

A TRIPLE ALIEN INTROGRESSION IN DURUM WHEAT INCREASES DISEASE RESISTANCE, GRAIN QUALITY AND YIELD UNDER MEDITERRANEAN RAINFED CONDITIONS

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Increase of genetic variability of cultivated wheats is an essential requisite for today's breeding strategies. This is true for any trait and especially yield, given the urgent need for substantial gains in the face of ongoing and future challenges, including climate changes. Resorting to the rich and largely untapped gene pools of wild wheat relatives via chromosome engineering (CE) represents a validated strategy to succeed in this endeavour. So far, introgression of useful alien genes into wheat by CE has usually involved single chromosomal segments of a given donor species and targeted a corresponding (homoeologous) wheat chromosome. Attempts to stack multiple alien segments from more than one alien species within a single wheat genotype are limited to a couple of cases in bread wheat, while none is reported in durum wheat. Here we present the first of such multiple introgression events into tetraploid durum wheat (*Triticum durum*, $2n = 4x = 28$), a species with lower tolerance than hexaploid bread wheat (*T. aestivum*, $2n = 6x = 42$) towards genome manipulations. We combined into a single durum wheat genotype, with the prevailing background of cv. Karur, three different individual transfers, involving 7AL, 3BS and 1AS wheat arms. In them, the telomeric end was replaced by a homoeologous portion of *Thinopyrum ponticum* 7AgL, *Aegilops longissima* ($2n = 2x = 14$) 3S¹S, and *T. aestivum* 1DS, respectively. Each of these segments, not exceeding a 23% of the physical arm length, harbours the following respective genes of interest: *Lr19+Sr25+Yp* (leaf and stem rust resistance, and a gene increasing semolina yellowness), *Gli-D1+Glu-D3* (genes affecting gluten properties) and *Pm13* (powdery mildew resistance). Following assessment of normal transmission of recombinant chromosomes and overall genome stability in early generations, the BC₃F₄₋₆ progenies of single, double and triple recombinants were tested across three years in Viterbo (Central Italy) in small and large scale field trials. The results showed that besides being well tolerated in a tetraploid background, the simultaneous presence of the three segments made the most stable recombinant R11-20 a superior and functional CE product, ready to be included in breeding pipelines. Depending on the season, significant yield advantage of the R11-20 vs. control genotypes ranged 33%-52%, primarily due to increases in biomass and grain number m⁻², harvest index and grain yield per spike. Moreover, the presence of resistance genes for relevant diseases, such as leaf or stem rust (including resistance towards Ug99 pathotypes for the latter), represents an additional breeding value of R11-20, which can contribute to the crop security and safety. The three segments analysed here did not negatively affect grain quality of the recombinants, but, instead, positively impacted on their yellow and gluten indexes when compared to the recurrent parent Karur.

