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Pyramiding resistance genes to Fusarium head blight and rusts from *Thinopyrum ponticum* into durum wheat

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Abstract. Taking advantage of climate changes, unfamiliar pests and diseases are challenging wheat crop species. This is the case for Fusarium Head Blight (FHB), which has recently become a threat in unusual environments, including those where durum wheat is traditionally cultivated. Since currently available durum wheats are largely suscept ble to FHB, new varieties are needed capable of maintaining yield capacity and grain quality under the disease pressure. A sustainable approach to achieve this aim is represented by transfer of resistance genes/QTL from related Triticeae species by means of "chromosome engineering". We resorted to this cytogenetic strategy, efficiently complemented with advanced characterization and selection systems, to transfer into durum a gene/QTL for FHB resistance (provisional designation *Fhb-7el$_2$*) located on the 7el$_2$L arm of the wild *Thinopyrum ponticum*. A bread wheat 7DS.7el$_2$L translocation line was employed as donor of the trait in crosses with previously developed durum wheat 7AS.7AL-7el$_1$L recombinant genotypes, carrying additional resistance genes (*Lr19+Sr25*) deriving from a different *Th. ponticum* accession. Given the nearly complete homology between the 7el$_1$L and 7el$_2$L arms, and in spite of some pairing reduction in the pentaploid F1's, pyramiding into durum of target genes/QTL from the two *Th. ponticum* accessions was successfully achieved. The selected multiple recombinant lines exhibited up to 80% reduction of suscept bility following *Fusarium* inoculation. The present proof of the *Fhb-7el$_2$* efficacy also in durum wheat opens the way for its straightforward breeding exploitation.

Keywords. Chromosome engineering – Wheat-alien transfer – *Triticum durum* – FHB – Scab – Lr19 + Sr25 genes.

Pyramidage des gènes de résistance à la fusariose de l'épi et à la rouille de Thinopyrum ponticum dans le blé dur

Résumé. *A la suite des changements climatiques, des ravageurs et des maladies auparavant inconnus chez le blé ont fait leur apparition sur cette culture. Tel est le cas de la fusariose de l'épi (FHB), qui représente une nouvelle menace pour certains environnements, y compris ceux où le blé dur est traditionnellement cultivé. Puisque les blés durs disponibles aujourd'hui sont très sensibles à la FHB, il est nécessaire d'obtenir de nouvelles variétés capables de maintenir le potentiel de rendement et la qualité du grain sous pression de maladie. Une approche durable pour atteindre cet objectif est le transfert de gènes de résistance/QTL à partir d'espèces apparentées à Triticeae par le biais de « l'ingénierie chromosomique ». Nous avons eu recours à cette stratégie cytogénétique, complétée efficacement par des systèmes de caractérisation et de sélection avancés, pour transférer chez le blé dur un gène/QTL pour la résistance à la FHB (désignation provisoire Fhb-7el2), situé sur le bras 7el2L de l'espèce sauvage Thinopyrum ponticum. Une lignée de translocation de blé tendre 7DS.7el2L a été utilisée comme donneur de ce caractère dans les croisements avec les génotypes recombinants de blé dur 7AS.7AL-7el1L développés précédemment, portant des gènes de résistance supplémentaires (Lr19 + SR25) issus d'une accession différente de T. ponticum. Compte tenu de l'homologie presque complète entre les bras 7el1L et 7el2L, et malgré une certaine réduction d'appariement dans les pentaploïdes F1, le pyramidage dans le blé dur des gènes cibles/QTL des deux accessions de Th. ponticum a été réalisé avec succès. Les lignées recombinantes multiples sélectionnées affichaient jusqu'à 80% de réduction de la vulnérabilité après l'inoculation de Fusarium. Cette preuve de l'efficacité du Fhb-7el2 aussi chez le blé dur ouvre la voie à son exploitation directe dans la sélection.*

