[a]	Ceoloni et al. (2014b), Forte et al. (2014)
PSR680	STS-RFLP	L	7B, $7el_1$	Xue et al. (2008), Zhang et al. (2011), Forte et al. (2014)
$STSLr19_{130}$	STS	L	$7el_1$	Prins et al. (2001)
$AG15_{601}$	STS	L	$7el_1$	Gennaro et al. (2009)
PSR129	RFLP-CAPS	L	7A, 7B, 7D, $7el_1$	Marais et al. (2001), Forte et al. (2014)
GWM282	SSR	L	7A, 7E	Shen et al. (2004)
BE424364	EST-SSR	L	7A, 7B, 7D, 7E[a]	Mullan et al. (2005)
TNAC1957	PLUG[b]	L	7A, 7B, 7D, $7el_1$, 7E[a]	Ishikawa et al. (2009), Hu et al. (2012a), Forte et al. (2014)
BF200943	EST	L	7B, $7el_1$	Zhang et al. (2011), Forte et al. (2014)
M7E_No. 2, 5, 15, 35, 86	SLAF-seq[c]	L	7E[a]	Chen et al. (2013)

[a]Newly established polymorphism and mapping (mapping only for MAG1932, BE474428, BE424364, and M7E markers)

[b]*PLUG* PCR-based landmark unique gene (EST-PCR)

[c]*SLAF-seq* specific length amplified fragment sequencing

Marker (s)	Type	Arm	Allele(s)	References
GWM333	SSR	L	7B, 7E	Shen et al. (2004)
BARC1075	SSR	L	7D, $7el_1$[a]	Song et al. (2005)
GWM573	SSR	S	7B, 7D, 7E	Shen et al. (2004)
GWM471	SSR	S	7A, 7E	Shen et al. (2004)
[a]Newly established polymorphism and mapping (mapping only for MAG1932, BE474428, BE424364, and M7E markers)				
[b]*PLUG* PCR-based landmark unique gene (EST-PCR)				
[c]*SLAF-seq* specific length amplified fragment sequencing				

This was also the case for the STS-*Psy1*, tagging the *Psy1* (*Phytoene synthase 1*) gene, a likely candidate for the "yellow pigment" phenotype in durum wheat (Pozniak et al. 2007; Singh et al. 2009) and other Triticeae species including *Th. ponticum* (Zhang and Dubcovsky 2008). A PCR assay was previously set up which enabled distinction between *Psy1* 7AL and $7el_1$L alleles (Forte et al. 2014). Using the same primer pair but reducing the annealing temperature from 60 to 56 °C, a 7EL-specific band was amplified (Fig. S3a); however, digestion of amplification products with *DraI* allowed to better discriminate the 7EL from the similarly sized $7el_1$L band and from the other allelic variants (Fig. S3b).

For PCR reactions, DNA was extracted from young leaves or half-kernels according to Dellaporta et al. (1983). Primer pairs and amplification conditions were mainly retrieved from GrainGenes database (http://wheat.pw.usda.gov/GG3/), and, where needed, optimized to maximize marker polymorphism in the specific experimental materials.

Amplified products were separated on 1–2% agarose gel (occasionally on 1.5–2% MetaPhor™ Agarose), visualized by ethidium bromide staining and images captured with Kodak EDAS 290 digital system.

Fusarium inoculation assays and quantification of fungal biomass

Inoculations with *F. graminearum*

Homozygous (HOM+) F_2 or BC_1F_2 plants, representative of 7EL-7el$_1$L recombinant genotypes (based on marker analyses) from [CS7E(7D) × T4] × *T. aestivum* crosses, together with null segregates (HOM−) from each progeny, as well as the CS7E(7D) FHB resistance donor parent and FHB-susceptible bread wheat lines (CS, T4 and Blasco), were employed for single-floret *F. graminearum* inoculation experiments. These were conducted under controlled conditions, with 16 h light/8 h dark photoperiod and 22–24 °C/20 °C corresponding temperature regimes, when plants were at mid-anthesis stage. Five plants per genotype were inoculated by spore injection onto the basal floret of one central spikelet from the tip of the first spike of each plant. The inoculum consisted of 1000 fungal macrospores, freshly grown on synthetic medium (Brown et al. 2010), suspended in 20 µl of sterile distilled water (5×10^4 mL^{-1} concentration) containing 0.05% Tween-20. The inoculum source was a mixture of *F. graminearum* strains, deriving from FHB symptomatic wheat spikes collected in a high-infection site of northern Italy (Emilia-Romagna region). Inoculated spikes were covered with a plastic bag for 2 days to maintain a high relative humidity. Disease symptoms were assessed at ~~7-, 14-, and 21-day~~7, 14 and 21 days post-inoculation (dpi), by calculating the percentage number of visually diseased florets (NDF) out of the total number of florets per spike. Differences in disease severity between genotypes were estimated by means of NDF ± SE (standard error).

Inoculations with *F. culmorum* and *F. pseudograminearum*

Homozygous BC_2F_3 derivatives of one of the 7EL-7el$_1$L FHB resistant recombinants (see “Results”), and Blasco as a

control were used in two independent infection experiments (replicas) performed with both *F. culmorum* and *F. pseudograminearum*. Twenty plants per genotype were included in each experiment. Seeds were surface sterilized by immersion in sodium hypochlorite (0.5% vol/vol) for 20 min and then rinsed thoroughly in sterile water. Seedlings were individually grown in 5 × 5 × 5-cm pots, arranged in plastic trays, at 23 °C, with a 14-h light period. Macroconidia of *F. culmorum* strain UK99 and *F. pseudograminearum* strain CBS 109956 were produced by culturing the fungi on Petri dishes containing SNA medium or potato–carrot agar medium (ATCC 335), respectively, and were harvested by washing the culture surface with 2 mL of sterile water. Conidia concentration was adjusted to 2×10^6 mL^{-1} for *F. culmorum* (Beccari et al. 2011) and 10^6 mL^{-1} for *F. pseudograminearum* (Mitter et al. 2006). Tween 20 was added to a final concentration of 0.05% to aid inoculum adhesion to the plant tissue. For inoculation, the method described by Mitter et al. (2006) was used with some modifications. The seedling stem base leaf sheath of recombinant and control genotypes at the first-leaf stage (Zadoks stage 11), about 10 days after emergence, were inoculated with 20 µL of conidia suspension, evenly spread with the help of a small paintbrush. Plots were covered with a plastic film for 2 days to maintain high relative humidity.

Fusarium crown rot (FCR) disease symptoms were assessed at ~~7- and 14-day~~7 and 14 days post-inoculation (dpi) using two parameters: symptom extension (SE; cm) and browning index (BI) of the infected tissue (visual rating of the degree of extension of necrosis, as indicated by brown discoloration), both based on a five-point scale (0, symptomless; 1, slightly necrotic; 2, moderately necrotic; 3, severely necrotic; 4, completely necrotic). Disease index (DI) was subsequently determined as SE × BI (Beccari et al. 2011).

Infection data were subjected to analysis of variance (ANOVA) by using SYSTAT12 software (Systat Software Incorporated, San Jose, CA, USA). The DI variable was entered as a ~~‘~~"dependent~~’~~" factor against ~~‘~~"independent~~’~~" factors, represented by genotype (G) and replica (R). The G × R interaction was also analysed. Three levels of significance ($P < 0.05$, $P < 0.01$, and $P < 0.001$) were considered to assess significance of the F values.

F. graminearum biomass assay

As previously described (Moscetti et al. 2013; Forte et al. 2014), a real-time qPCR assay for the *TRI6* gene, highly conserved among trichothecene-producing *Fusarium* spp. and positively correlated with DON accumulation in wheat grains (Horevaj et al. 2011), was performed to quantify *F. graminearum* biomass. Briefly, total DNA was extracted with DNeasy plant mini kit (Qiagen) from whole flour obtained by milling dry kernels of resistant and susceptible genotypes, harvested at the end of *F. graminearum* infection experiments. qPCR experiments were performed as described in Moscetti et al. (2013), using *ACTIN* as the housekeeping gene. The relative expression of susceptible (taken as 1) vs. resistant genotypes was determined by using the ~~2~~$^{-\Delta\Delta CT}$$2^{-\Delta\Delta C_T}$ method (Livak and Schmittgen 2001). Calculations were performed by Gene Expression Macro Version 1.1 (Bio-Rad Laboratories). Two biological and four technical replicates per genotype were statistically analysed by one-way ANOVA. Three levels of significance were considered, corresponding to $P < 0.01$, $P < 0.05$, and $P < 0.001$. When significant ~~F~~*F* values were observed, a pairwise analysis was carried out by the Tukey honestly–significant–difference test (Tukey test) at 0.99 confidence level.

