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**Registration of an hexaploid wheat translocation line carrying a short segment of chromosome 5A<sup>m</sup> 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<sup>m</sup>S-5AS·5AL R#45 carries the distal region of *Triticum monococcum* L. chromosome 5A<sup>m</sup>S 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<sup>m</sup>S segment in T5A<sup>m</sup>S-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<sup>m</sup>S-5AS·5AL R#45 has a shorter segment of *T. monococcum* chromosome 5A<sup>m</sup> relative to previous translocations, minimizing linkage drag and the probability of negative effects associated with the introgression of alien genes.

Translocation line T5A<sup>m</sup>S-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<sup>m</sup>S translocated to the 5AS chromosome of *T. aestivum* L. cultivar Chinese Spring.

The translocated 5A<sup>m</sup>S 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<sup>m</sup>. 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<sup>m</sup>

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<sup>m</sup> chromosome segment from 40 cM to 8 cM in T5A<sup>m</sup>S-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<sup>m</sup>S-5AS-5AL R#45 was derived from a backcross population where 5A/5A<sup>m</sup> homoeologous recombination was induced by the *ph1b* mutation. The backcross population involved an F<sub>1</sub> between the Chinese Spring (CS) line carrying the *ph1b* mutation (Sears 1977) and the CS 5A/5A<sup>m</sup> 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<sup>m</sup>1*, *Pinb-A<sup>m</sup>1*, and *GSP-A<sup>m</sup>1*). This F<sub>1</sub> was backcrossed to CS.

Molecular markers were used to construct a genetic map for the 5AS/5A<sup>m</sup>S recombinant lines and to select the lines with the shortest 5A<sup>m</sup> 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<sup>m</sup>S-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<sup>m</sup>S-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<sup>m</sup> segment on grain texture described before (Tranquilli et al. 2002), and demonstrates that the genes affecting hardness are still present in the reduced 5A<sup>m</sup> segment. Therefore, the shorter *T. monococcum* segment present in T5A<sup>m</sup>S-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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