Mots-clés. *Ingénierie chromosomique – Transfert de gènes étrangers chez le blé – Triticum durum – FHB – Fusariose – Gènes Lr19 + SR25.*

I – Introduction

In recent years, climatic changes have favoured the spread of previously uncommon fungal diseases, including Fusarium Head Blight (FHB), in several wheat growing areas, resulting in damage to wheat production and quality (Chakraborty and Newton, 2011). An efficient and sustainable strategy to counter the spread of the pathogen is development of resistant/tolerant varieties able to respond to the current and future demand for high-yielding and low-impact crops. Given the scarcity of resistance sources in cultivated *Triticum* species and even among their close relatives (Buerstmayr *et al*., 2009, 2012, 2013), we have looked outside the primary gene pool and targeted perennial wheatgrass species belonging to the *Thinopyrum* genus. *Thinopyrum* possesses a considerable array of genes for disease and pest resistance as well as for tolerance to environmental stresses, and even yield-related traits (Kuzmanović *et al*., 2013), some of which have been exploited in wheat breeding (reviewed in Ceoloni *et al*., 2013). *Thinopyrum* species are also valuable donors of effective resistance to FHB (Cai *et al*., 2005). Both the diploid *Th. elongatum* (2n = 14) and the decaploid *Th. ponticum* (2n = 70) were shown to harbour a major gene/QTL for FHB resistance on the long arm of a homoeologous group 7 chromosome, namely on 7EL and on $7el_2L$ respectively. While the $7el_2L$ gene/QTL has been mapped toward the distal end of the arm in close association with *XBE445653* and *Xcfa2240* marker loci (Shen and Ohm, 2007; Zhang *et al*., 2011), position of the 7EL locus(i) along the arm has not been determined so far (Shen *et al*., 2004; Shen and Ohm, 2006). The $7el_2L$ arm also carries the effective, but still unmapped, stem rust resistance gene *Sr43* (Kibirige-Sebunya and Knott, 1983; Xu *et al*. 2009), whereas it lacks any major leaf rust resistance gene (Kim *et al*., 1993).

On the other hand, on the $7el_1L$ arm (Sharma and Knott, 1966; Dvorak and Knott, 1977), also called 7AgL (Sears, 1973), originating from a different *Th. ponticum* accession, the leaf and stem rust resistance genes *Lr19* and *Sr25* are distally located (Ceoloni *et al*., 2005, 2013; Gennaro *et al*., 2009), in close linkage with a *Yp* gene contributing to yellow endosperm pigmentation (similarly present on $7el_2L$, see Kibirige-Sebunya and Knott, 1983). Both *Lr19* and *Sr25* are highly valuable resistance sources effective against a large majority of races of the corresponding fungal pathogen that has spread worldwide (Singh *et al*., 2008; Gennaro *et al*., 2009; Jain *et al*., 2009). Notably, they display their full efficacy in areas where durum wheat is the main cereal crop (such as central Italy) and rust diseases represent a constant challenge (leaf rust e.g., Gennaro *et al*., 2007) or tend to re-emerge (stem rust, see Nocente *et al*., 2011).

As $7el_1L$ proved to be fully homologous to $7el_2L$ (Forte *et al*., 2011; Zhang *et al*., 2011) and closely homoeologous to 7EL (Dvorak, 1975; Forte *et al*., 2011), pyramiding of the different *Thinopyrum* genes was considered a feasible target. Chromosome engineering strategies have been undertaken for the recombination-based pyramiding of resistance genes/QTL from both of the above-mentioned *Thinopyrum* species into bread and durum wheat recombinant lines. While the work involving the *Th. elongatum*-derived FHB resistance is underway, we present here the results of successful pyramiding of FHB resistance from *Th. ponticum* $7el_2$ chromosome into durum wheat lines already carrying the $7el_1L$-derived *Lr19* and *Sr25* rust resistance genes.