Pigment content

Yellow (carotenoid) pigment content (YPC) of recombinant and control lines was determined following the AACC International Method 14-50.01 (AACC International 2013) with some modifications, mainly corresponding to reduction of sample size and of solvent volume (see, e.g., Beleggia et al. 2010). Briefly, total pigments were extracted from 0.5 g of whole grain flour samples using water-saturated 1-butanol. Spectrophotometric measurements of pigment contents, using β-carotene as the reference (absorbance reading at 435.8 nm), were then converted to p.p.m. of dry matter (μg/g). For each genotype, final values corresponded to averages of duplicate analyses. Most grain samples derived from plants grown under the same, controlled conditions. For one specific recombinant line, also grown under field conditions, a control genotype grown under such conditions and in the same season was

employed for a correct comparison.

Agronomic evaluation

BC_1F_3 plants from the cross of one recombinant line, R74-10 with bread wheat Blasco, as well as Blasco plants, were field grown under common cultural practices for 1 year and in one locality (Viterbo, Central Italy). Plants were organized in duplicate rows (1 m long), at a 25-cm distance between rows and 10-cm distance along the row. Data were collected for plant height (PH), days to heading (DTH), spike number/plant (SNP), grain yield/plant (GYP), and spike traits, including grain number/spike (GNS), spikelet number/spike (SPS), grain number/spikelet (GNSP), 1000 grain weight (TGW), and grain yield/spike (GYS). Values recorded on 20 plants of R74-10 and of Blasco were used for one-way ANOVA. Each variable (i.e., trait measured) was entered as a "dependent" factor against genotype as "independent" factor. Three levels of significance ($P < 0.05$, $P < 0.01$, and $P < 0.001$) were considered. To confirm the efficacy of *Lr19*-based resistance to leaf rust, visual rating of disease severity, based on the modified Cobb scale (Peterson et al. 1948), was performed on R74-10 and Blasco plants, exposed to natural, high-infection pressure. Under the same conditions, other recombinant types besides R74-10 were also evaluated for this trait, but not for the yield-related traits described above, given the limited number and variability of plants available (early stage of backcrossing process to adapted cultivars).

Results

Meiotic pairing and isolation of 7EL-7el$_1$L bread wheat recombinants

To evaluate the pairing ability of *Th. elongatum* 7EL and *Th. ponticum* 7el$_1$L chromosome arms, and estimate the frequency of 7EL/7el$_1$L recombinant types, meiotic metaphase I pairing was analysed in pollen mother cells (PMCs) of [CS7E(7D) × T4] F_1 plants. Detection of 21 bivalents in contrast to 20 bivalents + 2 univalents, already feasible in

freshly stained PMCs, was indicative of pairing vs. non-pairing of the two critical chromosomes, i.e., the complete 7E and the 7DS·7DL-7el$_1$L translocated chromosome of line T4. However, it was from fluorescent GISH preparations that a precise calculation of their pairing frequency could be accomplished (Fig. 1). As expected in the absence of any pairing-promoting genetic condition, pairing between the two chromosomes invariably involved their long (L) arms only, with 7EL-7el$_1$L closely homoeologous portions being more frequently bound by a distal (Fig. 1b) than by a more proximal (Fig. 1c) chiasma. Twenty-two (19.6%) out of the one hundred and twelve PMCs analysed (pooled from 3 F_1 plants) showed the 7E/7DS·7DL-7el$_1$L rod bivalent labelled by the fluorescent *Thinopyrum* DNA on most of its length (except for the 7DS and part of the 7DL of the T4 chromosome, see Fig. 1b, c), while the remaining 80.4% exhibited the two critical pairing partners as univalents (Fig. 1d) . Please, insert Fig. 1 after this paragraph

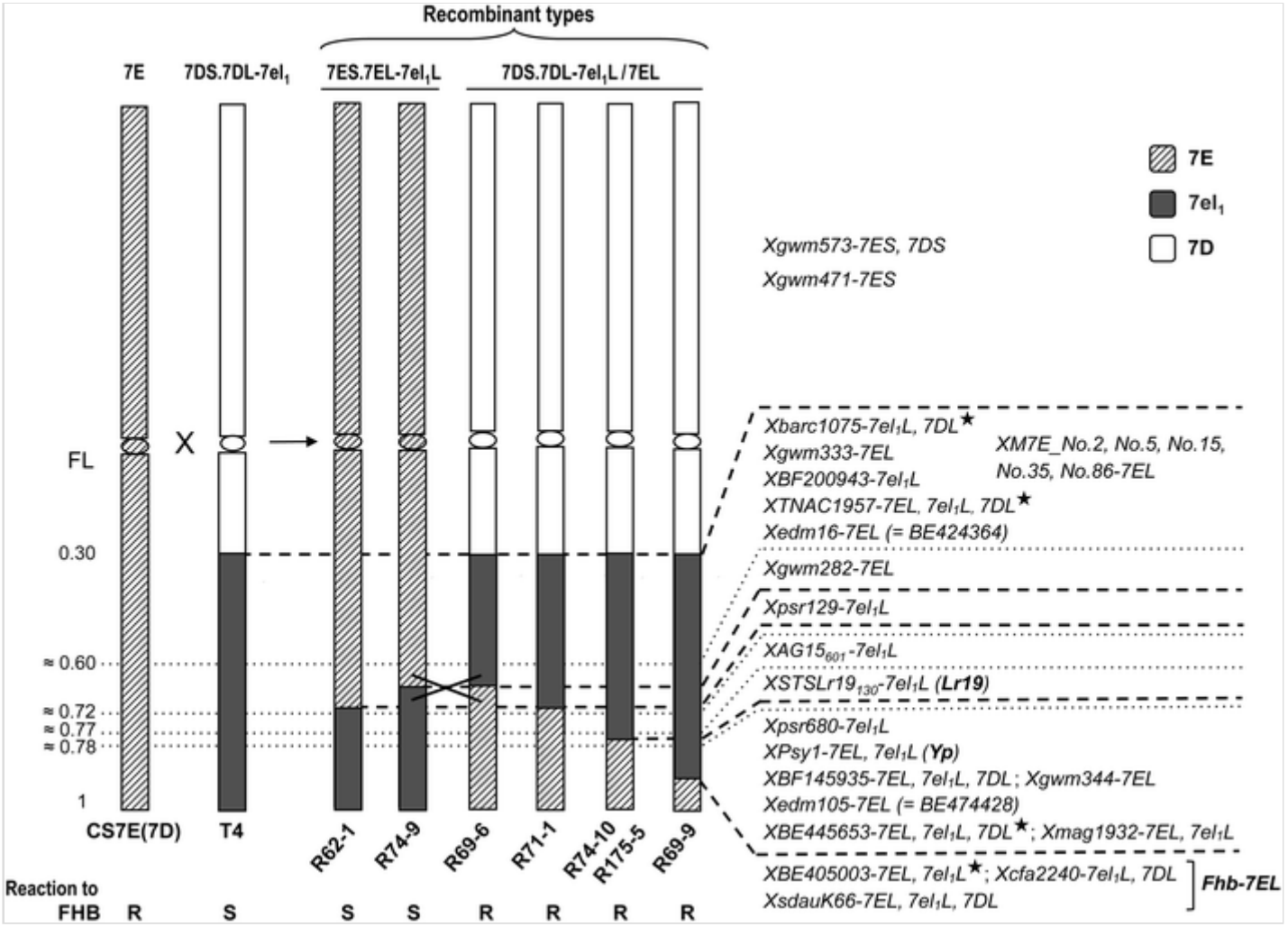
Suitable polymorphic markers allowed genotyping of the cross progeny of [CS7E(7D) × T4] F_1s with normal bread wheat cultivars (used as pollen parents). In line with the observed pairing frequency, occurrence of 7E − 7el$_1$ recombinants totalled 10.6% (Fig. 1). Of these, 5 (7.6%) were of the T4 chromosome type (70% *Thinopyrum* chromatin on 7DL), while 2 (3%) had a complete *Thinopyrum* chromosome, as shown by different alleles at marker loci on the short arm (*Xgwm573*, *Xbarc184*), and in the most proximal fraction of the 70% *Thinopyrum* segment of the long arm (e.g., *Xbarc1075*, *Xgwm333*, *XTNAC1957*, *XBE424364*, see Fig. 2). Confirmation of the different chromosome types was obtained by GISH analysis. 7EL/7el$_1$L polymorphism for more distally located marker loci allowed identification of recombinant types and definition of their respective 7EL-7el$_1$L genetic breakpoints (BPs). An approximate physical location of the various BPs was inferred from 7AL/7el$_1$L/7el$_2$L and 7DL/7el$_1$L/7el$_2$L cytogenetic maps previously produced (Ceoloni et al. 2014a, b; Forte et al. 2014), sharing several markers with current 7DL/7EL/7el$_1$L maps. Based on this, recombinant chromosomes of the 7E type, named R74-9 and R62-1, had their BPs between FL (fractional arm length) 0.60 and 0.72 (Fig. 2). Marker order in R74-9, which had the largest

