# Proceedings of the 2020 National Fusarium Head Blight Forum



## VIRTUAL December 7-11, 2020

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### IMPACT ON DURUM WHEAT OF SMALL INTROGRESSIONS FROM WILD *THINOPYRUM* SPECIES CONFERRING EFFECTIVE RESISTANCE TO *FUSARIUM* DISEASES: BREEDING PERFORMANCE AND METABOLIC RESPONSES Ljiljana Kuzmanović<sup>1</sup>, Giuseppina Fanelli<sup>2</sup>, Silvio Tundo<sup>1,3</sup>, Giulia Mandalà<sup>1,4</sup>, Gloria Giovenali<sup>1</sup>, Alessandra Capoccioni<sup>1</sup>, Sara Rinalducci<sup>2</sup> and Carla Ceoloni<sup>1\*</sup>

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### ABSTRACT

Durum wheat (*Triticum durum*, 2n = 4x = 28, DW) covers only 8% of the global wheat surface, yet it represents a strategic commodity for countries spanning many and diversified world areas, primarily the Mediterranean Basin. Recent climate changes have not only contributed to modify the DW conventional distribution areas, but also exposed the crop to unfamiliar pathogens. This is the case for fungal pathogens of the Fusarium genus, responsible for some of the most threatening diseases of wheat and other cereals, namely Fusarium head blight (FHB), or scab, and Fusarium crown rot (FCR). For DW, which results more vulnerable to both FHB and FCR than 6x bread wheat (BW), this condition is particularly worrying. In fact, being typically devoted to human consumption, the DW crop greatly suffers, besides yield and quality reduction, the safety problems associated with health-dangerous Fusarium mycotoxins, such as deoxynivalenol (DON). Since effective resistant sources are not available in breeding pools, a profitable and sustainable approach to arm the species with appropriate defense means consists of the exploitation of genetic variability present in related gene pools, such as those of the *Thinopyrum* genus. Of specific interest is the distal end of 7L arm of several congeneric species, enriched with genes/QTL for resistance to relevant wheat diseases. Among them, the Lr19+Sr25 genes (leaf and stem rust resistance, respectively), located on the 7el,L arm of 10x Th. ponticum, and also major QTL contributing resistance to both FHB and FCR, namely Fhb7, identified on the 7el, L arm of a different Th. ponticum accession, as well as its likely ortholog present on 7EL of the 2x Th. elongatum (1, 2, 3, 4, and refs. therein). The extraordinary efficacy against FHB of both Fhb7 QTL (indicated hereafter as Fhb7el, and Fhb7E for the sake of distinction), was initially established in laboratory BW lines, such as Thatcher 7DS·7el<sub>2</sub>L centric translocation line, and Chinese Spring (CS) 7E(7D) substitution line. Besides contributing to cytogenetic mapping of Fhb7 QTL (e.g. 2), we have successfully exploited chromosome engineering strategies to incorporate firstly Fhb7el, (1) and more recently Fhb7E (3) into DW. In both transfer schemes, previously produced DW recombinant genotypes, with 7el,L segments (including Lr19, Sr25 and Yp genes) spanning 23% (named R5) and 28% (R112) of their 7AL arms, were used as recipient lines. As donor of Fhb7el,, the 7DS·7el, LBW-Th. ponticum translocation line was used, whereas previously obtained BW 7DS · 7DL-7el, L/7EL recombinant types (named R69-9 and R74-10), carrying a terminal 7EL portion, including Fhb7E, embedded into a 7el,L Th. ponticum segment present on wheat 7DL

#### Durum Coordinated Project

(2), were employed. In both cases, transfer of the *Fhb7* QTL was achieved by homologous pairing and recombination between the 7el<sub>1</sub>L portions shared by donor and recipient recombinant chromosomes in 5x F<sub>1</sub>s. Stable DW recombinants with either *Fhb7* QTL combined with valuable 7el<sub>1</sub>L genes were isolated in BC<sub>1-2</sub> progenies to the Italian DW cv. Simeto of all cross combinations. Further BCs to the same DW yielded near-isogenic recombinant lines (NILRs, HOM+), whose spikes and seedlings were inoculated with *F. graminearum* and *F. culmorum*, respectively, for assessment of their FHB and FCR phenotypes compared with those of HOM– sibs. Following *F. graminearum* point inoculation, NILRs of both 7el<sub>1</sub>L+7el<sub>2</sub>L DW recombinant types, named R216 and R193 (same telomeric 7el<sub>2</sub>L portion with *Fhb7el*<sub>2</sub>, but inserted into the 7el<sub>1</sub>L segments of either R5 or R112, respectively), exhibited an average 75% reduction in FHB severity vs. HOM– controls (1). Similarly, 7el<sub>1</sub>L+7EL DW recombinants, possibly due to some contribution of the CS donor background, showed an even stronger resistance, with > 90% reduction of disease severity (3). Moreover, unlike the case of *Fusarium* spp. resistance QTL native to wheat, both *Fhb7el2* and *Fhb7E* showed to confer tolerance to FCR as well, with disease index reduced by over 50% (3).

