

Engineered Durum Wheat Germplasm

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Durum wheat (*Triticum durum* var. *durum*, $2n = 4x = 28$, AB genomes) is a major staple crop in the Mediterranean Basin, where its cultivation largely replaced that of tetraploid emmer, *T. dicoccum*, by the first millennium B.C.

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1. Introduction

If genetic gains in wheat yield are to be achieved in today's breeding, increasing genetic variability of cultivated genotypes is an essential requisite to meet. To this aim, alien gene transfer through chromosome engineering (CE) is a validated and sound strategy. Attempts to incorporate more than one alien segment into cultivated wheat have been rare, particularly for tetraploid durum wheat. Here we present the agronomic and quality performance of the first successful CE-mediated multiple introgression into the latter species. By assembling into 7AL, 3BS and 1AS arms of a single genotype homoeologous segments of *Thinopyrum ponticum* 7eL₁L, *Aegilops longissima* 3S^LS, and *Triticum aestivum* 1DS arms, respectively, we have stacked several valuable alien genes, comprising *Lr19+Sr25+Yp* (leaf and stem rust resistance and high semolina yellowness), *Pm13* (powdery mildew resistance) and *Gli-D1/Glu-D3* (genes affecting gluten properties), respectively. Advanced progenies of single, double and triple recombinants were field-tested across three years in a typical durum wheat growing area in Central Italy. The results showed that not only all recombinants had normal phenotype and fertility, but also that one of the triple recombinants had the highest yield through all seasons compared with all other recombinants and control cultivars. Moreover, the multiple introgressions enhanced quality traits, including various gluten characteristics and semolina yellow index. Presence of effective disease resistance genes added breeding value to the novel and functional CE products, which can greatly contribute to crop security and safety.

2. Description

Durum wheat (*Triticum durum* var. *durum*, $2n = 4x = 28$, AB genomes) is a major staple crop in the Mediterranean Basin, where its cultivation largely replaced that of tetraploid emmer, *T. dicoccum*, by the first millennium B.C. [1]. In keeping with the crop's geographical origin, i.e., adaptation to mild winters and dry summers, the Mediterranean region represents the largest world's growing area (60%) for durum wheat [2]. Other major cropping regions are in Northern United States of America, Canada, and Northern Mexico, besides minor ones in Southern Eurasia, India, South Australia, and Argentina [3,4,5]. Durum wheat is mostly used for pasta making, but it is also the raw material for producing other traditional foods, mostly typical of Mediterranean countries, such as flat breads, couscous, and bulgur. The current re-discovery of traditional foods on one hand, and, on the other hand, the new consumption habits of the growing urban populations, particularly in Asian and African developing countries [6,7,8], are boosting the popularity and demand for wheat- and specifically durum wheat-derived products, such as pasta and couscous [5,9]. Strong value chains for such products, already in place in traditional durum wheat growing countries and interestingly emerging for unconventional territories and markets [5], lead to the forecast of an increase in the global durum wheat cropping over the current 5–6% of total wheat production [10,11]. Moreover, with respect to the more worldwide spread bread wheat (*T. aestivum*, $2n = 6x = 42$, ABD), durum wheat exhibits an exceptional adaptation to most booming and threatening climatic stresses, notably heat and drought. In a future perspective, this feature will be able to make durum wheat, a strategic crop and commodity for marginal land farmers in the Mediterranean environment [5], together with other few drought tolerant species [12,13]. Therefore, interventions aimed at maintaining and broadening the durum wheat genetic basis are highly required and beneficial to sustainably cope with current and forthcoming limitations to secure and safe yields [14].