7el$_1$L piece inserted into 7EL, was suggestive of a paracentric inversion, involving marker loci *Xpsr129* and *Xgwm282*: on 7el$_1$L, the former locus appears to be more distal than the latter (see recombinant types R71-1 vs. R69-6), whereas the *Xpsr129* locus maps in the 7el$_1$L most distal portion of R74-9, and the *Xgwm282* locus results to reside in the proximally adjacent 7EL stretch (Fig. 2). Of the T4-type recombinants, R69-6 and R71-1, at least on the basis of the resolution allowed by the present maps, had an opposite distribution of 7EL and 7el$_1$L chromatin in their distal portions as compared to that of R74-9 and R62-1, respectively. Both R69-6 and R71-1 lacked the ~~STS*Lr19*$_{130}$ marker~~*XSTSLr19*$_{130}$ marker locus (Fig. S2), closely linked to the leaf rust resistance gene (Prins et al. 2001), which was instead present in the remaining three recombinant chromosomes, i.e., R74-10, R175-5, and R69-9 (Fig. 2). The first two had coinciding BPs located proximal to the *Psy1* locus, at which they possessed a 7EL allele (Fig. S3). In both lines, the presence of the *Sr25* stem rust resistance gene from 7el$_1$L could be excluded. This conclusion follows from the previous evidence indicating a mutant T4 (=Agatha) line, i.e., Agatha 235-6, with a shorter 7el$_1$L segment than that of the original T4 (Friebe et al. 1994), to possess a *Psy1-7el$_1$L* allele (Zhang and Dubcovsky 2008), while lacking *Sr25* (Knott 1984). Based on this, gene order in this 7el$_1$L region (previously undefined) was proved to be: centromere — Can this and the following two dashes be reduced in length? *Lr19—Psy1 (Yp)—Sr25*. Dissociation between 7el$_1$L and 7EL alleles occurred even more distally for the R69-9 recombinant, which had the smallest 7EL portion of all, including only marker loci *XBE405003*, *XsdauK66,* and *Xcfa2240* (Fig. 2; Fig. S1). As confirmed by STS or closely linked markers, this line possessed 7el$_1$L genes *Lr19* and *Yp* (*Psy-7el$_1$L*, Fig. S3), while the presence/absence of *Sr25* could not be ascertained on the basis of mapping data. Not only the *XBF145935* marker locus, loosely associated with *Sr25* in a previous study (Liu et al. 2010), but also *XBE445653* and even the most distal *XBE405003* (Fig. 2) were missing in Agatha mutant 235-6 (Fig. S4), preventing a reliable marker-*Sr25* association.

Fig. 2

Cytogenetic maps of group-7 chromosomes of parental and recombinant genotypes identified in the cross progeny of [CS7E(7D) × T4] F$_1$s with normal bread wheat cultivars, based on molecular marker analysis and GISH. Order of

marker loci is based on the largest consensus among published maps (see “Materials and methods”). Critical alleles are indicated for each marker. Some codominant and particularly user-friendly markers are starred. *FL* fractional arm length. 7EL-7el$_1$L and 7DL-7el$_1$L BPs are indicated by *dashed thick lines*. *Dotted lines* indicate the approximate physical position (FL) of 7EL-7el$_1$L BPs of the various recombinants, based on cytogenetic maps of group-7 recombinant chromosomes previously produced (see text). *Crossed solid lines* indicate a probable inversion, involving marker loci *Xpsr129* and *Xgwm282*, interrupting gross collinearity between 7EL and 7el$_1$L



As an expected consequence of the over 80% frequency of PMC's with unpaired 7E and 7D/7el$_1$ chromosomes (Fig. 1), non-recombinant types represented nearly 90% of the cross progeny of [CS7E(7D) × T4] × normal bread

wheat. Of these, around 50% contained either a complete 7E (25.8%) or a 7D/7el$_1$ (T4 type; 24.2%) in addition to a normal 7D from the wheat pollen parent. The remaining progeny was indicative of abnormal segregation of the two critical chromosomes, leading to aneuploid and/or misdivision types. In the majority of the cases (21.2%), both 7E and the T4 7D/7el$_1$ were evidently lost in the course of meiosis and absent in female gametes which, upon pollination with normal bread wheat (CS or Blasco), gave rise to $2n = 41$ plants. An additional 9.1% of the progeny represented the reciprocal type, i.e., plants with $2n = 43$, shown by marker and GISH analyses to carry both 7E and 7D/7el$_1$, besides a normal 7D from the wheat parent. Finally, in a further 9.1% of progeny (6 plants), the unpaired chromosomes underwent misdivision events, which gave rise to either telocentric chromosomes (3 out of the 6 total cases) or centric break-fusion (CBF) products (the remaining 3 individuals), as clearly shown by GISH (Fig. S5). Molecular marker analysis indicated the telocentric chromosome to correspond to the 7ES arm in two cases (Fig. S5a) and the 7EL arm in the third one; likewise, 7EL was involved in one CBF-derived chromosome (Fig. S5b), while 7ES was involved in the other two such types.

Reaction to inoculation of different *Fusarium* species and mapping of the resistance QTL on 7EL

Reaction to *F. graminearum* inoculation

Having no prior knowledge of the location along the 7EL arm of the major QTL determining FHB resistance, all recombinant lines except R69-6 were subjected to *F. graminearum* inoculation. Being altogether representatives of an array of different 7EL-7el$_1$L breakpoint positions, the resulting reaction to the fungal infection was expected to indicate with good approximation the position of the resistance locus along 7EL. Inoculation of CS7E(7D), used as a resistant control line, confirmed its excellent resistance, with only 3.6% of diseased florets at 21 dpi, with most plants showing a small lesion only on the inoculated floret and, at the most, one adjacent floret of the same spikelet (Fig. 3a); moreover, disease progress was very minor through the monitored time points (Table 2) and beyond (not

shown). By contrast, susceptible control lines, including normal CS, line T4, and Blasco, all exhibited heavy symptoms of fungal spreading along the infected spike, which peaked at 100% of diseased florets in some plants, mainly of Blasco at 21 dpi (Fig. 3e). While the latter and T4 averaged 86–87% of diseased florets, CS infection was less severe, averaging 75.5% (Table 2; Fig. 3b). This result is in line with the previous observations (Shen et al. 2004; Fu et al. 2012; Fedak et al. 2015), confirming the probable existence in CS of minor FHB resistance QTL (Shen et al. 2004 and references therein). Consistent with the lower susceptibility of CS compared to T4 and Blasco, F_2 null segregates (HOM−) of the ~~7E – 7el$_1$~~7E-7el$_1$ recombinants R69-9 (Fig. 3d), R175-5, R71-1, and R62-1, whose CS7E(7D) × T4 F_1s had been all crossed to CS, showed percentages of diseased florets ranging from 48.8 to 70%; conversely, R74-10 null segregates, deriving from BC_1 to Blasco, showed a 95.3% mean score.

