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Registration of an hexaploid wheat translocation line carrying a short segment of chromosome 5A^m including softness genes *Pina* and *Pinb* from *Triticum monococcum*

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Abbreviations: CS= Chinese Spring

Running head: Translocation of *Triticum monococcum* softness genes

Abstract

Translocation line T5A^mS-5AS·5AL R#45 carries the distal region of *Triticum monococcum* L. chromosome 5A^mS translocated into the 5AS chromosome of *T. aestivum* L. cultivar Chinese Spring (Reg. No -----, PI 651012). This translocation line was developed in collaboration by the Instituto de Recursos Biológicos, INTA, Argentina and the University of California, Davis, USA. The distal *T. monococcum* 5A^mS segment in T5A^mS-5AS·5AL R#45 is approximately 8 cM long and carries the active softness genes *Pina* and *Pinb* in the genetic background of Chinese Spring. This translocation replaced the deleted *Pina* and *Pinb* puroindoline genes from *T. aestivum* by the functional orthologues from *T. monococcum*, significantly reducing the hardness of the grain. Translocation line T5A^mS-5AS·5AL R#45 has a shorter segment of *T. monococcum* chromosome 5A^m relative to previous translocations, minimizing linkage drag and the probability of negative effects associated with the introgression of alien genes.

Translocation line T5A^mS-5AS·5AL R#45 (Reg. No -----, PI 651012) was developed in collaboration by the Instituto de Recursos Biológicos, INTA, Argentina and the University of California, Davis, USA. This line carries a distal segment of *Triticum monococcum* L. chromosome 5A^mS translocated to the 5AS chromosome of *T. aestivum* L. cultivar Chinese Spring.

The translocated 5A^mS segment from *T. monococcum* is approximately 8 cM long and carries the active puroindoline genes *Pina* and *Pinb* in the genetic background of Chinese Spring. The objective of this translocation was to replace the deleted puroindoline genes from *T. aestivum* chromosome 5A by the functional orthologues present in *T. monococcum* chromosome 5A^m. The puroindoline genes are responsible for the softness of the grain and the addition of functional puroindoline genes was expected to reduce the hardness of the grain.

Triticum monococcum chromosomes do not recombine well with *T. aestivum* chromosomes (Dubcovsky et al. 1995; Luo et al. 2000) and, therefore translocated 5A^m

segments block or significantly reduce recombination in those regions. We induced homoeologous recombination by the *ph1b* mutation and reduced the length of the translocated 5A^m chromosome segment from 40 cM to 8 cM in T5A^mS-5AS·5AL R#45. This shorter segment is expected to reduce linkage drag and the risk of negative effects associated with the introduction of other alien genes.

Methods

Translocation line T5A^mS-5AS·5AL R#45 was derived from a backcross population where 5A/5A^m homoeologous recombination was induced by the *ph1b* mutation. The backcross population involved an F₁ between the Chinese Spring (CS) line carrying the *ph1b* mutation (Sears 1977) and the CS 5A/5A^m recombinant substitution line number 25, which carries a 40-cM distal chromosome segment from *T. monococcum* (Luo et al., 2000) including the *Ha* locus (*Pina-A^m1*, *Pinb-A^m1*, and *GSP-A^m1*). This F₁ was backcrossed to CS.

Molecular markers were used to construct a genetic map for the 5AS/5A^mS recombinant lines and to select the lines with the shortest 5A^m segment including the *Hardness* locus from *T. monococcum*. Recombinant 45 (R#45) has a distal translocation (approximately 8 cM) with a recombination event between the *XBggp* and *XBG606847* loci (Bonafede et al. 2007). This translocation replaced the deleted *Pina* and *Pinb* puroindoline genes from *T. aestivum* 5AS arm by the functional orthologues from *T. monococcum*. The recombinant line was self-pollinated and plants homozygous for the T5A^mS-5AS·5AL translocation and homozygous for the wild type *Ph1b* allele were selected with molecular markers.

Characteristics

Grain hardness was measured in 12 plants homozygous for the T5A^mS-5AS·5AL_R#45 line and 12 sister lines without the *T. monococcum* translocation grown in a completely randomized design in the greenhouse. Hardness was evaluated using 300 kernels per line and a Single Kernel Characterization System Model 4100 (Perten Instruments). Seeds from R#45 (scores 30.1 ± 0.7) were significantly softer (*P*<0.0001) than those from Chinese Spring (53.2 ± 0.9).

This result parallels the effect of the larger *T. monococcum* 5A^m segment on grain texture described before (Tranquilli et al. 2002), and demonstrates that the genes affecting hardness are still present in the reduced 5A^m segment. Therefore, the shorter *T. monococcum* segment present in T5A^mS-5AS·5AL can be a useful tool to reduce grain hardness in common wheat.

A high-throughput codominant marker for the *XBggp* locus, which is tightly linked to the *Pin* genes, can be used to select the *T. monococcum Ha* allele in marker assisted selection programs. However, it is worth to check the final homozygous lines with the *T. monococcum Pin* markers as a final confirmation for the transfer of the genes responsible for softer grains (Bonafede et al. 2007).

Availability

Seed samples of the stabilized R#45 line (homozygous for the *Ph* gene and *T. monococcum Ha* locus) have been deposited in the USDA-ARS National Small Grain

Collection P.O. Box307, Aberdeen, ID 83210, USA, and in the INTA Germplasm Bank – Castelar, Argentina. Small quantities can be obtained for research and breeding purposes from the curator of the National Small Grain Collection or from the senior author.

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