II – Material and methods

The KS24 bread wheat 7DS.$7el_2L$ centric translocation line (Kibirige-Sebunya and Knott, 1983; Shen and Ohm, 2007; Fig. 1) was used as FHB resistance donor (type II resistance, i.e., inhibition of disease spreading after infection) in crosses with durum wheat recombinant lines, named R5-2-10, R112-4 and R23-1 (Fig. 1). The latter genotypes have 23%, 28% and 40%, of $7el_1L$ replacing corresponding portions of their 7AL arms, respectively (Ceoloni *et al*., 2005). Meiotic metaphase I chromosomes of pentaploid F1 plants were subjected to Genomic In Situ Hybridization (GISH) to assess the frequency of $7el_1L$/$7el_2L$ pairing. F1's were backcrossed to normal durum cultivars

to recover the 2n = 28 chromosome number in the target genotypes. Selection for the desired loci was aided by use of polymorphic SSR, EST and STS markers in the regions of interest (Fig. 1; see also Ceoloni *et al*., 2013). Further characterization was carried out by GISH on somatic chromosomes of the selected genotypes. Selected plants carrying $7el_2L$ markers linked to the FHB resistance locus (here provisionally designated *Fhb-7el*$_2$) were subjected to infection with *Fusarium graminearum*. A pair of central spikelets of each ear (one ear/plant) was inoculated by spore injection, and the disease spreading followed at 7, 14 and 21 days post-inoculation (dpi). The KS24 line, previously proved to be highly resistant also toward Italian *Fusarium* pathotypes, was included in the infection test in addition to several susceptible controls including the 7AL-$7el_1L$ recombinant lines R5-2-10 and R112-4, as awell as various durum wheat cultivars.

III – Results and discussion

GISH analyses on meiotic metaphase I cells confirmed the considerable pairing affinity between the largely homologous chromosomes $7el_1$ and $7el_2$. However, in contrast to their virtually complete pairing observed in bread wheat F1's from the cross of the KS24 line with the T4 translocation line (70% of 7DL replaced by $7el_1L$) (Shen and Ohm, 2007; Forte *et al*., 2011), $7el_1L/7el_2L$ pairing in KS24 x R5-2-10/R112-4/R23-1 pentaploid F1's, always detected in the form of a 7AL.7AS/7AS.7AL-$7el_1L/7el_2L$.7DS trivalent configuration, dropped to less than 40% frequency. This can probably be attributed to the fact that the homologous $7el_1L$ and $7el_2L$ portions lye on otherwise homoeologous chromosomes of the durum wheat parent (7A) and of the bread wheat parent (7D), the former having its complete 7A also present in the same cell.

In line with the observed pairing frequency, around 18% $7el_1L$-$7el_2L$ recombinants were identified in the progeny from the cross of (KS24 x R5-2-10/R112-4/R23-1) F_1 plants x durum cv. Ariosto, analysed with suitable molecular markers (Fig. 1). GISH applied to somatic chromosomes of the putative recombinant types revealed that only a minority of them had the desired combination of $7el_1$ and $7el_2$ target loci on wheat 7AL arm, the remaining ones showing $7el_1L/7el_2L$ recombined chromatin onto the 7DL arm. Of two 7AL recombinants, R85, like R23-1, has 40% of distal 7elL (Fig. 1), while R129, like R5-2-10, has 23% distal 7elL (Fig. 1). Molecular markers revealed that both R85 and R129 recombinants carry *Lr19* ($7el_1L$), as well as the $7el_2L$ allele for the most distal CFA2240 marker, to which the FHB resistance QTL seems to be more tightly associated (Zhang *et al*., 2011). However, the longer $7el_2L$ segment of R129 also includes $7el_2L$ alleles for the more proximal *XBE445653* and *XBF145935* EST marker loci, besides that for the *Yp* gene-linked *XSTSPsy1* locus (Fig. 1).