Fig. 3

Phenotypes of *F. graminearum* inoculated spikes (21 dpi) of bread wheat 7EL-7el$_1$L recombinant lines and control lines: **a** CS7E(7D) substitution line; **b** CS; **c** resistant recombinant line R69-9 HOM+; **d** R69-9 HOM−; **e** cv. Blasco. *Arrows* point at the inoculated floret, which essentially remained the only visible site of fungal presence, in both the donor line of the FHB QTL on 7EL (**a**) and the 7EL-7el$_1$L recombinant line (**c**)

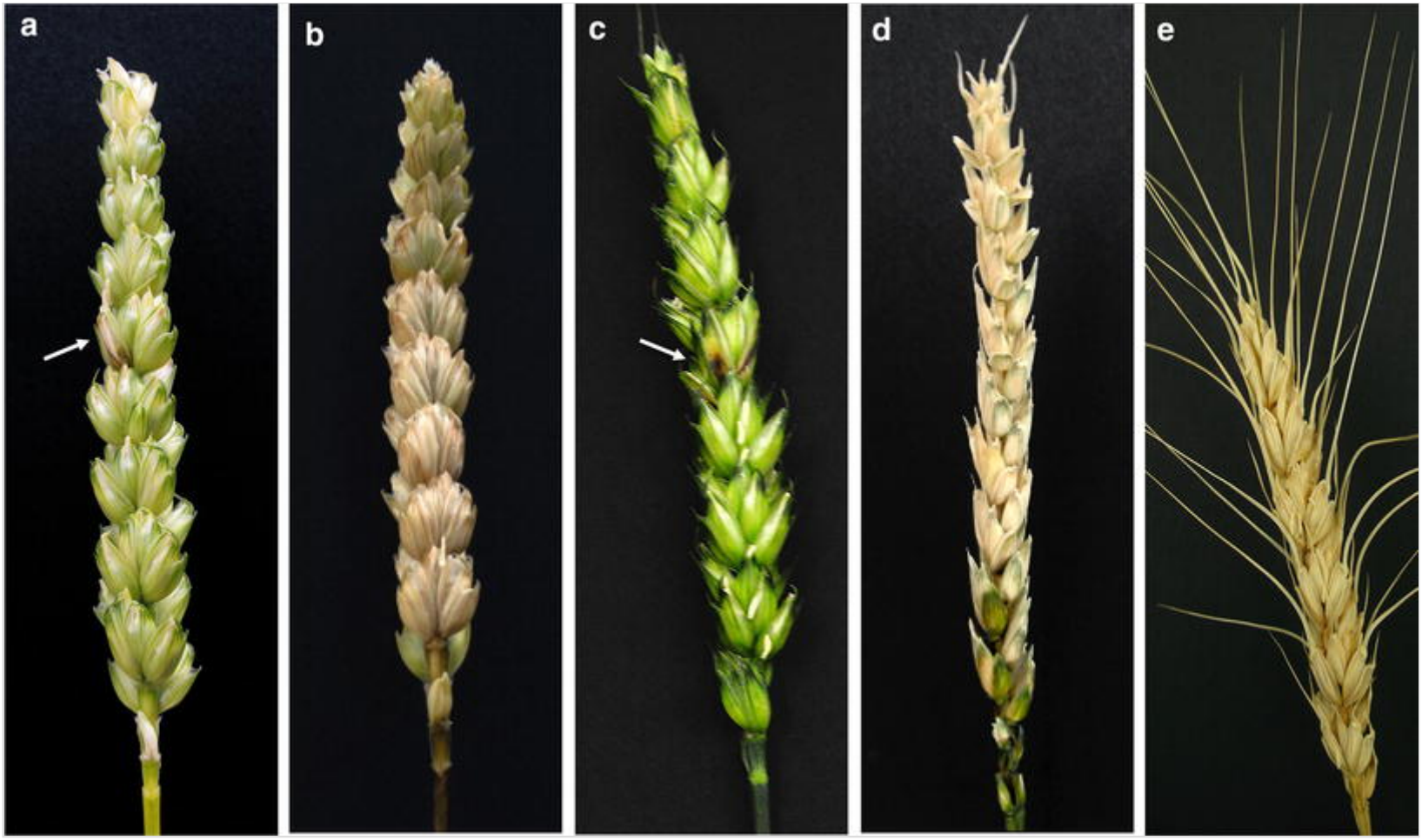

a
b
c
d
e

Table 2 In this (Table 2) as well as in Table 4, the pairs of acronyms, i.e. HOM+ and HOM-, each pair being associated to the number/symbol (e.g. R69-9, R175-5, etc.) of the first column, should be put in a separate column (as in the original manuscript). If this is not possible, each acronym pair should be given more indentation toward the right, to mimic the original table format (please, have a look at it!!)

Means and standard errors (SE) of percentages of diseased florets at ~~7 , 14 , and 21 day~~7, 14 and 21 days post-*F. graminearum* inoculation (dpi) in bread wheat 7EL-7el$_1$L F_2 homozygous recombinant types (HOM+)[a], in corresponding null segregates (HOM−)[a], and in control lines, i.e., CS, CS7E(7D), T4, and Blasco. Allelic variants at the *XBE405003* and *XBE445653* loci are indicated.

Line	*XBE405003* allele	*XBE445653* allele	No. diseased florets (% mean ± SE)		
			7 dpi	14 dpi	21 dpi
R69-9					
HOM+	7E	7el$_1$	2.0 ± 0.0	4.0 ± 2.0	4.9 ± 1.1
HOM−	7D	7D	9.9 ± 2.4	20.4 ± 2.6	48.8 ± 10.0
R175-5					
HOM+	7E	7E	2.2 ± 0.2	4.5 ± 0.3	5.5 ± 0.7
HOM−	7D	7D	–	–	–
R74-10					

[a] HOM+ and HOM-inoculated plants were F_2 segregates from the cross CS7E(7D)/T4//CS for all recombinant types, except for R74-9 [CS7E(7D)/T4//Blasco] and R74-10 [CS7E(7D)/T4]/2*Blasco

~~Allelic variants at the *XBE405003* and *XBE445653* loci are indicated~~

Line	*XBE405003* allele	*XBE445653* allele	No. diseased florets (% mean ± SE)		
			7 dpi	14 dpi	21 dpi
HOM+	7E	7E	3.9 ± 0.7	4.3 ± 0.6	4.3 ± 0.6
HOM−	7D	7D	14.7 ± 1.9	62.9 ± 1.4	95.3 ± 0.1
R71-1					
HOM+	7E	7E	1.8 ± 0.9	3.1 ± 1.4	4.2 ± 1.1
HOM−	7D	7D	10.0 ± 2.5	32.2 ± 8.9	70.0 ± 4.7
R62-1					
HOM+	$7el_1$	$7el_1$	10.6 ± 1.5	20.9 ± 5.0	62.0 ± 15.4
HOM−	7D	7D	5.9 ± 0.1	38.4 ± 11.6	53.8 ± 13.8
R74-9					
HOM+	$7el_1$	$7el_1$	15.5 ± 3.4	42.3 ± 3.1	91.7 ± 8.3
HOM−	7D	7D	–	–	–
CS 7E(7D)	7E	7E	2.0 ± 0.4	3.0 ± 0.7	3.6 ± 0.7
CS	7D	7D	8.8 ± 3.4	36.1 ± 8.8	75.5 ± 7.7

[a] HOM+ and HOM-inoculated plants were F_2 segregates from the cross CS7E(7D)/T4//CS for all recombinant types, except for R74-9 [CS7E(7D)/T4//Blasco] and R74-10 [CS7E(7D)/T4]/2*Blasco

~~Allelic variants at the *XBE405003* and *XBE445653* loci are indicated~~

Line	*XBE405003* allele	*XBE445653* allele	No. diseased florets (% mean ± SE)		
			7 dpi	14 dpi	21 dpi
T4	$7el_1$	$7el_1$	7.7 ± 0.5	41.9 ± 7.3	87.1 ± 3.2
Blasco	7D	7D	9.7 ± 1.3	37.9 ± 5.3	86.0 ± 5.3

[a] HOM+ and HOM-inoculated plants were F_2 segregates from the cross CS7E(7D)/T4//CS for all recombinant types, except for R74-9 [CS7E(7D)/T4//Blasco] and R74-10 [CS7E(7D)/T4]/2*Blasco

~~Allelic variants at the *XBE405003* and *XBE445653* loci are indicated~~

Disease scores of F_2 HOM+ recombinant lines gave clear results: lines R62-1 and R74-9 were both evidently FHB susceptible, albeit disease scores differed in relation to the respective background. HOM-+ plants of the former, having a prevailingly CS background, (see footnote to Table 2), did not differ significantly from corresponding HOM− segregates, and their disease severity at 21 dpi was in the range of 50–60% (Table 2). HOM+ plants of R74-9 exhibited a much more severe disease incidence (91.7%), probably ascribable to the presence of the highly susceptible Blasco in its genealogy (Table 2). By contrast, lines R69-9, R175-5, R71-1, and R74-10, irrespective of the variable background, exhibited a high degree of resistance, corresponding to about 95% reduction of FHB severity (from 4.2 to 5.5% of diseased florets), of the same order of magnitude of the CS7E(7D) donor line (Table 2; Fig. 3c).