The breeding potential of the various DW recombinant types equipped with either *Fhb7el2* or *Fhb7E* was preliminarily evaluated, on the basis of plant and spike traits, in small-scale field tests carried out during the 2018-19 and 2019-20 seasons at Viterbo experimental site (Central Italy). Natural disease pressure was negligible in both experimental years. Instead, weather conditions were quite different, with 2019-20 being much drier than 2018-19, characterized by a huge amount of rainfall and lower mean temperatures in coincidence with the critical phases of anthesis and grain filling. Whereas an overall absence of negative effects on yield and related traits was observed for all recombinant types (HOM+) vs. their controls (HOM-), the marked between-year difference was helpful at highlighting the best suited recombinant genotypes to the contrasting environmental conditions. Thus, between R216 and R193 (7el,L+7el,L), the 2018-19 season resulted more favourable to the latter, in which the 7el,L portion is part of the R112 longer 7el,L segment compared with R5. The better yield performance of R193 is in line with previous observations from multi-year/environment trials, which suggested R112 to carry in its most proximal 7el,L fraction (retained in R193) genes/QTL for morpho-physiological and agronomical attributes of the aerial and root plant portions that are best expressed in environments with optimal thermo-pluviometric patterns (5). On the other hand, R216 HOM+ had comparable or higher values than R216 HOM- for various yield and fertility traits in the hotter and drier 2019-20 season, thus mimicking the behaviour of its R5 parent (5). As to 7el,L+7EL recombinants, assessment of spike traits in R74-10/R112, R74-10/R5 and R69-9/R112 HOM+ genotypes and HOM- controls in the 2018-19 season, showed that presence of any of the three alien segments did not appreciably impair spike fertility and yield. However, R69-9/R112 was the best performing out of the three recombinants, and R74-10/R5 the least productive. This outcome might be associated with a different 7el,L/7EL ratio in the various recombinant types, with that of R74-10/R5 (longer 7EL segment than in R69-9, and shorter 7el, L segment than in R112) causing more disturbance to the recipient genome. Thus, the R69-9 derivatives appeared more appropriate for DW breeding, also because their 7EL portion does not replace the 7el,L allele of the Yp gene conferring higher semolina yellowness vs. the corresponding 7EL allele (2, 3). Consequently, subsequent work was focused on such recombinant types (R69-9/R5 and R69-9/R112), which exhibited a good performance at the plant and spike levels also in the drier 2019-20 season. Confirming their better adaptability to reduced rainfall, R69-9/R5 plants expressed higher values than their HOM- controls for a number of characters (mainly thousand grain weight and grain yield/spike), while R69-9/R112 was inferior to its HOM- controls for some spike traits. Based on 2019-20 dataset, an overall comparison of all DW-Thinopyrum spp. HOM+ recombinants, including R5 and R112 (7el<sub>1</sub>L only), as well as DW controls, Simeto and IDYT22 (an ICARDA line involved in

R216 and R193 pedigree), was carried out. ANOVA did not show statistically significant differences in grain yield/plant (GY). However, recombinants containing the  $7el_1L+7EL$  segment assembly, particularly R69-9/R5, turned out to be the best performing with respect to control varieties and to all other recombinant types. To a minor extent, also R216 and R193 ( $7el_1L+7el_2L$ ) showed higher GY than R5/R112 and Simeto, while being comparable to IDYT22. These results indicate that, even disregarding the specific effects of the *Fhb7* QTL introgression, the presence of 7EL or  $7el_2L$  chromosomal portions onto  $7el_1L$  segments has a favorable impact on yield potential of the recipient DW, even improving the positive contribution known to be associated with the original  $7el_1L$  segments (5 and refs. therein).

An additional research activity enabled by the novel recombinant types was undertaken to get insights on the mechanisms underlying the *Fhb7*-mediated resistance. To this aim, the untargeted metabolomic profile of the rachis tissue sampled at 2-4 days following F. graminearum (Fg) inoculation of spikes of R69-9/R5 (Fhb7E) HOM+ NIRLs was recently compared with that of HOM- Fg-inoculated sibs and of mock-inoculated plants. Extracted metabolites from the 4 genotype x treatment combinations were analyzed by LC/MS. Preliminary evidence from MetPA (Metabolic Pathway Analysis) shows main changes between HOM+ and HOM- Fg-inoculated samples at the level of phenylalanine metabolism, phenylpropanoid and diterpenoid biosynthesis, known to be main routes the plant activates in response to Fusarium inoculation. Particular interest arouses the Vitamin B6 metabolism, specifically induced in Fg-inoculated Fhb7E+ genotypes, which provides supporting evidence for B6 vitamers' role as potent antioxidants in plants subjected to abiotic and also biotic stresses. As a further contributor to the antioxidant capacity of stressed plants, the glutathione (GSH) metabolism was also up-regulated in Fg-inoculated Fhb7E+ and, to a minor extent, Fhb7E- rachises. Of a particular GSH adduct, i.e. a de-epoxidated DON-GSH adduct, whose formation was recently associated with a peculiar, xenobiotic mechanism at the base of Fhb7 resistance (4), clear proof of exclusive production in the rachis tissue of Fhb7E+ plants was obtained by LC-MS/MS analyses. Whereas the GSH-mediated event appears to be a major determinant in DON detoxification brought about in the presence of Fhb7 genes/QTL, additional detoxifying strategies, such as DON glycosylation, may contribute to an expected composite resistance response. In fact, the DON-3-glucoside (D3G) metabolite was solely detected in Fhb7E+ Fg-inoculated rachises. In line with this, D3G was four times more abundant in seeds harvested from the latter plants than in Fhb7E- sibs. Combined with the nearly 800 times lower DON content found in HOM+ vs. HOM- seeds, these results confirm DW lines carrying Fhb7E, and Fhb7 QTL in general, to be highly valuable also for the crop safety, thus enhancing its market/trade potential.

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