Based on selection by molecular markers homozygous plants, both carriers and non-carriers of the distal *Thinopyrum* segment, were isolated in F_2 progeny of R85 crossed with normal durum wheat, and these were subjected to infection with *Fusarium* ssp. to assess their resistance/susceptibility against Italian pathotypes. A pair of central spikelets of each ear (one ear/plant) was inoculated by spore injection and the disease spreading followed at 7, 14 and 21 days post-inoculation. As susceptible controls, plants of the R5-2-10 and R112-4 recombinant lines and durum wheat varieties Simeto and Duilio were also included in the experiment.

The phenotypic assay confirmed the tight association of the *Fhb-7el*$_2$ QTL with the CFA2240 marker (the *XBE445653* marker locus has a $7el_1L$ allele in R85), and for the first time it showed its efficacy, previously reported only in bread wheat (Shen and Ohm, 2007), to be fully displayed in durum wheat as well. In fact, the selected R85 homozygous plants showed a significant reduction of susceptibility to FHB, ranging between 60 and 80%.

In F_2 progeny of R85 recombinant heterozygous for a normal 7A, some deviation from normal transmission was observed, likely attributable to the known presence of a Segregation distortion (*Sd*) gene in its most proximal portion, i.e. comprised between its $7el_1L$-7AL breakpoint (= R23-1)

and that of line R112-4 (see Fig. 1). In order to eliminate drawbacks associated with presence of the *Sd* gene (Ceoloni *et al*., 2013), R85 was crossed with R112-4 and R5-2-10 recombinants. This allowed isolation of secondary recombinant types, named R193 and R216, with the same $7el_1L/7el_2L$ content of target loci as R85, but with overall shorter 7elL segments (Fig. 1), hence undergoing normal transmission (not shown). Homozygous plants of such recombinants, as well as of R129, are currently isolated and will be subjected to *Fusarium* infection to corroborate previous evidence on R85. Resistance to leaf rust conferred by *Lr19* was also validated in these materials, both in seedlings and adult plants, while presence of *Sr25* remains to be ascertained.

In conclusion, the recombinant durum wheat genotypes identified in this work represent novel and highly valuable material to be introduced in durum wheat breeding programs aimed at enhancing and widening the spectrum of resistance to a variety of relevant diseases, both traditional and newly emerged that are greatly challenging the crop.

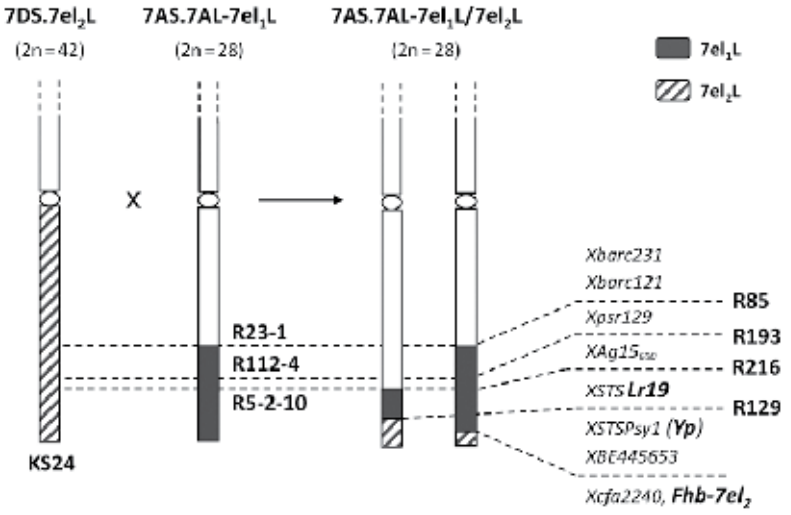

Figure 1. Pyramiding genes/QTL from *Th. ponticum* $7el_1L$ and $7el_2L$ chromosome arms: parental lines and their durum wheat recombinant products carrying different amounts of total 7elL chromatin and combinations of target and marker loci.

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