Correlating disease scores to mapping data, it was evident that the resistance was associated with the most distal markers, in particular to presence of a 7E allele at the *XBE405003* and *Xsdauk66* loci (Figs. S1a, b) and absence of the $7el_1$ allele at the *Xcfa2240* locus. This allelic makeup, followed by either $7el_1$ or 7E alleles at proximally adjacent

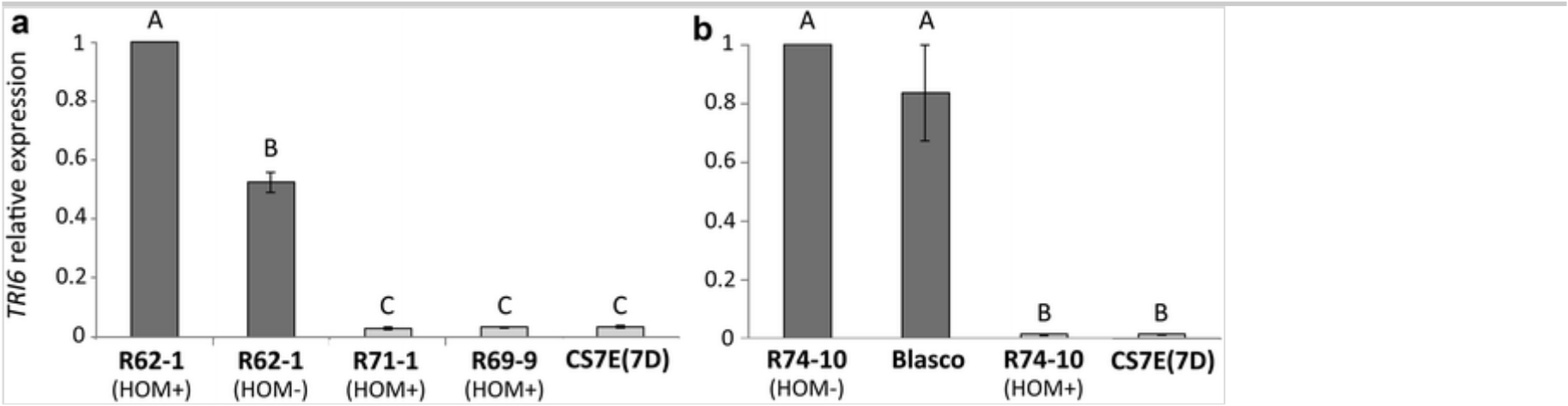
loci (e.g., *XBE445653*, *Xmag1932*, see Fig. 2 and Figs. S1c), was in all cases associated with the same resistant phenotype. On this basis, the major FHB resistance QTL can be allocated to the most distal end of the 7EL arm. Among the recombinant lines, R69-9 possesses the smallest 7EL segment still including the FHB resistance QTL (Fig. 2), in association with the three marker loci (Fig. S1). Notably, *Xcfa2240* and *Xsdauk66* loci are also closely linked to *Fhb7* on *Th. ponticum* $7el_2L$ arm (Guo et al. 2015).

Real-time qPCR on DNA extracted from kernels of recombinant and control lines fully confirmed the results of visual assessment of head blight. Estimates of fungal biomass, based on *Fusarium TRI6* transcript levels, not only showed the same, overall sharp difference between resistant and susceptible genotypes as resulted from single-floret inoculation, but also enabled distinction between the relative effect of the same 7EL FHB resistance QTL in different genetic backgrounds. When in fully [CS7E(7D) substitution line] or prevailingly CS background, as in lines R71-1 and R69-9 (HOM-+ F_2's from CS7E(7D)/T4//CS), fungal biomass was reduced by 97% with respect to R62-1 HOM+ (same background but FHB susceptible), carrying a $7el_1L$ allele(s) at the critical locus/i (see Fig. 2). When a 7DL allele(s) is present at this locus/i, as in the null segregate (HOM−) of R62-1, *TRI6* expression is about half that of R62-1 HOM+ (Fig. 4a), exactly corresponding to the inoculation assay (see above). When compared to susceptible segregates (HOM−) of R74-10 and to Blasco, the R74-10 recombinant (HOM + from [CS7E(7D)/T4]/2*Blasco BC_1F_2) and the CS7E(7D) FHB resistant donor line exhibited over 99% reduction of *TRI6* relative expression and hence fungal biomass (Fig. 4b).

Fig. 4 Can you please leave MORE SPACE between the two graphs (section **a** and section **b**) of the figure? Also, can you put the letters **a** and **b** more distant (vertically) from the edge of the graphs?

Fungal biomass evaluation, based on qPCR assay of relative expression of *Fusarium TRI6* gene, in kernels of infected spikes of bread wheat ~~7E – $7el_1$~~ 7E-$7el_1$ homozygous recombinant and control lines: R62-1, R71-1, and R69-9 (**a**) have a prevailingly CS background [CS7E(7D)/T4//CS], while R74-10 (**b**) has a CS7E(7D)/T4//Blasco genealogy. HOM− sibs

of R62-1 and R74-10 are homozygous F_2 segregates for a normal 7D. The CS7E(7D) substitution line was included in both data sets as the FHB resistance donor. *Letters above histograms* correspond to ranking of Tukey test at $P < 0.01$



Reaction to *F. culmorum* and *F. pseudograminearum* inoculations

In infected seedlings of the R74-10 FHB resistant recombinant and Blasco, FCR disease progress was monitored daily, and already at day 3, clear symptoms on the stem base leaf sheaths, with necrotic lesions typical of FCR, were observed on Blasco plantlets. When disease spreading was scored at 7 dpi, markedly different symptoms were found to be associated with the presence/absence of the 7EL introgression in materials inoculated with both *Fusarium* species (Table 3). The majority of Blasco plantlets had extended brownish necrotic lesions at the stem base leaf sheaths (Fig. S6b), corresponding to a mean disease index (DI) of 5 and 3.11 across the two experiments with *F. culmorum* and *F. pseudograminearum*, respectively. Conversely, the recombinant genotype had a highly significant 75-80% reduction in disease severity (DI of 1.03 and 0.77 for the two pathogens, respectively), with only a few plants showing visual symptoms (Fig. S6a). At 14 dpi, when the final evaluation was carried out, infection of both

Fusarium species had progressed in recombinant and control plants, and more in the case of *F. pseudograminearum* than *F. culmorum*, but the difference in DI between the two sets of plants remained of the same order of magnitude (Table 3). Beyond the 14th day after inoculation, Blasco seedlings tended to rapidly wither, all eventually approaching the maximum DI scores, while in R74-10 individuals, symptom intensification was minor, if any, and in all cases, discrimination between genotypes continued to be obvious (not shown).