Among advanced plant breeding strategies, chromosome engineering (CE) represents an effective approach to achieve genetic gains in wheat by resorting to its related gene pools, including those of the wild Triticeae species (reviewed in [14,15,16,17,18]). Through CE, chromosomal segments harbouring useful genes can be transferred from related (e.g., wild) genomes into those of cultivated wheats with high precision [19,20,21,22]. In most cases, CE-based transfers rely on

the promoting effect on pairing and recombination between corresponding, albeit not fully homologous (i.e., homoeologous), chromosomes of different Triticeae species exerted by mutations for wheat *Ph* (Pairing homoeologous) genes, mainly *Ph1* [23,24]. Whatever the intergenomic/interspecific cytogenetic relatedness, CE is inherently based on sexual gene transfer, hence representing an excellent non-GMO, yet non-conventional breeding option. The CE approach, in particular, offers a sustainable way to effectively use the still little exploited exotic genes from secondary and tertiary gene pools, and make them relevant for agriculture. Many genes have been so far transferred in wheat by means of CE, mainly disease resistance genes, but also genes for abiotic stress tolerance, grain quality and yield-related traits (reviewed in [14,16,25]). Nevertheless, a major impact on the breeding of genotypes created through CE has been rare so far, and mainly regarded hexaploid bread wheat, due to its bigger economic importance and better tolerance to chromosome manipulations with respect to tetraploid durum wheat. In the latter, phenomena such as sterility, reduced seed germination, segregation distortion, and anomalies of plant habit are more often observed than in the former upon alien transfers, with linkage drag more dramatically worsening with increasing segment size (e.g., [15,25,26,27,28,29,30,31]).

The transfer of alien chromatin in wheat through CE generally involves single segments deriving from one donor species targeting single wheat chromosomes. However, the possibility of combining, i.e., pyramiding, useful genes from different alien sources in a single genotype represents an appealing target, potentially enabling simultaneous enrichment of crop genotypes with a variety of novel, valuable features. This can be achieved either by “nesting” chromosome portions of different but closely related alien sources in a single alien segment of a given wheat recipient chromosome, or by stacking multiple alien segments on different wheat chromosomes. The former approach implies that the alien chromatin of primary and subsequent transfers shares homologous or homoeologous relationships, hence being capable of recombination. It was through homologous recombination between the 6RL arms of different rye (*Secale cereale* L.) cultivars, each inserted in wheat genetic backgrounds, that the *Pm20* powdery mildew resistance gene of one rye source was combined with residing genes of the other rye accession [32]. Several examples of recombination-based wheat-alien gene pyramiding involve group seven chromosomes of perennial grass species belonging to the *Thinopyrum* genus [15,16]. In bread wheat, “composite” alien segments, including chromatin from hexaploid *Th. intermedium* and decaploid *Th. ponticum* were generated on the 7DL arm, contributing the *Bdv2* BYDV resistance gene and the leaf rust (*Lr19*) and stem rust (*Sr25*) resistance genes, respectively [33,34]. Furthermore, two effective Fusarium head blight (FHB) resistance QTL, one from *Th. ponticum* accession “el₂” (*Fhb-7el₂* or *Fhb7*) and the other from diploid *Th. elongatum* (*Fhb-7EL*), were combined with *Lr19* and other valuable genes of the “el₁” accession of *Th. ponticum* onto the 7DL arm [35,36,37,38]. The same pyramiding of *Fhb-7el₂* or *Fhb-7EL* with *Lr19* and other 7el₁ genes was also realised in durum wheat, within a single *Thinopyrum* segment distally located on the 7AL arm [35,39]. Since the size of the alien block introgressed in all the quoted cases remains within tolerable amounts by the recipient genome, such recombinant genotypes represent promising materials for use in breeding and cultivation.