Table 3

Disease indexes (DI) detected in two independent infection experiments with *F. culmorum* and *F. pseudograminearum* of BC_1F_3 R74-10/Blasco homozygous 7EL-7el$_1$L recombinant plants and Blasco plants, and *P* values of the ANOVA analyses

Line	**DI (mean ± SE)**			
	F. culmorum		***F. pseudograminearum***	
	7 dpi	**14 dpi**	**7 dpi**	**14 dpi**
R74-10	1.03 ± 0.4	1.18 ± 0.4	0.77 ± 0.3	3.08 ± 0.5
Blasco	5.00 ± 0.8	6.14 ± 0.9	3.11 ± 0.6	10.21 ± 1.1
P genotype (G)	0.000***[a]	0.000***	0.000***	0.000***
P replica (R)	0.91	0.93	0.74	0.29
P G × R	0.38	0.38	0.27	0.79

[a] *** Indicates significant *F* values at $P < 0.001$

Analysis of yellow pigment content expressed by ~~7E – 7el$_1$~~ 7E-7el$_1$ recombinant lines

Spectrophotometric determination of yellow pigment content (YPC) of ~~7E = 7el$_1$~~7E-7el$_1$ recombinants and of control lines confirmed the strong association between the *Psy1* genotype and the YPC phenotype. Furthermore, a clear picture of the relative effect of the different *Psy1* alleles (either 7EL or 7el$_1$L, see Fig. S3) on YPC could be delineated (Table 4). All bread wheat lines involved in the background of recombinant lines, i.e. CS, Thatcher and Blasco, showed similar, low YPC values, ranging from 2.46 in Blasco #2 (plants grown under field conditions), to a maximum of 3.45 in CS. YPC increased when *Thinopyrum Psy1* alleles were substituted for the 7DL allele of wheat. Introgression of a *Th. ponticum* segment containing the *Psy1-7el$_1$L* allele (see Fig. 2) caused a conspicuous YPC increase in recombinant lines R62-1 (+123%) and R69-9 (+177%) vs. their HOM− controls, although not as high as that of T4 (7el$_1$L) and KS24-1 (7DS·7el$_2$L translocation line) vs. their Thatcher control (+259 and ~~242~~+242%, respectively; Table 4). By contrast, the presence of *Th. elongatum* chromatin at the *Psy1* locus corresponded to a lower YPC increase in lines R71-1 (+91%), R175-5 (+57%) and R74-10 (+61%). Seeds of homozygous F$_3$ field-grown plants of R74-10, deriving from BC$_1$ to Blasco, showed an even lower YPC increase (+26%) as compared to Blasco #2 (Table 4). The highest increase associated with the presence of the *Psy1-7EL* allele was exerted by the CS7E(7D) substitution line vs. normal CS (+141%), possibly indicating the existence of more than one QTL affecting YPC along 7E or 7EL, as suggested for 7el$_1$L as well (Ceoloni et al. 2000; Zhang and Dubcovsky 2008).

Table 4 In this (Table 4) as well as in Table 2, the pairs of acronyms, i.e. HOM+ and HOM-, each pair being associated to the number/symbol (e.g. R62-1, R69-9, etc.) of the first column, should be put in a separate column (as in the original manuscript). If this is not possible, each acronym pair should be given more indentation toward the right, to mimic the original table format (please, have a look at it!!)

Yellow pigment content (YPC) of 7E-7el$_1$ recombinant (R) lines (HOM+), carrying a different *Thinopyrum* allele at the *Psy1* locus, of sib lines lacking any *Thinopyrum* segment (HOM−), as well as of additional genotypes, including cross parents of R types, i.e., CS, CS7E(7D), T4 and Blasco, the KS24-1 bread wheat-*Th. ponticum* 7DS·7el$_2$L centric translocation line, and Thatcher (prevailing background of lines T4 and KS24-1)

Line[a]	**Generation**	**Background genotype**	***Thinopyrum Psy1* allele**	**YPC**	**Increment (%) of YPC vs. control** [b]
R62-1					
HOM+	F_2	CS^2/T4	7el$_1$L	8.38	123
HOM−			–	3.76	
R69-9					
HOM+	F_2	CS^2/T4	7el$_1$L	9.68	177
HOM−			–	3.49	
R71-1					
HOM+	F_2	CS^2/T4	7EL	7.62	91

[a]Except for the field-grown R74-10 (BC_1F_3) and Blasco #2, all other lines were grown under the same controlled conditions

[b]Except where differently indicated, increment of YPC values of HOM+ lines is relative to the corresponding HOM− sibs

Line[a]	**Generation**	**Background genotype**	***Thinopyrum Psy1* allele**	**YPC**	**Increment (%) of YPC vs. control** [b]
HOM−			–	3.98	
R175-5					
HOM+	F_2	CS[2]/T4	7EL	8.16	57
HOM−			–	5.19	
R74-10					
HOM+	BC_2F_3	CS/T4/Blasco[3]	7EL	5.86	61
HOM−			–	3.64	
R74-10 (field)					
HOM+	BC_1F_3	CS/T4/Blasco[2]	7EL	3.09	26 (vs. Blasco #2)
CS7E(7D)		CS	7EL	8.30	141 (vs. CS)
T4		Thatcher	$7el_1L$	11.84	259 (vs. Thatcher)
KS24-1		Thatcher	$7el_2L$	11.28	242 (vs. Thatcher)
Thatcher			–	3.30	
Blasco #1			–	3.10	

[a]Except for the field-grown R74-10 (BC_1F_3) and Blasco #2, all other lines were grown under the same controlled conditions

[b]Except where differently indicated, increment of YPC values of HOM+ lines is relative to the corresponding HOM− sibs

Line[a]	Generation	Background genotype	*Thinopyrum Psy1* allele	YPC	Increment (%) of YPC vs. control [b]
Blasco #2 (field)			–	2.46	
[a]Except for the field-grown R74-10 (BC_1F_3) and Blasco #2, all other lines were grown under the same controlled conditions					
[b]Except where differently indicated, increment of YPC values of HOM+ lines is relative to the corresponding HOM− sibs					

Impact of a 7EL + $7el_1L$ introgression on agronomic performance

Among the newly obtained bread wheat recombinant lines, R74-10 (and also R175-5, based on mapping data, Fig. 2) showed an appealing allelic combination for genes/QTL from the two *Thinopyrum* sources. It contained *Lr19* and yield-related loci from *Th. ponticum*, as well as *Psy1-7EL* and the QTL for FHB and FCR resistance from *Th. elongatum*. R74-10 was the first 7EL-$7el_1L$ recombinant line to be backcrossed to the adapted variety Blasco. As a preliminary assessment of behaviour under field conditions, homozygous BC_1F_3 R74-10/Blasco plants were grown for 1 year and in one location (Viterbo, Central Italy) alongside Blasco plants, and yield-related traits were evaluated (Table 5). Despite the still limited contribution of the adapted cultivar to the recombinant genotype, the latter had an overall phenotype closely resembling that of Blasco. There were no significant differences between the variety and the recombinant line for most traits and improved values for several of them (Table 5). Only TGW was 4.7% lower in R74-10 plants; however, because of the concomitant increase of spike number/plant (4.4%) and grain number/spike (5.6%) in the recombinant plants, their mean grain yield/plant was 7.8% higher than that of Blasco.

Table 5

Mean values, standard error (SE), ANOVA probability (*P*) values, and percentage (%) differences of field-grown homozygous recombinant (BC_1F_3 R74-10/Blasco derivatives) vs. Blasco plants for yield-related traits

Trait[a]	R74-10		Blasco		ANOVA	% Difference
	Mean	SE	Mean	SE	*P* value[b]	vs. Blasco
SNP	7.1	0.34	6.8	0.76	0.63	+4.4
GNS	32.1	1.07	30.4	1.62	0.37	+5.6
SPS	17.3	0.25	16.1	0.33	0.005**	+7.5
GNSP	2.1	0.09	2.1	0.06	0.93	0
TGW	42.3	0.65	44.3	0.51	0.03*	−4.7
GYS	1.4	0.05	1.4	0.08	0.92	0
GYP	9.7	0.55	9.0	1.02	0.54	+7.8
PH	75.8	1.12	72.6	0.50	0.03*	+4.4
DTH	146.3	0.33	145.6	0.19	0.11	+0.5

[a] *SNP* spike number/plant, *GNS* grain number/spike, *SPS* spikelet number/spike, *GNSP* grain number/spikelet, *TGW* thousand grain weight, *GYS* grain yield/spike, *GYP* grain yield/plant, *PH* plant height, *DTH* days to heading

[b] *, ** Indicate significant *F* values at $P < 0.05$ and < 0.01, respectively

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The R74-10 plants showed complete resistance to natural infection by leaf rust pathogen, whose epidemics recurrently occur throughout Italy and the Mediterranean region, thereby proving the maintenance of the *Lr19* gene efficacy in these environments. By contrast, Blasco plants, grown in adjacent rows, were rated as highly susceptible, averaging 80% in disease severity, based on the modified Cobb scale (Peterson et al. 1948). The same, sharp difference (Fig. 5) was observed in HOM-+ plants of other recombinant lines, previously proved to carry the $XSTSLr19_{130}$ locus, as compared to corresponding HOM− plants or to recombinant types lacking this *Lr19* tightly linked marker (Fig. 2; Fig. S2).

Fig. 5

Reaction to natural infection by leaf rust pathogen in field-grown plants of 7EL-7el$_1$L recombinant and control lines, carrying or lacking the *Lr19*-tightly linked locus $XSTSLr19_{130}$ (Fig. 2): **a** adjacent rows of a highly resistant (*right*) and a highly susceptible (*left*) genotype (R175-5 HOM+ and HOM−), with and without the $XSTSLr19_{130}$ marker locus, respectively (see Fig. 2; Fig. S2), respectively); **b** a heavily attacked leaf of a susceptible plant; **c** a detail of the healthy phenotype of a HOM+ R74-10 derivative

Discussion

In the present research, we have further enriched the already positive gene/QTL content of the *Th. ponticum* T4

segment with a highly effective QTL for resistance to wheat threatening diseases caused by *Fusarium* spp., i.e., FHB and FCR, deriving from the 7EL arm of *Th. elongatum*, a close relative of *Th. ponticum* (Ceoloni et al. 2015). The location of such QTL was not known prior to our work, which has thus produced the first, important result of assigning the *Fusarium* resistance locus to the telomeric ~~end~~portion of 7EL, in a distal position with respect to all $7el_1L$ target genes/QTL (Fig. 2). This evidence, derived from the correlation of *Fusarium* spp. disease scores with genetic mapping results of recombinant lines with different 7EL-$7el_1L$ breakpoints, was prerequisite for the feasibility of 7EL + $7el_1L$ gene pyramiding. This was successfully accomplished without resorting to any pairing promotion between the two *Thinopyrum* arms, which, based on early evidence (Dvorak 1975), were expected to behave as "close" homoeologs. The nearly 20% 7EL-$7el_1L$ metaphase I pairing that we detected (Fig. 1) is even higher than the 13.6% reported between complete 7E and $7el_1$ chromosomes in the F_1 between the corresponding CS addition lines (Dvorak 1975), and the pairing frequency matches the frequency with which recombinant types were isolated (10.6%, Fig. 1). Such frequency remains lower than that preliminarily estimated between 7EL and 7DL when present in a *ph1b* background (Gou et al. 2016). However, it was sufficient to successfully meet our pyramiding goal, allowing relatively rapid isolation of promising breeding materials.

A close affinity undoubtedly relates the group 7 *Thinopyrum* chromosomes involved in the present research; the genetic map of their 70% distal portion shows a high degree of synteny and collinearity, only interrupted, at least at the current resolution, by a small inversion (Fig. 2). Chromosome $7el_1$ was suggested to belong to the J genome (Ayala-Navarrete et al. 2007, and references therein), sometimes used as synonym of E to indicate one of the component genomes of *Th. ponticum* (reviewed in Ceoloni et al. 2015). Although the genome composition of polyploid perennial Triticeae is still controversial, it is believed that the genomes they carry represent modified forms of those present in their diploid, putative genome donors, as a result of various rearrangements that occurred at various stages in the course of their "reticulate" evolution (see Ceoloni et al. 2015 and references therein). Our results suggest substantial regional homology in the case of the $7el_1L$ vs. 7EL relationship.

As expected for its presumed higher fitness, of the two recombinant chromosome types, i.e., 7E with distal $7el_1L$ portions vs. T4 with distal 7EL segments, the latter prevailed. Moreover, irrespective of the size of their 7EL fraction (estimated to span from less than 20 to over 30% of the arm, see Fig. 2), the novel T4 recombinant types displayed normal segregation in F_2 progenies from the cross of CS7E(7D) × T4 F_1's with normal wheat (not shown). Three out of the five such recombinants, namely R74-10, R175-5 and R69-9, exhibit useful combinations of 7EL and $7el_1L$ genes/alleles for wheat improvement, as they all possess the *Lr19* gene for leaf rust resistance and the newly transferred locus for *Fusarium* spp. resistance (Figs. 2, 3, 5). R69-9, with the most distal 7EL-$7el_1L$ BP (Fig. 2), carries a *Psy-7el$_1$L* allele, associated with a considerable increase in YPC (Table 4), while the presence/absence of the *Sr25* stem rust resistance gene remains to be established. R74-10 and R175-5, with a coinciding 7EL-$7el_1L$ BP distally adjacent to *Lr19* (Fig. 2), contain the *Psy1-7EL* allele for the YPC candidate gene (Fig. S3). Early work (Dvorak 1975), briefly reported the *Th. elongatum* chromosome 7E as carrier of "a recessive allele for white flour color". Evaluation of total carotenoid pigments extracted from the parental CS7E(7D) and T4 lines as well as most 7EL-$7el_1L$ recombinant lines (Table 4) did not show the equivalent of a "null" or non-contributing *Psy1-7EL* allele; nonetheless, increment of YPC in HOM+ segregates of recombinants carrying a *Psy1-7EL* allele vs. their HOM− controls (wheat background only) was largely inferior to that of lines carrying a *Th. ponticum Psy1* allele, from either $7el_1L$ or $7el_2L$ sources (Table 4; Fig. S3). Although higher carotenoid content in the wheat grain is more often perceived as a desirable trait by breeders and consumers than in the past (reviewed in Ravel et al. 2013), the aesthetic preference for a not-too-yellow flour and bread maintains its considerable market share. Field-grown HOM+ R74-10 plants from the BC_1F_3 progeny after BC to Blasco not only confirmed the minor YPC increase compared to Blasco (Table 4), but also displayed very positive values for main yield parameters (Table 5), evidently contributed by the $7el_1L$ yield-related QTL, which altogether confer very good breeding potential to this material.

The QTL for resistance to *Fusarium* spp. is expected to give a further burst to this potential, both for its intensity and

for the wide spectrum of its efficacy. *F. graminearum*, *F. pseudograminearum,* and *F. culmorum* are major pathogens of wheat worldwide (Chakraborty et al. 2006; Gilbert and Haber 2013; Scherm et al. 2013). They are all causal agents of two devastating diseases of wheat and other small-grain cereals, Fusarium crown rot (FCR) and Fusarium head blight (FHB). FCR is a chronic problem in many semi-arid cereal growing regions worldwide, notably Australia and the Pacific North-West of USA, and it is becoming increasingly important in South and North Africa, Argentina, China, Italy, Turkey, and Syria (Chakraborty et al. 2006; Li et al. 2010). In contrast, the environments where humid and warm conditions prevail around the flowering stage are more prone to FHB, with North America, Canada, China, and northern Europe being amongst the most severely affected areas. Both diseases are expanding in recent years, as a result of climate changes and adoption of conservation farming practices favouring permanence of fungal inoculum on crop residues (Chakraborty and Newton 2011; Steenwerth et al. 2014). Although on a world scale, FCR remains predominantly caused by *F. pseudograminearum* and *F. culmorum*, while *F. graminearum* is the main agent of FHB (e.g., Chakraborty et al. 2006; Ma et al. 2010; Gilbert and Haber 2013; Matny 2015), recent epidemiological surveys indicate that climatic conditions play a major role in driving the prevalence of each *Fusarium* species, sometimes drastically reversing the previous reports on species distribution. For instance, *F. culmorum*, traditionally reported as the main inciter of FHB in northern, central, and western Europe, is recently seldom isolated in these regions compared with *F. graminearum*, while it is frequently reported as the main agent of FHB in the Mediterranean area (Scherm et al. 2013).