On the other hand, stacking multiple alien segments from more than one alien species within a single wheat genotype has more unpredictable outcomes in view of practical exploitation. Examples are limited to a few even for the more amenable hexaploid bread wheat genome. One such case is that of Singh et al. [40], who combined the widely exploited whole-arm 1RS:1BL translocation (1RS from rye, *S. cereale*; [41]) with the 7AgL (= 7el₁L) sizable translocation from *Th. ponticum* (named T4 or Agatha, reviewed in [16]) on the 7DL arm. In addition to the *Lr19*+*Sr25* rust resistance genes and yield-contributing genes of 7AgL derivation (see [16]), the 1RS arm was known to carry multiple disease resistance genes [42] and to determine positive effects on yield, both in bread wheat [41,43] and durum wheat [31]. Apart from the lateness defect and some grain yield penalty associated with the double translocation, its breeding potential, as of any 1RS:1BL translocation, was limited by the presence on 1RS of the secalin *Sec-1* locus, which negatively affects dough quality [44]. Another example of multiple alien segment stacking was reported by Ali et al. [45], who identified bread wheat lines with enhanced resistance to wheat streak mosaic virus (WSMV), due to the contemporary presence on wheat 4D and 1B chromosomes, respectively, of two short arm centric translocations, one from *Th. intermedium* (4Ai#2S), bearing the *Wsm1* gene, and the other from rye (1RS), probably carrying an enhancer of the WSMV resistance. Good field performance of the isolated recombinant and the absence of any meiotic instability associated with the alien chromatin presence, gave hope for their use in breeding programs [45].

A remarkable example of multiple alien introgressions is represented by the Chinese cultivar Xiaoyan 6, widely cultivated in the 1980s and 1990s and later used as a core parent for bread wheat breeding in China. In Xiaoyan 6, at least two wheat chromosomes (2A and 7D) carry chromosomal segments from *Th. ponticum*, with genes contributing tolerance to diseases and stressful environmental conditions, as well as good quality and yield stability [46]. Interestingly, Xiaoyan 6 derivatives, in which the rye 1RS arm or even the entire 2R chromosome were introduced in place of wheat 1BS and 2D, respectively, are cytogenetically stable, have additional disease resistances and beneficial agronomic attributes, including high seed-set, making them readily usable in production [47].

To our knowledge, only one case of multiple segment stacking can be recorded for durum wheat. This resulted from a successful attempt to combine in a single tetraploid genotype three different individual transfers, involving the 7AL, 3BS and 1AS wheat arms, each bearing homoeologous portions of *Th. ponticum* 7eL₁L, of *Aegilops longissima* (2n = 2x =14) 3S¹S, and of *Triticum aestivum* 1DS arms, respectively [48,49]. The individual transfer lines were selected among an array of wheat-alien recombinant types, obtained by *ph1*-induced homoeologous recombination, as bearing the respective target genes associated with alien segments of minimal length and exhibiting satisfactory agronomic performance. With all three alien segments being inserted at the most distal ends of the respective wheat arms, the *Th. ponticum* 7eL₁L portion spans 23% of the recombinant 7AL and harbours the *Lr19+Sr25* resistance genes, but also the *Yp* gene, increasing endosperm and semolina yellow pigmentation [49,50], along with several QTL enhancing yield-related traits [28,29,51]. The *Ae. longissima* 3S¹S segment contains *Pm13*, a highly effective resistance gene to powdery mildew, and replaces around 20% of the 3BS arm [26,52,53]. Finally, the 1DS chromosome segment derived from *T. aestivum*, harbours the *Gli-D1/Glu-D3* storage protein genes, and replaces 17% of durum wheat 1AS arm, containing the *Gli-A1/Glu-A3* homoeoloci [26,54,55]. Introduction of the *Gli-D1/Glu-D3* genes into the tetraploid context resulted in improved SDS, gluten index and dough strength (W) values, as well as in a good dough tenacity-to-elasticity (P/L) ratio, potentially suitable for both pasta and bread making [49,56]. Meiotic stability of the triple introgression line was shown not to be upset, and the simultaneous transmission of the three alien segments to be normal through both germplines [25,48]. Notwithstanding, the potential use in breeding and cultivation of any type of wheat-alien recombinant line depends upon its overall agronomic performance validated under field conditions. Some preliminary small-scale evaluation of the original durum wheat-triple alien recombinant, developed in the background of the Italian cv. Simeto, was previously carried out, and gave promising results for both yielding capacity and grain quality [49]. In our durum wheat pre-breeding program, we have transferred the three alien segments in different varietal backgrounds (Figure 1).

Figure 1. Field view of a high yielding triple recombinant of durum wheat (right), obtained through chromosome engineering, and its recurrent variety Karur (left).