In view of future environmental changes, which may further modify pathogen distribution, it is of particular significance to have incorporated into bread wheat the *Th. elongatum* 7EL QTL, able to provide resistance to all the three main *Fusarium* species responsible for FHB (Table 2) and FCR (Table 3). Despite several shared features in the aetiology, pathogen biology, and epidemiology between the two diseases, including the ability of all the three causal agents to produce harmful mycotoxins (e.g., Gilbert and Haber 2013; Matny 2015), in most cases, different host genes appear to be involved in wheat resistance against FHB and FCR. A significant correlation between reactions to

these two diseases was not found in a panel of wheat genotypes, including Sumai 3, nor in a doubled haploid population derived from a bread wheat variety with the dual resistance phenotype (Li et al. 2010). Similarly, a previous study on two cross progeny segregating for allelic differences at the major Sumai 3 FHB resistance QTL on the 3BS arm, failed to reveal such correlation (Xie et al. 2006). On the other hand, what is probably the most effective QTL for resistance to FCR in wheat identified so far, mapped on the 3BL arm (Bovill et al. 2010; Li et al. 2010; Ma et al. 2010, 2014; Zheng et al. 2015), was shown to provide resistance to infection of both *F. graminearum* and *F. pseudograminearum* following seedling assays (Bovill et al. 2010; Li et al. 2010; Ma et al. 2010), and also significantly reduced whitehead incidence under field conditions in some trials (e.g., Liu and Ogbonnaya 2015), though not in others (e.g., Martin et al. 2015). While spike inoculation for assessment of FHB is widely recognized as a robust assay, to estimate FCR severity, several methods have been adopted, involving different inoculation techniques, plant organs, and developmental stages, as well as varying growing conditions (reviewed in Liu and Ogbonnaya 2015). The validity of the visual scoring of brown discoloration of seedling stem base leaf sheaths, used here to assess the reaction to *F. culmorum* and *F. pseudograminearum* of wheat recombinant lines bearing the *Fhb-7EL* QTL, is supported by a recent study, where this assay was combined with a specific qPCR estimate of degree of colonization of the same tissues by *F. pseudograminearum* (Knight et al. 2012). Not only did this study highlight that visual assessments of disease symptoms reflected the extent of tissue colonization by the pathogen, but also showed the time point of closest correlation between the two tests to be much earlier than the 35 dpi most frequently used in FCR visual assays, i.e., at 14 dpi, coinciding with our final time point (Table 3).

A similarly strong correlation was detected in the present work between visual assessment of FHB symptoms and accumulation of fungal biomass in caryopses of infected plants, with and without the *Fhb-7EL* QTL: the considerably reduced FHB symptoms in genotypes with distal 7EL portions (Table 2) were invariably associated with a reduced fungal biomass (Fig. 4), measured by a qPCR assay for the *Fusarium* spp. *TRI6* gene (Horevaj et al. 2011). This assay turned out to be more sensitive than visual assessment in cases where the reduced FHB symptoms were only

evident in the initial phases of fungal infection. The qPCR values were clearly indicative of the permanence of the effects of resistance factors up to full grain maturity (Moscetti et al. 2013). Moreover, *TRI6* qPCR values were closely correlated with DON levels in the grain, resulting in a better predictor of DON accumulation than the visible disease symptoms on the spike or grain (Horevaj et al. 2011). *Fusarium* mycotoxin contamination, for which stringent limits on allowable levels have been enacted in several countries worldwide (Gilbert and Haber 2013), is a major concern for safety of the wheat crop, mostly used for food, not last for the economic impact of the quality downgrade (Matny 2015). Thus, the combined evidence of greatly reduced symptoms at the spike level, as well as dramatically decreased quantity of *F. graminearum* biomass in kernels of infected recombinant genotypes with the *Fhb-7EL* QTL, is a noteworthy result of the present study.

The magnitude of the effect of the *Th. elongatum* QTL on FHB symptoms and fungal biomass in bread wheat appears to be even higher than that exerted by the *Th. ponticum* 7el$_2$L QTL. Following infection with the same type of inoculum used in the present work, the latter reduced FHB severity and fungal biomass by around 80% in both bread and durum wheat (Forte et al. 2014), while *Fhb-7EL* reduced by over 95% symptoms at the spike level (Table 2) and by up to 99% fungal biomass (Fig. 4). Whether the two QTL from the different *Thinopyrum* species are allelic is an intriguing issue. Their location does appear to coincide in the corresponding genetic maps that we have developed (Fig. 2; see, for comparison, Fig. 2 and Supplementary Fig. 1 in Forte et al. 2014), and in that of Guo et al. (2015). However, much more marker-dense molecular maps and adequate plant materials (e.g., lines with a high degree of background isogeneity) are needed for a reliable allelism test, and, in general, to tag the targeted locus or loci within a given QTL. Resolution of a resistance locus into discrete genetic determinants and assessment of their respective functional contribution to the resistance phenotype are complicated tasks. Following several attempts, this target has recently been met for the most exploited wheat QTL for FHB resistance, *Fhb1*, originating from the Chinese cultivar Sumai 3 (Rawat et al. 2016). By mutation analysis, gene silencing, and transgenic overexpression, it has been shown that, among several candidates, a pore-forming toxin-like (*PFT*) gene at the *Fhb1* QTL confers FHB resistance.

Although the mechanism of *PFT* action remains to be elucidated, it was hypothesized that the predicted *PFT*-encoded chimeric lectin might participate in the recognition of pathogen-specific carbohydrates, causing toxicity and growth arrest to the fungus by interacting with its cell wall (Rawat et al. 2016). In the same study, *PFT* was proved to have no role in the detoxification of DON, and the DON-detoxification-controlling locus, also associated with the *Fhb1* QTL (Lemmens et al. 2005), was shown to be independent of *PFT*, albeit located near the same genetic block. The proposed action of *PFT* was considered to align with the broad spectrum of resistance conferred by *Fhb1* to different isolates and species of *Fusarium*, though only assessed, to our knowledge, for effectiveness toward the FHB disease (Stack et al. 1997; Mesterházy 2002).

Similar to the *Fhb1* case, the *Fhb-7EL* QTL mapped here is expected to be a complex locus, likely with multiple genetic determinants of the resistance response(s). Miller et al. (2011) described the 7EL-associated resistance as “multifaceted”, involving both physical and chemical interacting factors. In particular, they indicated that the rachis tissue played a key role in stopping the progression of *F. graminearum* beyond the node of the inoculated spikelet; this was also the place where extensive deposition of brown matter, possibly contributing to the block of fungal spreading, was concomitantly observed in the resistant genotype. Both RT-qPCR (Wang et al. 2010) and, more recently, RNA-seq combined with advanced bioinformatics strategies (Gou et al. 2016) have been applied to identify candidate genes underlying the 7EL *Fusarium* resistance QTL.

While awaiting this important piece of knowledge, exploitation of this rather exceptional source of resistance to *Fusarium* diseases in bread wheat breeding has become feasible, and made particularly attractive by its pyramiding with other valuable *Thinopyrum* genes/QTL. Given the expected inheritance as a unit of the composite *Thinopyrum* segment in cross progeny with wheat, either of the several codominant PCR-based markers identified in the course of the work, of which some particularly user-friendly (see Fig. 2; Fig. S1a, c), will enable easy tracking of the novel assembly in transfer programs into adapted cultivars. Recently, we have undertaken introgression of the same

gene/QTL combination into durum wheat, a species for which enhancement of resistance to *Fusarium* diseases is particularly desirable, as very few, effective sources are available (Forte et al. 2014; Prat et al. 2017, and references therein). The first stable tetraploid recombinants are going to be subjected to specific bioassays to verify expression of the acquired 7EL *Fusarium* spp. resistance QTL in the new genomic context.

Author contribution statement CC conceived the project and coordinated the research; PF, LK, and MEV performed the molecular marker and GISH analyses, as well as the *F. graminearum* bioassay; ST carried out the *F. culmorum* and *F. pseudograminearum* bioassays; IM performed the real-time qPCR assay for the *TRI6* gene; PDV performed the pigment content analyses; RD participated in coordinating the research; CC, LK, ST, and RD participated in writing the manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare that they do not have conflict of interest.

Electronic supplementary material

Below is the link to the electronic supplementary material.

Supplementary material 1 (PDF 672 kb)

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